



Complex coacervation between flaxseed protein isolate and flaxseed gum



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ABSTRACT

Flaxseed protein isolate (FPI) and flaxseed gum (FG) were extracted, and the electrostatic complexation between these two biopolymers was studied as a function of pH and FPI-to-FG ratio using turbidimetric and electrophoretic mobility (zeta potential) tests. The zeta potential values of FPI, FG, and their mixtures at the FPI-to-FG ratios of 1:1, 3:1, 5:1, 10:1, 15:1 were measured over a pH range 8.0–1.5. The alteration of the secondary structure of FPI as a function of pH was studied using circular dichroism. The proportion of α -helical structure decreased, whereas both β -sheet structure and random coil structure increased with the lowering of pH from 8.0 to 3.0. The acidic pH affected the secondary structure of FPI and the unfolding of helix conformation facilitated the complexation of FPI with FG. The optimum FPI-to-FG ratio for complex coacervation was found to be 3:1. The critical pH values associated with the formation of soluble (pH_c) and insoluble (pH₁) complexes at the optimum FPI-to-FG ratio were found to be 6.0 and 4.5, respectively. The optimum pH (pH_{opt}) for the optimum complex coacervation was 3.1. The instability and dissolution of FPI–FG complex coacervates started (pH₂) at pH 2.1. These findings contribute to the development of FPI–FG complex coacervates as delivery vehicles for unstable albeit valuable nutrients such as omega-3 fatty acids.

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1. Introduction

The process of complex coacervation or associative phase separation in protein–polysaccharide mixtures occurs due to electrostatic attraction of oppositely charged molecules, eventually leading to a solvent-rich and a biopolymer-rich phase (Schmitt & Turgeon, 2011; Tolstoguzov, 1998). Other factors influencing the complex coacervation are charge density, relative ratio, and total concentration of biopolymers, pH, and temperature of the solvent (Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998). Protein–polysaccharide mixtures form electrostatic complexes in a narrow pH range. Proteins are positively charged below their isoelectric point (pI) and can undergo complexation with negatively charged polysaccharides, resulting into soluble complex coacervates at pH_c, where pH_c is defined as the pH at which noncovalent interaction between protein and polysaccharide initiates (Aryee & Nickerson, 2012). Further reduction in mixture pH results into the formation of insoluble complexes at pH₁, where pH₁ is defined as the pH at which interaction between protein and polysaccharide is strong enough to cause macroscopic phase separation (Turgeon, Beaulieu, Schmitt, & Sanchez, 2003). The yield of complex coacervates is highest at pH_{opt}, where the net charge on the system is zero. The dissolution of complex coacervates back to solution state due to the protonation of polysaccharide occurs at pH₂, where pH₂ is

defined as the pH beyond which interaction between protein and polysaccharide starts decreasing. (Elmer, Karaca, Low, & Nickerson, 2011). The determination of these important pH values (pH_c, pH₁, pH_{opt}, and pH₂) for any protein–polysaccharide combinations provides better understanding of complexation behavior as a function of pH. De Kruif, Weinbreck, and de Vries (2004) suggested that pH induced changes in the conformation of protein also influence the complexation of polymers with proteins.

A number of studies have reported that plant proteins are capable of forming complex coacervates in the presence of polysaccharides. Pea protein is the most widely studied protein for complex coacervation (Ducel, Richard, Saulnier, Popineau, & Boury, 2004; Elmer et al., 2011; Klemmer, Waldner, Stone, Low, & Nickerson, 2012; Liu, Elmer, Low, & Nickerson, 2010; Liu, Low, & Nickerson, 2009a, 2009b). Other plant proteins considered appropriate for coacervation include soy protein (Jaramillo, Roberts, & Coupland, 2011), canola protein (Klassen, Elmer, & Nickerson, 2011) and corn protein (Quispe-Condori, Saldana, & Temelli, 2011).

