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Effects of high pressure on the physicochemical and functional properties of peanut protein isolates

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ABSTRACT

This study aimed to investigate the effects of high pressure (HP) on the physicochemical and functional properties of peanut protein isolates (PPI). The properties studied were surface hydrophobicity (H_0), contents of sulfhydryl group (SH) and disulfide bond (S–S), sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) characteristics, differential scanning calorimetric(DSC) characteristics, protein water-holding capacity (WHC), oil-binding capacity (OBC), and heat-induced gelling property. HP treatment from 50 MPa to 200 MPa for 5 min gradually increased the WHC and OBC, and significantly increased the H_0 ($p < 0.05$). The hardness of the heat-induced gelling increased by 50% after HP treatment at 100 MPa, but gradually decreased with further increased pressure. HP treatment at 50–200 MPa significantly increased the S–S content ($p < 0.05$) but decreased the SH content. The content of conarrachin II significantly changed with HP treatment in SDS-PAGE. These results suggest that HP treatment can be used to modify the properties of PPI at the appropriate pressure within a short time.

1. Introduction

Peanut is one of the main oil crops in China. Its planting area is the world's second, reaching about 4.55 million hm. in 2010. Its yield is also the world's highest, reaching 15.7 million tons and accounting for about 42% of the total yield worldwide, according to the statistical data of the Food and Agriculture Organization (http://faostat.fao.org). Peanut has become one of the few agricultural exports with international competitiveness in China. About 50–60% of peanuts grown in China are used to produce edible oil. The remaining meal, also called as defatted flour, is a protein-rich, inexpensive, and underutilized byproduct of peanut which contains approximately 50% high quality protein. Therefore, research on defatted peanut flour especially peanut protein is important to improve the added value of peanut (Wang, 2012, p. 26).

Peanut protein has high nutritional values, close to animal proteins, and contains no cholesterol (Zhou, Zhou, & Jiang, 2012, p. 7). Peanut protein not only contains many essential amino acids that are easily uptaken by the human body, but also possesses an attractive aroma and white color, which make it superior to soybean protein. Therefore, it is widely used in the food industry as a kind of plant protein resources. To optimize the application of peanut protein in food processing, many physical and chemical modifications have been applied to peanut protein. Food treated with high pressure (HP) is a brand-new high technology with bright prospects. High pressure (HP), also can be called the ultra high-pressure (UHP), and belong to the non-thermal processing techniques, using the fluid medium transmission pressure, can make the water, also can be oil, so they called high hydrostatic pressure (HHP). The mechanism of action is instantaneous and evenly throughout all parts of the material, neither depend on its size, shape, and composition of materials, also do not depend on the size, shape and composition of the package. In recent years, it has been widely used in the property modification of food proteins (Apichartsrangkoon, 2003; Ibanoglu & Karatas, 2001; Messens, Van Camp, & Huyghebaert, 1997; Molina, Defaye, & Ledward, 2002; Molina, Papadopoulou, & Ledward, 2001; Puppo et al., 2004, 2005; Torrezan, Tham, Bell, Frazier, & Cristianini, 2007; Wang et al., 2008; Zhang, Li, Tatsumi, & Kotwal, 2003). Treatment with HP reported changes the functional properties, such as protein emulsifying activities, solubility, and foaming, and the components of soybean protein, whey protein and lactalbumin (He, Liu, Liu, Hu, & Wang, 2013).

Recent studies have shown that HP treatment can change not only the functional characteristics of food proteins, but also their physical and chemical properties as well as molecular conformation (Kiffer & Schurer, 2007; Puppo et al., 2004; Zhang et al., 2003). However, only a few studies have investigated the modification of

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peanut protein by the HP technology (Zong & Chen, 2007, 2008). In these studies, mainly for its solubility and emulsifiability, didn't research gelling property, water-holding capacity and oil holding capacity, and the pressure used was high. In the meat products production, such as ham sausage production, mostly added a certain amount of plant protein, such as soybean protein, so as to improve the quality of products at the same time can save the cost, and in the application, it mainly used gelling property, water-holding capacity and oil holding capacity of protein etc. Therefore, this study main aimed to investigate the influences of HP (in the lower pressure range) on prior to freeze-drying on some physico-chemical and gelling property, water-holding capacity and oil holding capacity of peanut protein isolates (PPI) to optimize their application in the food industry.

