



Effects of high hydrostatic pressure treatments on haemagglutination activity and structural conformations of phytohemagglutinin from red kidney bean (*Phaseolus vulgaris*)

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ABSTRACT

Red kidney beans were subjected to high hydrostatic pressure (HHP) treatment (50, 150, 250, 350, 450 MPa) and phytohaemagglutinin (PHA) was then extracted by affinity chromatography. It appeared that HHP treatment could increase crude extract yield and decrease its haemagglutination activity. For purified samples, PHA yield was not affected at pressures <450 MPa while the haemagglutination activity was noticeably reduced at 450 MPa. The structural changes were investigated using electrophoresis, size exclusion chromatography (SEC), Fourier transform infrared (FTIR) and differential scanning calorimetry (DSC). Electrophoresis and SEC profiles revealed a new high molecular weight polymer after 450 MPa treatment. At pressures <450 MPa, FTIR showed an increase in β -sheet structure and a decrease in α -helix. At 450 MPa, the bands at 1688 cm^{-1} , representing aggregate strands and random coils, increased. The conclusions are that pressures <450 MPa can cause PHA unfolding and induce PHA aggregation at 450 MPa.

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1. Introduction

Legumes are widely consumed and are a major food source worldwide. One reason for this is based on the nutritional benefits derived from their high quality proteins, high fibre and low fat. (Peñas, Gomez, Frias, Baeza, & Vidal-Valverde, 2011; Sathe, 2002). Today many bean products are available and many have been associated with health-promoting properties, including anti-tumour activity, reduced risk of obesity, less hypercholesterolemia, as well as reduced coronary heart disease. In spite of these benefits that bean-rich diets offers, anti-nutritional factors such as legume lectins may also have a negative dietary impact.

Lectins are carbohydrate-binding proteins of non-immune origin possessing at least one non-catalytic domain that agglutinate cells or precipitate glycoconjugates (Blake & Goldstein, 1980). Members of the legumes are notably rich in lectins and the content of lectin in kidney bean is higher than in any other beans (Rüdiger & Gabius, 2001). Phytohaemagglutinin (PHA), the lectin extracted from red kidney beans (*Phaseolus vulgaris*), is a tetrameric combination of two subunits, lymphocyte-specific (L) and erythrocyte-specific (E) (Leavitt, Felsted, & Bachur, 1977). The anti-nutritional effects of PHA are manifest by a loss of epithelial resistance and

resistant to digestive enzymes, occasionally leading to an acute gastroenteritis (Liener, 1994), and PHA can sometimes be lethal if ingested at high concentrations (Lis & Sharon, 1998). Therefore, additional processing methods must be used to treat raw beans to make them safe to eat.

The traditional processing step to control the toxicity of PHA is cooking, but PHA is partially resistant to thermal denaturation and therefore beans need to be cooked for a long time (Shi, Xue, Kakuda, Ilic, & Kim, 2007). However, the prolonged thermal treatment will decrease the nutritional content and require more energy. High hydrostatic pressure (HHP), a promising new technology with outstanding advantages including a reduction in nutritional and flavor loss and shorter processing time, has been widely applied in the food industry after its first use by Hite (1899) for the treatment of milk. Numerous reports have discussed the functionality of proteins and carbohydrates in food treated with high hydrostatic pressure, such as the aggregation of proteins (Wang et al., 2011; Yin, Tang, Wen, Yang, & Li, 2008). These changes seem to be related to a HHP-induced disordering of the protein's three dimensional structures that are held together by various non-covalent interactions (Kieffer, Schurer, Kohler, & Wieser, 2007). To date, few studies have reported the effect of HHP on the conformation of lectin.

Thus, the aim of this work was to investigate the effects of HHP treatment at different pressure levels on the structural conformations (analysed by SEC and FTIR) and haemagglutination activity and thermal behaviour (DSC) of PHA.