There are plant proteins that are theoretically known to possess favorable characteristics for coacervation but are unexplored practically. Dickinson (2003) reported that charge density and droplet size are two important characteristics required for the stabilization of an emulsion. Wang, Li, Wang, Adhikari, and Shi (2010) observed that flaxseed protein concentrate when compared to soy protein concentrate, possessed higher surface charge and smaller emulsion droplet size. Recently,

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Kuhn, e Silva, Netto, and da Cunha (2014) found that flaxseed protein isolate (FPI) based emulsion are more stable than mixed FPI–whey protein isolate stabilized emulsions. In addition, the amino acid profile of flaxseed protein is nutritionally desirable, and it is considered nutritionally similar to other oil seed proteins such as soybean (Oomah, 2001). However, the complexation behavior of flaxseed protein with its own polysaccharide or with other polysaccharides has not been studied.

Flaxseed gum (FG) is another plant polymer identified as a good emulsifier (Cui, Ikeda, & Eskin, 2007). FG is a heteropolysaccharide composed of xylose, arabinose, glucose, galactose, galacturonic acid, rhamnose, and fucose (Cui, Mazza, Oomah, & Biliaderis, 1994). Functional properties of flaxseed gum are comparable to those of gum Arabic, and hence it can be used to replace gum Arabic in emulsions (Mazza & Biliaderis, 1989). Moreover, the consumption of flaxseed gum as dietary fiber is reported to reduce the blood glucose level thereby reducing the risk of coronary artery disease (Oomah & Mazza, 2000).

The important nutritional characteristics of flaxseed protein and gum mean that they can be economical source of functional foods (Oomah, 2001). A thorough study on the complexation behavior of these two biopolymers would help produce novel FPI–FG complex coacervates, which can be preferentially used to microencapsulate active bio-ingredients such as omega-3 oils. This study determines the optimum pH range, the FPI-to-FG ratio, and total biopolymer concentration required for the formation of soluble and insoluble complexes between FPI and FG. In order to gain greater insight into the formation of these complex coacervates, the underlying structural change of flaxseed protein as a function of pH was also investigated. Except for this work, the complexation behavior of flaxseed protein and flaxseed gum has not, so far, been reported.

2. Materials and methods

2.1. Materials

Golden flaxseeds (*Linum usitatissimum*) were received from Stoney Creek Oil Product Pty. Ltd (Talbot, VIC, Australia). FG and flaxseed protein isolate (FPI) were extracted in the laboratory at Federation University, Australia. All other chemicals used in this study were purchased from Sigma-Aldrich Australia (Sydney, New South Wales, Australia) and were analytical grade.

2.1.1. Extraction of flaxseed gum

FG was extracted from whole raw flaxseed using the method of Cui et al. (1994) with slight modification (Fig. 1). Briefly, the flaxseed was soaked in Milli-Q water at a flaxseed-to-water ratio of 1:18 at 50 °C with continuous and gentle stirring for 2 h for each of two consecutive cycles of extraction. The soaked seeds were filtered, and the water containing the dissolved gum was treated with three volumes of 95% ethanol to precipitate the gum. The precipitated gum was collected by centrifuge at 4,000g for 10 min. The precipitated gum was vacuum dried at 50 °C and stored at 4 °C.

2.1.2. Extraction of flaxseed protein isolate

Flaxseed protein was extracted from whole raw flaxseed following the method of Oomah, Mazza, and Cui (1994) with minor modifications. First, the flaxseeds were demucilaged as described in Section 2.1.1. The demucilaged seeds were dried in a hot air oven at 50 °C for 24 h and pulverized using a coffee grinder (EM0415, Sunbeam Corporation Ltd, NSW, Australia). The crushed meal was defatted for 3 h using hexane at a flaxseed-to-hexane ratio of 1:6. The hull was separated from the kernel by screening the tailings using a 0.15 mm sieve to further reduce the interference of the mucilage during protein extraction. This fat extracted powder was subsequently soaked in 0.1 M Tris buffer (pH 8.6 with 0.1 M NaCl) for 6 h at a powder-to-buffer ratio of 1:16. The large residues were then separated from the protein extract using double layered cheesecloth. This filtered sample was centrifuged at 9,000g for 20 min using an ultracentrifuge (Sorvall Instruments, Wilmington,

