Food Hydrocolloids 61 (2016) 119–127

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Characterization and antioxidant activities of phenolic interactions identified in byproducts of soybean and flaxseed protein isolation

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ARTICLE INFO

Article history:
Received 12 April 2015
Received in revised form
30 January 2016
Accepted 2 May 2016
Available online 3 May 2016

Keywords:
Soybean
Flaxseed
Protein isolation residue
Protein isolation supernatant
Antioxidant activity
Protein-phenolic interactions
Protein-lipid-phenolic interactions

ABSTRACT

This study aimed to characterize the phenolic interactions generated from by-products of the protein isolation process from flaxseed and soybean flour. Protein isolates were removed from defatted and full-fat soybean and flaxseed using sodium hydroxide extraction and isoelectric precipitation. The residue and supernatant remaining after protein isolation were subjected to extraction of the free and bound phenolic compounds, which were evaluated for their phenolic profiles and interactions as related to their antioxidant activity. Analysis of residues obtained after isolation of the proteins showed the large phenolic contents of bound form in flaxseed (56–62%) and free form in soybean (59–85%). The supernatant remaining after precipitation of protein isolates of soybean and flaxseed revealed a relatively large proportion of phenolics in the free form ranging from 87 to 95%. The profile of free phenolics extracted from the residue and supernatant remaining after extraction and precipitation of protein from soybean and flaxseed differed from the profile of bound phenolic compounds. The measurement of degradation of a β-carotene-linoleic acid emulsion showed that the extracted bound phenolics from residue remaining after protein extraction had antioxidant activities ranging from 27 to 34% for full-fat soybean residue and 18–24% for full-fat flaxseed residue. No antioxidant activities were noted following for bound phenolics extracted after base hydrolysis from the residue remaining after protein isolation from defatted soybean and flaxseed. The occurrence of protein–lipid–phenolic interactions in flaxseed protein residue and protein–phenolic interactions in soybean protein residue likely play a role in the observed antioxidant activities.

1. Introduction

Phenolic content is well recognized as a significant functional minor food constituent in legumes, oil-seeds, fruits and vegetables (Bendini, Toschi, & Lercker, 2001; Escarpa & Gonzalez, 2001; Pelillo et al., 2002). The occurrence of phenolics as a food ingredient part may affect and improve its biological, nutritional and functional properties (Alu'datt, Rababah, & Alli, 2014). In that regard, oil-seeds are an important human food source for lipids, proteins and phenolics (Escarpa & Gonzalez, 2001). Phenolics can exert pharmaceutical, biological, nutraceutical and therapeutic effects via their antiinflammatory, antioxidant, antiviral, hypolipidemic, anticancer and hypoglycemic properties (Bravo, 1998; Hollman et al., 1996). Beauchamp and Maller (1977) indicated that phenolics as a food constituent can enhance food palatability. Arai, Suzuki, Fujimaki, and Sakurai (1966) labelled the taste characteristics of phenolic acids extracted from oil-seed as bitter, astringent and sour.

The presence of aromatic ring with hydroxyl group and carboxylic acids in phenolics increase their affinity to conjugate with major food components such as proteins, carbohydrates, lipids and minerals (Bravo, 1998; Escarpa & Gonzalez, 2001; Alu'datt, Rababah, Ereifej, Brewer, & Alli, 2013). Bravo (1998) reported that the interaction of tannins with both carbohydrates and proteins occurs frequently in foods and various food products. The chemical

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http://dx.doi.org/10.1016/j.foodhyd.2016.05.002
0268-005X/

nature of functional groups in phenolics and protein may increase their affinity to form complexes by covalent bonding, hydrophobic interactions, ionic bonds and hydrogen bonds (Hagerman & Butler, 1978; Loomis & Battaile, 1966; Mason, 1955; Rubino, Arntfield, Nadon, & Bernatsky, 1996). Ratty and Das (1988) and Sabally (2006) reported the interaction of the phenolic compounds in food systems rich with carbohydrates and lipids. Alu'datt, Rababah, Ereifej, Brewer, et al (2013) found that the bound phenolic content in isolated proteins from full fat and defatted flaxseed was higher than isolated proteins from full-fat and defatted soybean. Alu'datt, Rababah, Ereifej, Brewer, et al. (2013) suggested the naturally occurrence of protein-lipid-phenolic and protein-phenolic interactions in flaxseed and soybean. Until recently, most of the nutritional concern of phenolics pertained to their deleterious effects in reducing food digestibility caused by the capability of phenolics to conjugate and precipitate protein, lipids, minerals and carbohydrates (Bravo, 1998). Bravo, Saura-Calixto, and Goni (1992) and Alu'datt, Rababah, Ereifej, Brewer, et al. (2013) stated, however, that there is relatively little research literature regarding the biofunctional impact of the naturally occurring interactions between phenolics with food constituents such as antioxidant effects.

