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Cruciferin nanoparticles: Preparation, characterization and their potential application in delivery of bioactive compounds

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ABSTRACT

Encapsulation of bioactive compounds is an emerging technique to provide protection against food processing and digestion as well as to increase their bioavailability. In this study, cruciferin, a major canola protein, was used to prepare calcium-induced nanoparticles by a cold gelation method. The particles were spherical in shape with ~200 nm diameter and polydispersity index (PDI) of 0.2–0.3. Alpha-helix structure was decreased while disordered structure and β-sheet/turn were increased respectively for heated protein and calcium-induced particles based on circular dichroism, FTIR and fluorescence studies. Driving forces for the particles formation were hydrophobic and electrostatic interactions. The particles did not show toxicity to Caco-2 cells at concentrations of up to 2.5 mg/mL. The cell uptake of labelled nanoparticles was also observed using confocal microscopy after 6 h incubation with Caco-2 monolayer. Release studies showed that the particles were resistant to pepsin and low pH in simulated gastric fluid, but released the encapsulated compounds (brilliant blue and β-carotene) in simulated intestinal conditions. Encapsulation also significantly increased the stability of β-carotene in a heat treatment (75 °C and 30 min) compared to unencapsulated form. Our results suggested that cruciferin can be used for preparing particles and delivery of bioactive food components.

1. Introduction

Canola including rapeseed, with an annual production of 70 million metric tonnes, now ranks as the second most abundant oilseed in the world (USDA, 2013). The meal after oil extraction contains 35–40% proteins; canola proteins are potential food proteins due to its well-balanced amino acid composition, high amount of lysine (6.0%) and sulphur-containing amino acids (3–4%), and high protein efficiency ratio (2.64) (Tan, Mailer, Blanchard, & Agboola, 2011; Wu & Muir, 2008). Furthermore, canola proteins were reported to show good emulsifying and gelling properties, or can be used as a precursor for bioactive peptides (Wu & Muir, 2008; Wu, Aluko, & Muir, 2009; Yamada et al., 2010; Yoshie-Stark, Wada, & Waesche, 2008). Canola contains two major proteins, cruciferin (12S globulin) and napin (2S albumin), accounting for 65% and 25% of total canola proteins, respectively. Cruciferin, with an isoelectric point (PI) of around 7.2 and a molecular weight of 300 kDa is composed of six subunits. Napin, a strongly basic protein (isoelectric point around 11), is composed of two subunits of 4.5 and 9.5 kDa (Wanasundara, 2011).

Proteins showing good emulsifying and gelling properties might also be used in delivery system. Food proteins are generally recognized as safe (GRAS), biocompatible, and biodegradable natural polymers. In addition to their ability to interact with different drug or nutraceutical compounds via multiple functional groups, proteins have capacity to protect the compounds within their three-dimension gel networks (Elzoghby, Samy, & Elgindy, 2012). To the best of our knowledge, the potential use of canola proteins in delivery systems has not been explored. Most of food proteins form heat-set hydrogels where different compounds can be entrapped and slowly be released. However, heat-set gels may not be suitable for encapsulation of heat-sensitive compounds. An alternative method is protein cold gelation where protein solution is preheated and then a network of the soluble protein aggregates is formed using multivalent ions; the bioactive compounds are added before forming the network. This method was first explored with whey protein (Barbut & Foegeding, 1993) and then effects of different salts (Bryant & McClements, 2000), sucrose (Kulmyrzaev, Cancelliere, & McClements, 2000) and pH and ageing time on the gelation (Cavallieri & Da Cunha, 2008) were studied. This method

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was further applied to study other proteins such as β-lactoglobulin (Remondetto & Subirade, 2003), soy protein (Maltais, Remondetto, Gonzalez, & Subirade, 2005; Maltais, Remondetto, & Subirade, 2008), and bovine serum albumin (Kundu, Chinchalikar, Das, Aswal, & Kohlbrecher, 2013). Using this method, proteins can form gel individually or in combination with other polymers. This method has been used for preparing cold, gel-like soy protein emulsions (Tang & Liu, 2013), delivery of probiotics using whey protein micro-beads (Doherty et al., 2011), forming ion-induced whey protein aerated gel (Tomczynska-Mleko, 2013), and whey protein/alginate hydrogel microparticles for oral delivery of insulin (Deat-Laine, Hoffart, Garrait, & Beyssac, 2013).

Due to resistance of cruciferin to gastric enzymes (Bos et al., 2007), it was hypothesized that cruciferin particles can protect bioactive compounds in the gastric conditions. The objectives of this study were to prepare canola proteins nanoparticles using the cold gelation method and to evaluate their potential for encapsulation and protection of model compounds. We showed that calcium-induced cruciferin (Cru/Ca) particles are promising carriers for delivery of nutraceutical compounds.

2. Materials and methods

2.1. Materials

Commercial canola meal, obtained from Richardson Oilseed Company (Lethbridge, AB, Canada) was ground, passed through a 35-mesh screen and stored at −20 °C for further use. Caco-2 cells (HTB37) were obtained at passage 19 from the American type culture collection (Manassas, VA). Dulbecco's modified eagle medium (DMEM), 0.25% (w/v) trypsin-0.53 mM EDTA, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), fetal bovine serum, 1% nonessential amino acids, and 1% antibiotics were all procured from Gibco Invitrogen (Burlington, ON, Canada). (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT), dimethyl sulfoxide (DMSO), 1-anilinonaphthalene-8-sulfonic acid (ANS), coomassie brilliant blue G-250, β-carotene, urea, sodium dodecyl sulphate (SDS), dithiothreitol (DTT), pepsin, pancreatin, Coumarin 6 and 4′,6-diamidino-2-phenylindole (DAPI) were obtained from Sigma (Oakville, ON, Canada).

