



Effect of high pressure treatment on functional, rheological and structural properties of kidney bean protein isolate



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ABSTRACT

Impact of high pressure (HP) treatment (200, 400 and 600 MPa for 15 min) on the functional, structural and rheological properties of red kidney bean protein isolate (KBPI) was investigated. HP treatment significantly influenced the water holding capacity, foaming, emulsifying properties, at above 600 MPa. FTIR spectroscopy traced the changes in the secondary structure (β -sheets) with the pressure treatment. Oscillatory rheological measurement of the HP-treated KBPI dispersions showed a significant increase in the G' and the G'' with increasing frequency, and a distinct thermal denaturation temperature (T_d). DSC measurement showed a slightly higher T_d values (105 °C) than rheometry. SDS-PAGE profiles revealed that the HP had a little effect on the protein structure when samples were treated below 600 MPa. The potential of plant-derived proteins have increased tremendously, and the developed KBPI could be used as one of the functional and nutritional ingredients in the food industry.

1. Introduction

Among legumes, kidney bean (*Phaseolus vulgaris* L.) is one the important produce, and is consumed as a cheap protein source in many developing countries. Kidney beans contain 20–30 g/100 g protein on a dry weight basis with a balanced amino acid composition; nonetheless, it is low in sulfur-containing amino acids, namely, methionine and tryptophan (Sathe, Iyer, & Salunkhe, 1981). Epidemiologic studies have revealed that consumption of kidney beans has several health benefits including the reduction of the risk of cardiovascular diseases, type II diabetes, obesity, and several/specific types of cancer (Dueñas, Martínez-Villaluenga, Limón, Peñas, & Frias, 2015).

Phaseolin or vicilin or 7-8S globulins, is the major storage protein from the kidney bean, and it constitutes about 75 and 82 g/100 g of the total seed proteins (Yin, Tang, Wen, Yang, & Yuan, 2009). It is an oligomeric protein, comprising of three polypeptide subunits namely α -, β -, and γ -phaseolin, and the molecular weight distribution ranged between 43 and 53 kDa (Romero, Sun, McLeester, Bliss, & Hall, 1975). Interestingly, vicilin present in kidney beans shows the unique structural peculiarity by low susceptibility to trypsin digestion and greater subunit homogeneity, than other vicilin components (Jivotovskaya, Senyuk, Rotari, Horstmann, & Vaintraub, 1996; Yin, Tang, Wen, Yang, & Li, 2008). Because of the unique peculiarity of vicilin, the kidney bean protein isolate (KBPI) exhibits a good gelation and emulsifying ability compared to other bean isolates (Kimura et al., 2008; Tang,

2008). Additionally, KBPI have potential to be applied as an excellent food functional ingredient in various food formulations, in particular, baking, meat, and extruded products.

High-pressure (HP) treatment – a novel processing technology is commonly employed for the protein gelation purpose as an alternative of heat processing. Application of HP denatures proteins, and forms gel. Influence of HP treatment on functionality and in vitro trypsin digestibility of KBPI has been reported (Yin et al., 2008). Soy protein forms gel at and above 300 MPa by rupturing noncovalent interactions within protein molecule, and it was found that the produced gel is relatively softer than the thermally treated gel (Ahmed, Ayad, Ramaswamy, Alli, & Shao, 2007; Dumoulin, Ozawa, & Hayashi, 1998). The gel rigidity during pressure-assisted gelation, is mostly, measured through the evolution of the complex viscosity or by measuring the elastic modulus, G' . However, HP-induced changes in rheological and structural properties of KBPI has not been investigated so far.

The objective of this work was to study the effect of high-pressure treatment (200, 400, and 600 MPa) on the physicochemical, functional, thermal, rheological, and structural properties of freeze-dried kidney bean protein isolate.

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2. Materials and methods

2.1. Materials

Red kidney beans (*Phaseolus vulgaris*), cultivated in India (Cv. VL Rajma 125) were procured locally. The beans were ground using a laboratory mill (Quadrumat Junior, Brabender, Germany) followed by passing through a 297- μ m sieve to obtain uniform particle size. The screened flour samples were packed in air-tight plastic containers, and stored until further use.

2.2. Preparation of kidney bean protein isolates

The kidney bean protein isolate (KBPI) was prepared following the method described by Fan and Sosulski (1974), with a minor modifications. The kidney bean flour (100 g) was dispersed in distilled water (1000 mL), and the pH of the dispersion was adjusted to 8.0 with 0.5 mol equi/L NaOH. The dispersion was continuously stirred followed by centrifugation at $4000 \times g$ (Beckman GS-6R, Hampton, NH, USA) at 25 °C. The supernatant was collected as a protein source, pH adjusted to 4.5, and further centrifuged to precipitate the protein. Protein curd was washed three times with distilled water and centrifuged at $4000 \times g$ for 5 min. The resultant supernatant was lyophilized (Virtis Genesis, Gardiner, NY, USA) to produce the KBPI.

2.3. Sample preparation for pressure treatment

KBPI dispersions were prepared in a glass beaker by dispersing the required amount of isolates into the water in a weight ratio of 1:4 and 1:5, followed by 1-h hydration. About 25 mL dispersions were transferred into low-density polyethylene bags (Whirl-Pak®, USA), and vacuum packaged before the HP treatment.

