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Improved thermal gelation of oat protein with the formation of controlled phase-separated networks using dextrin and carrageenan polysaccharides

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ABSTRACT

The thermal gelation of oat protein (OP) was investigated in the presence of polysaccharides at different pHs. The compressive stress dramatically increased in these phase-separated protein–polysaccharides gels due to an apparent increase in protein concentration. The polysaccharide structure significantly affected the degree of phase-separation and gel mechanical properties. The observed two-fold increase in gel compressive stress can be attributed to strong repulsive forces caused by carrageenan molecules. These resulted in a greater degree of phase-separation with the formation of carrageenan rich domains embedded in the protein phase, and a highly ordered protein network, stabilized by hydrogen and hydrophobic interactions. In the case of OP-dextrin gels, the rate of phase separation was slower than the rate of protein aggregation, thus the dextrin particles were uniformly distributed within the protein network. This research contributes to the basic understanding required for designing textures for novel plant-based protein products.

1. Introduction

Alternative protein sources are gaining importance as consumers are incorporating more plant-based proteins in their diets and in some cases, opting for a full replacement of animal protein. Currently soy and wheat proteins dominate this market. As the plant-based protein ingredient sector grows, there is the opportunity to meet the increasing market requirements for novel functionalities and sensory attributes of plant-protein sources. Our previous work revealed that oat protein could form polymer-like gels with percolating networks, opening several potential gelling applications in various foods (Nieto-Nieto, Wang, Ozimek, & Chen, 2015).

Previous studies have demonstrated that blends of proteins and polysaccharides can create gels with different microstructure, which can be applied to control or improve sensorial attributes in food products by providing texture and water retention (Baeza, Carp, Pérez, & Pilosof, 2002; Çakır et al., 2012; Pires Vilela, Cavallieri, & Lopes da Cunha, 2011; Uruakpa & Arntfield, 2006). Coacervate networks represent one continuous network formed when the attractive protein–polysaccharide interactions are favorable. Phase-separated networks are formed when protein–polysaccharide interactions are segregative, resulting in a bi-continuous phase. Interpenetrating networks are formed when no interactions are established between polymers; here each polymer forms an independent network (Morris, 1986). The charge density of the polysaccharide, as well as processing conditions such as pH and ionic strength, have a direct influence on the gel structure, and consequently also the gelling properties (De Jong, Klok, & Van de Velde, 2009). By modulating protein–polysaccharide interactions, a synergistic effect can be achieved. To date, most of the research has focused on improving gelling properties of dairy protein–polysaccharide mixtures (Çakır et al., 2012; Croguennoc, Nicolai, Durand, & Clark, 2001; Weinbreck, Nieuwenhuijse, Robijn, & de Kruif, 2003). Research efforts on plant-protein gels have included soy (Baeza et al., 2002; Pires Vilela et al., 2011), pea (Liu, Elmer, Low, & Nickerson, 2010) and canola (Uruakpa & Arntfield, 2006). Yet the knowledge acquired is still very limited.

The objective of this work is to systematically study how the presence of different polysaccharides may impact the microstructure of oat protein gels and their subsequent gelling properties. Three polysaccharides were selected, including dextrin, carrageenan and chitosan. Dextrin is a low molecular weight carbohydrate (~3 kDa) produced by the partial acid hydrolysis of starch, thus exhibiting the α-(1 → 4)-Glc structure of amylose and the α-(1 → 4)- and α-(1 → 4,6)-Glc branched structure of amylopectin, but with a lower polymerization (Secundo & Guerrieri, 2005, Silva et al., 2014). Carrageenan is a gel-forming polysaccharide derived from a number of seaweeds, and is a sulfated polygalactan with 15 to 40% ester-sulfate content, which makes it an anionic polysaccharide. It has an average relative molecular weight well above 100 kDa and is formed by alternate units of D-galactose and 3,6-anhydro-galactose (3,6-AG) joined by α-1,3 and β-1,4-glycosidic linkage (Necas & Bartosikova, 2013). Chitosan is a

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linear-cationic polysaccharide derived from partial N-deacetylation of chitin and is normally obtained from crustacean shells with the structure α(1 → 4)-linked 2-amino-2deoxy-β-D-glucan (Shahidi, Arachchi, & Jeon, 1999). The impact of adding different polysaccharide over the bulk properties of the gels was evaluated at different pHs. The oat protein gel formation mechanism in the presence of polysaccharide is discussed with special emphasis on phase-separated networks.

2. Materials and methods

2.1. Materials

Using naked oat grains (*Avena nuda*) from Wedge Farms Ltd., Manitoba, Canada (protein content = 16.6% ± 0.64), oat protein isolate (OP) was extracted according to our previous work (Nieto-Nieto, Wang, Ozimek, & Chen, 2014) and the protein content was determined to be 89.57% ± 0.73 using a Leco nitrogen analyzer (FP-428, Leco Corporation, St Joseph, MI) using a protein calculation factor of 6.25. Urea, 2-mercaptoethanol, sodium dodecyl sulfate, Rhodamine B, Fluorescein 5-isothiocyanate (FITC), carrageenan (molecular weight of 300–400 kDa and composed predominantly by κ-carrageenan as reported by supplier) and chitosan from shrimp shells (molecular weight of 150 kDa and 75–85% deacetylation degree) were obtained from Sigma-Aldrich Canada (Oakville, ON, Canada). Food grade dextrin Capsul 2730 (molecular weight > 10 kDa) was obtained from Ingredion Canada Inc. (Mississagua, ON, Canada).

