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Introducing Speckled sugar bean (*Phaseolus vulgaris*) protein isolates as a new source of emulsifying agent

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ABSTRACT

The present work was carried out to investigate the secondary structure of Speckled sugar bean protein isolates (SSBPI) as a new source for the formation of oil in water emulsions. For this purpose the structure and emulsifying properties of SSBPI were compared with other common bean protein isolates namely, Red mexican bean protein isolates (RMBPI) and Great northern bean protein isolates (GNBPI). Results showed that all proteins were rich in globulin with Phaseolin being the major protein fraction. These bean proteins had high acidic amino acids contents of glutamic and aspartic acids. The major building fractions for the secondary structure of all bean protein isolates were beta form structures (turn and sheet) and SSBPI had the highest and GNBPI possessed the lowest turn substructure. Comparison between different bean proteins exhibited that RMBPI had more fluorescence intensity yield followed by SSBPI and GNBPI. Among bean proteins, SSBPI had less surface hydrophobicity while GNBPI had the highest hydrophobic surface at pH 3 and 7. Surface tension was influenced by protein sources and RMBPI was more effective in the reduction of water surface tension compared to other bean proteins. Although SSBPI had better surface activity at oil-water interface no significant differences were observed between bean proteins. Among bean proteins, SSBPI had the highest electrical charge on the surface which promoted enough repulsion between emulsions oil droplets. Emulsions prepared with SSBPI had lower droplet size with narrow distribution compared to other bean proteins. Results revealed that SSBPI has a great potential to be used as a new source of food emulsifier.

1. Introduction

Proteins are surface-active molecules often used to increase the emulsion stability against flocculation and creaming (Dickinson & Golding, 1997). During emulsion formation, proteins are adsorbed at the surface of oil droplets in the form of a densely packed layer (Dickinson, 2010). Therefore, protein facilitates the formation of smaller oil droplets during homogenization and prevents droplets coalescence over storage through increasing the repulsion forces between droplets (McClements, 2005; Ruttarattanamongkol, Nor Afizah, & Rizvi, 2015; Trentin, De Lamo, Güell, López, & Ferrando, 2011).

The demand for novel proteins with specific functionality has been recently increased and food scientists are always searching for new sources of proteins. Accordingly, there is still a place for the new sources of plant proteins to meet this demand (El Nasri & El Tinay, 2007). However, for a novel protein to be useful for food processing application, it should possess desirable functional and nutritional qualities.

Protein extracts from legumes are environmentally conservative protein sources that have significant economic advantages over more expensive meat and dairy proteins (Jafari, Rajabzadeh, Tabtabaei, Marsolais, & Legge, 2016). However, except for soy, due to the inadequate information about their structural and functional properties, they do not have sufficient or special application as functional ingredients in food industries (Karaca, Low, & Nickerson, 2011).

Beans (*Phaseolus vulgaris*) are amongst the most widely cultivated and consumed legumes of the world (Tiwari & Singh, 2012) which are a great source of nutrients such as protein, carbohydrate, dietary fiber, minerals and vitamins (Sathe, 2002). They are the second group of legume seeds, after soy, cultivated throughout the world (Xu & Chang, 2008). Beans have high protein content (15–25%) (Sathe, 2002) which is twice the level found in cereal

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grains and significantly more than the level in conventional root crops (Ustimenko-Bakumovsky, 1983). Several bean proteins such as common bean, black bean and kidney bean have excellent emulsifying properties (high emulsifying capacity and good stabilizing effect), and therefore can be used successfully to prepare food emulsions (Evangelho et al., 2017; Makri & Doxastakis, 2006; Sathe, 2002; Shevkani, Singh, Kaur, & Chand Rana, 2015; Wani, Sogi, Shivhare, & Gill, 2015).

Speckled sugar bean is a grain legume which is very nutritious and rich in protein. The grain is easily stored, making it a useful crop for subsistence use, while is a high-yielding variety with very acceptable seed appearance (Wortmann, 2004). It also has resistance to some of the important diseases. Although the protein extracted form Speckled sugar bean is nutritionally rich, to date there is no information regarding its structure or emulsifying properties.