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2. Materials and methods

2.1. Materials

Dried red kidney beans were purchased from a local supermarket in Guangzhou, China. Red blood cells in buffer (Human group B, glutaraldehyde treated) were obtained from Sigma–Aldrich (St. Louis, MO, USA). The trypsin (2×10^3 U/g) was purchased from Sanye Biochemistry Co. (Hangzhou, Zhejiang, China). Affi-gel blue gel and dialysis membrane (D36 mm, 6000–8000 Da) were obtained from Bio-Rad Laboratories (Shanghai) Co. Ltd., (Shanghai, China). Low molecular weight protein markers were purchased from Dinguo Biotechnology Co. Ltd., (Shanghai, China). All other chemicals used in the present study were of analytical or higher grade.

2.2. High hydrostatic pressure (HHP) treatment

The beans were soaked in 10 mM Tris–HCl buffer (pH 7.4, 1:10, w/v) at 4 °C for 6 h to soften the beans, and then homogenised in a blender (National Co. Ltd., Osaka, Japan). The homogenate was divided into six aliquots, placed in double polyethylene sealable bags, and then vacuum sealed in a DZ-280/2SD multi-functional vacuum packaging machine (Jinqiao Technology Electronic Equipment Manufacturing Co. Ltd., Dongguan, Guangzhou, China), leaving no headspace. The vacuum sealed packages were subjected to HHP-treatment at ambient temperature in a high-pressure vessel (KEFA Hitech Food Machine Co. Ltd., Baotou, Neimenggu, China) with a filling volume of 5 L. Oil was used as the pressure transmitting medium. Untreated samples of homogenate were used as a control (atmospheric pressure 0.1 MPa at ambient temperature). HHP-treatment samples also in triplicate were performed at 50, 150, 250, 350 and 450 MPa for 15 min holding time. The rates of pressure increase and release were about 100 MPa/min and 200 MPa/min, respectively. After the HHP treatment, the contents in the plastic bag were stored at 4 °C for 36 h before extraction of crude PHA.

2.3. Extraction and purification of PHA

After sitting for 36 h at 4 °C, the homogenates were filtered through four-layers of gauze and then centrifuged in a CR22G high-speed centrifuge (Hitachi Co. Tokyo, Japan) at 8000g for 30 min at 4 °C. The supernatants were lyophilized and the protein concentration of lyophilized samples was determined by Bradford assay in 96-well microtiter plate (Bio-Rad Protein Assay).

The affinity chromatography purification on an Affi-gel blue column was applied according to the method of Ye, Ng, Tsang, and Wang (2001) with some modifications. The lyophilized samples at 10% (w/v) were dissolved in 10 mM Tris–HCl buffer (pH 7.4) and then the entire sample loaded on an Affi-gel blue gel column (2.5×20 cm) which had been equilibrated with the same buffer. The column was then washed with 10 mM Tris–HCl buffer (pH 7.4) until the unbound proteins were removed. The adsorbed proteins were eluted with 1.4 M NaCl dissolved in 10 mM Tris–HCl buffer (pH 7.4) and then dialyzed against double distilled water at 4 °C for 36 h with changes at approximately 4 h intervals. Fractions were lyophilized and preserved in a desiccator for further analyses within 3 months. The protein concentration of these lyophilized fractions was determined by Bradford assay.

2.4. Haemagglutination assay

The suspension of red blood cells (type B) were incubated with 2 U/mL trypsin reagent mixed with 10 mM potassium phosphate buffer (pH 7.4) (1:1, v/v) for 15 min at 37 °C and then centrifuged at 2500g for 10 min at 37 °C. The precipitate was suspended in

10 mM potassium phosphate buffer (pH 7.4) followed by centrifugation and washing with the same buffer three times to obtain a 2% (w/v) suspension of red blood cells which were pipette into a 96-well microtiter V-plate (Dinguo Biotechnology Co. Ltd., Shanghai, China) for the haemagglutination assays. The lyophilized fractions of crude and purified samples were dissolved in the phosphate buffer containing 0.15 M NaCl to obtain a concentration of 6 mg/mL. A 25 μ L aliquot of each sample was serially diluted two-fold, and 25 μ L of blood were added to each of the wells. The agglutination was determined visually after incubation at 37 °C for about 40 min. The erythrocytes in the negative control (phosphate buffered saline and blood cells) had fully sedimented by then and formed a small dot at the bottom of the well. The haemagglutination titer was defined as the reciprocal of the highest dilution exhibiting haemagglutination. This was assigned a value of one haemagglutination unit (Khan, Ahmad, & Khan, 2007).

