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Effects of high pressure homogenization on faba bean protein aggregation in relation to solubility and interfacial properties

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ABSTRACT

High pressure homogenization profoundly impacted the globular protein aggregation, and subsequently also its solubility and interfacial properties, using faba bean protein as model. The aggregate size and apparent molecular weight were studied by dynamic laser scattering and size exclusion chromatography before and after high pressure homogenization. High pressure homogenization dissociated large insoluble protein aggregates (> 1 μm), leading to soluble supramolecular aggregates. Accordingly, 15 kpsi homogenization dramatically improved faba bean protein solubility (10 mg/ml) from 35 to 99% at neutral pH. High pressure homogenization resulted in certain level of protein unfolding with increased surface hydrophobicity. High pressure homogenized proteins adsorbed at the air-water interface faster than the untreated ones as a result of their higher surface hydrophobicity and the dissociation of insoluble protein aggregates. However, the supramolecular aggregates may compete with protein molecules at the interface, which then impaired the viscoelasticity of the interfacial network. This phenomenon observed may be related to the slower rate of rearrangement for supramolecular aggregates at interface due to a more complex structure. Consequently, the foaming capacity of 30 kpsi treated faba bean protein improved from 91 to 260% with 95% retention for 30 min. However, high pressure homogenization had negative effects on protein emulsifying property. This research revealed that plant globular protein aggregation status can determine both protein solubility and functionality. It has also provided insight how high pressure homogenization can be used strategically to modify protein functionality by modulating protein aggregation.

1. Introduction

The use of high pressure is a technology widely employed in food, pharmaceutical, cosmetic and various industries (Galazka, Dickinson, & Ledward, 2000; Masson, Tonello, & Balny, 2001). A great deal of research is underway to understand the effect of high pressure process on the structure and functions of biomacromolecules and other bioactive compounds (Galazka et al., 2000; Paquin, 1999). Proteins are one of the many pressure sensitive biomacromolecules (Masson et al., 2001; Mozhaev, Heremans, Frank, Masson, & Balny, 1996). Significant research progress has revealed how static high pressure influences protein structure and functionality depending on protein structure, pressure level and other external parameters such as pH, temperature and solvent composition (Balny & Masson, 1993). Pressure disturbs the balance of intramolecular and solvent-protein interactions, leading to a change in the 3 dimensional structure of a protein molecule (Jaenicke, 1991). Static high pressure (> 100 MPa) has a strong influence on the hydrophobic and electrostatic interactions which stabilized the quaternary and tertiary structures of globulin proteins (Galazka et al., 2000). On the other hand, changes of secondary structure, stabilized by hydrogen bonds, take place at higher static pressures (> 200 MPa) since hydrogen bonds are less affected by pressure (Carrier, Mantsch, & Wong, 1990). Static high pressure treatment may significantly change protein functional properties. For example, Molina et al. found that soy protein isolate showed optimum emulsifying activity after treatment at 400 MPa, which may be related to the dissociation of soy protein 7S into denatured subunits (Molina, Papadopoulou, & Ledward, 2001).

The high pressure homogenization technique is commonly used to stabilize milk product by disrupting dairy fat globules (Diels, Callewaert, Wuytack, Masschalck, & Michiels, 2005). This technology is also an important operation to obtain fine emulsions, especially protein stabilized emulsions (Keerati-u-rai & Corredig, 2009). Different from static high pressure treatment, during high pressure homogenization, protein molecules not only experience high pressure but also the combined force-induced phenomena of cavitation, shear, turbulence and heating simultaneously over short period of time (Bouaouina,