The ability of a protein to aid in the preparation of an emulsion depends on the molecular characteristics of the protein such as size, solubility, surface hydrophobicity and structural flexibility, as these characteristics determine the protein adsorption properties. The emulsifying properties of bean proteins are also dependent on environmental parameters such as pH. Therefore, the main object of the present research was to evaluate the structure and emulsifying properties of Speckled sugar bean proteins compared to other common bean proteins (Red mexican and Great northern bean). Furthermore, possible relationship between structure and emulsifying properties were considered.

2. Materials and methods

2.1. Materials

Three types of common bean seeds (*Phaseolus vulgaris*) including Speckled sugar (cultivar red mottled Pache baghala), Red mexican (cultivar Goli) and Great northern (cultivar Dorsa) were kindly supported by Arak Agriculture Research Center, Iran (Fig. 1). Commercial sunflower oil (Nina brand, Iran) was purchased from local market and all chemicals used in this study were of analytical grades (Mojallali (Iran), BioRad, Sigma and Merck companies (USA)).

2.2. Protein extraction

The bean proteins were extracted according to the method described by Du et al. (2018). In brief, beans were first ground into flour and dispersed in Millipore water (1:10 bean flour to water ratio) with the pH adjusted to 9 for 45 min. The slurry was then centrifuged at 15000 × g at 20 °C for 15 min, and the precipitates were discarded. The supernatant obtained was subjected to acid precipitation by adjusting the pH to 4.5 using 1 M HCl solution and the precipitate was recovered after centrifugation at 7000*g* for 15 min. The protein precipitate was washed twice and neutralized to pH 7 (1 M NaOH and HCl were used for pH adjustments), freeze dried and stored at 4 °C.

Total protein content was measured using Kjeldahl method and considering 6.25 as the conversion rate of nitrogen to crude protein (Kjeldahl, 1883). Speckled sugar, Red mexican and Great northern bean protein isolates had 92.37%, 90.54% and 85.89% protein content, respectively.

2.3. Protein solubility

Following the method of Owusu-Apenten (2002) with slight modifications, the solubility of the bean proteins were determined at various pH values (from 3.0 to 9.0). After preparation of protein dispersions in deionized water (1%), slurries were adjusted to pH 3–9 with 1 M HCl or NaOH and stirred at 1000 rpm for 30 min. Solutions were then centrifuged at 5000 g for 15 min at room temperature. Protein solubility was then determined by the Biuret method using a spectrophotometer (UV-2601; RayLeight, China) at 540 nm wavelength with bovine serum albumin (BSA) as the standard.

2.4. Amino acid analysis

To determine the amino acid composition, 30 mg of been proteins were hydrolyzed under nitrogen in 4 mL of 6 mol L^{-1} HCl at 110 °C for 22 h. Derivatization was carried out using isothiocianate. Chromatography was performed using a Knauer high-performance liquid chromatography (HPLC) system (Berlin, Germany) which consisted of a gradient controller (Manager 5000), UV detector (UV 2500) set at 254 nm and a Eurospher C18 column (250 mm × 4.6 mm, with 5 μm particle size) maintained at 25 °C. The solvent system consisted of two eluents. Solvent A was a solution of 50 mmol L^{-1} sodium acetate containing 0.4 mL L^{-1} triethylamine. The pH was adjusted to 6.8 using glacial acetic acid. Solvent B was a blend of 60 mL acetonitrile and 40 mL water.

2.5. Electrophoresis pattern (SDS-PAGE)

The fractions of three protein isolates were analyzed by gel electrophoresis pattern evaluation. Reducing SDS-PAGE analysis

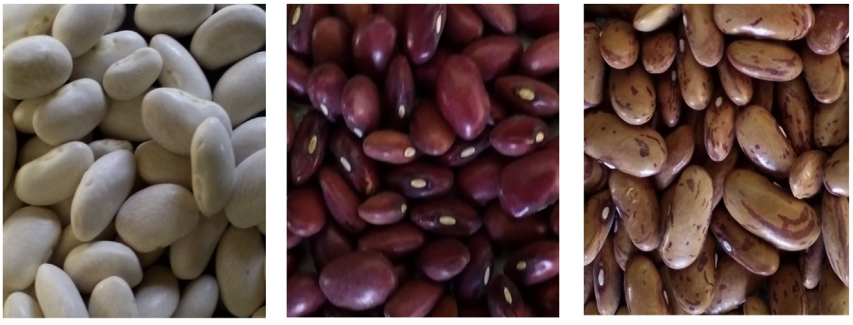

Fig. 1. Pictures for Great northern, Red mexican and Speckled sugar beans. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)