2.5. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and native-PAGE

A 12% acrylamide separating gel and a 5% acrylamide stacking gel containing 0.1% SDS were used in an electrophoresis apparatus (Bio-Rad Laboratories, California, USA) to separate the PHA constituents according to the method of Laemmli (1970) with slight modifications. The lyophilized red kidney bean PHA samples were dissolved in 60 mM Tris–HCl buffer (pH 8.8), containing 2% SDS, 5% 2-mercaptoethanol, 25% glycerol and 0.1% bromophenol blue. The solutions were heated in boiling water for 5 min and then centrifuged at 10,000g for 3 min prior to application to the electrophoresis gel. Fifteen microliter of each sample was loaded onto a gel lane. A cocktail of protein standards containing rabbit phosphorylase b (97.4 kDa), bovine serum albumin (66.2 kDa), rabbit actin (43.0 kDa), bovine carbonic anhydrase (31.0 kDa), and trypsin inhibitor (20.1 kDa) was used for MW determination. After electrophoresis the gel was stained with 0.25% Coomassie Brilliant Blue-R250 in 50% trichloroacetic acid and then destained in methanol/acetic acid/water (1:1:8, v/v/v).

The assay for native-PAGE was according to the method of Shi and Jackowski (1998). The main differences from the Laemmli assay were the absence of SDS and 2-mercaptoethanol in the sample buffer and no thermal treatment before electrophoresis.

2.6. Size exclusion chromatography (SEC)

The SEC experiment was performed using a Waters HPLC 600 system (Waters, Division of Millipore, Milford, MA, USA) fitted with a TSK-GEL G4000SW column (0.75×60 cm, Tokyo, Japan). The PHA samples at 1% (w/v) were dissolved in 50 mM phosphate buffer (pH 7.2) containing 0.2 M NaCl (1%, w/v), and then filtered with 0.45 μ m membranes (Millipore, Billerica, MA, USA). The following chromatographic conditions were applied: (1) injection volume, 20 μ L; (2) elution rate, 1 mL/min; (3) elution solvent: 50 mM phosphate buffer (pH 7.2) containing 0.2 M NaCl; (4) elution time: 32 min. The UV absorbance was recorded at 280 nm and the data were collected and analysed using Breeze software (Waters).

2.7. Fourier transform infrared (FTIR) spectroscopy

FTIR spectroscopy was carried out on a Nicolet 8210E FTIR spectrometer (Nicolet, Madison, WI, USA) equipped with a deuterated triglycine sulphate detector. The spectrometer was continuously purged with dry air from a Balston dryer (Balston, Janesville, IA, USA). One mg of sample was mixed with 100 mg KBr and ground gently with an agate pestle and mortar under an infrared lamp, and then pressed into a 13 mm diameter disc by applying 15 tons

Table 1

The yield and haemagglutination activity of the crude extract from red kidney bean and the PHA purified by Affi-gel blue gel.

