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Nature of protein-protein interactions during the gelation of canola protein isolate networks

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ABSTRACT

The nature of interactions involved during the gelation of a canola protein isolate was investigated using rheology and fractal imaging at neutral pH as a function of protein concentration (5.0–9.0% w/w). The onset of denaturation and the denaturation temperature by differential scanning calorimetry for canola protein isolate (CPI; 98.2% protein) was 78.6 °C and 87.1 °C, respectively. Rheological testing determined the gelation temperature (T_{gel}) to be ~87–90 °C for all concentrations. The log % strain at break increased from 1.70 to 1.80 as CPI concentration increased from 5.0 to 7.0% (w/w). Rheological testing of CPI in the presence of destabilizing agents, NaCl (0.1 and 0.5 M), urea (0.1, 0.5, 1 and 5 M) and 2-β-mercaptoethanol (0.1 and 2%), was performed. Samples with NaCl and urea (0.1–1 M) had similar temperature profiles and T_{gel} values to CPI alone whereas no gel was formed with the addition of 5 M urea and 2-β-mercaptoethanol reduced the strength of the gel network. Fractal dimension and lacunarity was analyzed using CLSM imaging. The fractal dimension value for all CPI concentrations was ~1.5. The lacunarity of the gel decreased from 0.62 to 0.41 as the concentration of CPI increased from 5 to 7% (w/w). Mechanistic understanding of CPI aggregation and network formation will enable the food industry to better tailor food structure when CPI is present as ingredient.

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1. Introduction

The use of plant protein ingredients as an alternative to animal derived sources by the food industry is growing due to their lower cost, availability, and consumer perception of health and sustainability (Stone, Karalash, Tyler, Warkentin, & Nickerson, 2015). A protein's ability to gel is an important functional attribute required to maintain food quality and structure in a wide range of food applications, for use in controlled delivery systems for carrying bioactive compounds and in the development of edible films/packages (Doi, 1993; Akbari & Wu, 2016). During heat-set gelation, proteins partially denature to allow for aggregation and the formation of a gel network (Zheng, Matsumura, & Mori, 1993). Gel formation and the final structure depend on: a) intrinsic protein factors, such as thermal stability, size and composition; and b) external factors, such as temperature, pH, ionic strength, and the type of salts present (Doi, 1993; Zheng et al., 1993). Understanding the nature of interactions during canola protein gel formation is important for the development of novel uses for canola protein and low value canola meal.

Canola (*Brassica napus* L.) is an important oilseed crop grown primarily for its high oil content and contributes 19.3 billion/year to the Canadian economy (LMC, 2013). During processing, seeds are crushed to remove the oil leaving a protein-rich meal as the by-product. This meal, consisting of up to 50% protein has been used mostly as animal feed but due to its nutritional and functional properties it is being investigated for its use in the food industry and also the bioproduct industry (Aider & Barbana, 2011). Canola protein has a balanced amino acid profile and a high protein efficiency ratio (PER = 3.29) (Khattab & Arntfield, 2009). Canola protein has been studied for its bioactive peptides, functional properties (solubility, gelation, foaming and emulsion capacity and stability), for the protection and release of bioactive compounds, and as a protein bioplastic (Akbari & Wu, 2016; Manamperi et al., 2015; Wu, Aluko, & Muir, 2009). It consists of mainly two proteins, cruciferin (~65%) and napin (~25%). Cruciferin (12 S) is a 300–310 kDa globulin protein containing multiple subunits stabilized primarily by non-covalent linkages, whereas napin (2 S, albumin) has a lower molecular weight (12–14.5 kDa) and is composed of two polypeptides stabilized primarily by disulfide bonds (Aider & Barbana, 2011; Aluko & McIntosh, 2001; Berot, Compoint, Larre, Malabat, & Gueguen, 2005; Monsalve & Rodriguez, 1990). A minor protein fraction, oleosin, is a

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low molecular weight amphiphatic protein found in the oil bodies of the seed (Tzen, Lai, Chan, & Huang, 1990). The amount of each type of protein found in canola is dependent on the climate, the environment, the growing conditions and the protein extraction process (Aider & Barbana, 2011).

