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Inulin at low concentrations significantly improves the gelling properties of oat protein – A molecular mechanism study

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ABSTRACT

The effect of inulin addition at low concentrations (0.1–0.5%) on the thermal gelation of oat protein gels was investigated using textural profile analysis, rheological measurements and microstructure observation through scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM). Results indicate that oat protein hexamers were dissociated with heat into monomers, which then became the reactive units involved in the development of a unique percolating network. The major forces responsible for the establishment of the structure were disulphide bonds, as well as hydrogen bonds and hydrophobic forces. A small amount of inulin can greatly increase the compressive stress of the gels prepared at pH 7 from 13.93 to 22.98 kPa. This is related to the phase separation phenomena produced during heating, which increased the apparent protein concentration. Moreover, inulin formed nanoparticles in the void spaces of the protein network performing a filling effect and creating junction zones. Localized interactions such as hydrogen and hydrophobic bonds were possible between protein and inulin at the borders of junction zones. This research has provided a new approach to make strong oat protein gels at neutral pH. Future applications may promote the utilization of oat protein as a plant derived gelling ingredient in a wide range of food applications.

1. Introduction

Oats are an important crop worldwide, with an annual production of approximately 21 million tonnes. Canada is a major supplier of oats, making up the majority of world oat trade (Food and Agricultural Organization (FAO), 2012). This grain has recently attracted research and commercial attention mainly due to the growing public awareness of the health benefits of β-glucan, which is known to reduce blood cholesterol and glucose levels. Several techniques have been developed to isolate β-glucan from oat grain as a health ingredient in food products. Protein is the second largest component (12–20%) in oats after starch. Oat protein have a superior amino acid profile due to a higher content of lysine because globulins represent 70–80% of the total protein in oats, whereas alcohol-soluble prolamines are the major storage proteins in other cereals (Robert, Nozzolillo, Cudjoe, & Altosaar, 1983). The 12S globulin is the major oat protein fraction, which resembles the structure of 11S globulin of soy (glycinin). Thus, oat protein possesses gelling potential (Ma, Khanzada, & Harwalkar, 1988; Ma and Wood, 1987; Nieto-Nieto, Wang, Ozimek, & Chen, 2014). Plant proteins are normally considered inferior to animal proteins (e.g. gelatin, egg white and whey protein) in terms of gelling properties. In our recent work, trypsin treated oat protein could form gels with comparable mechanical strength to egg white protein at pH 9 (Nieto-Nieto et al., 2014). This has provided opportunity for oat protein to be used a new gelling ingredient from plant resources in food formulations such as meat binder and fat replacer, or used in meat analogues for vegetarian foods. However, strong gels could be only obtained at alkali pH when heated to 110–120 °C. The gels were weak when formed under acidic and neutral pH at 100 °C. This has significantly limited the application of oat protein in food systems that normally have pH values in the range of 2.5–7 and heating temperature at 100 °C or lower. Therefore novel approaches to enable formation of stronger oat protein gels within a more appropriate pH and temperature for food processing are necessary to promote the utilization of oat protein as a gelling agent.

Inulin is a non-digestible polysaccharide naturally occurring in several edible fruits and vegetables. It is formed by fructose molecules linked by β-(2–1) glycosidic bonds, generally with a terminal

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glucose unit connected to the last fructose by an α-(1–2) bond (Roberfroid, 2007). Due to the unique nature of inulin bonds, digestive enzymes in the human gut cannot hydrolyze this polysaccharide. Thus, inulin reaches the colon undigested and produces a prebiotic effect (Blecker et al., 2001). Additionally, inulin has other interesting biological properties such as enhancing mineral absorption, and reducing blood lipid levels and the risk of colon cancer (Roberfroid, 2007). The utilization of inulin in the food industry is not limited to its biological properties; it is also incorporated in food formulations as a fat replacer or bulking agent, such as in baked goods, sauces and yogurt (Blecker et al., 2001; Bot, Erle, Vreeker, & Agterof, 2004). Previous reports have investigated the influence of inulin addition in milk (Arango, Trujillo, & Castillo, 2013), soy protein gels (Tseng, Xiong, & Yang, 2009), yogurt (Guggisberg, Cuthbert-Steven, Piccinali, Bütikofer, & Eberhard, 2009) and cheese (Giri, Kanawjia, & Khetra, 2014), finding that the protein-inulin system had improved gelling properties. Nonetheless the effect of inulin addition on the gelation properties of oat protein has never been reported. Thus it is hypothesized that inulin addition can produce a synergistic effect which will enable the formation of strengthened oat protein gels.

