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Comparative studies on the physicochemical properties of peanut protein isolate–polysaccharide conjugates prepared by ultrasonic treatment or classical heating

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ABSTRACT

Peanut protein isolate (PPI) was glycated with dextran or gum Arabic through ultrasonic treatment or classical heating. The physicochemical properties of PPI–polysaccharide conjugates prepared by ultrasonic treatment were compared to those prepared by classical heating. Compared with classical heating, ultrasound could accelerate the graft reaction between PPI and polysaccharides. PPI could glycate more dextran than gum Arabic under both ultrasonic treatment and classical heating. Despite the higher degree of graft, ultrasonic treatment was able to prepare PPI–polysaccharide conjugates with higher color lightness as well as lower yellow tones than classical heating. During glycation, high molecular weight component was formed, and conarachin mainly participated in glycation reaction instead of arachin. Solubility and emulsifying properties of the conjugates prepared through ultrasonic treatment were both improved as compared to conjugates obtained by classical heating and PPI. The solubility of PPI–dextran was improved for pH range 3 to 9, while that of PPI–gum Arabic was improved for pH > 7. Meanwhile, the emulsifying properties of PPI–gum Arabic were better than those of PPI–dextran conjugates. Decrease of lysine and arginine contents suggested these two amino acids attended the glycation between PPI and polysaccharides. Structural feature analyses suggested that conjugates obtained by ultrasonic treatment had less α-helix and more β-structures, higher surface hydrophobicity and less compact tertiary structure as compared to conjugates obtained by classical heating and PPI.

1. Introduction

Peanut is one of the most important oil crops in China. Low-temperature peanut meal is the by-product of cold pressed peanut oil, which is gradually popularized among consumers because of its light color and flavor. For cold pressed peanut oil production, the seed is peeled, degerminated, cold pressed at 60 °C and then filtrated. These steps would avoid the residual aflatoxin contained in the oil and meal, and protein in the low-temperature peanut meal is only slightly denatured compared with the protein in high-temperature peanut meal. However, low-temperature peanut meal is mainly used as animal feed and fertilizer due to its poor protein solubility and high residual oil ratio.

Various methods were developed to prepare peanut concentrates and isolates from peanut seeds. These products possessed much better properties compared with those of other legume seeds when used as ingredients in foods such as cereals and synthetic meat products (Basha & Cherry, 1976). Peanut protein isolate (PPI), extracted from peanut meal, has higher protein content (>85%), and better functional properties, such as emulsifying properties, foaming and gelling properties than other peanut protein products (Zhao, Liu, Zhao, Ren, & Yang, 2011). However, more research is needed to improve functional properties and understand physiochemical properties of PPI. Physical, chemical and enzymatic treatments have been done to improve PPI with better functional properties and better utilization in food industry (Dong, Zhao, Shi, et al., 2011; Dong, Zhao, Yang, et al., 2011; Hu, Zhao, Sun, Zhao, & Ren, 2011; Lawal, Adebowale, & Adebowale, 2007). Conjugating proteins with polysaccharides through glycation could improve the emulsifying properties, heat stability, antimicrobial activity and many other functional properties of proteins (Kato, 2002). Moreover, previous studies suggested that the allergenicity of proteins could be reduced drastically through glycation, and this reduction was correlated with the structural change of allergenic proteins (Nakamur et al., 2008; Nakamura et al., 2006; Yang, Li, Li, & Wang, 2013). Therefore, glycation has attracted much attention nowadays and could be one of the most promising approaches to produce peanut proteins applied in food formulations.

Generally, glycation of proteins is carried out by using either dry-heated treatment (heating the freeze-dried powder of protein–polysaccharide mixed solution at certain temperature and humidity for several days or weeks) or wet-heated treatment (heating protein–polysaccharide mixed solution at certain temperature) (Chevalier, Chobert, Popineau, Nicolas, & Haertlé, 2001; Kato, Mifuru, Matsudomi, & Kobayashi, 1992; Shepherd, Robertson, & Ofman, 2000). The use of

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ultrasound in food industry has increased recently due to its promising effects in food preservation and product modification. High energy ultrasound applications are usually found at intensities higher than 1 W/cm^2 and at frequencies between 18 and 100 kHz, which has been applied for degassing of liquid foods, the induction of oxidation/reduction reactions, extraction of enzymes and proteins, enzyme inactivation and the induction of nucleation for crystallization (Knorr, Zenker, Heinz, & Lee, 2004). Previous study compared ultrasonic treatment with classical heating in preparing soybean protein isolation and gum Arabic conjugation, and discovered that ultrasound could accelerate larger degree of the graft reaction (Mu et al., 2010). Moreover, researchers found that ultrasound, especially at higher intensities, could potentially be a means to promote the Maillard reaction in the glycine–maltose solution (Guan, Wang, Yu, Xu, & Zhu, 2010). However, there is little study on the effects of ultrasound on glycation reactions, and none of the researches was carried out regarding PPI glycation.