2.4. High hydrostatic pressure treatment

The pressure treatment was carried out using a laboratory-scale HP equipment (QFP 2L-700 Avure Technologies, OH, USA), with a pressure vessel 100 mm in diameter and 254 mm in height. KBPI dispersions were treated at selected pressures (200, 400 and 600 MPa) for 15 min. Untreated KBPI sample or control sample was considered as 0.101 MPa. Details of the HP-treatment are described elsewhere (Ahmed, Mulla, & Arfat, 2017). In brief, the pressurization was performed at selected pressure levels (200, 400 and 600 MPa for 15 min) at 23 °C. The ‘come-up’ and depressurization times were excluded in the treatment time. The average pressurization rate was 20–25s per 100 MPa and depressurization time was approximately < 5 s. The initial temperature of the water was 20 °C, and it reached to 26 °C at the highest pressure level of 600 MPa due to adiabatic heating. After the HP treatment, the samples were freeze-dried (FD) for about 72 h and ground to powder for further use. All of the pressure treatments were performed in duplicates.

2.5. Physicochemical properties

The moisture, ash, and crude protein ($N \times 6.25$) content of the kidney bean isolate were determined by the AACC method (AACC International, 2011). The water holding capacity (WHC) of KBPI samples was determined following the method described by Ahmed, Thomas, and Arfat (2016).

2.6. Foaming capacity (FC) and foaming stability (FS)

Foaming capacity was measured as the percentage increase in foam volume of the KBPI after blending. The foam stability was determined by measuring the foam volume at selected time period (30, 60, and 90).

2.7. Emulsifying activity index (EAI) and emulsion stability index (ESI)

The EAI and ESI were measured following the method described by Pearce and Kinsella (1978), with a slight modification. Dispersions containing 5 mL of 0.5 g/100 g protein dispersion were homogenized with 5 mL of corn oil at 7200 rpm for 5 min.

The sample collected from the bottom of the emulsion was diluted in sodium phosphate buffer containing 0.1% SDS at pH 7. The absorbance of the diluted emulsion was measured at 500 nm. EAI and ESI were calculated by the following equations:

$$EAI \left(\frac{m^2}{g} \right) = \frac{(2.2303 \times A_0 \times N)}{c \times \varphi \times (1 - \theta) \times 10000} \quad (1)$$

$$ESI \text{ (min)} = \left(\frac{A_0}{\Delta A} \right) \times t \quad (2)$$

where A_0 is the absorbance of the diluted emulsion immediately after homogenization, N is the dilution factor ($\times 150$), c is the weight of protein per volume (g/mL), φ is the optical path (0.01 m), θ is the fraction of oil used to form the emulsion (0.25), ΔA is the change in absorbance between 0 and 10 min, and t is the time interval, 10 min.

2.8. Differential scanning calorimetric (DSC) measurement

A DSC (TA Q 2000, TA Instruments, New Castle, DE) was employed to measure the thermal analysis for the KBPI samples. The DSC was calibrated with indium and sapphire for temperature and heat capacity calibration. The KBPI dispersions (≈ 10 mg) were heated from 0 to 150 °C at a heating rate of 10 °C/min in heating/cooling cycles in a nitrogen atmosphere to detect the protein denaturation. Instrument software was used to calculate thermal properties.

2.9. Rheological measurement

Oscillatory rheological measurements of KBPI dispersions (pressure treated/untreated) were carried out using a Discovery Hybrid Rheometer HR-3 (TA Instruments, New Castle, DE, USA) with a plate and cone geometry (40 mm and cone angle 2°). KBPI dispersions were placed in a 54- μ m gap between geometries, and the perimeter was covered with a thin layer of silicone oil to prevent the sample dehydration. The sample temperature was controlled by a peltier system. Nonisothermal heating of all dispersions was carried out from 50 to 115 °C at a heating rate of 5 °C/min to detect the extent of protein denaturation after HP-treatment. Frequency sweep tests (0.1–10 Hz) were carried out in the linear regime, at constant strain (0.1%) at 25 °C before and after nonisothermal heating. Rheological parameters were obtained directly from the manufacturer supplied computer software (TRIOS, TA Instruments, NewCastle, DE, USA).

2.10. Fourier transfer infrared (FTIR) spectroscopy

The FT-IR analysis was performed with a Nicolet iS5 FTIR Spectrometer (Thermo Scientific, Madison, WI, USA). A total of 32 scans were accumulated in absorption mode with a resolution of 4 cm^{-1} . The spectrum was obtained from a range of 4000 to 550 cm^{-1} .

2.11. SDS-PAGE experiments

SDS-PAGE was carried out following the method described by Laemmli (1970) using 12 and 4 g/100 g resolving gel and stacking gel, respectively. Slab gels (1-mm thick) were run at a constant voltage of 100 V. The electrophoresis was performed with a Bio-Rad Mini Gel Apparatus. Trans blotting system. The analyses of the electrophoretic patterns to obtain the protein profiles were carried out by using Red Imaging system equipped with Alpha-View SA Software.

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