2.2. Canola protein extraction

Canola proteins, cruciferin and napin, were extracted using the method we previously developed (Akbari & Wu, 2015). A slurry of canola meal:water (1:10, W:V) was acidified to pH 4, stirred for 2 h and centrifuged at 10,000 rpm for 20 min at 4 °C. The resultant pellet was mixed with 10× volume of water, and the pH was adjusted to 12 while stirring at room temperature for 1 h. After centrifugation, the supernatant was adjusted to pH 4 to precipitate proteins. The collected precipitate was washed twice with acidified water (pH 4), centrifuged and freeze-dried as cruciferin isolate. The supernatants obtained from acidic washing and protein precipitation were combined (pH 4) and subjected to an ultrafiltration system (Millipore, Bedford, MA, US) to remove salts and anti-nutritional compounds using a 10 kDa membrane; the protein solution was concentrated and filtered at the concentration factor of 20 and diavolume of 10, respectively. The obtained retentate was freeze dried as napin.

2.3. Preparation of particles

Canola protein nanoparticles were prepared using the cold gelation method (Marangoni, Barbut, McGauley, Marcone, & Narine, 2000). Effects of preheating temperatures, pHs and calcium concentrations on cold gelation of cruciferin and napin were studied. In brief, 18 mL cruciferin and napin dispersions (10 mg/mL) in MQ-water were stirred for 1 h at 500 rpm and then the pH was adjusted to 12 using 1 M NaOH. After stirring for 1 h, the protein solutions were heated at 95 °C and 120 °C for 30 min in tightly closed tubes. After 0.5 h cooling at room temperature, the protein solutions were diluted with 12 mL water containing 0.06% w/v sodium azide and the pH was adjusted to 7, 8, 9 and 10 using 1 M HCl. Afterward 6 mL of 9, 18, 27 and 36 mM $CaCl_2$ was added drop by drop to the protein solutions (at final $CaCl_2$ concentrations of 1.5, 3, 4.5 and 6 mM, respectively) while stirring to induce particle formation. The stability and turbidity of the particle suspensions were assessed to determine the appropriate conditions for particles formation. The stability of the particles was studied for a period of 3 weeks stored at 4 °C. The turbidity was measured at 600 nm using a UV/VIS spectrophotometer (V-530 Jasco, Japan). The dispersions containing stable particles with at least 50% increase in the turbidity compared to the initial protein solution turbidity were considered as appropriate particle dispersions. Effects of cruciferin concentrations (5, 10, 15, and 20 mg/mL), final $CaCl_2$ concentrations of 1.5 and 3 mM, and pHs 8, 9, 10 on the particle formation at preheating temperature of 120 °C were also studied.

2.4. Particles characterization

2.4.1. Size, surface charge and morphology of particles

Size of the particles was determined by dynamic light scattering using Malvern Nanosizer ZS (Malvern, Worcestershire, UK). Zeta potential of the particles was also measured by laser doppler velocimetry using the Nanosizer. Prior the measurements, samples were diluted in 10 mM phosphate buffers (the same pH of particle suspensions) to obtain a slight opalescent dispersion and prevent multiple scattering effects. Morphology of the prepared particles was studied using a transmission electron microscopy (TEM, Philips Morgagni 268, FEI Company, The Netherlands) at 80 kV. The particles were freshly prepared, put on Formvar-covered copper grids, negatively stained with phosphotungstic acid, and then air dried.

2.4.2. Protein conformation studies

The conformational changes of cruciferin before and after heating as well as after particle formation were characterized using Circular Dichroism (CD), fourier transform infrared (FTIR) and fluorescence spectrophotometer. The far-UV CD spectra of the protein samples (1 mg/mL) at pH 9 were measured using Olis DSM 17 Circular Dichroism (GA, USA). The path length of the quartz cell was 0.2 cm and the spectra represented an average of five scans collected in 1-nm steps at a rate of 20 nm/min over the wavelength range of 190–250 nm.

For FTIR, the freeze-dried samples was dried in a vacuum desiccator using phosphorous pentoxide for 48 h. FTIR spectra of the milled samples in KBr pellets were recorded in the range of 4000–400 cm^{-1} using a Thermo Fisher Scientific Nicolet 8700 FTIR spectrophotometer (Madison, WI, USA). The recorded spectra were deconvoluted in amide I band region (1700–1600 cm^{-1}) using Omnic 8.1 software at a bandwidth of 25 cm^{-1} and an enhancement factor of 2.5. The detected amide I bands in the spectra were assigned to protein secondary structures using previously established wavenumber ranges reported by Kong and Yu (2007) and Pelton and McLean (2000).

The intrinsic fluorescence emissions of the samples were measured using a Shimadzu RF-5301PC spectrofluorophotometer (Kyoto, Japan) equipped with a 1-cm path length quartz cell. The protein concentration and pH of the samples were adjusted to 5 mg/mL and pH 9, respectively. The fluorescence measurements