2. Materials and methods

2.1. Preparation of peanut protein isolate

Defatted peanut flour was obtained from Gaotang Lanshan Co., Ltd. (Shandong province, China, and the content of protein was 45.90 ± 0.13%). PPI was prepared at room temperature as follows. Briefly, the defatted peanut flour was mixed with 10-fold (w/v) deionized water with 1 N sodium hydroxide (Sinopharm Chemical Reagent Co., Ltd., Beijing, China) at pH 9.0. The proteins were then extracted with stirring with 150 rpm for 2 h at room temperature and centrifuged at 3500 *g* for 10 min. The supernatant was adjusted to pH 4.5 with 2 N hydrochloric acid (HCl, Beijing Chemical Factory, China), centrifuged at 3500 *g* for 10 min, and discarded. The precipitants were then thoroughly stirred with deionized water and freeze dried until further use. For HP treatment, the protein concentration was adjusted to 43.23 g/L (concentration obtained through preliminary experiments, in preliminary experiments, changes in the hardness of the heat-induced gelling of various protein concentration (4.323, 8.65, 43.23, 86.46, 129.69, 172.92 g/L) treated with high pressure have been studied, and results shown the hardness of the heat-induced gelling of 4.323, 8.65, 86.46, 129.69, 172.92 g/L protein (99.43–128.13 g) were smaller than that of 43.23 g/L. So 43.23 g/L protein concentration was studied in this paper), and the content of protein was 86.46 ± 0.09%, which was determined by micro-Kjeldahl method using a nitrogen conversion factor of 5.46, the contents of ash, moisture, crude fat and total carbohydrate were 3.35 ± 0.25%, 4.12 ± 0.13%, 0.60 ± 0.08% and 5.47 ± 0.10%, respectively, which were determined using AOAC methods (AOAC, 1990, 1996), and the control samples (un-treated) were prepared by freeze-drying the PPI without HP treatment.

2.2. High pressure processing

HP treatment was carried out in a 0.6 L reactor unit (HPP.L2-600/2 ultra HP systems, Huatai Sen Miao Biological Engineering Technology Co., Ltd., Tianjin, China) equipped with temperature and pressure regulators. Water was used as the pressure transmitting medium in the vessel. Prior to pressure processing, 5% (w/v) (concentration obtained through preliminary experiments, in preliminary experiments, changes in the hardness of the heat-induced gelling of various protein concentration (0.5%, 1%, 5%, 10%, 15%, 20% w/v) treated with high pressure have been studied, and results shown the hardness of the heat-induced gelling of 0.5%, 1%, 10%, 15%, 20% w/v protein (99.43–128.13 g) were smaller than that of 5% w/v. So 5% w/v protein concentration was studied in this paper) of PPI solutions with a suitable volume were vacuum-conditioned in a polyethylene bag. The PPI solutions were then subjected to HP treatment at 50, 80, 100, 150, and 200 MPa for 5 min (pressure fluctuation range ± 10 MPa), and its temperature was kept at 25 ± 2 °C during processing. After HP treatment, the PPI solutions were freeze-dried.

2.3. Preparation of peanut protein isolate gels

The preparation of PPI gels followed the method by Wu, Wang, Ma, and Ren (2009), with some modifications. About 14% (w/v) of PPI solutions for untreated and HP-treated samples (50–200 MPa) were heated for 1 h at 95 °C and cooled immediately in a cold water bath. The heated samples were stored at 4 °C for 24 h until test.

2.4. Gelling property

To evaluate the texture of PPI gels, a uniaxial compression test was performed with a TA-TX2i texture analyzer (Stable Micro System Ltd., Godalming, England) according to the procedure of Guo et al. (2005) and Pinterits and Arntfield (2008) with some modifications. A spherical plunger (12 mm) was utilized. The samples were compressed to 50% at a rate of 0.1 mm/s. The trigger point was 100 g. The resulting data were interpreted using Texture Expert Version 1.22 analysis software (Stable Micro System Ltd., Godalming, England). The program produced a force–displacement curve. Two parameters, the springiness and hardness of the material, were determined.

2.5. Water-holding capacity and oil-binding capacity

WHC of samples was determined as described by Beuchat (1977). The sample (1.000 g) was placed in a centrifugal tube, weighed, added with 10 mL of distilled water, and mixed using a Fisher Gene II vortex at the highest speed for 5 min. After the mixture was thoroughly wetted, the samples were allowed to stand at room temperature for 30 min and centrifuged at 3000 *g* for 20 min. The supernatant was decanted and the centrifuge tube containing the sediment was weighed. The WHC (gram of water per gram of protein) was calculated as: $WHC = (W_2 - W_1)/W_0$, where W_0 is the weight of the dry protein (g), W_1 is the weight of the tube plus protein samples (g), and W_2 is the weight of the centrifuge tube with the sediment (g).