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Desrumaux, Loisel, & Legrand, 2006; Grácia-Juliá et al., 2008). Bouaouina et al.(Bouaouina et al., 2006) reported that high pressure homogenization (up to 300 MPa) improved the foaming properties of whey proteins, which may be related to the dissociation of large protein aggregates (> 1 μm). Maresca et al.(Maresca et al., 2017) found that the foaming capacity of 100 MPa treated bovine serum albumin was increased as a result of protein conformational changes. Grácia-Juliá et al.(Grácia-Juliá et al., 2008) found that high pressure homogenization (250–300 MPa) induced aggregation of whey protein isolates through hydrophobic interactions. On the other hand, Liu et al.(Liu et al., 2011) showed that the negative effects of high pressure homogenization (160 MPa) on whey protein emulsifying property are related to the dissociation and reaggregation of whey proteins. Shen et al. (Shen & Tang, 2012) suggested that sulfhydryl group and disulfide bond interchanges might involved in the formation of soy soluble aggregates induced by dynamic high pressure (120 MPa). Keerati-u-rai et al. (Keerati-u-rai & Corredig, 2009) reported that high pressure homogenization (65 MPa) caused changes in soy protein aggregation status due to protein partial unfolding. From these previous studies, high pressure homogenization has been shown to impact protein aggregation and conformation, especially at the interfaces, to interfere with the emulsifying and foaming properties. However, more researches are required to understand how high pressure homogenization impacts protein aggregation and conformation in aqueous solutions, and how such changes may impact protein solubility and functionalities. Furthermore, most of the previous research has focused on dairy proteins and enzymes. Research on plant proteins is still limited, with focus on soy and lupin proteins (Bader, Bez, & Eisner, 2011; Floury, Desrumaux, & Lardieres, 2000; Floury, Desrumaux, & Legrand, 2002; Martinez, Ganesan, Pilosof, & Harte, 2011).

Legume proteins have gained particular attention in recent years in food and bio-based material applications due to their sustainability, high nutritive value and low allergenicity. Globulins, mainly legumin (40–45%, 11S, M_w 200–500 kDa) and vicilin (20–25%, 7S, M_w 150 kDa) are the major storage proteins in faba bean seeds (60–80%, dry weight) (El Fiel, El Tinay, & Elsheikh, 2002). Different from the well-characterized β-lactoglobulin (M_w 18 kDa), legumin and vicilin have a greater molecular weight and more complex quaternary and tertiary structure (Schwenke, 2001). The legumin proteins (11S) are hexamers and each subunit consists of one acidic (about M_w 40 kDa) polypeptide and one basic (about M_w 20 kDa) polypeptide linked via disulphide bond (Gueguen & Cerletti, 1994). The vicilin proteins (7S) are trimetic proteins composed of 3 subunits (about M_w 50 kDa each) (Gueguen & Cerletti, 1994). 15S and > 15S components were also found in faba bean proteins through ultracentrifuge investigation. Since 15S and > 15S components showed the same sodium dodecyl sulfate gel electrophoresis pattern as 11S, 15S and > 15S components are considered to be dimeric or polymeric forms of 11S (Mori & Utsumi, 1979). Some studies on pulse 11S type proteins, such as soy glycinin and pea legumin, found that environment factors such as pH and ionic strength can influence the association and dissociation of the subunits (Gueguen, Chevalier, Barbot, & Schaeffer, 1988; Ruiz-Henestrosa, Martinez, Patino, & Pilosof, 2012; Wolf, 1970). For example, pea legumin (11S) could dissociated into 3.5S when the pH was lower than 3.4 or higher than 10 with ionic strength (I) of 0.1 (Gueguen et al., 1988). Ruiz-Henestrosa revealed that soy glycinin shifted to less assembled structure at pH 7.0 (I = 0.05) as compared to pH 7.6 (I = 0.5) (Ruiz-Henestrosa et al., 2012). The dissociation-reaggregation behaviour of the polymorphic structure of legumin and vicilin, along with their conformation are important for their functionalities (Schwenke, 2001). Faba bean is a pulse crop increasingly grown in western Canada which is capable of growing in cool, wet environments and is used for both human and animal consumption. Faba beans contain a relatively large protein content (26–39%) among pulse crops with a well-balanced amino acid composition (Bramsnaes & Olsen, 1979; Gueguen & Cerletti, 1994). Faba bean proteins also possess good functional properties; for example, Tsoukala et al. (Tsoukala, Papalamprou, Makri, Doxastakis, & Braudo, 2006) and Karaca et al. (Karaca, Low, & Nickerson, 2011) showed that faba bean proteins have excellent emulsifying properties, comparable to lupin, pea and other legumin proteins. Gumus et al. (Gumus, Decker, & McClements, 2017) used faba bean proteins as emulsifier to prepare ω-3 oil emulsion. In this work, faba bean protein was used as a model protein to investigate how high pressure homogenization impacts pulse globulin protein structure, aggregation and their functional properties. Faba bean protein structure was systematically characterized and correlated with its surface tension and interfacial dilation rheology as well as the subsequent solubility, foaming and emulsifying properties. Here we studied the influences of different pressures on the aggregation status change and the corresponding mechanisms, protein conformation and surface hydrophobicity.