The gelation properties of canola proteins have typically involved the addition of fixatives (e.g., transglutaminase) (Pinterits & Arntfield, 2008), the use of chemically modification (e.g., succinylated and acetylated proteins) (Paulson & Tung, 1988; Schwenke, Dahme, & Wolter, 1998), and mixtures involving anionic polysaccharides (Uruakpa & Arntfield, 2004). In general, cruciferin forms a heat-set gel under alkaline conditions, producing stronger gels than when prepared under acidic conditions (Léger & Arntfield, 1993). Napin on the other hand, does not have the same gelation properties as cruciferin and is only able to form a weak gel (Tan, Mailer, Blanchard, Agboola, & Day, 2014). Schwenke et al. (1998) reported that gelation temperature of salt extracted CPI that was comprised of 70% cruciferin and 30% napin to be 69 °C at pH 9.0 with 15% CPI. Also, the author reported that 12.5% purified cruciferin protein isolate-formed stronger gels with higher shear modulus than 12.5% canola proteins isolate between pH 6.0 and 8.0.

Léger and Arntfield (1993) studied gel formation involving 6% of 12S CPI that was extracted using a protein micellar mass method. The authors investigated the gelling properties of CPI as a function of pH, and with the addition of different concentration of salts, dithiothreitol, and guanidine hydrochloride. As pH range varied from pH 4.0 to 11.0, the authors found that stronger gels formed under alkaline conditions relative to acidic ones. The authors also reported that at pHs close to the isoelectric point, gel networks displayed the highest G′ values (describes the elastic component of the gel). The addition of salt was found to contribute to the thermal denaturation properties of 6% 12S canola globulin. At pH 9.0, the 12S canola globulin denatured at 81 °C, however with the addition of 0.1 M sodium salts (e.g., sodium sulfate, sodium acetate, sodium chloride, and sodium thiocyanate) thermal of the proteins increased leading to higher denaturation temperatures (i.e., 85.75 °C–87.4 °C). Moreover, the addition of the aforementioned sodium salts to the 12S canola globulin solution resulted in similar cooling curves when a temperature ramp was performed from 90 °C to 25 °C at 2 °C/min, indicating a similar gelation mechanism. The addition of guanidine hydrochloride also led to alteration of the protein conformation by disrupting the covalent bonds causing weakened gel network (Léger & Arntfield, 1993).

Paulson and Tung (1988) studied the use of succinylation of CPI to enhance gelation, where unmodified canola protein gelled at pHs only above 9.5, but succinylated canola protein was able to gel over a much wider pH range (pH 5.0–11.0). The presence of insoluble particulates in the unmodified canola resulted in the formation of opaque gel, whereas the succinylated CPI produced translucent gel except at pH 5.0. The authors reported that opaque gels had the characteristic of having a pasty precipitate with weak elasticity, whereas the translucent gel was firm and springy. Schwenke et al. (1998) investigated acetylated CPI on their gelation properties. The authors reported that the acetylated CPI consisting of 70% cruciferin and 30% napin showed strong pH dependence for gelation temperature due to an increase in the number of negatively charged carboxyl groups. Acetylated CPI (12.5%) showed the highest dynamic storage modulus (G′) values at pH 6–6.3, whereas napin showed highest the G′ at ~pH 9.

The overall goal of this study was to investigate the nature of interactions involved during the gelation of a canola protein isolate without any form of modification using primarily rheology and fractal imaging (based on network morphology). Findings from this study will shed new light into mechanisms driving canola protein gel formation. To date, work has been limiting in terms of gaining a better understanding of the nature of interactions occurring within the network.