The OBC was determined following the method of Chakraborty (1986). The sample (1.000 g) was placed in a centrifugal tube, weighed, added with soybean salad oil (obtained from a commercial supermarket in Beijing, Beihai COFCO Grain and oil industry (Tianjin) Co. Ltd., China) of 5 mL, and mixed for 5 min using a vortex mixer. The samples were allowed to stand for 30 min. The protein–oil mixtures were then centrifuged at 3000 *g* (Feige Centrifuge 4500 R) for 20 min. The supernatant of salad oil was decanted and the centrifuge tube containing the sediment was weighed. The OBC (gram of oil per gram of protein) was calculated as: $OBC = (W_2 - W_1)/W_0$, where the symbols have the same meaning as those in the WHC formula.

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

SDS-PAGE was performed following the method of Wang and Zou (2004, pp. 100–120) as well as Laemmli (1970). For sample preparation, the protein samples (0.1000 g) were dissolved in 20 mL of phosphate-buffered saline (PBS; 0.01 M, pH 7.2), mixed for 10 min using a vortex mixer, and centrifuged at 10 000 *g* for 10 min. Up to 10 μL of supernatant were then added with 10 μL of sample buffer (0.08 M Tris–HCl buffer, pH 6.8), 1% (w/v) SDS, 2% (v/v) 2-β-mercaptoethanol, 5% (v/v) glycerol, and 0.025% (w/v) bromophenol blue and mixed well. The samples were then heated in a boiling water bath for 5 min and cooled down before 8 μL of samples were

added, and commercial molecular weight markers covering the range of molecular weights from 14 400 to 97 200 were used (Sigma, St. Louis, MO, USA). Electrophoresis was conducted with tank buffer (0.025 M Tris, 0.192 M glycine, pH 8.6–8.7) at 80 mA until the blue reached the separation gel into 110 V. The gels were stained with 0.25% coomassie brilliant blue (R-250) for 30 min and destained by acetic acid solution with high concentration of methanol (methanol/glacier acetic acid/deionized water = 227:37:236 (v/v/v)) overnight for 12 h. Images for the protein electrophoresis spectrum were taken using an AlphaEase FC gel imaging system.

2.7. Contents of sulfhydryl group and disulfide bond

The contents of SH and S–S were determined according to the methods of Beveridge, Toma, and Nakai (1974) and Luo, Luo, and Wu (1986) with some modifications. PPI powder (75 mg) was mixed with 1 mL of 0.086 M Tris-glycine buffer (pH 8.0) on a magnetic blender, and then 4.7 g of guanidine hydrochloride were added and the volume was maintained to 10 mL with the buffer.

To measure the content of SH, 4 mL of 8 M urea–5 M guanidine hydrochloride solution (8 M urea plus 5 M guanidine hydrochloride solution) and 50 μL of Ellman's reagent (4 mg/mL) were added to 1 mL of the above solution. The absorbance of mixture was measured at 412 nm in an ultraviolet–visible spectrophotometer (Beijing BeiFen TinanPv Science Instrument Co., Ltd., China) and a solution without protein was used as blank control.

To measure the content of S–S, 15 μL of β-mercaptoethanol and 4 mL of 5 M urea–8 M guanidine hydrochloride solution were added to 1 mL of the above solution. The mixture was kept at 25 °C for 1 h and then 10 mL of 12% trichloroacetic acid (TCA) was added. The mixture was kept again at 25 °C for another 1 h and centrifuged at 4200 *g* for 10 min. The precipitate was added three times with 5 mL of 12% TCA, dissolved in 10 mL of 8 M urea, and added with 40 μL of Ellman's reagent (4 mg/mL). The absorbance was measured at 412 nm and a solution without protein was used as blank control.

The content of SH was calculated as: SH (μM SH/g) = $73.53\ A_{412} \times D/C$, where A_{412} is the absorbance at 412 nm, D is the dilution coefficient, and C is the sample concentration (mg (dry protein)/mL). The content of S–S was calculated as: S–S (μM S–S/g) = $(Q_1 - Q_2)/2$, where Q_1 and Q_2 are the content of SH before and after deoxidation, respectively.

2.8. Surface hydrophobicity

H_0 of PPI was determined according to the method of Kato and Nakai (1980) using 1-anilinonaphthalene-8-sulfonic acid (ANS) as the fluorescence probe. Protein solution (1 mg/mL) was prepared in PBS (0.01 M pH 7.0). After treatment at 20 °C for 2 h, the mixture was centrifuged at 10 000 *g* for 20 min. Protein concentration of the supernatant was determined with Folin phenol protein quantitation method. The obtained supernatants were diluted to 0.15, 0.075, 0.038, and 0.019 mg/mL. About 20 μL of ANS reagent (8.0 mM in the same buffer) were added to 4 mL of the protein solutions. Fluorescence intensity was measured with a Hitachi F2500 fluorescence spectrometer (Tokyo, Japan), at wavelengths of 390 nm (excitation) and 490 nm (emission). The initial slope of fluorescence intensity versus protein concentration plot was used as an index of H_0.