2. Materials and methodologies

2.1. Materials

Dry faba bean seeds of mixed varieties were kindly provided by W.A. Grain & Pulse Solutions (Alberta, Canada). Canola oil was purchased from a local market (Superstore, Edmonton, AB, Canada). Protein standards (Sigma-Aldrich Co., Oakvillem Ontario, Canada) including thyrogloulin (660 kDa), γ-globulin (150 kDa), albumin (43 kDa), ribonuclease A (14 kDa) and *p*-aminobenzoic acid (0.14 kDa) were used for M_w calculation. D_2O, DOH, DCl, 1-anilino-8-naphthalenesulfonate (ANS), urea, sodium dodecyl sulfate (SDS), β-mercaptoethanol and hexadecane were purchased from Sigma-Aldrich Co. (Oakville, Ontario, Canada). All chemicals were reagent grade and used without further purification. Water used in this study was purified by Milli-Q Advantage A10 system (EMD Millipore Corporation, MA, USA).

2.2. Faba bean proteins extraction

Faba bean proteins were extracted by alkaline solution followed by isoelectric precipitation. Briefly, faba bean grains were dehulled by a Forsberg Model 2 Huller (Forsbergs, Inc., Thief River Falls, MN, USA) and then milled into flour (Retsch ZM 200, Retsch GmbH, Germany). The dehulled faba bean flour was dispersed into water at a flour-to-water ratio of 1:10. The mixture was adjusted to pH 11 with 1 mol/l NaOH and stirred vigorously for 1 h (Karaca et al., 2011; Makri, Papalamprou, & Doxastakis, 2006). The suspension was then centrifuged at 8000 g for 15 min at 4 °C. The supernatant was adjusted to pH 4.5 using 1 mol/l HCl to precipitate the proteins which were then collected by centrifugation at 8000 g for 15 min at 4 °C. The protein precipitate was neutralized using 1 mol/l NaOH. After freeze-drying, the dry protein powders were stored in plastic bottle in 4 °C before analysis. The protein content was 76% (w/w, dry weight) as determined by nitrogen analyzer (FP-428, Leco Corporation, St. Joseph, MI, USA) using a protein conversion factor of 5.4 (Mosse, 1990).

2.3. High pressure homogenization treatment

A faba bean protein solution was prepared by dispersing protein powders into water at 22 °C to a concentration of 1% (w/v). The pH was adjusted to 7 by 0.1 mol/l NaOH, followed by vigorous stirring for 1 h. Then the protein solution passed through high pressure homogenizer (Nano DeBEE, Bee International Inc., MA, US) at 15,000 psi (103 MPa) and 30,000 psi (207 MPa) for 6 cycles, respectively. The protein samples treated by 15,000 and 30,000 psi were coded as 15 kpsi and 30 kpsi, respectively. The untreated protein sample was coded as 0 kpsi. Previous work showed that the number of cycles influenced the protein structure (Maresca et al., 2017). Thus, 6 cycles were applied to all the samples for the sake of consistency. The sample inlet reservoir, nozzle, emulsifying cell and outlet port of the high pressure homogenizer were all cooled by an ice bath. Samples were collected in sterile tubes placing