Dextran is widely used in protein glycation. Conjugates of whey protein and dextran exhibited improved solubility and thermal stability, and conjugates with soy protein isolate formed highly stable emulsions (Diftis & Kiosseoglou, 2006a; Jiménez-Castaño, López-Fandiño, Olano, & Villamiel, 2005). Gum Arabic, on the other hand, has strong ability to stabilize oil–water interface due to its high solubility, low viscosity, good surface activity, as well as the ability to form protective film around emulsion droplets (Chanamai & McClements, 2002). Previous study suggested that emulsifying properties of soy protein isolate could be significantly improved after glycating with gum Arabic (Xue, Li, Zhu, Wang, & Pan, 2013). However, there is little information available on the comparisons among different protein–polysaccharide conjugates.

In this research, the effect of ultrasonic treatment on the glycation reaction between PPI and polysaccharides was compared with that of classical heating. The effect of two different polysaccharides, dextran and gum Arabic, on the solubility and emulsifying properties of PPI–polysaccharide conjugates was compared. Secondary, tertiary and other structure features of conjugates were also compared.

2. Materials and methods

2.1. Materials and chemicals

Low-temperature peanut meal was provided by Shandong Guangda Lvyuan Food Technology Co., Ltd (Shandong, China). The protein concentration in the meal was 43.6%, residual oil ratio was 5.6%, and moisture content was 5.5%. Dextran (MW 40,000) and gum Arabic (MW 240,000–580,000) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). 1,8-anilinonaphthalenesulfonate (ANS) reagent was purchased from Sigma-Aldrich Chemical Company (St Louis, MO, USA). Bovine serum albumin (BSA), Folin & Ciocalteu's phenol reagent, o-Phthaldialdehyde (OPA), and other reagents were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Soybean oil was purchased from local supermarket and used without further purification.

2.2. Preparation of peanut protein isolate (PPI)

PPI was prepared according to the method of Liu, Zhao, Ren, Zhao, and Yang (2011) with slight modifications. Low-temperature peanut meal was extracted with *n*-hexane until the final fat content was less than 1%, then dispersed in deionized water (1:20 w/v), and the pH was adjusted to 8.0 with 2 M NaOH. The dispersions were stirred for 2 h at room temperature, and then centrifuged at 8000 ×*g* and 20 °C for 20 min. The supernatants were adjusted to pH 4.5 with 2 M HCl, and centrifuged again at 5000 ×*g* and 20 °C for 10 min. The precipitate was washed and re-dispersed in deionized water, and the pH was adjusted to 7.0 with 2 M NaOH. The dispersions were then freeze-dried (PPI). The PPI yield was at least 70% (g/100 g of protein in meal) and the protein content of PPI was 86.7% (g/100 g of powder), determined by Kjeldahl method (Kjeldahl factor: 5.46) (Quist, Phillips, & Saalia, 2009).

2.3. Preparation of PPI–dextran and PPI–gum Arabic conjugates

PPI (1%, w/v) and dextran (1%, w/v) were dispersed in phosphate buffer solution (0.2 M, pH 7.5). Then the slurry was treated by ultrasonic equipment (VCX 500, Sonics Vibra Cell, USA) with a probe of a 13 mm titanium tip. A temperature of 80 °C, an ultrasonic power of 200 W (150.76 W/cm^2), a time of 40 min and pulse mode (2 s on and 2 s off) were chosen for the ultrasonic treatment. Thereafter, the slurry was cooled to ambient temperature and dialyzed at 4 °C for 24 h. Finally, the sample was freeze-dried and stored at −20 °C. The same slurry was conducted by classical heating in a water bath at 80 °C for 24 h and the subsequent procedure was the same to ultrasonic treatment. Furthermore, the slurry treated by heating (40 min at 80 °C) without ultrasonic treatment was used as control (PPI–dextran mixtures). The preparation of PPI–gum Arabic conjugates through ultrasonic treatment or classical heating and PPI–gum Arabic mixtures was the same to the preparation of PPI–dextran conjugates and mixtures.

2.4. Degree of graft (DG)

Free amino groups were determined by an o-phthaldialdehyde assay (Vigo, Malec, Gomez, & Llosa, 1992). OPA of 40 mg was dissolved in 1 ml methanol and mixed with 25 ml of 10 mM sodium tetraborate, 2.5 ml of 20% (w/w) SDS, and 100 μl of β-mercaptoethanol, and the solution was diluted to a final volume of 50 ml with distilled water to form the OPA reagent. 4 ml of OPA reagent was added into 200 μl of sample solution (2 mg protein/ml) and incubated 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 $(A_0 - A_t) \times 100\% / A$, where A_0 are the levels of free amino groups in mixtures of PPI–polysaccharides; A_t are the levels of free amino groups in conjugates of PPI–polysaccharides and A are the levels of free amino groups in PPI.

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

SDS-PAGE was carried out on a Bio-Rad Mini-Protein Tetra Electrophoresis System (Bio-Rad Laboratories, Inc., Hercules, USA) with a 4% stacking gel and 12% separating gel. The protein content of all samples was 2 mg/ml. After electrophoresis, gels were stained for protein with Coomassie R250 dye (Yadav, Parris, Johnston, Onwulata, & Hicks, 2010).

2.6. Protein solubility

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 (Lowry, Rosembroug, Lewis, & Randall, 1951) using BSA as the standard, and protein solubility was expressed as grams of supernatants protein per 100 g of protein.

2.7. Emulsifying properties

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 the solution (1:3, v/v) were homogenized at 24,000 rpm for 1 min with an Ultra-Turrax homogenizer (Ika T18 Basic, Staufen, Germany). After homogenization, the emulsion of 50 μl 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