Sample	Yield (g protein/ 100 g bean meal) ^a		Haemagglutination titer (U) [‡]	
	Crude extraction	Purified by Affi-gel blue gel	Crude extraction	Purified by Affi-gel blue gel
0.1 MPa	9.95 ± 0.08 ^a	1.23 ± 0.04 ^a	2 ³	2 ⁵
50 MPa	10.24 ± 0.11 ^b	1.17 ± 0.04 ^a	2 ³	2 ⁵
150 MPa	10.26 ± 0.18 ^b	1.21 ± 0.02 ^a	2 ³	2 ⁵
250 MPa	10.41 ± 0.18 ^b	1.16 ± 0.09 ^{ab}	2 ²	2 ⁵
350 MPa	10.75 ± 0.07 ^c	1.04 ± 0.06 ^b	2 ²	2 ⁵
450 MPa	11.15 ± 0.10 ^d	0.89 ± 0.10 ^c	2 ¹	2 ⁴

^a Expressed as means ± standard deviation of triplicate measurements. Means with different letters within a column are significantly different ($P < 0.05$).

[‡] Haemagglutination titer (U) was the reciprocal of the maximum dilution of the samples that gave visible agglutination.

pressure for 2 min. The spectrum for each sample was recorded in absorbance mode from 400 to 4000 cm^{-1} at a resolution of 2 cm^{-1} with 128 co-added scans. A straight baseline passing through the ordinates at 1700 and 1600 cm^{-1} was adjusted as an additional parameter to obtain a best fit. The curve-fitting procedure was carried out by stepwise iterative adjustment towards a minimum root-mean-square error of the different parameters determining the shape and position of the absorption peaks. It was performed by assuming an initial mixed Gaussian line-shape function, with a full width band at half-height (fwhm) of 11 cm^{-1} . Base line corrections, normalisation, derivation and curve fitting were analysed by the PeakFit Version 4.12 software (SPSS Inc., Chicago, IL, USA), and Origin 8.0 (OriginLab Corp., Northampton, MA, USA) software.

2.8. Differential scanning calorimetry (DSC)

The thermal behaviour was determined using DSC measurements performed on a TA Q100-DSC thermal analyzer (TA Instruments, New Castle, DE, USA). The calorimeter was calibrated using an indium standard. The dry PHA samples were accurately weighed (1.5–2 mg), placed into coated aluminium pans, and then 10 μL of 10 mM phosphate buffer (pH 7.2) was added and the pans hermetically sealed. Before the measurements the pans were equilibrated at 4 °C for at least 4 h. The pans were heated in the calorimeter from 25 to 120 °C at a heating rate of 5 °C/min. An empty pan was used as the reference. Denaturation temperature (T_d) and denaturation enthalpy (ΔH) were obtained from the thermograms using the Universal Analysis 2000 software, Version 4.1D (TA Instruments-Waters LLC). All experiments were conducted in triplicate.

2.9. Statistical analysis

Data processing was performed with one-way analysis of variance (ANOVA), using the SPSS Version 13.0 for Windows (SPSS Inc.). Significant differences between means ($P < 0.05$) were identified using the least significant difference (LSD) test.

3. Results and discussion

3.1. The yield and haemagglutination activities of crude extract

The ability of these samples to haemagglutinate is based on their capacity to bind to red blood cells and form a PHA-erythrocyte-PHA matrix, which prevented erythrocytes from sedimenting to the bottom of the well. The effect of different HHP treatments (50, 150, 250, 350, and 450 MPa) on the yield and haemagglutination activities of the crude extract of red kidney beans is shown in Table 1. A slight but significant ($P < 0.05$) increase of the crude extract yield was observed with increasing pressure from 50 to 450 MPa, showing that the HHP treatment contributed to the dissolution of the intracellular proteins within red kidney beans, as

proved by Butz, Koller, Tauscher, and Wolf (1994) who found that a pressure of 100 MPa was enough to cause rupture of intracellular vacuoles and plant cell walls in onions. In terms of haemagglutination activity, the titer remained constant (2³ U) as the pressure increased from 50 to 150 MPa, decreased to 2² U at 250 MPa and finally reduced to 2¹ U at 450 MPa. There were two possible factors that responsible for the decrease in haemagglutination activity. One factor could be a pressure induced extrusion of both PHA and non-PHA proteins through the cell wall with a selective increase in the level of non-PHA protein. This would make the crude extraction yields higher but will not increase the haemagglutination activity. The second factor could be an HHP induced disruption of the native three dimensional structure of PHA. This native structure is partially associated with the haemagglutination activity of lectin molecules (Khan et al., 2007; Kieffer et al., 2007).

