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Structural modification in condensed soy glycinin systems following application of high pressure

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ABSTRACT

The structural behaviour of soy glycinin systems following application of industrially relevant high hydrostatic pressure (600 MPa for 15 min at ambient temperature) was investigated throughout the experimental range up to 80% (w/w) solids content, and results were compared to conventional thermal treatment. Using small-deformation dynamic oscillation in shear, modulated differential scanning calorimetry, infrared spectroscopy and X-ray scattering, it was demonstrated that soy glycinin with twelve disulphide linkages displays extensive unfolding at low to intermediate solid levels (30–60%, w/w). In contrast, it largely maintains native conformation at 70 and 80% (w/w) solids showing about 20% denaturation, as compared to the thermal transition of native counterparts. Experimental data from infrared spectroscopy also argue for retention of the native conformation in condensed soy glycinin systems comprising mainly beta sheets in the secondary structure. At subzero temperatures, condensed glycinin at atmospheric and pressurised conditions undergoes vitrification phenomena recording experimental glass transition temperatures. Experimental data were successfully modelled using theoretical frameworks for mechanical studies on amorphous synthetic polymers.

1. Introduction

Soy proteins, being valuable food ingredients, are used in a wide range of industrial applications from techno- and biofunctional ingredients to mimetics of desirable organoleptic properties in processed foods (Kasapis & Tay, 2009; Tsumura et al., 2005). The major components of soy protein isolates are glycinin (11S) and β-conglycinin (7S), representing about 42% and 34%, respectively, of the proteinaceous material (Peng, Quass, Dayton, & Allen, 1984). The quaternary structure of native glycinin molecules is of interest being strongly folded with two identical annular-hexagonal monomers stacked on top of each other and linked by disulfide bonds (Hou & Chang, 2004; Keerati & Corredig, 2009; Kinsella, 1979; Nielsen, 1985).

Conventional heat treatment is used in the food industry to attain acceptable microbial safety hence extending the shelf life of products. Through the years, research in the structure–function relationship of soy 11S demonstrated that application of heat initiates dissociation of molecules followed by aggregation and gel formation at high temperatures (>70 °C). Thermal treatment causes unfolding of polypeptide chains in glycinin with subsequent exposure of buried hydrophobic residues (German, Damodaran, & Kinsella, 1982; Hermansson, 1986; Sorgentini, Wagner, & Aiidn, 1995). It has been further stated that the mechanism of gel formation in soy glycinin is based on a cluster–cluster aggregation of protein particles whereby the dimension of the clusters depends on the size and shape of heat-induced aggregates (Renkema, Lakemond, Jongh, Gruppen, & Vliet, 2000).

As a non-thermal technology, high-pressure processing has been of recent interest, since it appears to be promising in maintaining the sensory quality and nutritional profile of processed food materials. Comprehensive experimental studies on the effect of high pressure on globular protein systems have been conducted over the last few years (San Martin et al., 2002; Speroni et al., 2009; Wang et al., 2008; Zhang, Li, Tatsumi, & Kotwal, 2003). They have argued that pressure-denatured proteins, unlike the heat-denatured counterparts, maintain a relatively compact structure with water molecules being able at high enough levels to penetrate the hydrophobic core of the globular molecule (Galazka, Dickinson, & Ledward, 2000; Harano & Kinoshita, 2006; Hummer, Garde, Garcia, Paulaitis, & Pratt, 1998). Thus, high pressure processing is able to disturb primarily intramolecular hydrophobic clusters,

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induce disulfide-bond exchange and alter electrostatic interactions, whereas covalent linkages and hydrogen bonds are less affected, resulting in a negative volume change in the denatured molecule (Messens, Van Camp, & Huyghebaert, 1997; Minerich & Labuza, 2003; Yuste, Capellas, Pla, Fung, & Mor-Mur, 2001).

Studies have reported that glycinin is sensitive to high pressure treatment at values greater than 400 MPa. Under these conditions, the treatment results in a soft and smooth gelling texture while heat treatment at 80 or 90 °C yields a hard and firm gel. Circular dichroism analysis of these materials indicated partial conversion of the ordered structures of α-helix and β-sheet into random coil following processing at 500 MPa for 10 min (Apichartsrangkoon, 2003; Zhang et al., 2003). Work thus far focused on relatively dilute aqueous systems, i.e. up to 10 or 20% (w/w) solids content, and demonstrated the effect of level and time of pressurisation on reversible or irreversible conformational changes by altering the equilibrium between intermolecular and solvent–protein interactions.

There has been no fundamental work in the literature on higher levels of solids in glycinin systems (up to 80%) in relation to high pressure effects that may lead to a glassy consistency in the proteinaceous matrix. This study deals with the structural properties of condensed glycinin preparations following application of high pressure through thermomechanical analysis. Work is further aided by FTIR that identify changes in the secondary conformation of soy glycinin under both atmospheric and pressurised conditions. In the absence of extensive thermodynamic order in solute and solvent from ambient to subzero temperatures, materials at high levels of solids should exhibit glass transition phenomena and this type of behaviour is probed presently with theoretical frameworks from research in amorphous synthetic polymers.