2.9. Differential scanning calorimetry

DSC experiments were performed on a TA Q200-DSC thermal analyzer (TA Instruments, New Castle, NANO) according to the method described by Meng and Ma (2001). Briefly, 2.0 mg of protein samples were accurately weighed into aluminum liquid pans and 10 μL of 0.1 M PBS (pH 7.0) were added. The pans were then hermetically sealed and heated in a calorimeter from 20 to 120 °C at a rate of 10 °C/min (Wang et al., 2008; Yin, Tang, Wen, Yang, & Li, 2008). Peak or denaturation temperature (T_d), width at half peak height of endothermic peak ($\Delta T_{1/2}$), and enthalpy change of denaturation (ΔH) were computed from the thermograms by the universal analysis 2000 software (Universal V4.5A, TA Instruments-waters LLC). In all cases, the sealed pans containing protein samples and buffers were equilibrated at 25 °C for more than 2 h.

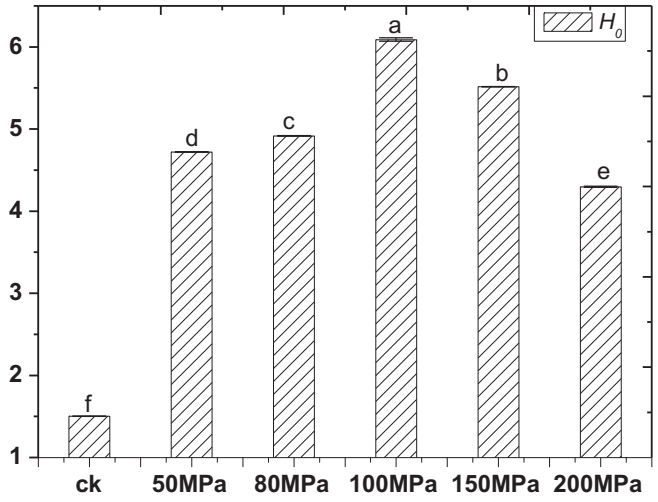

Fig. 1. H_0 of untreated (0.1 MPa) and HP-treated (50–200 MPa) PPI dispersions. Different characters (a–f) on top of a column indicate significant ($p < 0.05$) differences among samples treated under different levels.

2.10. Statistical analysis

Data collected from three replicate experiments were used for the analysis. Parameters determined include average, standard deviation, and least significant difference test with SPSS 18.0. A level of $p < 0.05$ was used to indicate significant differences.

3. Results and discussion

3.1. H_0 analysis

The fluorescence emission spectra of ANS (a polarity-sensitive fluorescent probe) were used to detect the index of the H_0 for untreated and HP-treated PPI samples. Given its extreme sensitivity to environmental changes, ANS is also very sensitive to the conformational changes of protein molecules (Yin, 2009, pp. 89–90; Yin, Xiang, & Tong, 2010). HP treatment increased H_0 significantly ($p < 0.05$) after the pressure was increased from 50 to 200 MPa compared with the untreated control (Fig. 1). These results indicated that the molecules of fluorescent aromatic amino acids were mostly located in the internal area of the protein and surrounded by a variety of non-polar amino acid residues in the untreated PPI. Therefore, the polarity of the local environment was weaker than that of the external water solution and the H_0 was low. However, the proteins were denatured after HP treatment (50–200 MPa). The side chains of aromatic amino acid molecules were gradually exposed to aqueous solution. The increasing environmental polarity increased the H_0 index (Yin et al., 2010). At treatment of 100 MPa, the H_0 index was largest, which inferred that the highest amount of the hydrophobic groups of the peanut proteins were exposed to the outside and the protein structure was the more relaxed conformation control other PPI samples. Puppo et al. (2004)

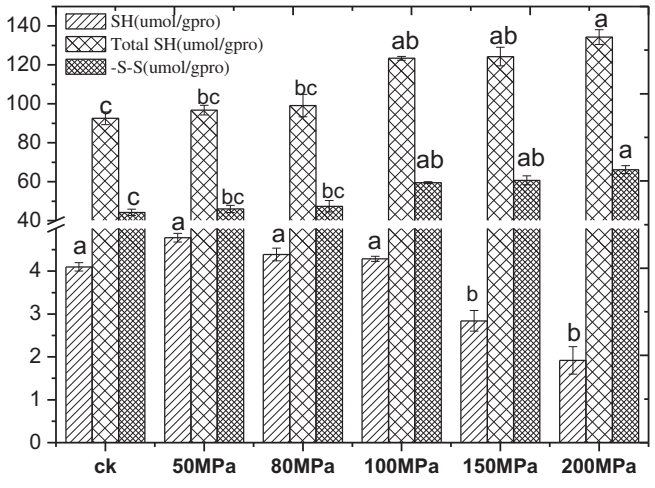

Fig. 2. SH content, total SH content, and −S−S content of untreated (0.1 MPa) and HP-treated (50–200 MPa) PPI dispersions. Different letters on top of a column indicate significant ($p < 0.05$) differences among samples treated under different levels.