3.2. Purification of PHA by affinity chromatography

Affi-gel blue gel is a beaded, cross-linked agarose gel with covalently attached sulphonated triazine dye (Cibacron Blue F3GA dye), which functions as an ionic, hydrophobic, aromatic, or sterically active binding site in various applications, and has been applied to the isolation of red kidney bean lectin (Ren et al., 2008). Moreover, Shi et al. (2007) reported that the breakdown products of the PHA formed during thermal processing still bind to the Affi-gel blue gel. As shown in Table 1, the yield of purified PHA was about one tenth of the original crude sample and the haemagglutination activity was significantly increased, indicating that the purification processing by affinity chromatography effectively enriched the PHA samples by removing large amounts of non-PHA components. The sample that underwent 450 MPa pressure treatment had the lowest yield after purification, which was probably due to the fact that the PHA structure was irreversible disrupted and could not absorb to the affinity gel. The decrease in the yield of purified PHA when the pressure was higher than 250 MPa, indicates that the HHP treatment was just improving the yield of non-PHA proteins. As for haemagglutination activity, it remained constant when the pressure increased from 50 to 150 MPa, the same phenomenon that was observed with the crude samples. When the pressure increased from 150 to 250 MPa or even to 350 MPa, the haemagglutination activity of the purified PHA did not change (remained at 2⁵ U), but the corresponding crude sample decreased by one titer. The reason could be that purification processing removed part of non-PHA proteins which has played an enrichment effect. Furthermore, the haemagglutination activity decreased at 450 MPa, probably linked to the partial denaturation of PHA treated at relative high pressure.

3.3. SDS- and Native-PAGE analysis

SDS-PAGE was underutilised to explore the subunit changes to HHP treated PHA samples. Fig. 1(A) shows the electrophoretic

patterns of 5 HHP treated PHA samples and one untreated PHA sample. Each sample has a major band with a molecular weight of about 31 kDa which corresponds to the basic subunit of PHA as previously reported (Ren et al., 2008). The HHP-treated samples revealed two additional bands, one with low intensity located between 43 and 66.2 kDa and another around 20.1 kDa, suggesting that they were probably formed under the high hydrostatic pressure. This result is consistent with a previous study with raw soybean seeds, confirming that HHP treatment is able to induce the formation of new protein bands (Peñas et al., 2011).

Native PAGE patterns shown in Fig. 1(B) did not detect any differences between the native and the HHP-treated samples when the pressure was less than 450 MPa. However, at 450 MPa, an additional band appeared in the high molecular weight fraction, probably due to the aggregation of proteins. Grácia-Juliá et al. (2008) have reported a similar result when they examined the effect of dynamic high pressure on whey protein.

3.4. SEC assay

SEC was used to better understand the changes to the conformation of the native proteins that could not be seen in the electrophoresis gel (Fig. 1). As shown in Fig. 2, there were two major peaks

referred to as P1 and P2 in the profile of the untreated PHA with elution times of 18.4 and 22 min, respectively. From a comparison with the marker profile (data not shown), the molecular weight of P1 was about 126 kDa, possibly corresponding to a tetramer of PHA. For the HHP treated samples, a small, gradual decrease was seen for P1 as the pressure increased. At the same time, the low molecular weight fraction P2 (<75 kDa) disappeared. A possible explanation for these phenomena is that low molecular weight proteins were more sensitive to HHP treatment. Shi et al. (2007) found the PHA in the intact beans was more stable suggesting that components in the kidney bean could partially protect the lectin. Furthermore, a small new peak with a larger molecular weight, was formed at 150 MPa, and was even more predominant at 450 MPa suggesting aggregation of the unfolded PHA, or the aggregation of unfolded PHA with other low-molecular-weight proteins through hydrophobic interactions and other weak bonds (Tang, Wang, Yang, & Li, 2009). However, Tang et al. (2009) found that soy protein isolate (SPI) initially aggregated at a low pressure (200 MPa) and then dissociated into smaller aggregates at pressures higher than 400 MPa. The discrepancy between our result and that report could be related to differences in the structural characteristic of the proteins and the HHP treatment conditions used in each study.

