



Fabrication, characterization and controlled release properties of oat protein gels with percolating structure induced by cold gelation



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ABSTRACT

Cold-set oat protein isolate (OPI) gels possessing a percolating network structure were successfully prepared using a glucono- δ -lactone (GDL) induced acidification method. The polymer-like percolating structures were established by active OPI monomers as small building block units through abundant cross-linking points. By increasing the GDL concentration, more intra-floc linkages and greater particle volume fractions were created at the supramolecular level, resulting in a dense rough gel wall with superior mechanical properties. In particular, at a 10% GDL content the gel was generated at the OPI isoelectric point (IEP). This OPI gel had a compact network structure with small pores and a thick wall, with an excellent water holding capacity (90%) and comparable mechanical strength (30 kPa) to egg white gel (22–32 kPa). Also, the cold-set OPI gels could resist acidic juice and pepsin digestion, which protected both α -amylase enzyme activity and the viability of probiotics in harsh gastric conditions. The *in vitro* release experiment demonstrated that OPI gels had the capacity to prevent the premature release of bioactive compounds in simulated gastric fluids, and yet still allowed their gradual release in a simulated intestinal environment where they normally would be absorbed. A convenient and non-toxic method to prepare OPI gels with superior performance has been described. These gels have the potential to act as delivery vehicles for sensitive compounds in food and non-food applications.

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1. Introduction

Protein gels have been extensively used in biomedical and food applications due to their renewable nature, superior nutritional value, inherent biocompatibility, and biodegradability (Chen, Remondetto, & Subirade, 2006; Wang, Bamdad, Song, & Chen, 2012). Over the past decade, applications of animal protein based gels (collagen, gelatin, albumin, whey protein, etc.) as wound dressings, scaffolds for cell growth in tissue engineering, and nutraceutical delivery systems have been widely studied (Dobaczewski et al., 2006; Chen & Subirade, 2006; Côté, Laroche, Gagnon, Chevallier, & Doillon, 2004; Cummings, Gawlitta, Nerem, & Stegermann, 2004; Gunasekaran, Ko, & Xiao, 2007; Thein-Han, Saikhun, Pholpramoo, Misra, & Kitiyanant, 2009). For globular proteins, either a particulate or filamentous gel is obtained by heating, depending on the environmental pH (Lefèvre & Subirade, 2000; Stading & Hermansson, 1990). At a pH near a protein isoelectric point (IEP), heating cannot split the globular subunits,

leading to the formation of spherical particles and a particulate gel structure. Whereas at a pH that deviates from the IEP, a strong repulsive force causes the dissociation of subunits into monomers and induces extensive protein unfolding. The association of these unfolded protein chains results in flexible linear strands and filamentous gel or fine-stranded structure. Collagen and gelatin exhibit triple-helix structure (Duconseille, Astruc, Quintana, Meersman, & Sante-Lhoutellier, 2015; Muyonga, Cole, & Duodu, 2004; Okuyama, Miyama, Mizuno, & Nachinger, 2012), and these helices lose their conformation during heating and partially recover during cooling to form junction zones containing triple-helix and coil structures. The nature of these flexible molecular structures means they eventually form a percolating gel network with a high degree of cross-linking (Coppola, Djabourov, & Ferrand, 2012; Duconseille et al. 2015; Oakenfull & Scott, 2003). This not only confers the gel many mechanical properties (Forte, D'Amico, Charalambides, Dini, & Williams, 2015), but is also of relevance when developing drug delivery systems with gel scaffolds (Gaowa et al., 2014; Kim, Furuya, & Tabata, 2014).

In recent years, proteins derived from plant sources are important due to health, religious and cost concerns of animal sources

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(Kim et al., 2014; Gaowa et al., 2014). Plant protein delivery vehicles have been developed for the controlled release of drugs, unsaturated fatty acids, vitamins, and other small bioactive compounds (Chen & Subirade, 2009; Lai & Guo, 2011; Wang & Chen, 2012; Yang, Zhou, & Chen, 2014b). Although most research has focused on the formation of plant protein coacervates and particles, gels have been underrepresented despite their potential. Plant protein gels have a high water holding capacity and good biocompatibility to provide a non-reactive environment for sensitive bioactive compounds, such as enzymes and probiotics. The gelation process of soy protein has been extensively studied (Maltais, Remondetto, Gonzalez, & Subirade, 2005; Maltais, Remondetto, & Subirade, 2008; Vilela, Cavallieri, & da Cunha, 2011), and to a less extent, plant proteins, such as from pea, canola, and oat, have also demonstrated gelling properties upon heating (Nieto-Nieto, Wang, Ozimek, & Chen, 2014; Sun & Arntfield, 2010; Yang, Wang, Vasanthan, & Chen, 2014a).

Cold-set gelation of whey and soybean proteins was achieved by adding Ca^{2+} ions or glucono- δ -lactone (GDL) to a preheated protein suspension (Barbut & Foegeding, 1993; Bryant & McClements, 2000; Cavallieri & da Cunha, 2008; Maltais et al., 2005). This method requires a heating step during which proteins are denatured and then polymerize into soluble aggregates. It is followed by a cooling step and then the addition of Ca^{2+} or GDL, resulting in the formation of a network of soluble aggregates. Cold-gelation also generates filamentous and particulate gels depending on the Ca^{2+} or GDL concentration (Maltais, Remondetto, & Subirade, 2009). For example, at higher Ca^{2+} concentration, the electrostatic repulsive force among protein aggregates rapidly diminishes, promoting the fast and random growth of protein aggregates in all directions resulting in a particulate gel structure. On the other hand, lower Ca^{2+} concentration promotes the formation of a filamentous gel with a fine-stranded structure. Here, strong repulsive forces facilitate interactions among hydrophobic patches that gradually approach one and grow into a linear aggregate of compatible inter- and intra-floc links (Maltais et al., 2008; Remondetto & Subirade, 2003). These cold-set gels offer opportunities to prepare the means to carry and protect sensitive nutraceutical compounds and develop innovative functional food ingredients. However, cold-set gels from other plant proteins other than soy protein have seldom been reported.

