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Effect of the molecular weight of a neutral polysaccharide on soy protein gelation

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ABSTRACT

The effects of galactomannans with different molecular weights on the heat–induced gelation characteristics of soybean protein were investigated using dynamic small-strain rheometry, under conditions where the proteins carry a net negative charge (pH 7). Microstructure of the resulting gels was investigated by confocal laser scanning microscopy. Phase-separated systems were obtained with different morphologies and degree of phase separation, depending on both biopolymer concentrations and polysaccharide molecular weight. In general, a gelling enhancing effect on soy proteins was verified, despite extensive phase-separation processes observed at the higher polysaccharide molecular weight. This effect was demonstrated by an increase of the gelation rate, a decrease in the temperature at the onset of gelation, and an increase of gel stiffness and elastic character, with the length of polysaccharide chains. Overall, the results obtained established that the judicious selection of the galactomannan molecular weight may be used to modify the structure and gelation properties of soy proteins, originating a diversity of rheological characteristics and microstructures that will impact on the design of novel food formulations.

1. Introduction

Soybean proteins have recognized nutritional and functional value and are now largely used in food formulation and processing (Sharma, Su, Joshi, Roux, & Sathe, 2010; Singh, Kumar, Sabapathy, & Bawa, 2008). The gelation potential of soybean proteins is one of their most important functional properties used in both traditional and processed foods, either as natural occurring food constituents or added ingredients. Soy protein gelation (Hermansson, 1985; Nagano, Fukuda, & Akasaka, 1996; Renkema, Gruppen, & van Vliet, 2002; Renkema, Knabben, & van Vliet, 2001) also follows the three-step gelation process generally accepted for heat-induced gelation of globular proteins (Clark, Kavanagh, & Ross-Murphy, 2001; Gosal & Ross-Murphy, 2000). Heating a globular protein in aqueous environment causes denaturation and partial molecular unfolding (with subsequent exposure of hydrophobic residues), which leads first to salt- and pH-dependent aggregation (intermolecular hydrophobic interaction of the unfolded proteins), and then to gelation, agglomeration of aggregates into a network structure when the amount of aggregated protein exceeds a critical concentration. Subsequent cooling is expected to cause structure development within the protein network, with additional short-range interactions being developed, mainly hydrogen bonding.

Controlled mixture of different food components/ingredients may be an advantageous strategy to improve protein functionality. In fact, protein-polysaccharide blends have been largely studied, among other objectives, also as a way to improve functionalities and to optimise new food ingredients (Kasapis, 2008; Turgeon, Beaulieu, Schmitt, & Sanchez, 2003). In addition, protein-polysaccharide interactions have an important role on the bulk and/or interfacial properties of multicomponent systems, impacting the structure, texture, functionality and stability of complex foods (Foegeding et al., 2011; van de Velde, de Hoog, Oosterveld, & Tromp, 2015).

Depending on biopolymer concentration, molecular weight, conformation, charge density, and on the solvent characteristics (pH, ionic strength, presence of co-solutes), different physical interactions can be developed within protein-polysaccharide systems, related with incompatibility and segregation, co-solubility, or complexation between both polymers (Grinberg & Tolstoguzov, 1997; Turgeon et al., 2003). In protein-neutral polysaccharide aqueous mixtures, miscibility between both biopolymers can occur at low polymer concentration and/or molecular weight, but incompatibility and phase separation are much more generally observed phenomena, often segregative and related to volume excluded effects between both biopolymers (Doublier, Garnier, Renard, & Sanchez, 2001; Grinberg & Tolstoguzov, 1997; Wang, van Dijk, Odijk, & Smit, 2001). Obviously, the molecular weight of each polymer is one of the most important factors influencing their mixing

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behavior. In polysaccharide/protein systems, increasing the molecular weight of the polysaccharide typically promotes phase separation, decreasing the concentration required to obtain demixing of the system (Bourriot, Garnier, & Doublier, 1999; Hoskins, Robb, Williams, & Warren, 1996; Tuinier, ten Grotenhuis, & de Kruif, 2000).

Given the increasing importance of soy proteins in food processing and product formulation, it is quite understandable that several studies have been already performed on the interactions between soy proteins and other food macromolecular components, namely polysaccharides. In fact, synergism/antagonism effects, co-solubility, complexation between both polymers, or incompatibility and segregation have been reported, for mixtures of soy proteins with charged and uncharged polysaccharides, with crucial effects on the bulk and/or interfacial properties of the multicomponent systems (Braga, Azevedo, Marques, Menossi, & Cunha, 2006; Cavallieri, Garcez, Takeuchi, & Cunha, 2010; Chang, Li, Wang, Bi, & Adhikari, 2014; Hua, Cui, & Wang, 2003; Li et al., 2008; Li, Yeh, & Fan, 2007; Ortiz, Puppo, & Wagner, 2004; Perrechil, Braga, & Cunha, 2013; Zhu et al., 2009).

Although the available information is still rather limited, a few studies have already focused on the influence of the size and degree of branching of the polysaccharide on the phase behavior and functionality of soy protein-polysaccharide mistures. Li et al. (2008) analyzed the effect of two dextrans with different molecular weight on the phase behavior and microstructure of preheated soy protein (aggregates)/dextran mixtures at room temperature and pH 7.0, below gelling protein concentrations. The results showed that molecular weight of dextran and size of soy protein aggregate shifted the boundary of the instability region and the gelation areas towards lower biopolymer concentration. Regarding the effects of the polysaccharide branching, galactomannans with different amounts of galactose side chains showed a different impact on the kinetics of the phase separation in polysaccharide-soy protein systems subjected to heat-induced gelation, with the low-branched galactomannan showing a more pronounced effect on the mixed gels' viscoelasticity and microstructure (Monteiro, Rebelo, Cruz e Silva, & Lopes da Silva, 2013).