2. Experimental section

2.1. Materials

Soy protein isolate (SPI) was obtained from Oppenheimer, Sydney, NSW, Australia. The composition of the defatted SPI was reported by the supplier as 90% protein, 5% moisture, 4% ash and 1% carbohydrate.

2.2. Extraction of glycinin from soy protein isolate (SPI)

SPI was used in this investigation to extract the glycinin (11S) fraction *via* the method of Wu et al. (1999) and Kasapis and Tay (2009) with minor modifications. In doing so, sodium metabisulphite (0.98 g/L) was added to 10% (w/w) SPI dispersion in deionised water with pH adjustments to 6.4 using 1 M HCl, and the preparation was kept in an ice bath overnight. This was then centrifuged at 6500 *g* for 20 min at 4 °C using Sorvall RC5B Refrigerated Superspeed Centrifuge (Bad Homburg, Germany). The pellet was dissolved in deionised water with pH adjustment to 7.5 and the solution was dialysed in semi-permeable membrane against deionised water for 24 h at 4 °C. The resultant salt-free dialysate was freeze dried and stored at 5 °C for further experimentation.

2.3. Preparation of soy glycinin samples

Soy glycinin protein dispersions of 10% (w/w) solids were prepared by mixing the freeze-dried powder in deionized water at neutral pH and room temperature. To ensure proper dissolution, the samples were stirred for 2 h using a magnetic stirrer and stored at 4 °C overnight. Following this, a series of concentrations for the glycinin samples (20–80%, w/w) were prepared by dehydrating the original material using a rotary evaporator at 40 °C (±1 °C).

Vacuum sealed packages of glycinin samples were treated with high hydrostatic pressure at 600 MPa at about 21 °C (original temperature which did not reach more than 40 °C during experimentation) for 15 min using a cylindrical vessel apparatus of high pressure with 35 L volume and 70 mm plunger diameter (Quintus Press-QFP 35L, Avure Technologies, Kent, WA, U.S.A). Demineralised water was the pressure transmission medium and the increment of pressure was at a rate of 100 MPa per 20 s. Both pressurized and atmospheric samples ware subjected to physicochemical characterisation over a broad temperature range of −70 to 90 °C.

2.4. Experimental analysis

2.4.1. Sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed according to the method of Laemmli (1970) in a Bio-Rad mini-Protein electrophoresis cell at a constant voltage (100 V) with a gel concentration of 6%. Glycinin samples for SDS-PAGE were prepared by mixing with sample buffer (60 mM Tris-HCl, 2% SDS, 14.4 mM β-mercaptoethanol, 25% glycerol, 0.1% bromophenol blue, pH 6.8). Proteins were resolved on a 10% separation gel, stained with 0.1% Coomassie Brilliant Blue for 30 min and stored overnight. The solution was homogenised by 30 s sonication cycles for 3 min. Two different concentrations of non-denatured glycinin samples (50 and 100%) and five different concentrations of heat-denatured protein samples (10, 30, 60, 90, and 100%) were prepared from the stock solution. Following migration, the protein fractions were further stained with 0.1% Coomassie Brilliant Blue.

Fig. 1 reproduces a typical electrophoresis run of native soy glycinin in an SDS-PAGE gel. The different components present in lanes can be identified as AB subunits, which involve acidic and basic polypeptides of the soy protein (Kasapis & Tay, 2009; Petruccelli & Anon, 1995). In thermally treated lanes, the soy glycinin fraction is also made of acidic and basic subunits of about 37 and 15 kDa, respectively. The work revealed that the extracted sample of glycinin was not entirely pure but contained β-conglycinin subunits as cross-contamination. As discussed in the

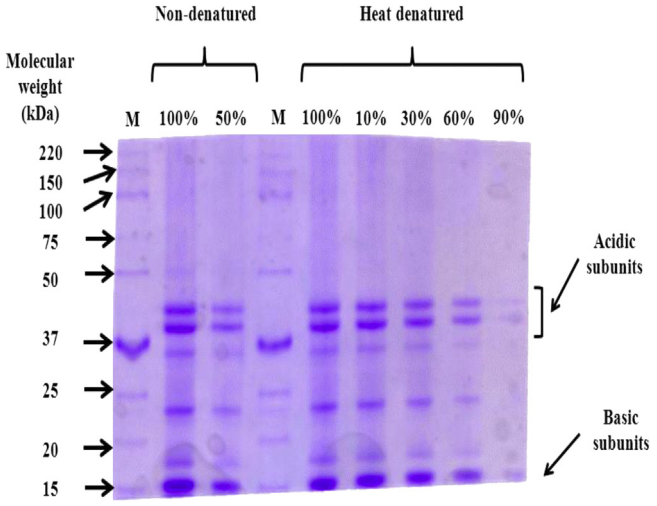

Fig. 1. SDS-PAGE of soy glycinin: lane M is the protein marker; lane 1 is the 100% non-denatured; lane 2 is the 50% non-denatured; lane 3 is the 100% heat-denatured; lane 4 is the 10% heat-denatured; lane 5 is the 30% heat-denatured; lane 6 is the 60% heat-denatured; lane 7 is the 90% heat-denatured (percentages refer to sample's relative concentration).