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Structural and functional characterization of kidney bean and field pea protein isolates: A comparative study

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ABSTRACT

Protein isolates were prepared from different kidney bean (KB) and field pea (FP) lines and their physicochemical (protein content, colour, electrophoretic profile & zeta potential), structural (thermal & conformational), dynamic rheological and functional (emulsification, foaming, water and fat absorption) properties were evaluated. These isolates differed significantly in colour-, structural-, thermal- and functional-properties. SDS-PAGE and size exclusion chromatography revealed that vicilins (~150 kDa) were prominent proteins in KB isolates, while FP protein isolates contained both legumins and vicilins (~330 and ~155 kDa, respectively) as major components. FTIR spectroscopy revealed that β-sheets, β-turns and α-helix were main secondary structures in the KB and FP proteins. KB proteins had relatively more β-sheets (38.6%) while less α-helix (22.8%) than FP proteins (30.0 and 28.0%, respectively). The rheological properties of the protein isolates were measured as gelation temperature (T_{gel}), gel reinforcement ($G_{reinforcement}$) and tan δ. KB proteins had higher thermal denaturation temperature (T_d), T_{gel} and $G_{reinforcement}$ while lower tan δ than FP proteins. Principal component analysis (PCA) revealed that T_d, T_{gel} and $G_{reinforcement}$ related positively, whereas tan δ related negatively with the proportion of β-sheets. Protein solubility, emulsion stability, foaming capacity and stability were positively related to the charge on the proteins.

1. Introduction

Legumes are the edible seeds of pod-bearing plants belonging to the order Leguminosae. The legumes are considered as the second most important source of human food after cereals (Tiwari, Gowen, & McKenna, 2011). They are inexpensive source of proteins and other nutrients such as resistant starch, dietary fibre, vitamins, minerals and poly-phenoles. In general, legumes are good sources of proteins (20–40%) especially when consumed in combination with cereals (Boye et al., 2010; Dave Oomah, Patras, Rawson, Singh, & Compos-Vega, 2011).

The proteins as concentrates or isolates are used as a functional ingredient primarily to increase nutritional quality and to provide desirable sensory characteristics such as structure, texture, flavour, and colour to formulated food products. The protein concentrates and isolates used by the food industry today are mostly derived from soybean, whey and wheat, but because of the dietary restrictions and preferences (related allergenicity, vegetarianism, Halal, etc.), food manufacturers and consumers are looking for alternative protein sources (Toews & Wang, 2013; Adebiyi & Aluko, 2011; Boye et al., 2010; Karaca, Low, & Nickerson, 2011a, 2011b). Legumes can be considered most suitable for the preparation of protein isolates because of their high protein content, low cost and wide acceptability. Beans and peas are amongst the most widely cultivated and consumed legumes of the world (Tiwari & Singh, 2012). Kidney bean (*Phaseolus vulgaris* L.) is widely grown and consumed in Africa, India, Latin America, Mexico and Egypt. This bean usually contains 20–30% protein, which has a good amino acid composition but is low in sulfur-containing amino acids notably methionine and tryptophan (Sathe, 2002). Peas (*Pisum sativum* L.) are the second most important leguminous crop which is grown over 25 million acres worldwide (Schatz & Endres, 2009, pp. 1–8). It is extensively grown and consumed in Canada, France, China, Russia and the United States (Simsek, Tulbek, Yao, & Schatz, 2009). Field pea contains 20–25% proteins with higher levels of lysine and tryptophan than cereal grains. Moreover, field peas contain 5–20% lesser trypsin inhibitors than soybeans (Schatz & Endres, 2009, pp. 1–8). Furthermore, the proteins from kidney bean and field pea have been found suitable for preparation of gluten-free

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muffins with the characteristics comparable to those made using wheat gluten (Shevkani & Singh, 2014, in press).

In spite of high protein content and low cost of the legume proteins, their application in food formulation depends upon functional properties which have been defined as the physical and chemical properties of proteins that influence their behaviour in food systems during processing, storage, cooking and consumption (Kinsella & Melachouris, 1976). The functional properties of proteins have been classified according to the mechanism of action on three main groups: (i) properties related with hydration (water and oil absorption, solubility, thickening & wettability) (ii) properties related with the protein structure and rheological characteristics (viscosity, elasticity, adhesiveness, aggregation & gelification), and (iii) properties related with the protein surface characteristics (emulsifying and foaming, formation of protein-lipid films, whippability) (Moure, Sineiro, Dominguez, & Parajo, 2006). However, the most important functional properties of protein in food include its solubility, water- and fat-binding capacities, gel forming and rheological behaviours, emulsifying capabilities, foaming and whipping abilities, which are related to molecular size, structure, charge distribution of the protein molecules as well as environmental factors, and processing conditions (Day, 2013; Tang & Sun, 2011).

The proteins from different legumes and cultivars/lines differ in physicochemical and structural characteristics which affect their functionality and applications in the food industry. The present investigation would provide information on the relationships between protein characteristics (physicochemical & structural) and the functional properties (gelation, solubility, emulsification, foaming, water- and fat-binding capacities) of protein isolates from different kidney bean (KB) and field pea (FP) lines. The information on protein characteristics amongst different lines would assist in identifying their targeted applications in the food industry (Rui, Boye, Ribereau, Simpson, & Prasher, 2011).

