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# Heat-induced modifications in the functional and structural properties of vicilin-rich protein isolate from kidney (*Phaseolus vulgaris* L.) bean

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ABSTRACT

Heat-induced changes in the physico-chemical (and/or functional) and structural properties of protein isolate from kidney beans (KPI) were characterised. The extent of protein denaturation, free sulphydryl contents, surface hydrophobicity, as well as structural characteristics of the proteins were evaluated. Analyses of size-exclusion chromatography combined with laser scattering showed that the heating at 95 °C led to transformation of 7S-form vicilin to its 11S-form, and even higher molar mass (MW) oligomers or polymers. Moderate heating (for 15–30 min) significantly improved protein solubility, emulsifying and foaming activities (at neutral pH), whilst extensive heating (for 60–120 min) on the contrary decreased these properties. Spectral analyses of fluorescence and/or Raman spectroscopy showed that tertiary and secondary conformations of protein in KPI were remarkably affected to a varying extent by the heating. The results suggested a close relationship between functional properties of the vicilin from kidney bean and its conformational characteristics.



## 1. Introduction

There is a growing interest in the utilisation of proteins from different legumes, due to increased needs for protein nutrition and food formulation. Kidney (*Phaseolus vulgaris* L.) bean is the most widely produced and consumed food legume in Africa, India, Latin America and Mexico. The protein (20–30%) from this legume has been shown to have a good amino acid composition but be lack of sulphur-containing amino acids (notably methionine) and tryptophan (Sathe, 2002). Vicilin, sometimes also named as phaseolin (or G1 globulin), is the major storage protein. It is an oligomeric protein consisting of three polypeptide subunits α-, β- and γ-phaseolin with molecular weight distribution from 43 to 53 kDa (Romero, Sun, McLeester, Bliss, & Hall, 1975).

The flour from kidney bean has been proved to have higher functional properties, e.g., gelation capacities, emulsifying activity and emulsion stability, relative to soybean flour (Chau & Cheung, 1998). Clearly, these good properties of the flours are largely contributed by the proteins contained in this flour. However, the thermal and surface active properties (e.g., foaming) of proteins from kidney beans were different depending on the method of preparation. The alkali-extracted protein isolates (amorphous) had lower denaturation enthalpy change ($\Delta H$), and higher surface hydrophobicity and foam expansion than acid-extracted protein isolates (crystalline) (DiLollo, Alli, Biliarderis, & Barthakur, 1993). In our previous study, it was found that the alkali-extracted protein isolate of kidney bean (KPI) showed much higher heat-induced gelation properties than those from other *Phaseolus* legumes, and their thermal denaturation and gelation seemed to be largely related to their free sulphydryl and/or disulfide bonds (Tang, 2008).

The heat treatment has been widely applied to improve the nutritional properties of legume proteins, e.g., especially *in vitro* protease digestibility, by means of inactivating protease inhibitors or denaturing the proteins. For example, it was observed that heating treatment (at 100 or 121 °C for 15 min) could remarkably improve the *in vitro* protease digestibility of the major globulin (vicilin or phaseolin) of kidney bean (Romero & Ryan, 1978). In this case, the improvement of *in vitro* digestibility was attributed to the disruption of conformational constraints (on protease hydrolysis) of the native molecule. Although the influence of the thermal treatment on the properties of soy protein or its purified glycinin (11S) and β-conglycinin (7S) was well recognised, little information is available concerning about the effects on other legume proteins.

In our previous study (Yin, Tang, Wen, Yang, & Li, 2008), we investigated the influence of high pressure treatment on the functional and nutritional properties of KPI, and found that the treatment at 200–600 MPa led to gradual unfolding of protein structure and subsequent aggregation, in a pressure dependent manner, and the emulsifying activities were improved only at pressures of 200 or 400 MPa. However, the *in vitro* trypsin digestibility and thermal stability of proteins was unaffected by high pressure treatment. Thus, the high pressure treatment is not a good choice to modify these vicilin-rich protein isolates from legumes. In the

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present study, we applied a thermal treatment (at 95 °C for 15–120 min) to modify the physico-chemical and functional properties of KPI. The heating temperature (95 °C) applied was slightly higher than the denaturation temperature (about 94 °C) of the vicilin component in KPI (Yin et al., 2008). Additionally, the heat-induced structural conformational changes were also characterised.

## 2. Materials and methods

### 2.1. Preparation of KPI

Red kidney bean (*Phaseolus vularis* L.) seeds, cultivated in Gangshou province of China were purchased from a local supermarket (Guangzhou, China). The preparation of KPI was according to the same process as described in our previous paper (Tang, 2008). After soaked, de-hulled and dried in the oven, the de-hulled beans were smashed and passed an 80-mesh to produce the flour. The flour was defatted by solvent extraction with *n*-hexane (the ratio of the flour to *n*-hexane = 1:5 g/ml) for three times. The defatted flour was then dispersed with 10-fold distilled water. The dispersions were adjusted to about pH 8.0 with 2 mol/l NaOH, and magnetic-stirred at room temperature for more than 2 h, and then centrifuged at 9000g for 30 min to obtain the protein supernatant. The supernatant were adjusted to pH 4.6 using 2 mol/l HCl, and placed at 4 °C for 2 h, and then centrifuged at the same condition. The obtained precipitate was washed with pre-cooled water for several times, and re-dispersed in distilled water, and adjusted to neutral pH. Last, the protein dispersion was dialysed three times at 4 °C against desalted water (1:100 v/v, 3 times), and then lyophilised to yield KPI. The protein content of this protein was about 92.5% (wet basis), as determined by micro-Kjeldahl method, using a nitrogen conversion factor of 6.25.