Oat has the highest protein level (12–20%) among cereals and a superior amino acid profile containing high quantities of essential amino acids lysine and threonine (Klose & Arendt, 2012; Mohamed, Biresaw, Xu, Hojilla-Evangelista, & Rayas-Duarte, 2009). The major fraction of oat protein is 12S globulin. It consists of A- and B-subunits with molecular weight of about 32 and 22 kDa respectively, where the A-subunit is an acidic polypeptide and the B-subunit is a basic polypeptide. These two subunits are disulfide bonded to form a dimer, which further associates into a hexamer through non-covalent forces (Burgess, Shewry, Matlashewski, Altosaar, & Mifflin, 1983). Our previous work revealed that heat-induced oat protein gels exhibited a percolating network structure, different from many other globular proteins but similar to gelatin (Nieto-Nieto et al., 2014). These gels had good mechanical properties and were comparable to that of egg protein. In spite of oat's potential, cold-set gelation of oat protein has never been reported. This work aimed to develop cold-set oat protein gels using GDL. The effect of various GDL levels on gel structure, mechanical properties, and water holding capacity was investigated. The gel formation mechanism was discussed by correlating the gel microscopic structure with macroscopic properties. The protection of enzymes and probiotics their controlled release were evaluated in a simulated human gastro-intestinal tract.

2. Experimental section

2.1. Materials

Naked oat grain (*Avenanuda*) (crude protein 17.2%) was purchased from Wedge Farms Ltd., Manitoba, Canada. Glucono- δ -lactone (GDL), amylase, and an amylase activity assay kit were obtained from Sigma-Aldrich Canada (Oakville, ON, Canada). *Lactobacillus acidophilus* (ATCC4536) was obtained from American Type Culture Collection (ATCC, MD, USA). MRS broth and other chemicals (analytical grade) were purchased from Fisher Scientific (Whitby, ON, Canada). Milli-Q water was used in all experiments. Oat protein isolate (OPI) was extracted from defatted oat flour using an alkaline and isoelectric point precipitation method according to our previous work (Nieto-Nieto et al., 2014). The protein content of OPI was $85.07 \pm 2.4\%$ determined by Leco nitrogen analyzer (FP-428, Leco Corporation, St Joseph, MI) and a nitrogen to crude protein conversion factor of 5.83 was used.

2.2. Gelation process of OPI solutions with GDL addition

A dynamic rheology experiment was carried out on a DHR-3 rheometer (TA Instruments, DE, USA) to study the gelation process of OPI solutions with addition of GDL. Parallel plate geometry with a gap of 1 mm was used to measure dynamic viscoelastic parameters (shear storage modulus G' and loss modulus G''). The value of the strain amplitude for all samples was set as 1%, which was within a linear viscoelastic regime. The preheated OPI (5 and 7%, w/v) solutions with different GDL contents (3, 5, 10, and 15%, w/w, based on the dry weight of protein) were placed on the plate immediately after addition of GDL and the dynamic time sweep measurements were performed at an angular frequency of 1 Hz at 25 °C over a period of 1200 min. A frequency sweep was subsequently conducted as a function of angular frequency (ω) from 0.1 to 100 rad s^{-1} at 25 °C to study gel shear strength. A thin layer of low-viscosity silicone oil was applied to prevent dehydration during the test. The change of pH value during gelation was monitored simultaneously after GDL addition using a pH meter (Thermo Scientific Orion 3 Star pH Meter, MA, USA).

2.3. Cold-set OPI gel preparation

OPI (5 and 7%, w/v) was dissolved in water and stirred overnight at room temperature. The solutions were then adjusted to pH 8 using 1 M NaOH. OPI solutions were tightly sealed in glass vial and heated at 115 °C (above denaturation temperature) in an oil bath for 15 min. Subsequently, they were cooled down to room temperature and different amounts of GDL (3, 5, 10, 15% w/w, based on the dry weight of protein) were added to the OPI solutions. The solutions were stored at 4 °C for 1200 min to form OPI gels, and the obtained gels were coded as OG5-3, OG5-5, OG5-10, OG5-15, OG7-3, OG7-5, OG7-10, and OG7-15, corresponding to the different OPI and GDL concentrations, respectively. For example, OG5-3 gel was prepared from 5% (w/v) OPI solution with 3% (w/w) GDL addition.

2.4. OPI gel properties

Mechanical properties of OPI gels were determined using an Instron 5967 Universal testing instrument (Instron Corp., Norwood, MA, USA) equipped with a 50 N load cell. The cylindrical gels had the height of about 10 mm and the diameter of around 12 mm. All the samples were compressed twice to 50% of their original height at room temperature with the constant crosshead speed of 1 mm/min. Two texture parameters including compressive stress and springiness were determined from the typical force-time curves.

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