Therefore, the present investigation was undertaken to: (1) study and compare physicochemical, structural, thermal, rheological and functional properties of protein isolates from different KB and FP lines and (2) to establish possible relationships between these properties.

2. Materials and methods

2.1. Materials

Five lines of each kidney bean (EC 572723, Pi 244719, Pi 312296, Pi 249554 & PLB 10 1) and field pea (IC 394027, IC 342028, IC 291541, IC 381453 & IC 299013) were grown at Regional Centre, National Bureau of Plant Genetic Resources, Shimla, India. The grains were cleaned and ground to pass all through a sieve with 250 μm aperture widths. The flours were defatted thrice using hexane (1:4) for 8 h (total defatting time 24 h). Finally, the solvent was separated and residue was dried at 35 °C for 24 h (Shevkani, Singh, Kaur, & Rana, 2014).

2.2. Preparation of protein isolates

Protein isolates from different KB and FP lines were prepared using alkaline extraction and isoelectric precipitation technique as described elsewhere (Shevkani & Singh, 2014, in press). The defatted flours were dispersed in deionized water and pH of the dispersions was raised to 9.0 using 1 N NaOH. The dispersions were stirred for 1 h at room temperature and then centrifuged at 8000 *g* for 20 min. Following centrifugation, supernatants were collected and sediments were re-suspended in deionized water, stirred for 1 h at pH 9.0 and centrifuged (8000 *g* for 20 min). The two supernatants were pooled and the pH of supernatants was lowered to 4.5 with 1 N HCl. The precipitated proteins were recovered by centrifugation at 8000 *g* for 20 min at 4 °C. The precipitates were re-suspended in deionized water, neutralized and freeze-dried. The protein isolates from different KB and FP lines were referred as KBIs and FPIs, respectively.

2.3. Protein and ash content

The protein- (nitrogen × 6.25) and ash-content of KBIs and FPIs were determined using standard methods (AOAC, 1990).

2.4. Colour properties

Colour parameters (L^*, a^* and b^*) were measured using Ultra Scan VIS Hunter Lab (Hunter Associates Laboratory Inc., U.S.A.). L^*, a^* and b^* values of the isolates were recorded following the method elaborated elsewhere (Shevkani, Kaur, Singh, Singh, & Singh, 2014). Whiteness index (WI) of these isolates was also calculated using the equation $WI = L^* - 3b^*$ (Marcone & Kakuda, 1999).

2.5. Sodium dodecyl sulphate poly-acrylamide gel electrophoresis (SDS-PAGE)

The SDS-PAGE was performed according to Laemmli (1970) using 12% resolving gel and 5% stacking gel. KBIs and FPIs were dissolved in 1 ml of Laemmli buffer pH 6.8, containing Tris, SDS, β-mercaptoethanol, bromophenol blue and glycerol. The isolates were boiled for 5 min and then centrifuged at 13,600 *g* for 10 min at room temperature. The proteins were resolved at a constant current of 35 mA. The gel was stained overnight with Coomassaie Brilliant Blue R 250 (0.25%) and de-stained using 20% methanol and 10% acetic acid solution.

2.6. Size exclusion chromatography

Size exclusion chromatography was carried out on FPLC system (AKTA purifier, GE Healthcare) equipped with a Superose 12 10/300 GL column (GE Healthcare Bio-Sciences, Uppsala, Sweden). KBIs and FPIs from different lines (100 mg) were dissolved in 1 ml borate buffer (0.1 M sodium borate, 0.2 M sodium chloride, pH 8.3). The proteins were eluted at a flow rate of 0.4 ml per min. The aforementioned buffer was used as mobile phase/eluent. The eluate was continuously monitored at 280 nm. Molecular weight standard (12,000–200,000) kit for gel filtration chromatography (Sigma–Aldrich, St. Louis, MO, USA) was used for calibration.

2.7. Fourier-transform infrared (FTIR) spectroscopy and protein secondary structure

Infrared spectra of KBIs and FPIs were recorded using FTIR spectrometer (Vertex 70, Bruker Optics Inc., Germany) equipped with Attenuated Total Reflectance (ATR) cell (PIKE Technology Inc., USA). Protein isolates were stored in desiccators over P_2O_5 for more than two week in order to remove moisture. The moisture-free isolates were placed on the ATR crystal and pressed down to ensure good contact. The spectrometer was continuously purged with dry air. The spectra in the range of 4000–600 cm^{-1} were recorded (average of 120 spectra at 4 cm^{-1} resolution) and referenced against that of empty cell. No evidence of any water signal was found in the IR spectra of KBIs and FPIs. The spectrum were subjected to Fourier self-deconvolution (FSD), second derivative (SD) analysis and curve fitting procedures to locate overlapping peaks in amide-I (1700–1600 cm^{-1}) region (Fig. 1) using Omnic software (Thermo Nicolet Cooperation, Madison, WI). The relative proportions of different secondary structures of KBIs and FPIs were