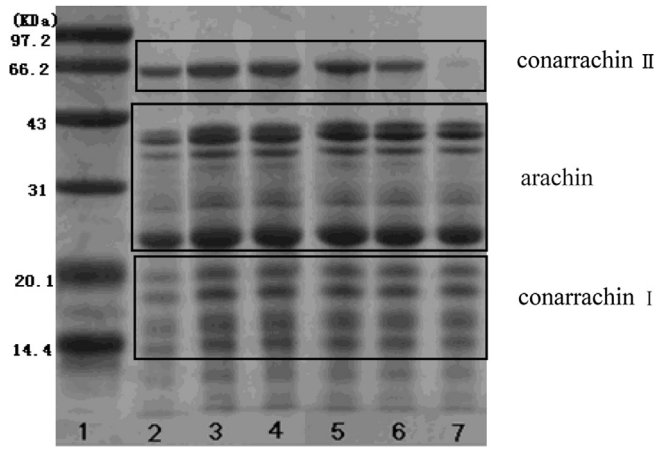

Fig. 3. Conarrachin II, arachin, and conarrachin I of untreated (0.1 MPa) and HP-treated (50–200 MPa) PPI dispersions. 1 − marker, 2 − 0.1 MPa, 3 − 50 MPa, 4 − 80 MPa, 5 − 100 MPa, 6 − 150 MPa, 7 − 200 MPa.

and Wang et al. (2008) used 1% soybean protein isolate (SPI) for HP treatment at 200–600 MPa, and showed that SPI treated at 400 MPa exhibited the maximum H_0 value. After treatment over 100 MPa, the H_0 index of PPI gradually dropped, which indicated that the structure of peanut protein isolates was not stable after pressure treatments. Molina et al. (2001) used 10% SPI solutions for HP treatment, and explained that H_0 value decreased of SPI due to partial denaturation and subsequent aggregation of hydrophobic groups. The unfolded protein aggregated with the increasing pressure. Based on the changes between the H_0 indices of the untreated and HP-treated PPI samples, the HP can lead to the conformational change of PPI.

3.2. Contents of SH and S–S

S–S and SH groups are important functional groups in peanut protein molecules. They belong to weak secondary bonds and maintain the tertiary structure of the proteins. Their changes reflect the degree of protein denaturation (Tian & Du, 2007). Therefore, they have crucial influences on the protein functional properties. The SH and S–S bonds in PPI samples were significantly increased by HP treatment (Fig. 2). With increased pressure, the total SH content (including SH and reduction of disulfide bond) in PPI gradually increased after HP treatment (50–200 MPa). Compared with the control, SH contents decreased after the PPI were treated at 150 and 200 MPa, which indicated that significant changes occurred in the PPI conformations at HP. PPI were unfolded and the buried cysteine residues in PPI molecules were exposed. However, SH content in PPI decreased gradually above 100 MPa. This result might be attributed from the formation of S–S bonds between SH group with other groups. Therefore, also gradually increased the content of S–S bonds in PPI at elevated pressure (Wu, 2010, pp. 24–25; Yin, 2009, pp. 89–90). Kiffer and Schurer (2007) found that the SH content increases in the protein molecules of wheat gluten after treatment above 200 MPa. Zhang et al. (2003) showed that the SH content in the soybean protein rapidly increases after treatment at more than 200 MPa, but tends to decrease after treatment beyond 500 MPa. Puppo et al. (2004) observed the content of SH of SPI (pH 8) decreased as the pressure increased, while that of SPI (pH 8) began to decrease at pressures higher than 200 MPa. These results showed that though the HP facilitated the exposure of SH group in protein molecules, the unstable structure of protein molecules caused reactions between exposed SH and other groups in protein molecules. These results showed that though the HP facilitated the exposure of SH group in protein molecule, the unstable structure of protein molecule caused reactions between exposed SH and other groups in protein molecule.

