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Effect of extraction method on functional properties of flaxseed protein concentrates

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ABSTRACT

Physicochemical (zeta potential (ζ), conductivity, surface hydrophobicity (H_0), protein solubility (PS)) and emulsifying (emulsion capacity (EC), droplet size, polydispersity (PDI), emulsifying activity (EAI), and stability (ESI) indexes) properties of alkali-(A-FPC), enzymatic-(E-FPC), and enzymatic-solvent-(ES-FPC) extracted protein concentrates from flaxseed meal (FM) were investigated and compared to commercial pea protein concentrate (PPC). The yield, composition, and properties of the protein concentrates were significantly influenced by the methods of extraction. All emulsions were similar in polydispersity with mono-modal droplet distribution and size of $\leqslant$0.43 μm that carried a net negative charge at neutral conditions (pH 7.0). A-FPC showed significantly higher H_0 (66.14) than that of ES-FPC (52.63), and E-FPC (43.27) and was comparable to PPC (68.47). The highest solubility was found for E-FPC followed by A-FPC at neutral pH. A-FPC displayed significantly ($p < 0.05$) the highest EC (87.91%), EAI (87.18 m^2/g) and ESI (12.51 min) compared to the other protein concentrates.

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1. Introduction

Flaxseed (*Linum usitatissimum* L.) is a unique plant source of polyunsaturated fatty acids and phytoestrogens that has been consumed all over the world for centuries (Omar, Shan, Zou, Song, & Wang, 2009). Flaxseed consists of 40–45% lipid, 20–25% protein, 20–25% fibre and 1% lignans (Hall, Tulbek, & Xu, 2006). To date, flaxseed use for protein and dietary fibre has not been valued as highly as the oil fraction, and has not been used as a source of protein for human consumption (Martínez-Flores et al., 2006). In New Zealand, the protein-rich defatted meal following oil extraction is one of the major by-products, and is being used primarily as animal feed (Teh, Bekhit, Carne, & Birch, 2014). Therefore, using flaxseed meal (FM) as a valued source of protein has potential to be an alternative food supply resource to help meet food requirements in the world (Omar et al., 2009). There are only a few studies on the protein components of flaxseed. These studies reported that flaxseed consists of two different protein fractions; a high molecular weight fraction (11–12 S; globulins) and a low molecular weight fraction (1.6–2 S; albumins) which are salt- and water-soluble respectively (Chung, Lei, & Li-Chan, 2005). The amino acid profile of FM is comparable to that of soybean and FM contains no gluten protein (Oomah & Mazza, 1993). Studies of flaxseed amino acid composition have shown that they have a beneficial influence on coronary heart disease and cancer (Wang, Li, Wang, & Özkan, 2010).

Flaxseed protein has been usually obtained by aqueous extraction of the seed meal involving solubilisation of proteins at high pH, followed by precipitation of the proteins at their isoelectric point (Dev & Quensel, 1988). The method of protein extraction from oilseeds has an effect on the quality, composition and functional properties of the extracted proteins (Aluko & McIntosh, 2001). Compared to other oil seeds, the extraction of flaxseed protein is complicated by the presence of mucilage, which is the soluble dietary fibre of flaxseed. Mucilage makes up almost 8% of the seed dry weight and may contribute useful emulsifying properties (Wang et al., 2010). However, the high viscosity of mucilage reduces protein extraction efficiency and impedes the sedimentation of protein during separation (Dev & Quensel, 1988; Mazza & Biliaderis, 1989). Flaxseed protein was first extracted by Osborne (1892), who reported the presence of an albumin-like protein and a globulin with 17.7% and 18.6% nitrogen content respectively. However, the Osborne (1892) methodology was not commercially practical (Oomah & Mazza, 1993). To date several methods for extraction of flaxseed protein have been investigated, such as isoelectric precipitation (Dev & Quensel, 1988; Karaca, Low, &

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Nickerson, 2011a; Krause, Schultz, & Dudek, 2002; Martínez-Flores et al., 2006; Silva, O'Callagahan, O'Brien, & Netto, 2013; Teh et al., 2014), using buffer (Kaushik et al., 2016; Oomah, Mazza, & Cui, 1994), Hexametaphosphate-assisted (Wanasundara & Shahidi, 1996), micellisation (Krause et al., 2002), salt extraction (Karaca et al., 2011a), and acid precipitation (Teh et al., 2014). Previous studies have either de-mucilaged flaxseed protein before extraction (Kaushik et al., 2016; Oomah et al., 1994), or used low values of the flaxseed protein concentrate with high viscosity (Wang et al., 2010). Moreover, flaxseed proteins produced mostly by alkali extraction have reported on the functionality with contradictory results (Krause et al., 2002; Martínez-Flores et al., 2006; Mueller, Eisner, & Kirchhoff, 2010). According to the literature, there is no study on extraction, physicochemical and emulsifying properties of FPC, produced by enzyme assisted (E-FPC) and enzyme-solvent assisted (ES-FPC) extractions, and these properties have not been compared with commonly used pulses emulsifier, such as pea protein concentrate (PPC). The objectives of the present study were to extract of FPCs using alkali isoelectric precipitation (A-FPC), enzymatic (E-FPC) and enzyme-solvent assisted (ES-FPC) extractions in order to produce protein concentrate with low viscosity (E-FPC, ES-FPC), evaluate the physicochemical (zeta potential, conductivity, surface hydrophobicity, protein solubility) and emulsifying (emulsion capacity, droplet size, polydispersity, emulsifying activity, and stability) properties of extracted FPCs, and compare these properties of FPCs with pea protein as one of the common plant-based emulsifier with no allergen concern.