Here we report the influence of the molecular weight of an uncharged polysaccharide on the heat-induced gelation of soy proteins, including the incipient behavior around the gelation threshold and gelation kinetics, focusing on the changes imparting by the presence of the polysaccharide on the gelation process, and on the viscoelasticity and microstructure of the produced soy protein/galactomannan networks.

2. Materials and methods

2.1. Samples

The soy protein (SP) isolate was obtained from defatted soybean meal (Iberol, Portugal) after oil extraction, based on methods previously described (Renkema & van Vliet, 2002). The defatted flour was dispersed in 10-fold water and stirred for 2 h at room temperature, with pH adjusted periodically to 8 with 2 M NaOH. The suspension was centrifuged at 12000*g* for 30 min at 10 °C and the supernatant was subjected to isoelectric precipitation by adjusting the pH to 4.7 with 2 M HCl. The suspension was maintained at 4 °C for 2 h and then centrifuged (12,000 *g*, 30 min, 10 °C). The protein precipitate was re-suspended in water at room temperature, the pH was adjusted to 7 with 1 M NaOH, and freeze dried. The laboratory prepared SP isolate had protein, ash and lipid contents of 89%, 2.9% and 1.9% (w/w), respectively, as determined by standard analytical methods (American Association of Cereal Chemists, 1995), with an 11S/7S ratio of 1.18 as determined by SDS-PAGE electrophoresis (Monteiro et al., 2013).

The native galactomannan was obtained from a commercial sample of locust bean gum provided by Danisco Portugal - Industrias de Alfarroba, Lda. (HG M200), by dissolution in water, precipitation in 80% ethanol, filtration, washing with ethanol and acetone, and drying under vacuum overnight at 30 °C. Galactomannan samples with different molecular weights were prepared by controlled enzymatic depolymerisation using an *endo*-β-mannanase from *Aspergillus niger* (Megazyme International Ireland, Wicklow, Ireland) as previously described (Monteiro, Tavares, Evtuguin, Moreno, & Lopes da Silva, 2005). Mannose-to-galactose (M/G) ratios, intrinsic viscosities and the relative average molecular weights (M_w, M_n and M_w/M_n) for the galactomannan samples were determined as described elsewhere (Monteiro et al., 2005; Tavares, Monteiro, Moreno, & Lopes da Silva, 2005).

2.2. Preparation of solutions

SP dispersions were prepared in Milli-Q ultrapure water by stirring gently at 4 °C overnight. Galactomannan samples were first dispersed at room temperature for 1 h, followed by heating at 90 °C for 30 min under magnetic stirring, and then centrifuged (24,400*g*, 30 min, 20 °C) after cooling. Mixtures of soy proteins and galactomannan solutions were prepared at room temperature by gentle stirring for 60 min (pH adjusted to 7.0 if needed). All solutions were degassed under vacuum during 1 h before testing. Mixtures were studied at two soy protein (SP) concentrations, 6 and 10 g/dL, and also for two different galactomannan concentrations, 0.2 and 0.5 g/dL.

2.3. Rheological characterization

Dynamic rheological measurements were performed using a CVO HR 120 rheometer (Bohlin Instruments), equipped with a cone (4°, Ø 40 mm) and plate geometry (gap 150 μm) for isothermal time experiments or with a ridged parallel plate geometry (rotating plate diameter 40 mm and gap 1 mm) for temperature sweep experiments. The sample temperature was kept within 0.1 °C of the required temperature using a Peltier element at the bottom plate. A low-viscosity mineral oil was spread on the exposed sample surface and a solvent trap was used which sealed the sample against the environment using silicone, in order to keep solvent evaporation from the sample to a minimum all through the duration of each test.

Dynamic oscillatory tests were conducted by applying a sinusoidal shear stress in order to achieve a constant applied strain within the linear region. The measured response of the material was the phase difference between the applied stress and the resulting strain wave. Data were simultaneously converted into frequency-dependent material parameters used to characterize the viscoelastic properties of the system, the complex modulus ($G^* = [(G')^2 + (G'')^2]^{½}$), and its real and imaginary counterparts, the storage modulus (G') and the loss modulus (G'').

Each sample was placed onto the rheometer measuring system and submitted to a temperature ramp from 40 °C to 95 °C at 1 °C/min, and maintained at 95 °C for 10 min. The temperature was decreased back to 20 °C (rate 1 °C/min), and the viscoelastic properties of the final gel at 20 °C were assessed by frequency sweep experiments, after a short equilibration period (30 min) at this temperature. Isothermal curing experiments were carried out by performing a 3 h time sweep test at 80 °C. Time and temperature sweep tests were performed at an angular frequency of 2 rad/s and at low strain amplitude of 0.4% selected after preliminary tests in order to keep the measurements within the linear viscoelastic regime.

2.4. Gelation kinetics analysis

Heat-induced gelation of globular proteins is also under kinetic control. A fundamental kinetic study is not easy to carry out considering the complex mechanism of protein gelation and the several events involved. In order to compare the effect of the polysaccharide on the SP gelation kinetics (at 80 °C), we used a kinetics approximation based on the following assumptions.

We assumed that the aggregation of the protein molecules and the