3.5. Secondary structure

The conformational changes in the secondary structures of the PHA samples were monitored by FTIR spectroscopy to gain information on the transformational processes and the structures evolving with varying levels of complexity. Amide I bands, at $1700\text{--}1610\text{ cm}^{-1}$, are principally attributed to C=O stretching vibrations with some N-H bending and to a small extent to C-H stretching vibrations (Byler & Susi, 1986). The stacked deconvoluted amide I bands of the FTIR spectrum of PHA are shown in Fig. 3. Nine major bands associated with the conformation of proteins were observed in the amide I bands, after deconvolution.

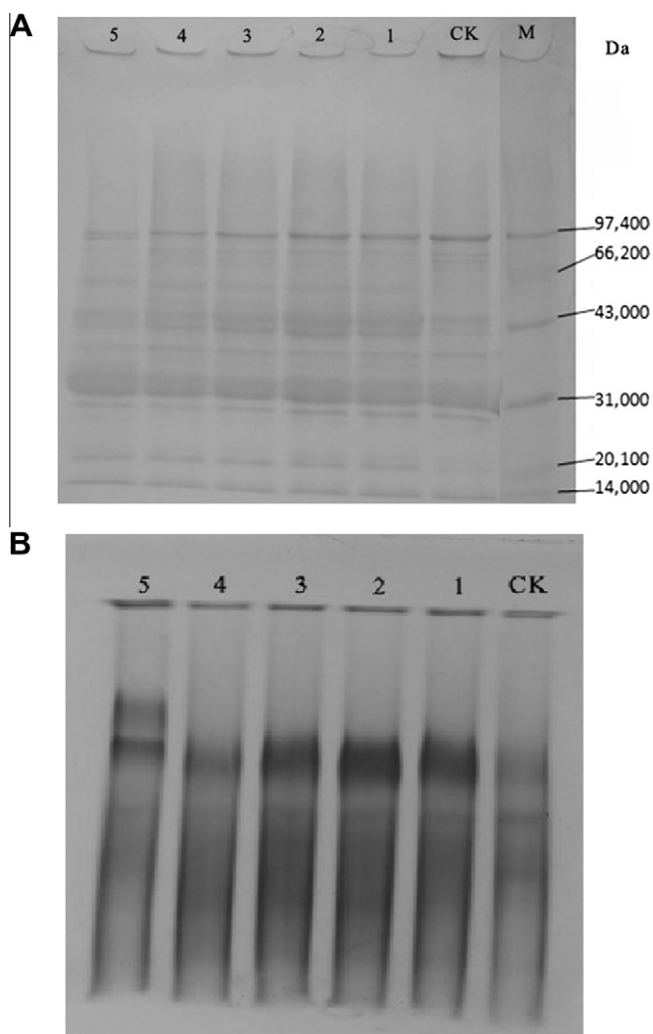


Fig. 1. SDS-PAGE (A) and native-PAGE (B) profiles of HHP treated samples (Lane 1, 50 MPa; Lane 2, 150 MPa; Lane 3, 250 MPa; Lane 4, 350 MPa and Lane 5, 450 MPa), native (Lane CK) and molecular markers (Lane M).

