Food Research International 81 (2016) 31–38

Contents lists available at ScienceDirect

Food Research International

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

The effect of pH on the gelling behaviour of canola and soy protein isolates

Jae Hee J. Kim, Natallia V. Varankovich, Michael T. Nickerson *

Food and Bioproduct Sciences, University of Saskatchewan, Agriculture Building, 51 Campus Drive, Saskatoon, SK S7N 5A8, Canada

ARTICLE INFO

Article history:
Received 31 August 2015
Received in revised form 28 December 2015
Accepted 28 December 2015
Available online 30 December 2015

Keywords:
Canola
Soy
Gelation
Fractal dimension
Rheology
Confocal laser scanning microscopy

ABSTRACT

The present study investigates the gelation mechanisms of a canola protein isolate (CPI) as a function of a pH (3.0–9.0), and compares it to that of a commercial soy protein isolate (SPI). A rheological investigation found that CPI was non-gelling at pH 3.0, and then formed a gel with increasing strength as pH was raised from pH 5.0 to 9.0. In contrast, the commercial SPI ingredient was found to be non-gelling at pH 9.0, but formed the strongest networks at pH 5.0 near its isoelectric point (pI = 4.6). Denaturation temperature as determined by differential scanning calorimetry were found to occur at ~78 °C for CPI at pH 5.0, then shifted to higher temperatures (~87 °C) at pH 7.0/9.0, whereas detection of SPI denaturation could not be obtained due to instrument sensitivity. Gelling temperatures were similar for both CPI and SPI (~82–86 °C) at all pHs, with the exception of SPI at pH 5.0 (~46 °C). Overall CPI networks were stronger than SPI, since the latter had weaker inter- and intramolecular junction zones. Confocal laser scanning microscopy images indicated that CPI gels became denser with lower lacunarity values as pH increased from 3.0 to 9.0. Moreover, the fractal dimension of CPI gels was found to increase from ~1.5-1.6 to ~1.8 as pH increased from 5.0/7.0 to 9.0, respectively suggesting diffusion-limited cluster-cluster aggregation. Images of SPI networks were not concurrent with fractal analysis under the conditions examined. Despite CPI having excellent gelling properties that are comparable to SPI, its need for alkaline pH conditions will limit its applicability in foods.

1. Introduction

Canola proteins are starting to gain tremendous interest from the food industry as a potential plant protein alternative to soy because of their blend of essential amino acids, functionality and availability (Canola Council of Canada, 2011; Ohlson & Anjou, 1979). Canola proteins are isolated from the meal once the oil has been extracted, as a means to enhance the value of the meal by-product beyond feed markets. Although soy proteins currently dominate the plant protein market, allergen concerns and its beany taste are driving the search for suitable replacements.

Canola proteins are dominated by two major proteins: napin and cruciferin, representing ~20% and ~60% of total protein, respectively (Aider & Barbana, 2011). Cruciferin (11/12 S; S is a Svedberg unit) is a salt soluble hexameric globulin protein made of six subunits and has a molecular mass of 300–310 kDa. Each subunit comprises of an acidic α-chain (~30 kDa) and a basic β-chain (~20 kDa) connected by one disulfide linkage (Aluko & McIntosh, 2001; Wanasundara, 2011). In contrast, napin (2 S) is a water soluble albumin protein with a molecular mass of ~12–17 kDa. Napin consists of 2 polypeptides held together by disulfide bonds (Salleh et al., 2002). A third protein, known as oleosin is also present within the oil bodies within the seed and hence the oil fraction, and makes up ~2–8% of the total proteins (Aider & Barbana, 2011; Höglund, Rödin, Larsson, & Rask, 1992; Salleh et al., 2002). In contrast, soy is dominated by two globulin proteins: glycinin and β-conglycinin (Arrese, Sorgentini, Wagner, & Añón, 1991). Glycinin (11 S) is hexameric protein comprised of six subunits, with each subunit consisting of an acidic and basic polypeptide chain held together by a disulfide linkage (Chen et al., 2013). β-Conglycinin is a 7 S globulin trimer comprised of three subunits (α′, α and β) held together by non-covalent interactions (Chen et al., 2013).

In order to enhance the utilization of canola proteins, a greater understanding of their functional attributes is needed in relation to soy. In terms of gel formation, a 3-dimensional gel network is formed when an infinitely branched protein aggregate occupies a given space (Rogovina, Vasil'ev, & Braudo, 2008). Typically, temperature is raised in globular proteins to induce some level of partial or complete denaturation of the quaternary and tertiary structure to expose buried hydrophobic moieties to the surface. Depending on the solvent conditions, fractal aggregates develop into a weak, less ordered particulate-type gel (random aggregation) or a stronger, more ordered fibrous ('tree-like branching' or 'string-of-bead-type' aggregates) network (Doi & Kitabatake, 1989). Growth typically occurs in 2-steps: first, proteins approach one another and interact via weak linkages (e.g., van der Waals

* Corresponding author at: Department of Food and Bioproduct Sciences, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK S7N 5A8, Canada.
E-mail address: michael.nickerson@usask.ca (M.T. Nickerson).

http://dx.doi.org/10.1016/j.foodres.2015.12.029
0963-9969/

forces); and second, stronger bonds (e.g., ionic and covalent) form to hold the larger structures firmly together (Meakin & Jullien, 1988). Aggregate growth can be described according to the material's fractal dimension (D_f) (or space filling capacity), which depending on its magnitude, suggests whether growth is diffusion limited ($D_f = 1.7–1.8$) or reaction-limited ($D_f = 2.0–2.2$) (Ikeda, Foegeding, & Hagiwara, 1999; Meakin & Jullien, 1988).