The aim of this work was to investigate the effect of oat protein and inulin interactions on the gelling properties of oat protein isolate. Mechanical and rheological properties of oat protein gels were determined and their microstructures were observed. We attempted to better understand gelling mechanisms of oat protein-inulin system by correlating protein structure changes during heating to gel microstructures and bulk properties. Improvement of the gelling properties of oat protein at acidic and/or neutral pH may create broad applications of this plant-sourced gelling ingredient in foods. These value-added opportunities may represent very significant sources of revenue to oats producers and processors to enhance their sustainability.

2. Experimental

2.1. Materials

Oat protein isolate (OPI) was extracted according to our previous work (Nieto-Nieto et al., 2014) and the protein content was determined to be 90.4% ± 0.6 using the Leco nitrogen analyzer (FP-428, Leco Corporation, St Joseph, MI). 2-mercaptoethanol, urea, sodium dodecyl sulfate, fluorescein isothiocyanate (FITC), Rhodamine B and dimethyl sulfoxide (DMSO) were obtained from Sigma–Aldrich Canada (Oakville, ON, Canada). Inulin was also purchased from Sigma–Aldrich Canada (Oakville, ON, Canada). According to producer's specifications, Inulin was obtained from chicory root by a hot aqueous extraction method and has an average polymerization degree of 25.

2.2. Gel preparation

Gels were prepared by heating the protein-inulin suspensions at pH 2.5, 5 and 7 adjusted with 0.1 N NaOH or HCl. The concentration of OPI in the mixtures was kept constant at 15% (w/v), as previous research confirmed the establishment of a self-supporting gel network at this protein content (Nieto-Nieto et al., 2014). The concentration of inulin varied from 0 to 0.5% (w/v) in the mixture, these values were selected based in preliminary trials. Samples were labeled as OPI, OPI-I 0.1%, OPI-I 0.25% and OPI-I 0.5%, representing inulin content of 0%, 0.1%, 0.25% and 0.5% respectively. Test tubes containing the suspension were tightly closed and placed in an oil bath at 100 °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. Textural profile analysis (TPA)

The mechanical properties of the gels were 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, ~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 to evaluate their mechanical properties. The textural profile parameters were determined from the typical Instron force–time curve. 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. Springiness is the distance calculated from the area under the second compression peak and gumminess is the product of peak compression force in the 1st bite cycle multiplied by cohesiveness.

2.4. Water holding capacity (WHC)

A gel sample (~1.0 g) was placed into a Vivaspin 20 centrifugal filter unit (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) and centrifuged at 290 × g for 5 min at 15 °C (Nieto-Nieto et al., 2014). The weight of the gel was recorded before (W_i) and after (W_f) centrifugation to the nearest 0.0001 mg and the percentage of water loss after centrifugation was expressed as:

$$\%WHC = 100 - \left(\left(\frac{W_i - W_f}{W_i}\right) \times 100\right)$$

2.5. Scanning electron microscopy (SEM)

The morphology observation of the gels was carried out with a Phillips XL-30 scanning electron microscope (FEI Company, Oregon, USA) at an acceleration voltage of 6 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.

2.6. Rheological measurements

The rheological measurements were done with a TA Discovery HR-3 rheometer (TA instruments, New Castle, DE, USA). 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. 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. To study the changes in viscoelastic properties as a function of temperature, OPI and OPI-inulin suspensions were subject to a temperature ramp from 25 to 95 °C, 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. The temperature ramp was not run up to the gelling temperature used in other experiments described in this paper (100 °C) as preliminary experiments reaching 100 °C produced unstable readings due to water boiling, thus the maximum temperature used was 95 °C. All rheological measurements were done within a predetermined linear viscoelastic region at the strain value of 0.05%. To study the molecular interactions involved in the formation of OPI and OPI-inulin gels, a frequency sweep analysis was conducted on gels compressed to 80% of its original height. Gels were prepared as previously described in the gel preparation section at pH 2.5, 5 and 7 and cut into approximately 1 cm (height) sections. The resulting gel disks were submerged for 48 h in