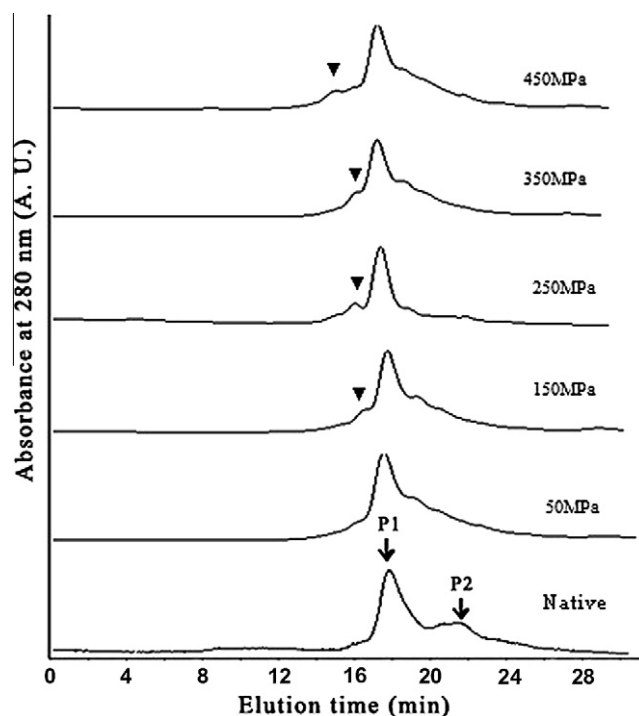


Fig. 2. HPLC elution profiles of native (0.1 MPa) and HHP treated samples. The symbol "▼" within the figure indicates the newly appeared polymer.

These major bands can be assigned as follows: 1694 and 1662 cm^{-1} , β -type turn and bend; 1688, 1676, 1635, 1628 cm^{-1} , β -sheet; 1653 cm^{-1} , α -helix; 1643 cm^{-1} , unordered structures (random coils), 1611 cm^{-1} , aromatic ring vibration of tyrosine residues (Law, Choi, & Ma, 2008). Compared with the native PHA, an intense IR band appearing around 1635 cm^{-1} , corresponding to intermolecular parallel β -sheet structures (Boye, Ismail, & Alli, 1996; Byler & Susi, 1986), accompanied with an obvious red shift (about 2 cm^{-1}) occurred at 50 MPa. This indicates a HHP-induced unfolding of protein structure (Lefèvre & Subirade, 2001). There was no significant difference in the total integrated intensity below 450 MPa, suggesting that the unfolding process dominated the structural changes after HHP treatment (50–350 MPa). This type of structural change is in agreement with the molten globule state which is defined as a protein structural state intermediate between the ordered and fully unfolded states, that usually retains most of the secondary structures associated with the ordered secondary state and also forms some internal hydrophobic interactions and other weak bonds (Yang, Dunker, Powers, Clark, & Swanson, 2001). According to Reddy, Srinivas, Ahmad, and Surolia (1999), the molten globule-like state of peanut lectin monomer retains its carbohydrate specificity. In the present work, PHA was found to keep its haemagglutination activity at low pressure treatment, which is in good accordance with the report of Reddy et al. (1999). However, the band at 1688 cm^{-1} , attributed to aggregate strands (Wang et al., 2011), was not seen at lower pressures, but was observed at 450 MPa. It may be possible that the unfolded proteins have undergone a re-association of their secondary structures. Moreover, this formation of new aggregates was followed by an increase in the relative amount of random coil (about 1643 cm^{-1}). Based on the decrease of haemagglutination activity at 450 MPa, these results suggest that a perturbed structure of PHA could hamper the interaction between lectin and carbohydrate ligands, which agrees well with studies performed with some other lectins (Gaikwad & Islam Khan, 2003; Khan et al., 2007).

3.6. Thermal behaviour

Endothermic effects seen with DSC thermograms are mainly attributed to the disruption of intra and intermolecular bonds. The denaturation peak temperature (T_d) can be used to monitor the thermal stability of protein, and the enthalpy value (ΔH) is correlated with the proportion of undenatured protein or the extent of

Table 2

Effect of HHP treatment on thermal properties of PHA.