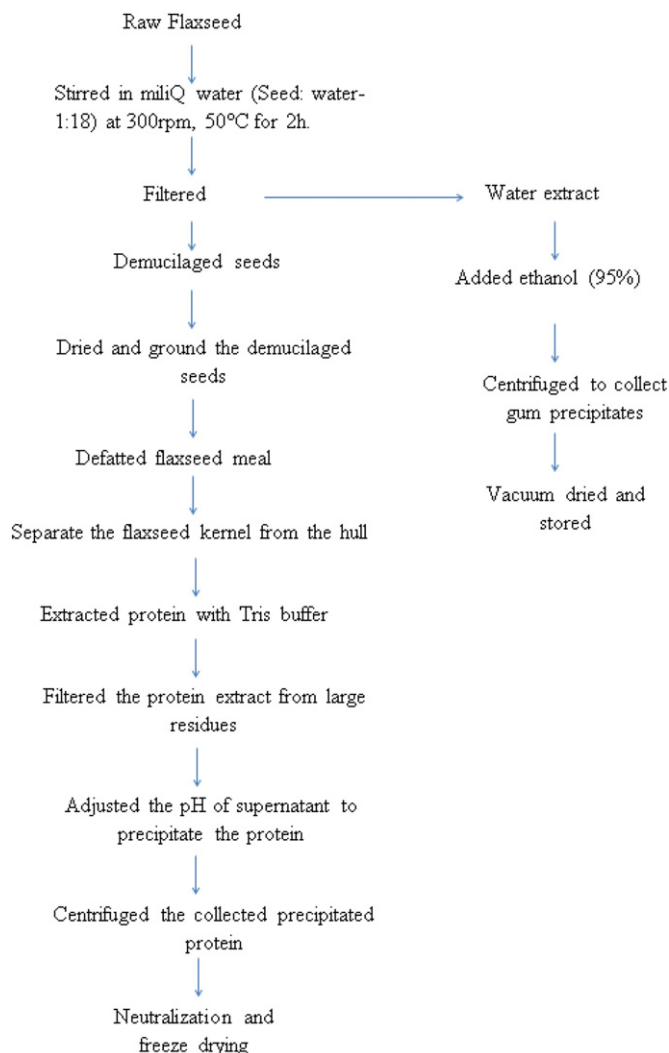


Fig. 1. The protocol of extraction of flaxseed gum (FG) and flaxseed protein isolate (FPI) from whole flaxseeds.

DE). The supernatant was collected and the pH was adjusted to 4.2 using 0.1 M HCl to precipitate the flaxseed protein. Once the pH was adjusted, the sample was stored at 4 °C for 16 h in order to provide sufficient time for protein to precipitate completely. The precipitated protein was recovered by centrifuging at 12,000g for 20 min. The recovered solid mass was redispersed in Milli-Q water and was neutralized using 0.1 M NaOH. Finally, the FPI was obtained by freeze drying the sample at −45 °C compressor temperature and 0.5 mm vacuum pressure using a freeze drier (DYNAVAC, Dynavac Engineering, Australia). The freeze-dried FPI was ground, vacuum sealed, and stored at 4 °C.

2.2. Chemical analysis of FPI and FG

Chemical analyses on all materials were performed according to AOAC Methods 925.10 (moisture), 923.03 (ash), 920.87 (crude protein), and 920.85 (lipid) (AOAC, 2003). Carbohydrate content was calculated on percent differential from 100%.

2.3. Identifying pH_c, pH₁, pH_{opt}, and pH₂ by turbidimetric analysis

FPI (1% w/w; pH 8.4) and FG stock solutions (0.3%, w/w; pH 7.0) were prepared by dispersing FPI and FG powders in Tris buffer (0.1 M pH 8.4) and Milli-Q water, respectively, followed by stirring at 500 rpm for 16 h at room temperature (21–22 °C), and 1 h at 40 °C to dissolve the protein. Tris buffer at pH 8.4 was used to prepare FPI

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