Food Hydrocolloids 33 (2013) 349–360

Contents lists available at SciVerse ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

# The influence of galactomannans with different amount of galactose side chains on the gelation of soy proteins at neutral pH

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ARTICLE INFO

*Article history:*
Received 7 February 2013
Accepted 15 April 2013

*Keywords:*
Soy proteins
Galactomannans
Protein–polysaccharide interactions
Dynamic rheological tests
Microstructure
Phase separation

ABSTRACT

The influence of diverse galactomannans, differing mainly on the degree of branching (amount of galactose side chains along the main mannan backbone), on the heat-induced gelation of soybean proteins at pH 7, was investigated using dynamic oscillatory rheological measurements at low strain amplitude and microstructural analysis by confocal laser scanning microscopy. Rheological tests were performed during gel formation, induced by either isothermal heating or by heating/cooling at a constant rate. Two different protein concentrations were analysed, one in the vicinity of the critical gel conditions and the other corresponding to a well developed gel, whereas the galactomannan concentration ranged from 0 to 0.5%. The presence of the galactomannan promoted the gelation to occur for protein concentration below the critical gelation of soybean proteins alone, decreased the gelling temperature and had a positive effect on the gel strength of the heat-induced gels. These effects were more pronounced as the degree of branching decreases. The consequence of demixing and phase separation was dependent on biopolymer concentration and galactomannan branching, resulting in an array of microstructures, spanning emulsion-like, bicontinuous and aggregated morphologies. Structure development within the galactomannan-rich phases, dependent on the branching degree and on the capability of the galactomannan for self-association, may have played a role in the phase separation and viscoelasticity of the final gels. It was demonstrated that by using soybean proteins and galactomannan mixtures at above phase separation concentrations and controlling the polymer concentration and the length of the unsubstituted polysaccharide backbone, tailor-made viscoelasticities and microstructures can be obtained with useful applications in food formulation.



## 1. Introduction

The complexity of food systems, recognized as multicomponent soft materials (Mezzenga, Schurtenberger, Burbidge, & Michel, 2005; van der Sman, 2012), is largely due to the interactions that take place between the macromolecular components, including proteins and polysaccharides. Actually, the importance of protein–polysaccharide interactions to understand structure and functionality of complex multicomponent food systems, to optimize food formulation and to design products with desired structure and consumer acceptance, or novel textures is well recognized (Dickinson, 2006; Semenova, 2007; Tolstoguzov, 2003; Turgeon, Schmitt, & Sanchez, 2007). Knowledge on phase separation between food system's components is also essential to avoid the detrimental effects of destabilization during storage, which is often accompanied by adverse changes in texture, aspect and in the general product quality.

The thermodynamic nature of the segregative or associative phase separations processes has been described as driven by enthalpy or entropy. In the case of neutral polysaccharides, such as the galactomannans studied in this work, their mixtures with proteins usually lead to phase separation through thermodynamic incompatibility, which arises mainly from the low entropy of mixing and segregative phenomena (Turgeon, Beaulieu, Schmitt, & Sanchez, 2003).

Most of the available information on protein–polysaccharide systems reports on milk proteins and gelatin. Much less is known about the structural and functional behaviour of these mixed systems considering proteins from vegetable sources. In recent years, proteins of plant origin have been preferred alternatives to animal-based sources for the food industry, mainly due to consumer dietary preferences and increased concerns regarding the safety of

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http://dx.doi.org/10.1016/j.foodhyd.2013.04.012

animal derived products. Soy proteins are among the most important proteins from vegetable sources, obtained from the relatively abundant byproducts of soybean oil industry, and with recognized high nutritional value and important functional properties in foods (Moure, Sineiro, Domínguez, & Parajo, 2006).

Therefore, as expected, functional properties of soybean proteins and polysaccharides mixtures have also been studied, including in specific food applications (Chin, Keeton, Miller, Longnecker, & Lamkey, 2000; Diftis, Biliaderis, & Kiosseoglou, 2005) with proved advantages.

A synergistic effect between soy protein and xanthan, guar or locust bean gum was suggested based on the increasing viscosity observed for the polysaccharide–soy protein mixtures, but also an antagonistic interaction between soy protein and locust bean gum at lower polysaccharide concentration (Sánchez, Bartholomai, & Pilosof, 1995). Further studies have provided insights into the probable mechanisms explaining the effect of different polysaccharides upon functionality of soy proteins. In this respect, starch and the sulphated polysaccharides, carrageenans, are among the most studied polysaccharides.

The addition of denaturated soy proteins to starch systems has shown to restrict the swelling and gelatinization of starch (Li, Yeh, & Fan, 2007; Ribotta, Colombo, León, & Añon, 2007) and has revealed the occurrence of two-phase networks.

Mixtures of k-carrageenan and soy proteins under certain ionic conditions and polymer proportions may result on complex formation and synergistic effects between both biopolymers, ensuing improved textures and gel viscoelasticities, with both polymers contributing to the network formation (Baeza, Carp, Pérez, & Pilosof, 2002; Ortiz, Puppo, & Wagner, 2004). Nevertheless, when both biopolymers carry a negative charge, mixtures of k-carrageenan and soy proteins (both native protein and heat-induced aggregates) showed segregative phase separation (Li, Hua, Qiu, Yang, & Cui, 2008).

