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Effect of phenolic compound removal on rheological, thermal and physico-chemical properties of soybean and flaxseed proteins

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ABSTRACT

This study aimed to investigate the effect of removal of phenolics on physico-chemical properties of protein isolates obtained from flaxseed and soybean. Proteins were isolated (I) from full-fat (F) and defatted (D) soybean (s) and flaxseed (f) using isoelectric precipitation. Free and bound phenolics were removed from the protein isolates. Thermal and gelation properties of protein isolates before and after removal of phenolics were investigated. Protein isolates from defatted soybean after removal of free and bound phenolics were showed a decrease in thermal stability of glycinin. For protein isolate from full-fat soybean, the results showed the removal of free phenolics increase thermal stability of glycinin with increase water holding capacity (WHC) and produce more viscous and less elastic gels as compared to protein isolate after removal of bound phenolics. Removal of free and bound phenolics from flaxseed protein isolates decrease thermal stability, WHC and viscoelastic properties as compared to protein isolate after removal of free phenolics.

1. Introduction

Little information has been reported about the on the interactions of phenolics in plants with the most other food components, e.g., proteins, carbohydrates and lipids, even though they are known to happen commonly (Bravo, Saura-Calixto, & Goni, 1992). The interactions of phenolic compounds with other food components in complex food systems affect the palatability of foods (Beauchamp & Maller, 1977). Interactions of tannins with other food components such as protein or polysaccharide components are known to occur (Bravo & Saura-Calixto, 1998). The chemistry of phenolics permit them to interact with other food components such as proteins due to the presence of hydrophobic interactions (Hagerman & Butler, 1978), ionic bonding (Rubino, Arntfield, Nadon, & Bernatsky, 1996), hydrogen bonding (Loomis & Battaile, 1966) and covalent bonding (Mason, 1955). These protein–phenolic interactions and lipid–phenolic interactions have been studied both in vitro and in vivo (Longstaff, Feuerstein, McNab, & McCorquodale, 1993; Quesada et al., 1996).

The contents of bound phenolic compounds in isolated protein from flaxseed were higher than isolated proteins from soybean (Alu'datt, Rababah, Ereifej, Brewer, & Alli, 2013). Alu'datt et al. (2013) found that the presence of protein–phenolic interactions in flaxseed and soybean protein isolates improved antioxidant properties of foods. Sinapic acid interacted in the protein isolate in isolated proteins from defatted flaxseed (Alu'datt et al., 2013). Little materials have been reported about naturally occurring interactions of phenolic compounds with proteins. Ratty and Das (1988) and Sabally (2006) reported that the protein–phenolic interactions may affect on functional properties and biological properties of the food containing proteins. Bendini, Toschi, and Lercker (2001) and Pelillo, Biguzzi, Bendini, Gallina Toschi, Vanzini, and Lercker (2002) found that carbohydrate–phenolic and lipid–phenolic interactions may enhance the antioxidant properties of food components. *p*-Coumaric acid, syringic acid and hesperidin were the major identified bound phenolic compounds identified in soybean protein isolates, while gallic acid, *p*-hydroxybenzoic acid, syringic acid, caffeic acid, ferulic acid and *p*-coumaric acid were the major bound phenolic compounds identified in flaxseed (Alu'datt et al., 2013). The overall objective of this research was to investigate the effects of removal of phenolics on physico-chemical properties of soybean and flaxseed protein isolates by measuring denaturation temperature, water holding capacity (WHC) and rheological properties.

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2. Materials and methods

2.1. Plant materials

Soybean (Cultivar SB, 210) was obtained by Great Lakes Organic Inc. (Ontario, Canada). Flaxseed was obtained from La Meunerie Milanaise Inc. (Milan, PQ, Canada). Samples were defatted using petroleum ether using a Soxhlet apparatus for 10 h (AOAC, 1995). The grains were cleaned and grounded and sieved to remove the bran and stored in refrigerator at 4° C for further analysis.

2.2. Preparation of protein isolates from oil-seeds

Isoelectric precipitation for flaxseed and soybean proteins was carried out according to the method described by Krase, Schultz, and Dudek (2002) with some modifications. Samples (10 g) of full-fat (F) and defatted (D) soybean (s) and flaxseed (f) were mixed with dilute NaOH (2.0 mol L^{-1}, 100 mL). The mixture was adjusted to pH 11.0 and stirred (23° C/60 min), then centrifuged (10,000×*g*, 30 min). The extract was filtered through cheese cloth and the residue was discarded. The extract was adjusted to pH 4.6 using dilute HCl (0.1 mol L^{-1}). The precipitated protein isolate was separated by centrifugation (10,000×*g*, 15 min), then freeze dried. The protein isolate obtained by this technique was designated as (I).

2.3. Removal of phenolics from protein isolates

2.3.1. Removal of 'Free' phenolics

A sample (1 g) was extracted with 25 mL methanol (24° C/1 h) then centrifuged (10,000×*g*, 10 min). Using methanol extraction for phenolics has been reported by van Ruth, Shaker, and Morrissey (2001). The supernatant was filtered using cheese cloth then flushed under a stream of nitrogen and stored at −18° C for further analysis.