2.2. Gel preparation

Dextrin, chitosan and carrageenan were selected for this study. Gels were prepared by heating the protein–polysaccharide suspensions at pH 3 and 7 adjusted with 0.1 N NaOH or HCl. Protein–polysaccharide suspensions were prepared by dispersing protein and polysaccharide powder in deionized water and the mixture was stirred at room temperature overnight using a stirring hotplate (Fisher Scientific, Markham, ON, Canada). The concentration of OP in the mixtures was kept constant at 15% (*w*/*v*) and the concentration of polysaccharide was 0.5% (*w*/*v*). These values were determined based on preliminary trials, which allowed gel formation. Samples were labeled as OP, OP-DE, OP-CH and OP-CA, representing gels containing oat protein, oat protein-dextrin, oat protein-chitosan and oat protein-carrageenan respectively. Test tubes containing the suspension were tightly closed and placed in an oil bath at 110 °C for 30 min. Once the heat treatment was completed, the tubes were cooled in an ice bath and stored in the refrigerator overnight.

2.3. Scanning electron microscopy (SEM)

Morphological observation of the gels was carried out with a Phillips XL-30 scanning electron microscope (FEI Company, Oregon, USA) at an acceleration voltage of 20 kV. The samples were frozen in liquid nitrogen and freeze-dried before observations. The cross-section and surfaces of the dry gels were sputtered with gold and platinum, observed and photographed.

2.4. Gel properties

The compressive stress of the gels was evaluated using an Instron 5967 universal testing machine (Instron Corp., Norwood, MA, USA). Gels were dismounted from test tubes and cut into cylindrical pieces (~10 mm height and ~14 mm diameter). A two cycle compression test using a 50 N load cell was performed at room temperature at a rate of 1 mm/min and 50% compression. The compressive stress was calculated as the peak compression force in the 1st bite cycle, divided by the initial cross-section area of the gel sample.

Additionally, the water holding capacity (WHC) of the gels prepared was evaluated. For this, a gel sample (0.9–1.2 g) was placed into a Vivaspin 20 centrifugal filter unit (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) and centrifuged at 453 ×*g* for 10 min at room temperature. The weight of the gel was recorded before (W_i) and after centrifugation to the nearest 0.0001 mg, considering ΔW_{tot} as the difference in water content of the sample, before and after centrifugation, the percentage of water loss was expressed as:

$$\%\mathrm{WHC} = \frac{\mathrm{W_i} - \Delta \mathrm{W_{tot}}}{\mathrm{W_i}} \times 100.$$

2.5. Rheological measurements

The rheological measurements were done with a TA Discovery HR-3 rheometer (TA instruments, New Castle, DE, USA). To compare the effects of different molecular interactions on the formation of OP and OP-polysaccharide gels, we used the methodology reported by Yang, Wang, Vasanthan, & Chen, 2014. Gels were prepared as previously described in the gel preparation section and cut into 1.0 ± 0.1 cm (height) sections. The resulting gel disk were submerged for 48 h in solutions of 2-mercapthoethanol (2-ME) (0.2 M), urea (6 M) and sodium dodecyl sulfate (SDS) (1% *w*/*v*), which could disrupt disulfide bonds, hydrogen bonds and hydrophobic interactions respectively. Since the storage modulus is sensitive to changes on the gel network, a frequency sweep test was done to evaluate the dependence of G' to frequency (0.1–100 rad/s) on gels compressed to keep 80% of its original height. A gap of 8.0 ± 0.8 mm was thus used to make sure the gel structure was well maintained during the test. To study the changes in viscoelastic properties as a function of temperature, OP and OP-polysaccharide initial suspensions were subject to a temperature ramp from 25 to 95 °C, hold at 95 °C for 5 min, and then cooled down to 25 °C at a rate of 1.5 °C/min. Sample conditioning took place before and after each temperature ramp for a period of 3 min. For these measurements, approximately 1 mL sample was loaded in the bottom plate of the parallel plate geometry; the upper plate was lower to the appropriate geometry gap (1 mm). To avoid evaporation during heating a solvent trap was used and a thin layer of silicone oil was applied. The temperature of the bottom plate was controlled with a Peltier system. All rheological measurements were done within a predetermined linear viscoelastic region, which was determined in preliminary experiments, setting the strain value at 0.05%.

2.6. Turbidity and particle size measurements

The turbidity of the samples was measured before and after heating (110 °C, 30 min) in order to follow the protein aggregation process. Changes in turbidity at 600 nm were recorded at a protein concentration of 0.1% at pH 3 and 7 on a Jasco V-530 UV/VIS spectrophotometer (Jasco Corporation, CA, USA). The absorbance was measured using quartz cuvettes with a 10 mm path length.

A Zetasizer Nano ZS ZEN1600 system (Malvern Instruments, U.K.) was used to study the change of particle size distribution caused by the heat treatment (110 °C for 30 min). Samples were immediately cooled in an ice bath to room temperature, after completing the heating period. Samples were then diluted to a total concentration of 0.1% (*w*/*v*) and passed through a 0.45 μm pore size filter prior to measurement. Number-based particle size distribution was measured to identify the total number of particles of a given size (Van der Meeren, Dewettinck, & Saveyn, 2004).

2.7. Surface hydrophobicity (H_o)

Protein surface hydrophobicity was determined using the apolar fluorescent dye, ANS (Alizadeh-Pasdar & Li-Chan, 2000). Protein and