In food industry practices, the residue and supernatant remaining after extraction and precipitation of protein isolates at their isoelectric points is typically discarded or used for animal feed. Such by-products of protein isolation can have a rich phenolic content, which is present either in the free form and bound with other food constituents such as carbohydrates and lipids. In that regard, phenolic interactions with other food constituents is also responsible for errors in the quantification of phenolics, if only the free phenolic form is assessed. The antioxidant properties of the complexes generated from the interaction between phenolics and food constituents from oil-bearing plants could have use in the animal feed and food preservative industry (Alu'datt, Rababah, Ereifej, Brewer, et al., 2013). Such complexes have also been indicated to exert anticancer, antiviral, antiinflammatory, hypolipidimic and hypoglycemic properties that could have potential pharmaceutical indications. The main objective of this study was to investigate and characterize the effect of phenolic interactions in the residue and supernatant remaining after extraction and precipitation of protein isolated from soybean and flaxseed flour.

2. Materials and methods

2.1. Materials

Soybean (SB, 210) and flaxseed were obtained from Great Lakes Organic Inc. (Ontario, Canada) and La Meunerie Milanaise Inc. (Milan, QC, Canada), respectively. Flaxseed and soybean were cleaned, dried and grounded using a coffee grinder and then stored at −18 °C for further analysis. A soxhlet apparatus was used to remove lipids from samples using petroleum ether at 60 °C for 10 h.

2.2. Production of the residue and supernatant after precipitation of protein isolates from soybean and flaxseed

Proteins were removed from full-fat and defatted soybean and flaxseed samples according to the method described by Alu'datt, Rababah, Ereifej, Brewer, et al. (2013). Ten grams of samples were added to 100 ml of a 2 M NaOH aqueous solution (pH 11.0) and then mixed at 25 °C for 60 min in a water bath followed by centrifugation at 10,000×*g* for 30 min. The aqueous extract was clarified through cheese cloth. The residues remaining after extraction of proteins were flushed under a stream of nitrogen followed by lyophilization. The dried residue (Re) obtained by this procedure was stored at −18 °C for further investigation. The NaOH extracts were adjusted to pH 4.6 (0.1 M HCl) and then separated by centrifugation at 10,000×*g* for 15 min in order to precipitate the protein isolates. The supernatant remaining after precipitation of proteins was flushed under a stream of nitrogen followed by lyophilization. The dried supernatant (Su) obtained by this method was stored at −18 °C for further investigation. Fig. 1 shows the procedure for the process and preparation of residue and supernatant after protein extraction and precipitation from the full-fat and defatted soybean and flaxseed meals.

2.3. Extraction of free phenolics

One gram of residue and supernatant remaining after protein extraction from the full-fat and defatted soybean and flaxseed

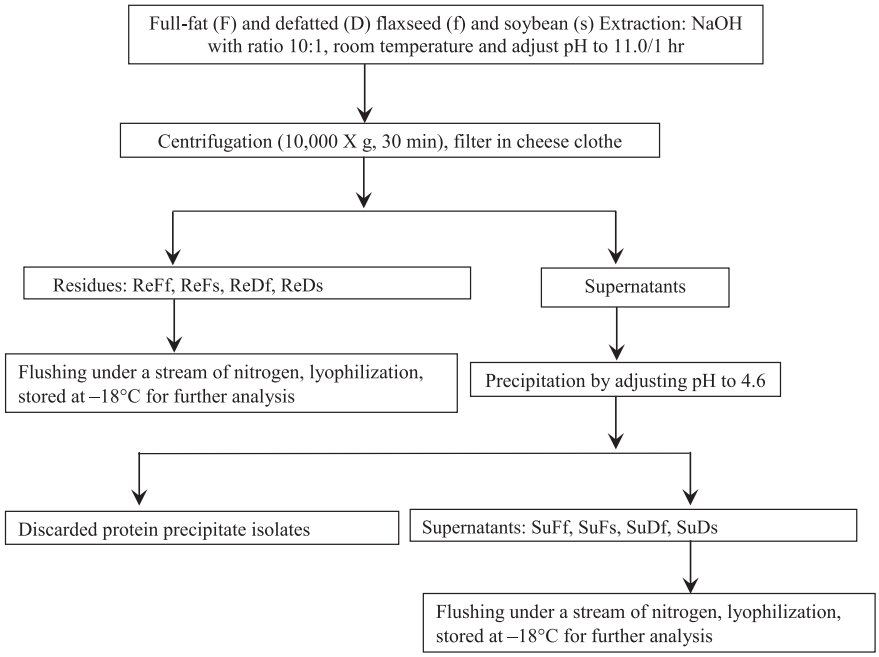

Fig. 1. Preparations of residues (Re) and supernatants remaining after precipitation of protein isolates (Su) from full-fat (F) and defatted (D) soybean (s) and flaxseed (f) samples.