3.3. SDS-PAGE

Fig. 3 shows the SDS-PAGE results of untreated and HP-treated PPI samples. The bands of every subunit in PPI corresponding to the molecular weight of 61.0, 40.5, 37.5, 35.5, 23.5, 18.0, 17.0, and 15.5 kDa. The band at 61.0 kDa belongs to the chaperon of conarrachin II; the bands at 40.5, 37.5, 35.5, and 23.5 kDa belong to arachin; and the bands at 18.0, 17.0, and 15.5 kDa belong to the chaperon of conarrachin I (Prakash & Rao, 1986). The solubility of un-treated and 50, 80, 100, 150, 200 MPa treatment were 73.88 ± 0.24%, 65.20 ± 0.23%, 65.45 ± 0.24%, 65.88 ± 0.24%, 65.03 ± 0.24%, 64.35 ± 025%, respectively, which were determined using the method of Wang et al. (2008). No significant difference for the bands of the chaperon subunits of conarrachin I in PPI was observed between the HP-treated and untreated PPI samples, which indicated that HP has no significant effect on the chaperon of conarrachin I. However, the intensity of the bands for the subunits of arachin increased after treatment with different pressures, which indicated that HP leads to the aggregation of arachin. Given that the β-mercaptoethanol used in SDS-PAGE can break the S–S in PPI, the appearance of the strips for aggregates demonstrated the formation of covalently cross-linked complexes without S–S. However, the intensity of the subunit band at 61.0 kDa showed a trend of increasing at first and then decreasing with the increasing pressure. This also indicated that the contents of conarrachin II had the similar changed trend with HP treatment and suggested proteins in PPI aggregated at the beginning and stretched later with the increasing pressure. Combined with the solubility and DSC results also inferred that the chaperon of conarrachin II was much more sensitive to pressure changes than peanut globulin and the chaperon of conarrachin I.

3.4. Gelling property

Fig. 4 shows the hardness and springiness of the heat-induced gels with the untreated PPI and treated PPI at 50 MPa–200 MPa. The springiness of gel was not improved after the pressure treatment of PPI (Fig. 4). However, the hardness of the gel significantly

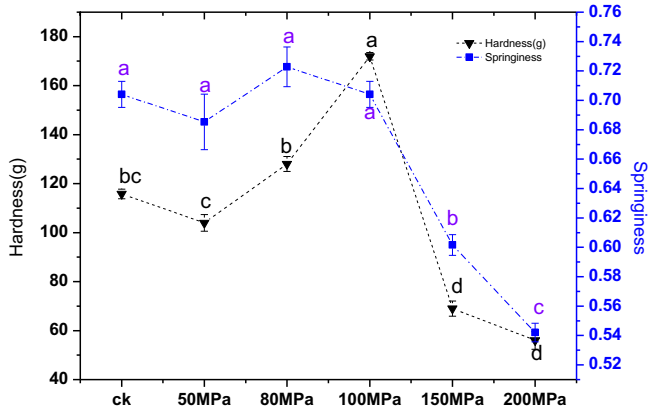

Fig. 4. Hardness and springiness of heat-induced gelation of untreated (0.1 MPa) and HP-treated (50–200 MPa) PPI dispersions. Different letters on top of a column indicate significant ($p < 0.05$) differences among samples treated at different levels.

increased, and showed a trend of increasing at first and then decreasing with increased pressure. The maximum hardness was achieved at 100 MPa, when the hardness of treated PPI showed about 50% of increase compared with the control. The hardness decreased significantly after treatment at 150 MPa. Because the pressure used in this study was relatively low, make it easier and more energy conservation for this condition applied to practical application. Peanut protein isolates treated at >200 MPa were not the study point as the heat-induced gelling property would be worse when treated at >200 MPa as showed in pre-study. Modification of functional properties of peanut protein isolates was attributed to the changes of peanut protein isolates resulted from HP treatment. Particularly, change in surface hydrophobicity (H_0) clearly proved there was change in conformation of peanut protein isolates. There were reports that change in surface hydrophobicity (H_0) of plant proteins may change its emulsifying activities (Wang et al. 2008; Yin et al. 2008). In this study, the change trends of both heat-induced gelling property and surface hydrophobicity (H_0) of peanut protein isolates were similar and reached the peaks in 100 MPa. In sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) suggested conarrachin II was more pressure-sensitive. Yang, Chen, and Zhao (2001) studies indicated that conarrachin II was less heat-sensitive, and given its change trend of heat-induced gelling property, it was safe to conclude that change in conarrachin II was one of the major factors contributed to