### 2.2. Heat treatment

The KPI solutions (1 or 2% w/v) were prepared with 50 mM phosphate buffer (pH 7.4) containing 50 mM NaCl. Aliquots of KPI solutions (50 or 100 ml) were sealed in plastic containers with thin wall, and incubated in a water bath at 95 °C. At accurate periods of heating time (15, 30, 60 and 120 min), the samples were immediately taken out and cooled in an ice bath. Then, the cooled samples (with plastic containers) were frozen in liquid nitrogen. The unheated KPI sample (control) was also frozen in the same mode. Last, the frozen KPI samples (unheated and heated) were lyophilised. The untreated and heat-treated (15, 30, 60 and 120 min) KPI samples were denoted as control, K-15, K-30, K-60 and K-120, respectively.

### 2.3. High-performance size-exclusion chromatography (HPSEC) combined with multi-angle laser light scattering (MALLS)

The HPSEC and the MALLS systems were the same as those described by Zhao, Mine, and Ma (2004). Two TSK columns (G4000 $PW_{XL}$ + TSK G6000 $PW_{XL}$) were connected in series (TOSOH Corp., Montgomeryville, PA). The fractionation range of these two columns was 2000–8000 000 for proteins. The mobile phase (50 mM phosphate buffer, pH 7.4, containing 50 mM NaCl) was filtered through 0.2 μm (Whatman International Ltd., Maidstone, England) and then 0.02 μm filters (Millipore Corp., Bedford, MA). The flow rate was 0.8 ml/min.

A Dawn EOS photometer (Wyatt Technology Corp., Santa Barbara, CA) was used. Two auxiliary analogue inputs enabled interfacing to external detectors such as refractive index (RI) and UV detectors. The instrument was placed directly before the RI detector and after the SEC columns and UV detector to avoid backpressure on the RI cell. Dynamic light scattering measurement was performed on-line in the flow cell using a QELS metre (Wyatt Technology Corp., Santa Barbara, CA). An optical fibre receiver was mounted in the read head of one of the MALLS detectors (detector 13 in our works). Chromatographic data were collected and processed by the ASTRA software (Wyatt Technology Corp.). The $M_w$ of protein eluting in small and individual slices of the SEC chromatogram was determined, based on the Debye plot (Zhao, Ma, Yuen, & Phillips, 2004b). The d*n*/d*c*, where *n* and *c* present the refractive index and sample concentration for each data slice, is reckoned to be constant (±0.185 ml/g) across the sample peak, and nearly independent of its amino acid composition. Bovine serum albumin monomer (Sigma, St. Louis, MO) was used for normalising various detectors' signals relative to the 90° laser light detector signal.

### 2.4. Differential scanning calorimetry (DSC)

DSC experiments were performed on a TA Q100-DSC thermal analyser (TA Instruments, New Castle, DE), according to the procedure of Meng and Ma (2001), with some modifications. Approximately 2.0 mg of untreated or heated KPI samples were accurately weighed into aluminum liquid pans, and 10 μl of 50 mM phosphate buffer (pH 7.4) was added. The pans were hermetically sealed and heated in the calorimeter from 20 to 120 °C at a rate of 10 °C/min. A sealed empty pan was used as a reference. Peak or denaturation temperature ($T_d$), enthalpy change of denaturation ($\Delta H$) and width at half peak height of endothermic peak ($\Delta T_{1/2}$) were computed from the thermograms by the universal analyser 2000, version 4.1 D (TA Instrument-Waters LLC, USA). All experiments were conducted in triplicate.

### 2.5. Free sulphydryl (SH) content

Free sulfhydryl groups (SH) contents of protein isolates, including total and exposed SH contents, were determined according to the method of Ellman (1959) and Beveridge, Toma, and Nakai (1974), as described by Yin et al. (2008). The SH contents were expressed as μmol/g of protein. The determinations were conducted in duplicate.

### 2.6. Surface hydrophobicity ($H_o$)

$H_o$ was determined using ANS, according to the method of Haskard and Li-Chan (1998). In brief, stock solutions of $8 \times 10^{-3}$ M $ANS^-$, and 1.5% (w/v) protein were prepared in 10 mM phosphate buffer (pH 7.0). To successive samples containing 4 mL of buffer and 20 μl of $ANS^-$ stock solution were added 10, 20, 30, 40, and 50 μl of 1.5% protein solution. The mixtures were shaken in a vortex mixer for 5 seconds. Fluorescence intensity (FI) was measured at wavelengths of 390 nm (excitation) and 470 nm (emission) using a RF-5301 PC spectrofluorometer (Shimadzu Corp., Kyoto, Japan) at 20 ± 0.5 °C, with a constant excitation and emission slit of 5 nm. The FI for each sample with probe was then computed by subtracting the FI attributed to protein in buffer. The initial slope of the FI versus protein concentration plot was calculated by linear regression analysis and used as an index of $H_o$. Additionally, the extrinsic fluorescence spectroscopy of ANS in the presence of proteins was obtained at a protein concentration of $7.5 \times 10^{-5}$ g/ml (or 20 μl of 1.5% protein solution mixed with 4 ml of the buffer). The determinations were conducted in duplicate.

### 2.7. Protein solubility (PS)

An aqueous solution (1% w/v) of samples in distilled water was stirred magnetically for 30 min, and then with either 0.1 N HCl or