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Emulsifying properties of proteins extracted from Australian canola meal

Siong H. Tan [a,b,*], Rodney J. Mailer [c], Christopher L. Blanchard [a,d], Samson O. Agboola [a,b]

[a] E.H. Graham Centre for Agricultural Innovation, Pine Gully Road, Wagga Wagga, NSW 2650, Australia
[b] School of Agricultural and Wine Sciences, Charles Sturt University, Private Bag 588, Wagga Wagga, NSW 2678, Australia
[c] NSW DPI, Pine Gully Road, Wagga Wagga, NSW 2650, Australia
[d] School of Biomedical Sciences, Charles Sturt University, Private Bag 588, Wagga Wagga, NSW 2678, Australia

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ABSTRACT

Canola protein albumin fraction, globulin fraction, and canola protein isolate (CPI) were compared to commercial soy protein isolate (SPI) in terms of their emulsifying properties at various pH values. The globulin fraction had higher emulsifying capacity (EC), higher emulsifying activity index (EAI), and the droplet size of emulsions it stabilized was consistently smaller irrespective of pH compared to albumin fraction or CPI. In comparison to SPI, globulin fractions also had higher EC at all pH values tested, higher EAI at acidic pH, and smaller or comparable average emulsion droplet size at both pH 4 and 7. The stability of canola protein based emulsions were comparable to those of SPI based emulsions at most pH values (except the emulsion stabilized by the CPI at pH 4), with no significant ($p > 0.05$) changes in droplet size during storage for up to 7 days at room temperature. These emulsions, however, experienced separation into the emulsion and serum phases after 24 h storage at room temperature with the exception of CPI- and SPI-stabilized emulsions at pH 9. This study demonstrates the comparable emulsifying properties (forming or stabilizing) of some canola proteins to commercially available SPI, suggesting the potential use of canola proteins in food applications.

1. Introduction

Production of canola in Australia was a record 2.2 mil ton in 2010/11 season due to an increase in plantation area (AOF Crop Report, August 2011). The average oil content for the harvest was 42.9% in whole seed, and the average protein content was 39.9% in oil-free meal (Seberry, Parker, & Ayton, 2010). The protein-rich canola meal left after the oil extraction is generally used as a protein source in livestock and aquaculture industries (Canola Council of Canada, 2009). The potential use of Australian canola meal has not been fully explored. The continuous growth in canola production worldwide implies that more meal will be produced as a result of the increased oil extraction. This signifies the need to re-assess the use of canola meal, possibly for the extraction of high market value products, such as functional protein extracts for use in human food applications.

Canola meal has been recognized as a potential alternative protein source for human consumption due to its nutritional value and technological functionalities (Aider & Barbana, 2011; Tan, Mailer, Blanchard, & Agboola, 2011a). However, the presence of antinutritional factors such as glucosinolates and major phenolic compounds including sinapine and tannins has restricted the incorporation of canola meal in human food. Our recent study on the extracted protein fractions has concluded that glucosinolate content of all the protein fractions was below the detection limit (<3 μmol/g), and sinapine was not detected in most of the extracted protein fractions (Tan, Mailer, Blanchard, & Agboola, 2011b). This finding suggested that canola protein extracts would be a safe ingredient in human food products.

As a food ingredient, technological functional properties of a protein are crucial. Proteins generally have excellent emulsifying properties (Smulders, Caessens, & Walstra, 1999). However, contradictory views on emulsifying properties of canola proteins have been documented (Tan et al., 2011a). Emulsifying properties in this case refer to the ability of proteins to form emulsions and to stabilize the emulsions formed. The ability of canola protein isolate to form emulsions are generally measured by means of turbidimetry

Abbreviations: Alb, albumin fraction; CPI, canola protein isolate; Glo, globulin fraction; EAI, emulsifying activity index; EC, emulsifying capacity; SPI, soy protein isolate.

* Corresponding author. E.H. Graham Centre for Agricultural Innovation, Pine Gully Road, Wagga Wagga, NSW 2650, Australia. Tel./fax: +61 269332085.
E-mail addresses: terran_t@hotmail.com, siongtan@csu.edu.au (S.H. Tan), rod.mailer@australian-oils-research.com (R.J. Mailer), cblanchard@csu.edu.au (C.L. Blanchard).

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(emulsifying activity index, EAI), and volume of oil emulsified per gram protein sample (emulsifying capacity, EC) (Tan et al., 2011a). The stability of emulsions is normally determined by measuring the changes in EAI over time, and by the volume of water separated within a given period of time. The study of emulsifying properties by measuring the changes in average droplet size and distribution is probably the most direct way of determining emulsification efficacy (Agboola, Ee, Mallon, & Zhao, 2007). Particles scatter light at an angle determined by the particle size providing a useful way to directly measure particle size (McCave, Bryant, Cook, & Coughanowr, 1986). This type of analysis is yet to be meaningfully applied to the study of the canola protein emulsifying property.

The majority of the investigations on canola protein functional properties so far have been conducted on meals or protein isolates (Aluko & McIntosh, 2001; Aluko, McIntosh, & Katepa-Mupondwa, 2005; Khattab & Arntfield, 2009). Canola protein isolates are normally prepared by direct alkaline extraction followed by acid precipitation (Tan et al., 2011a). However, the poor water solubility of such protein fractions (Yoshie-Stark, Wada, & Wasche, 2008) results in these isolates having unsatisfactory food technological functional properties (Kinsella, 1979). There is, however, an alternative method as described by Osborne and Mendel (1914) using water, salt solution, alkaline, and alcoholic solutions sequentially for the extraction of several protein fractions which have better solubility. The adaptation of this method for canola protein extraction could probably produce "Osborne" fractions of better characteristics and food functionality. However, to our knowledge, functionality studies on individual Osborne fractions have not previously been reported.