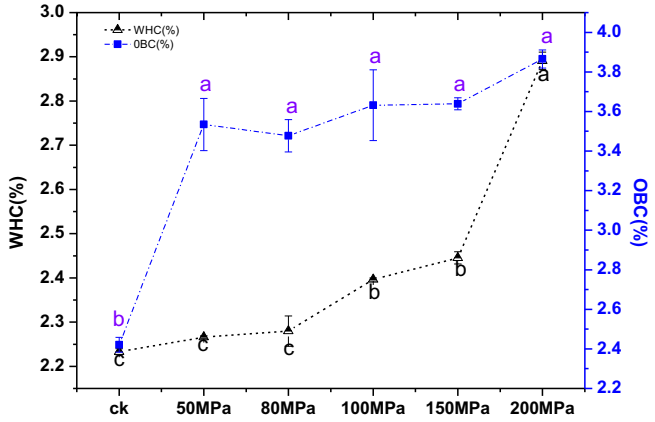

Fig. 5. WHC and OBC of untreated (0.1 MPa) and HP-treated (50–200 MPa) PPI dispersions. Different letters on top of a column indicate significant ($p < 0.05$) differences among samples treated at different levels.

the modification of functional properties of peanut protein isolates. Studies showed disulfide bond (S–S) played a critical role in formation of protein gelling, and disulfide bond could change with sulfhydryl group (SH) mutually (Molina et al., 2002). Through the structure analysis of PPI, the increased gel hardness resulted from the stretching of PPI after HP treatment, which made the PPI structure loose and more hydrophobic groups were exposed, which improved their gelling property. There were many factors including sample protein characteristics, concentration, heating temperature, heating time, pH, ion capacity and cooling condition that may affected the formation of gelling (Alvarez, Ramaswamy, & Ismail, 2008; Catsimpoolas and Meyer, 1990; Damodaran, 1988; Nakamura, Utsumi, & Mori, 1986; Petrucelli and Anon, 1994), so, even the functional properties of peanut protein isolates have been improved after high pressure treatment, the feasibility of application of modified peanut protein in food industries still need to be verified out of laboratory.

3.5. WHC and OBC

PPI are mainly applied as food supplementary materials to ham sausages. The properties of the plant proteins utilized mainly include the gelatin formation, WHC, and OBC. Poor functional characteristics limit the application of PPI. However, their functional properties were significantly improved through the modification by HP treatment. As shown in Fig. 5, both the WHC and OBC of PPI were increased after the HP treatment. They both tended to increase with the increasing pressure. After treatment with different pressures (50, 80, 100, 150 and 200 MPa), the WHC increased by 1.48%, 2.09%, 7.33%, 9.51%, and 29.46%, and the OBC increased by 43.65%, 45.98%, 50.01%, 50.31%, and 59.66%. The improvement in the OBC was more obvious than that of the WHC. The results indicated that the OBC of PPI after treatment at 50–200 MPa were remarkably higher than the commercial soybean protein isolates. However, although the WBC of HP-treated PPI improved significantly compared with the natural PPI, the improvement was much lesser than that of commercial soybean proteins. As for the rigidity of the gels formed, that of PPI after high-pressure treatment was significantly higher than that of commercial soybean proteins (the commercial soybean protein isolates obtained from Wandefu Co., Ltd. in Shandong province, China, and its WHC, OBC, hardness of heat-induced gel were 2.33 ± 0.03 g/g, 6.27 ± 0.47 g/mL, 97.59 ± 6.88 g).

3.6. DSC

Table 1 and Fig. 6 shows the DSC scanning results of untreated and HP-treated PPI samples. The untreated PPI showed two peaks of heat absorption at 93.53 and 107.25 °C, which correspond to the

Table 1
DSC characteristics of untreated (0.1 MPa) and HP-treated (50 MPa–200 MPa) PPI dispersions.

PPI sample	DSC characteristics			
	T_{d1} (°C)	T_{d2} (°C)	$\Delta T_{1/2}$ (C)	ΔH (J/g)
Control	93.53 ± 0.27^{a}	107.25 ± 0.09^{a}	6.35 ± 0.12^{a}	8.780 ± 0.58^{a}
50 MPa	93.4 ± 0.27^{a}	106.84 ± 0.01^{c}	6.86 ± 0.52^{a}	7.2 ± 0.74^{b}
80 MPa	93.01 ± 0.65^{a}	107.09 ± 0.0^{9b}	6.74 ± 0.20^{a}	6.99 ± 0.61^{c}
100 MPa	92.44 ± 0.31^{b}	105.74 ± 0.75^{d}	6.4 ± 0.07^{a}	6.44 ± 0.32^{d}
150 MPa	–	106.73 ± 0.22^{c}	6.33 ± 0.15^{a}	6.08 ± 0.5^{e}
200 MPa	–	107.09 ± 0.01^{b}	6.16 ± 0.02^{a}	6.05 ± 0.12^{e}

T_{d1}, T_{d2}, $T_{1/2}$, and ΔH indicate the peak temperature of conarrachin, peak temperature of arachin, width at half peak height of the peak for arachin, and enthalpy change of arachin at the peak, respectively. "–" means undetected. Different characters (a–e) of columns indicate significant ($p < 0.05$) differences among samples treated under different levels.