2. Materials and methods

2.1. Materials

Cold-pressed flaxseed (*Linum usitatissimum* L.) meal (FM) was supplied by Bio Oils (Ashburton Limited, New Zealand). Pea protein (S85F) from yellow pea (*Pisum sativum*) was donated by Roquette-frères SA (Lestrem, France). Cellulase (from *Aspergillus niger*, activity 0.8 Units.mg^{-1} powder, pH 5.0, temperature 37 °C), Bovine serum albumin (BSA), and 8-anilino-1-naphthalenesulfonic acid (ANS) were purchased from Sigma–Aldrich (St. Louis, MO, USA). All general chemicals were analytical grade and obtained from Thermo Fisher (Scoresby, VIC, Australia). Deionized water was obtained from a Milli-Q Advantage A10 Water System (©EMD Millipore Corporation, Billerica, MA, USA).

2.2. Experimental methods

Three methods comprising (A-FPC), (E-FPC), and (ES-FPC) extractions were used for the isolation of protein from FM, containing about 10–15% residual oil after cold pressing. Defatted flaxseed meal (DFM) was prepared by dispersing FM in hexane (1:5, w/v) at ambient temperature (21 ± 2 °C) and stirring using a digital overhead stirrer (IKA® RW20, Staufen, Germany) for 6 h with renewal of hexane every 3 h to obtain defatted flaxseed meal as described previously (Amza, Amadou, Zhu, & Zhou, 2011). The DFM was dried in a fume hood to remove any remaining organic solvent and stored at −20 °C in vacuum-packed bags until analysed.

2.2.1. Alkali isoelectric precipitation

Alkali flaxseed protein concentrate (A-FPC) was prepared from DFM using isoelectric precipitation according to the methods of Amza et al. (2011). DFM (5%, w/v) was mixed with deionized water in a 2 L beaker and stirred using an overhead stirrer (IKA® RW20, Staufen, Germany). The suspension was adjusted to pH 9.5 by adding 1 M NaOH and mixing was maintained at 1600 rpm for 1 h. The pH of the suspension was kept at pH 9.5 during alkali extraction and then centrifuged (Beckman J2-21 M/E, Palo Alto, CA, USA) at 15,300*g*, for 30 min at 4 °C. The extraction procedure was repeated on the residual solids and the supernatants of the two extractions were pooled. In order to obtain a flaxseed protein concentrate (FPC), the pH was adjusted to 4.0 with 1 M HCl (Silva et al., 2013). The precipitated flaxseed protein was separated by centrifugation at 30,100*g* for 1 h at 4 °C, re-suspended thrice in acidified water (pH 4.0) and centrifuged, the final washed protein concentrate was adjusted to pH 7.0 with 1 M NaOH (Silva et al., 2013). The resultant flaxseed fractions consisting of residual solids, mucilage (in the supernatant) and protein concentrate were freeze dried (Virtis 12SL, Gardiner, NY, USA), vacuum-packed and stored at −20 °C for further analysis (Krause et al., 2002).

2.2.2. Enzymatic extraction

Enzymatic flaxseed protein concentrate (E-FPC) was prepared according to the method of Udenigwe, Lin, Hou, and Aluko (2009). DFM was mixed with deionized water as per the alkali isoelectric precipitation method (2.2.1). The suspension was then adjusted to pH 5.0 with 1 M HCl, followed by the addition of cellulase (2%, w/w) to DFM to enable hydrolysis of the fibre content. The suspension was transferred to a 2 L conical flask and agitated at 37 °C, and 150 rpm for 4 h in an orbital mixer incubator (Ratek, Victoria, Australia). The hydrolysed suspension was cooled to 4 °C followed by alkali extraction as described previously (2.2.1). The resultant less viscous flaxseed mucilage and precipitated protein concentrate were freeze dried, vacuum-packed and stored at −20 °C for further analysis.

2.2.3. Solvent extraction

Flaxseed protein concentrate was prepared as per enzymatic extraction (2.2.2). Then the precipitated flaxseed protein was mixed with 100 ml ethanol (95%) and the suspension was kept overnight at 4 °C. The precipitated flaxseed protein (ES-FPC) was separated by centrifugation at 15,300*g*, 4 °C for 15 min, washed, freeze-dried, vacuum-packed and stored at −20 °C for further analysis.

2.2.4. Proximate analysis

Moisture, ash, protein, fat, and carbohydrate contents of the primary materials (FM and DFM), residual solids, mucilage, protein concentrates (A-FPC), (E-FPC), (ES-FPC), and pea protein concentrate (PPC) were measured based on AOAC official methods (AOAC, 2003). Protein content was determined by the Kjeldahl method using a conversion factor (N% × 6.25) for all samples. Carbohydrate content was calculated based on percent differential from 100% considering the other components.

2.3. Physicochemical properties

For all tests, 0.01 M phosphate buffer (pH 7.0) was used to prepare protein solutions, except for the protein solubility, whereby a range of phosphate buffers from pH 2.0 to 8.0 were used (Adebiyi & Aluko, 2011).

2.3.1. Zeta potential and conductivity

The zeta potential (ζ) and conductivity of the protein concentrates were measured by preparation of the protein solutions (0.05%, w/w) in 0.01 M phosphate buffer, pH 7.0, using a laser light scattering Zetasizer (Nano-ZS, Malvern, Grove wood, UK) (Karaca et al., 2011a).

2.3.2. Average surface hydrophobicity (H_0)

Surface hydrophobicity was determined using the fluorescent probe, 8-anilino-1-naphthalenesulfonic acid (ANS) based on the methods of Kato and Nakai (1980). Protein solutions (0.01%, w/v) were prepared in 0.01 M phosphate buffer, pH 7.0, to obtain

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