The residue remaining from methanol extraction described above was extracted with 25 mL methanol (60° C/1 h) and centrifuged (10,000×*g*, 10 min). Use of heat for phenolics extraction has been reported by Johnsson, Kamal-Eldin, Lundgren, and Aman (2000). The supernatant was filtered using cheese cloth then flushed under a stream of nitrogen, and stored at −18° C for further analysis. The phenolics obtained by this technique were designated as free phenolics.

2.3.2. Removal of 'Bound' phenolics

Basic hydrolysis to extract bound phenolics has been reported by Krygier, Sosulski, and Hoggie (1982) and Eliasson, Kamal-Eldin, Andersson, and Aman (2003). The residue remaining from methanol/heat extraction after removal of free phenolic compounds as described in Section 2.3.1 was hydrolysed with dilute alkaline solution (25 mL, pH 12.0, 0.1 mol L^{-1} NaOH) for 12 h at 24° C then centrifuged (10,000×*g*, 10 min). The supernatant was filtered with cheese cloth. The bound phenolic compound of lyophilized supernatant extracted with 25 mL methanol (23° C/1 h) and centrifuged (10,000×*g*, 10 min). The supernatant was filtered using cheese cloth then flushed under a stream of nitrogen and stored at −18° C for further analysis.

The residue remaining from alkaline extraction described above was hydrolysed with dilute acid solution (25 mL, pH 2.0, 0.1 mol L^{-1} HCl) for 12 h at 24° C. Several authors used dilute acid to extract bound phenolics Oomah, Der, and Godfrey (2003). The supernatant was filtered with cheese cloth. The bound phenolic compound of lyophilized supernatant was extracted with 25 mL methanol (23° C/1 h) and centrifuged (10,000×*g*, 10 min). The phenolics obtained by this technique were designated as bound phenolics.

2.4. Effect of removal of phenolics on thermal properties of protein isolates

Thermal denaturation temperature of protein isolates was carried out using the procedure described by Ahmed and Ramaswamy (2006). Protein isolates were subjected to the thermal denaturation. Dispersions (20% w/v, 5% w/v NaCl, pH = 8) of all protein isolates were prepared. Thermal analysis was performed using a differential scanning calorimeter (DSC) (TA Q100, TA Instruments). The instrument was calibrated for temperature and heat flow using indium and sapphire standards. Nitrogen was used as purge gas at a flow rate of 50 mL/min. Hermetically sealed aluminum pans were used to avoid moisture loss during the analysis; a small sample of dispersion was sealed in the sample pan, weighed, cooled to −10 °C in the DSC and held for 10 min for equilibration. The heating rate was 5 °C/min over a range of 0–150 °C. A four-axis robotic device was used to automatically load the sample and reference pans to the DSC. The DSC data were analysed translated into PRN format for Microsoft Excels manipulation and stored on disc. As reference, an empty aluminum pan used. All analysis was performed in duplicate.

2.5. Effect of removal of phenolics on gelation properties of proteins

2.5.1. Preparation of gels

The procedure for preparation of gels was carried out according to the method described by Boye, Alli, Ismail, Gibbs, and Konishi (1995). Aqueous dispersions (20% w/v) of protein isolates from were prepared in beakers using 5% w/v NaCl and mixed well using magnetic stirrer. The pH was adjusted to pH 8 by addition of 0.1 mol L^{-1} NaOH solution. The beakers were covered with aluminum foil to prevent moisture loss. The protein dispersions were heated at 95 °C for 30 min using water bath. The gels were cooled to 4 °C for 24 h before water holding capacity and gelation studies.

2.5.2. Water holding capacity (WHC) of protein gels

WHC of the gels prepared in Section 2.5.1 was determined for all protein isolates using a centrifugation technique (Kinsella & Morr, 1984). The gels were centrifuged at (10,000×*g*, 15 min) and the separated supernatants after centrifugation were measured. Water holding capacity was expressed as the water separated after centrifugation. All measurements were done in triplicate.

2.5.3. Rheological properties of protein gels

The rheological properties of the gels prepared in Section 2.5.1 were determined for all protein isolates according to the method described by Ahmed and Ramaswamy (2006) with modifications using a rheometer (AR 2000, TA Instruments, New Castle, DE, USA). A measured volume (approximately 2 mL) of sample was placed on the bottom plate of the rheometer. The experimental temperature was 22 °C. The rheological properties of the protein gels were determined using a controlled stress rheometer, and the experimental dynamic rheological data were obtained directly from the TA Rheology Advantage Data Analysis software (V 5.1.42). The samples were subjected to oscillatory shear between two 60-mm parallel plates with a gap of 1000 μm. The AR 2000 was equipped with an efficient Peltier Temperature Control System, and the sample temperatures were precisely controlled and monitored. Dynamic rheological tests were performed within the linear viscoelastic region in the frequency (ω) range between 0.063 rad/s and 62.800 rad/s. Results were expressed as the storage modules (G′), loss modules (G″) and tan delta over time. All the rheological measurements were carried out in duplicate.