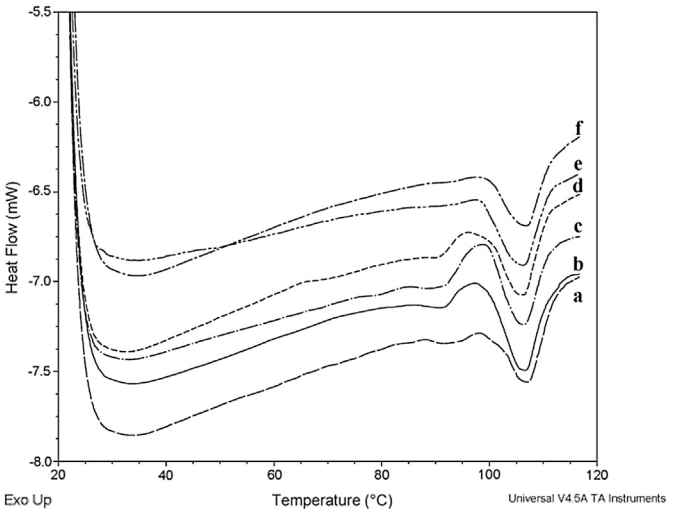

Fig. 6. DSC thermograms of PPI samples. (a) 0.1 MPa, (b) 50 MPa, (c) 80 MPa, (d) 100 MPa, (e) 150 MPa, (f) 200 MPa.

peaks for the chaperon of conarrachin (T_{d1}) and arachin (T_{d2}), respectively. As shown in Table 1, the temperature of the peak for the chaperon of conarrachin gradually decreased with the increasing pressure (50–100 MPa), while the peaks were invisible for the treatment at 150 and 200 MPa. The results demonstrated significant denaturation of the chaperons of conarrachin after the HP treatment. The structure of the protein became relaxed, and complete denaturation occurred at 150 MPa when the peak of heat absorption became invisible. On the contrary, the arachin was more stable than the chaperon of conarrachin and the peak was still visible after HP treatment. However, the temperature for the peak of heat absorption changed with treatments at different pressures. The minimum temperature was obtained after treatment at 100 MPa, which was 105.74 and 1.51 °C less than that of the control. These results showed that HP treatment had different effects on the chaperons of arachin and conarrachin.

Arntfield and Murray (1981) demonstrated that the total enthalpy (ΔH) of soybean globulins and their β-chaperon proteins, which represented the ratio of natural proteins or proteins with ordered structure, was significantly influenced after the HP treatment. The ΔH of PPI after the pressure treatment was lower than that of the control, and it gradually decreased with the increasing pressure. This result indicated that the conformation of the PPI changed with the elevated pressure. The PPI became more stretched and the three-dimensional structure was more disordered, which made the PPI more unstable, which reduced the needed enthalpy of the native proteins. The peaks of heat absorption for the chaperons of conarrachin disappeared after treatment above 150 MPa, while those for arachin remained visible. This result indicated that the chaperons of conarrachin were more liable to denature than arachin. The half-width of the peak for the arachin was related to the coordination of the switch between the natural and denatured states of the proteins (Privalov, 1982). As shown in Table 1, no difference was observed for the half-width after HP treatment at 50 MPa–200 MPa ($p > 0.05$). This result indicated that the pressure treatment did not change the switch coordination during peanut protein denaturation.

4. Conclusions

HP treatment at 50–200 MPa significantly improved water-holding capacity (WHC), oil-binding capacity (OBC) of peanut protein isolates, but in most cases, it does not improve heat-induced gelling property. The PPI reached maximum hardness of heat-induced gel after treatment at 100 MPa. Treatment with different pressure resulted in different on the proteins in aggregation, stretching and refolding extent of protein molecules, and more hydrophobic groups were exposed in the surface proteins molecules after treatment at 100 MPa. Additionally, HP treatment could result in intensity denaturation of conarrachin II. These changes seemed to largely account for the influence of HP treatment on the functional properties of PPI.

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Appendix. List of symbol and acronyms

PPI	peanut protein isolate
HP	high pressure
H_0	surface hydrophobicity
SH	sulfhydryl group
S–S	disulfide bond
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
DSC	differential scanning calorimetric
WHC	water-holding capacity
OBC	oil-binding capacity

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