2. Materials and methods

2.1. Materials

Defatted canola meal produced from *Brassica napus* L. was kindly donated by Agriculture and Agri-Food Canada (Saskatoon, SK, Canada) after being processed by POS BioSciences Corp. (Saskatoon, SK, Canada). All chemicals used in this study, unless otherwise stated were purchased from Sigma-Aldrich (Oakville, ON, Canada). Water used in this study was Milli-Q™ water (EMD Millipore, Billerica, MA, USA).

2.2. Preparation of canola protein isolates

A canola protein isolate (CPI) was prepared from defatted meal using slightly modified methods of Klassen, Elmer, and Nickerson (2011). In brief, 0.05 M Tris-NaCl buffer (Lot 103470, Fisher Scientific, Fair Lawn, New Jersey, USA) containing 0.1 M NaCl was prepared and adjusted to pH 7.0 using 1.0 N (HCl). The prepared buffer was then used to dissolve the defatted meal at a meal-to-buffer ratio of 1:10 for 2 h at room temperature (22–23 °C) under constant stirring (500 rpm) using a mechanical stir plate. The dispersion was then centrifuged (Sorvall RC Plus Superspeed Centrifuge, Thermo Fisher Scientific, Asheville NC, USA) at 3000 $\times g$ for 1 h to collect the supernatant, followed by a second centrifuge step after removal of the pellet (3000 $\times g$ for 1 h) to further clarification. The supernatant was then vacuum filtered using a #1 Whatman filter paper (Whatman International Ltd., Maidston, UK), dialyzed (Spectro/Por tubing, 6–8 kDa cut off, Spectrum Medical Industries, Inc., Rancho Dominguez, CA USA) at 4 °C where Milli-Q™ water was changed 3 times a day for 72 h to remove the salt, and then freeze-dried using a FreeZone 6 freeze drier for 24 h (Labconco Corporation, Kansas City, MO, USA) to produce a dry CPI powder. The powder was stored at 4 °C for later usage. The proximate composition of the CPI was determined using the Official Methods of Analysis of AOAC International, including methods 923.03, 920.87, 925.10 and 960.39 for ash, crude protein (%N $\times$ 6.25), moisture and crude fat, respectively (AOAC, 1990; AOAC, 2003). Percent carbohydrate was determined based on the difference from 100%. The proximate analysis was conducted in triplicate.

2.3. Amino acid composition

The amino acid profile for CPI was determined by POS (POS BioSciences Crop., Saskatoon, SK, Canada). High performance chromatography (HPLC) and pico-tab amino acid analysis system (Waters Corporation, Milford, MA, USA) was used to analyze the amino acid profile of CPI and SPI. In brief, it followed method developed by Bidlingmeyer, Cohen, Tarvin, and Frost (1987), where 15 mL of 6 N HCl was added to the CPI samples to hydrolyze the protein before HPLC separation. AOAC official methods 985.28 (AOAC, 2003) was used to determine the sulfur-containing amino acid where cysteine and methionine was oxidized with 10 mL of cold performic acid before hydrolysis of protein. AOAC method 988.15 (AOAC, 2003) was used to examine the amount of tryptophan where tryptophan was hydrolyzed with 10.0 mL of 4.2 M NaOH prior to HPLC analysis.

2.4. Differential scanning calorimetry

The thermodynamic properties of a 9.0% (w/w) CPI gel network at pH 7.0 was investigated using differential scanning calorimetry (DSC). This pH was chosen to be slightly by CPI's isoelectric point (pH 5.6, Klassen et al., 2011). CPI solutions that contain 9.0% (w/w) CPI were used instead of 7.0% CPI as the enthalpy of transition was greater providing more accurate analysis. Gel samples of approximately 10 mg were weighted into Tzero Alodined pans and hermetically sealed (TA Instruments, New Castle, DE, USA). Samples were heated at 5 °C/min from 25 to 110 °C using a Q2000 DSC (TA Instruments, New Castle, DE, USA). The

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