Sample	T_d ($^{\circ}\text{C}$) ^a	ΔH (J/g) [‡]
0.1 MPa	96.13 $\pm$ 0.03 ^a	3.16 $\pm$ 0.05 ^a
50 MPa	96.07 $\pm$ 0.03 ^a	2.69 $\pm$ 0.01 ^b
150 MPa	95.81 $\pm$ 0.16 ^b	2.58 $\pm$ 0.03 ^c
250 MPa	96.65 $\pm$ 0.27 ^c	1.70 $\pm$ 0.14 ^d
350 MPa	96.33 $\pm$ 0.11 ^a	1.32 $\pm$ 0.02 ^e
450 MPa	99.36 $\pm$ 0.04 ^d	2.81 $\pm$ 0.01 ^f

Values are means $\pm$ standard deviation of triplicate measurements. Means with different letters within a column are significantly different ($P < 0.05$).

^a T_d ($^{\circ}\text{C}$), thermal denaturation temperature.

[‡] ΔH (J/g), enthalpy change of endotherm.

ordered protein structure (Arntfield & Murray, 1981). A prominent endothermic peak was observed in the DSC profiles of all native and HHP treated samples (data not shown). It clearly indicated that the native structure of PHA had not been completely lost with HHP treatment, although changes in secondary structure were evident (monitored by FTIR spectroscopy). As shown in Table 2, the T_d in the DSC profiles decreased as the pressure increased to 150 MPa compared to the native PHA, while a further slight increase was seen at 250 MPa and then decreased at 350 MPa. In addition, a progressive decrease in enthalpy value (ΔH) from 3.16 to 1.32 J/g with increasing pressure from 50 to 350 MPa indicated HHP induced unfolding of undenatured protein (Arntfield & Murray, 1981; Wang et al., 2011). However, at 450 MPa, it appeared a significant increase in ΔH value and T_d , suggesting an increase in ordered structure and an increase in the protein's thermal stability. It could be speculated that higher pressure may induce rearrangements and aggregation, as previously shown by electrophoresis, SEC and secondary structure analysis.

4. Conclusions

It was found that HHP treatment at 50 MPa was sufficient to improve the yield of dissolvable proteins in red kidney bean, while it had little effect on the amount of extracted PHA. Based on the haemagglutination analyses, a pressure increase up to 450 MPa produced a noticeable decrease in the haemagglutination activity of PHA. Considering the results of electrophoresis, SEC, FTIR and DSC analyses, it is likely that low pressure (<450 MPa) leads to unfolding of protein (FTIR and DSC data) and subsequent aggregation at 450 MPa (electrophoresis, SEC, FTIR and DSC data). These structural changes induced by HHP treatment appears to be have considerable influence on PHA haemagglutination activity. More studies are needed to focus on the effect of HHP on the oligosaccharide chains of PHA.

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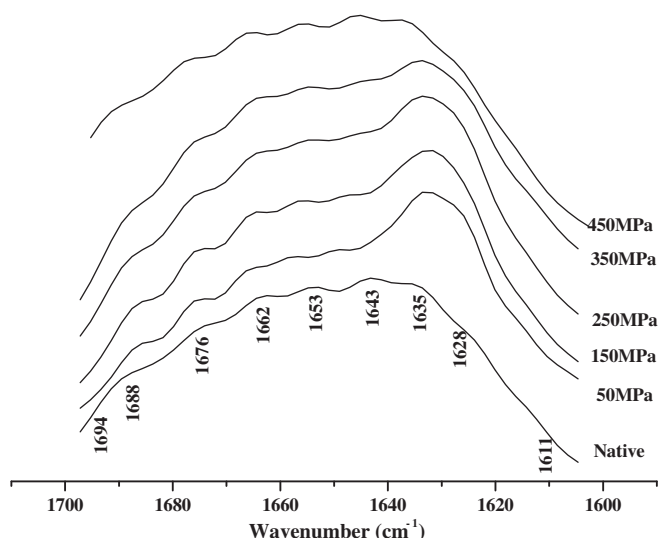


Fig. 3. Deconvoluted FTIR spectra of native (0.1 MPa) and HHP treated samples.

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