The overall goal of this research was to examine the gelation mechanism as a function of pH for a canola protein isolate (CPI), and to compare it to that of a commercial soy protein isolate (SPI) product.

2. Materials and methods

2.1. Materials

Defatted canola meal produced from *Brassica napus* (2012 crop year) was kindly donated by Agriculture and Agri-Food Canada (Saskatoon, SK, Canada) after being processed by POS BioSciences Corp. (Saskatoon, SK, Canada). The meal served as the starting material for protein extraction. A commercial soy protein isolate product was kindly donated by Archer Daniels Midland Company (PRO-FAM 974, Lot 13020412, Decatur, IL, USA) for this project. 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

Canola protein isolate (CPI) was prepared from defatted meal using slightly modified methods of Folawiyo and Apenten (1996) and 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 Super speed Centrifuge, Thermo Fisher Scientific, Asheville NC, USA) at 3000 × *g* for 1 h to collect the supernatant, followed by a second centrifuge step after removal of the pellet (3000 × *g* for 1 h) for 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. The protein solution was then frozen (−30 °C) within a chest freezer and then freeze dried using a temperature difference of 35 °C for 24 h (FreeZone 6 freeze dryer, Labconco Corporation, Kansas City, MO, USA) to produce a dry CPI powder. The powder was stored at 4 °C for later usage within a walk-in cold laboratory.

2.3. Proximate composition

The defatted canola meal, CPI and SPI proximate compositions were determined using the AOAC methods 923.03, 920.87, 925.10 and 960.39 for ash, crude protein (%N × 6.25), moisture and crude fat, respectively (AOAC, 1990, 2003). Percent carbohydrate was determined based on the difference from 100%. The proximate analysis was conducted in triplicate.

2.4. Differential scanning calorimetry

The thermodynamic properties of a 9.0% (w/w) CPI gel network was investigated using differential scanning calorimetry (DSC) as a function of pH (5.0, 7.0 and 9.0). 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 weight 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 instrument was calibrated using indium. From the heating curve, the onset temperature, denaturation temperature and the enthalpy associated with the denaturation were determined. Samples were measured in triplicate and reported as a mean ± one standard deviation. CPI at pH 3.0 was non-gelling and therefore was not tested, whereas exothermic events associated with soy proteins could not be detected by the instrument.

2.5. Surface charge (zeta potential)

Overall surface charge of CPI and SPI was determined by measuring the electrophoretic mobility (U_E) of 0.05% (w/w) protein solutions as a function of pH (2.0–9.5) using a Zetasizer Nano-ZS90 (Malvern Instruments, Westborough, MA, USA). Zeta potential (ζ) is calculated by applying U_E to the Henry's equation:

$$U_E = \frac{2\varepsilon\zeta f(\kappa\alpha)}{3\eta} \quad (1)$$

where ε is permittivity, $f(\kappa\alpha)$ is a function related to the ratio of particle radius (α) and Debye length (κ), and η is the dispersion viscosity. A Smoluchowski approximation $f(\kappa\alpha)$ of 1.5 was assumed for this study, as is convention when using a folded capillary cell, and with samples of particles sizes larger than 0.2 μm dispersed in a moderately electrolyte solution (>1 mM). The Smoluchowski approximation assumes that a) the concentration of particles (proteins) is sufficiently high such that such thickness of the electric double layer (Debye length) is small relative to the particle size ($\kappa\alpha \gg 1$); and b) ζ is linear related to U_E. All measurements were reported as the mean ± on standard deviation ($n = 3$).

2.6. Rheological properties of CPI and SPI solutions

The rheological properties of CPI and SPI solutions were examined using a 7.0% (w/w) CPI or SPI protein concentration at pH 3.0, 5.0, 7.0 and 9.0. Canola and SPI was prepared by dispersing their respective powders (adjusted for protein levels) into 0.1 M NaCl prepared with Milli-Q water (Millipore Corporation, MA, USA), and was then allowed to stir using a mechanical stir plate at 500 rpm for 1 h at room temperature (22-23 °C). The pH of the solution was adjusted to 7.0 using 0.5 M NaOH or HCl, and periodically checked during stirring.

All rheological measurements were made using an AR-1000 rheometer (TA Instrument, New Castle, DE, USA) equipped with a Peltier plate temperature control, and a 40 mm diameter −2° cone and plate geometry (with a gap of 51 μm). Each protein solution (~630 μL) was transferred onto the geometry, and allowed to equilibrate for 5 min prior to analysis. To prevent sample drying during heating, a light application of mineral oil was placed on the fringe of the geometry. The viscoelastic storage (G′) and loss (G″) moduli were initially followed during a heating-cooling cycle for each sample. Temperature was ramped upwards from 25 °C to 95 °C on a continuous basis at a rate of 1 °C/min, a frequency of 0.1 Hz and strain amplitude of 1%. The sample was then allowed to equilibrate at 95 °C for 5 min, and then ramped downwards from 95 °C to 25 °C at the same rate. The G′ was plotted vs. temperature on arithmetic coordinate to determine the heat setting temperature (or sol-gel transition temperature), taken by extending the tangent from the steepest part of the rise in G′ to the x-axis in the heating curve (Rogers & Kim, 2011; Winter & Chambon, 1986). Following the temperature cooling ramp, the sample was allowed to equilibrate at 25 °C for 1 min, followed by a time sweep measurement of G′ for 1 h at a frequency of 0.1 Hz to evaluate the level of structure formation over time. Once completed, both G′ and G