In this study, we compared emulsifying properties of the major canola protein fractions extracted by the Osborne method (albumin and globulin fractions), to the more common canola protein isolate (CPI), and to the commercially available soy protein isolate (SPI). Emulsifying properties were studied in terms of emulsifying capacity (EC), emulsifying activity index (EAI), and average droplet size at a range of pH 4 to 9. These results were compared and associated with the conformations of the protein samples which were determined by means of maximum fluorescence intensity. The stability of emulsions formed was also studied, in terms of changes in particle size and changes in physical attributes of the emulsions during storage.

2. Materials and methods

2.1. Sources of materials and chemicals

Industrial cold-pressed canola meal of mainly *Brassica napus* variety was supplied by Cootamundra Oilseeds Pty Ltd (Cootamundra, Australia). Soy protein isolate (SPI) was purchased from Natural Health Supplements (Burleigh Heads, Australia). Reagents and chemicals were supplied by Sigma–Aldrich (Castle Hill, Australia). Canola oil was purchased from a local supermarket.

2.2. Protein extraction

2.2.1. Osborne method

Albumin and globulin fractions were prepared using the classical procedure of Osborne and Mendel (1914) with modifications as described by Tan et al. (2011b). Canola meal was first ground into powder using a Breville Coffee 'n' Spice Grinder CG2B (Breville Pty Ltd, Sydney, Australia). The meal sample was then extracted with de-ionized water (1:10) for 1 h using a rotary shaker at room temperature. The samples were centrifuged at 3000 *g* for 10 min to obtain the water soluble albumin fraction (supernatant). This process was repeated twice on the meal pellet after which the albumin fractions were pooled, filtered, and concentrated by using a Rotavapor R-210 (Buchi Labortechnik, Flawil, Switzerland) at 40 °C. The residues (meal pellet) were then similarly extracted with 5 g/100 mL NaCl to obtain globulin fractions. Salt in the globulin extract was removed by dialysis at 4 °C using cellulose membrane (molecular weight cut off 12.4 kDa) using de-ionized water (1:20), for 72 h with water changes every 24 h. Both extracts were freeze–dried and kept at −20 °C until further analysis.

2.2.2. Direct alkaline extraction

Canola protein isolate (CPI) was prepared using Direct alkaline extraction. The canola meal was extracted three times with 0.1 mol/L NaOH solution (1:10) for 1 h using a Rotary Tube Mixer RSM7DC (Ratek Instruments, Boronia, Australia) at room temperature. Extracts (soluble fractions) were pooled and then pH adjusted to 4.0 with 1 mol/L HCl and centrifuged at 3000 *g* for 10 min to obtain the precipitates (CPI). The precipitate was washed with de-ionized water, freeze–dried and kept at −20 °C until further analysis.

2.3. Intrinsic fluorescence intensity

Intrinsic fluorescence intensity was determined as described by Agboola and Aluko (2009) with modifications. The fluorescence measurement was carried out using a Cary Eclipse Fluorescence Spectrophotometer (Varian Inc, Mulgrave, Australia). The excitation wavelength was fixed either at 280 nm (tyrosine and tryptophan fluorescence) and the emission measured between 290 and 400 nm, or fixed at 295 nm (tryptophan fluorescence only) and emission monitored between 300 and 450 nm. The effect of pH (4–9) was measured at 25 °C, at a protein concentration of 125 μg/mL.

2.4. Emulsifying properties

2.4.1. Emulsifying capacity (EC)

EC was determined according to the method described by Webb, Ivey, Craig, Jones, and Monroe (1970) with modifications. Protein sample (50 mg) was dispersed in 40 mL of 10 mmol/L phosphate buffer at pH 4, 7, and 9, and then stirred for 1 min using a Dumax Laboratory Stirrer (Betts & Co P/L, Milperra, Australia). The protein solution was added to 40 mL canola oil and the mixture was stirred for 1 min to form an emulsion. A volt-ohm meter with 2 electrodes immersed in the mixture was used to determine the electrical resistance of the emulsions. The mixture was continuously stirred while additional oil was added to the mixture using a burette until a sudden increase in electrical resistance of the dispersion occurred upon the collapse of the emulsion. EC was expressed as mL oil emulsified per g protein sample.

2.4.2. Preparation of emulsions

An oil-in-water emulsion was prepared using 20 ml canola oil as the dispersed phase and 80 ml phosphate buffer (10 mmol/L) as the continuous phase with 1g/100 mL protein concentration. Each preparation also contained 0.05 g/100 mL sodium azide to prevent microbial growth. The protein, sodium azide, and buffer solution were mixed for 1 h using a rotary shaker prior to the addition of canola oil. Then the whole mixture was first homogenized for 1 min using an Ultra-Turrax T25 homogenizer (Janke & Kunkel, Staufen, Germany) and the coarse emulsion was then passed through an EmulsiFlex-C5 homogenizer (Avestin, Ottawa, Canada) at an average pressure of 125 MPa. Each emulsion was passed through the homogenizer 3 times to ensure complete dispersion of the oil.

2.4.3. Emulsifying activity index (EAI)

EAI of protein samples at pH 4–9 was determined according to the spectroturbidimetric method of Pearce and Kinsella (1978) with