Mixtures of soy proteins with other anionic polysaccharides, such as pectins, have shown that the polysaccharide may exert a stabilizing effect through electrostatic interactions with the soy proteins (Giancone, Torrieri, Masi, & Michon, 2009; Lam, Shen, Paulsen, & Corredig, 2007), depending on polymer amount and different densities of ionizable carboxylic acid groups.

Recent contributions have focused on the effect of galactomannan on gelation and interfacial activity of soy proteins. The galactomannans are neutral polysaccharides, naturally occurring in the seeds of some *Leguminosae*, consisting of linear chains of 1,4-1inked β-D-mannopyranose residues to which varying proportions of α-D-galactopyranosyl residues are randomly attached at position 6 as side-chains. The degree of branching, *i.e.* the mannose-to-galactose (M/G) ratio, is dependent on the galactomannan origin and has revealed an important role in macromolecular interactions involving galactomannans, especially in polysaccharide–polysaccharide mixtures (McCleary, Amado, Waibel, & Neukom, 1981; Lopes da Silva, Gonçalves, Doublier, & Axelos, 1996; Schorsch, Garnier, & Doublier, 1997), but also on the influence of these polysaccharides on protein functionality (Tavares, Monteiro, Moreno, & Lopes da Silva, 2005).

Guar has shown to promote the aggregation of thermally denatured β-conglycinin, in conditions of thermodynamic incompatibility between the protein and the polysaccharide (Zhu et al., 2009). A decreased gel rigidity was shown to occur for soy protein–locust bean gum mixtures at low biopolymer concentrations, when compared with predictions from the behaviour of individual constituents by applying blending laws, explained by a low effective concentration due to the incomplete phase demixing (Hua, Cui, & Wang, 2003). A study on the interactions of soy proteins and non-surface active polysaccharides at the air–water interface, under conditions of neutral pH, revealed only a little influence of locust bean gum on the soy protein interfacial film properties, attributed to a limited incompatibility between the proteins and the non-surface active neutral polysaccharide (Martinez, Sanchez, Ruiz-Henestrosa, Patino, & Pilosof, 2007).

The aim of the present study was to characterize the influence of the degree of branching of neutral non-gelling polysaccharides on soy protein gelation. We report microscopy and rheometry results obtained for soy protein–galactomannan aqueous mixtures, at pH 7; the galactomannans studied differ mainly on the degree of branching, i.e. on the mannose-to-galactose ratio. This is, to our knowledge, the first time that the effects of polysaccharide structural details on soy protein gelation are reported.

## 2. Materials and methods

### 2.1. Materials

Soy protein isolate (SPI) was prepared in the laboratory from industrial defatted soybean meal by isoelectric precipitation to pH 4.7. The protein precipitate was re-suspended in water, the pH adjusted to 7, and freeze dried. The protein, lipid and ash contents of the SPI powder were 89% (using Nx6.25), 1.9% and 2.9% (w/w), respectively, as determined by standard analytical methods (AACC, 1995), with an 11S/7S ratio of 1.18 as determined by SDS-PAGE electrophoresis.

The native galactomannan samples were obtained from commercial samples of guar gum (Meypro Guar CSAA, M175, Meyhall Chemical AG, Switzerland), tara gum kindly provided by Dr. Cairns (IFR, Norwich, UK) and locust bean gum (HG M200, Danisco Portugal – Industrias de Alfarroba, Lda.), and purified by double precipitation with 80% ethanol.

All reagents and solvents (analytical grade) were obtained from Sigma–Aldrich (Sigma–Aldrich Co., Portugal).

### 2.2. Analysis of the galactomannan samples

The mannose-to-galactose (M/G) ratio was calculated with basis on the relative amounts of monosaccharides determined by gas–liquid chromatography after hydrolysis with sulphuric acid, and derivatisation to alditol acetates, as previously described (Tavares et al., 2005). The relative viscosities of galactomannan aqueous solutions were determined by capillary viscosimetry, at 25.0 ± 0.1 °C. The intrinsic viscosities, [η], were determined by extrapolating to infinite dilution, using the combined Huggin's and Kraemer's equations, and the relative average molecular weight was determined by gel permeation chromatography (Monteiro, Tavares, Evtuguin, Moreno, & Lopes da Silva, 2005).

### 2.3. Preparation of solutions

SPI dispersions were prepared in Milli-Q ultrapure water by stirring gently at 4 °C overnight; the pH was kept constant at 7. The galactomannan samples were first dispersed at room temperature for 1 h, heated to 90 °C, for locust bean gum (LBG) and tara gum (TG), and 70 °C for guar gum (GG), kept at this temperature for 30 min, and centrifuged (24,400 *g*, 30 min, 20 °C) after cooling. Mixtures of SPI and galactomannan solutions were prepared at room temperature, under gentle stirring (pH adjusted to 7.0 if needed) for 45 min. Sodium azide 0.02% (w/w) was used as a preservative. All solutions were degassed under vacuum during 1 h before testing. Mixtures were studied at two SPI concentrations, one close to the sol–gel transition threshold (6 wt.% protein) and the other corresponding to a well-developed gel (10 wt.% protein),