Food Hydrocolloids 42 (2014) 81–91

Contents lists available at ScienceDirect

Food Hydrocolloids

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

A micro- and macro-scale approach to probe the dynamics of sol–gel transition in cereal β-glucan solutions varying in molecular characteristics

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ARTICLE INFO

Article history:
Received 13 October 2013
Accepted 6 January 2014
Available online 3 February 2014

Keywords:
β-Glucans
Gelation
Confocal microscopy
Microstructure
Particle tracking microrheology

ABSTRACT

Cereal β-glucans are known to display functional properties with health benefits, such as reduction of plasma cholesterol and of postprandial serum glucose levels in humans and animals; such effects have been lately attributed to viscosity and gelation potential of these water soluble fibres. The local dynamics of β-glucan solutions differing in molecular size and the molar ratio of trimers to tetramers (DP3/DP4) chain segments, which undergo gelation upon ageing, was investigated. Confocal microscopy, particle tracking microrheology and conventional bulk shear rheological measurements have been employed to study the microstructure and mechanical properties of the polysaccharide networks on various length scales. The structural features of β-glucans, such as molecular weight and ratio of DP3/DP4, were found to be important determinants of their gelling properties and microstructure. For the β-glucan gels cured at 25 °C both the microrheology and the bulk rheology revealed that with decreasing molecular weight and increasing DP3/DP4 molar ratio the gelation time decreased, while the gelation rate increased along with the storage modulus. In all samples studied, particulate clusters of β-glucan assemblies were generated with size ~1–15 μm and the clusters were eventually interconnected to expand over the available space to form an elastic network. The pore size and the structural entities were found to increase in size at lower values of the DP3/DP4 molar ratio and with preparations of high molecular weight. The size and the compactness of the structural entities seem to play an important role in the network reinforcement. The embedded tracer particles were found to experience relatively homogeneous microenvironments at DP3/DP4 ~2.1, reflecting a rather slow rate of chain aggregates structuring. The behaviour of the polysaccharide network dynamics at a microscopic level does not always seem to match the overall bulk macroscopic response.

1. Introduction

Mixed-linkage (1 → 3), (1 → 4) β-D-glucans (β-glucans) are structural components of the subaleurone and endosperm cell walls of cereal grains, such as oat, barley, rye, wheat and sorghum (Lazaridou & Biliaderis, 2007; Lazaridou, Biliaderis, & Izydorczyk, 2007). β-Glucans which are either naturally present in foods or incorporated in formulated products exhibit all the functional properties of viscous and gel forming hydrocolloids. In addition, these indigestible polysaccharides display all the physiological properties of soluble dietary fibres, such reducing blood serum cholesterol, regulating blood glucose levels and improving the large intestine function. Such effects have been lately attributed to viscosity enhancement or gelation potential of these water soluble dietary fibres in aqueous media (Kwong, Wolever, Brummer, & Tosh, 2013; Lazaridou & Biliaderis, 2007; Shelat, Vilaplana, Nicholson, Gidley, & Gilbert, 2011; Wolever et al., 2010; Wood, 2007).

β-Glucans from cereals have a similar generalized chemical structure; they are linear homopolysaccharides of D-glucopyranosyl residues (Glcp) inter-linked via a mixture of β-(1 → 3) and β-(1 → 4) linkages and consists of consecutively (1 → 4)-linked β-D-glucose residues in blocks (i.e., oligomeric cellulose-like segments) that are separated by single (1 → 3)-linkages. Most of the cellulose segments are trimers and tetramers, however small amounts (~5–10%) of longer cellulosic oligosaccharides are also present in the polysaccharide chains (Lazaridou & Biliaderis, 2007; Lazaridou, Biliaderis, Micha-Screttas, & Steele, 2004; Wood, Weisz, & Blackwell, 1991). Significant differences in the trisaccharide to tetrasaccharide molar ratio occur among the different genera of cereals,

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0268-005X/$ – see front matter © 2014 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodhyd.2014.01.025

following the order of wheat (3.7–4.5), barley (1.8–3.5), rye (1.9–3.0) and oat (1.5–2.3) β-glucans. Moreover, the observed diversity in molecular weight (Mw) of cereal β-glucans, ranging from 20×10^3 to $> 10^6$, is attributed to variations in genetic and environmental factors as well as to different methods employed for extraction–isolation and Mw determination of these biopolymers (Lazaridou & Biliaderis, 2007; Lazaridou, Biliaderis, et al., 2007).

Polysaccharide concentration along with molecular weight and the ratio of tri- to tetra-cellulosic oligomers in the polymeric chains of cereal β-glucans govern their rheological behaviour (viscosity enhancement and gel forming properties) (Bohm & Kulicke, 1999; Lazaridou & Biliaderis, 2004, 2007; Lazaridou, Biliaderis, & Izydorczyk, 2003; Lazaridou et al., 2004; Tosh, Brummer, Wood, Wang, & Weisz, 2004; Vaikousi, Biliaderis, & Izydorczyk, 2004), which in turn, regulates the technological and nutritional functionality of these indigestible biopolymers (Lazaridou & Biliaderis, 2004; Wood, 2007). Despite the fact that fresh cereal β-glucan solutions exhibit typical random–coil flow behaviour, depending on the thermal history, gel network formation can take place due to interchain associations via hydrogen bonding (Lazaridou & Biliaderis, 2004, 2007; Lazaridou et al., 2003; Vaikousi et al., 2004). Thus, physically cross-linked cereal β-glucan gels have been obtained under isothermal conditions (at >0 °C curing temperatures and at > 4% w/v polymer concentration), as well as after repeated freezing and thawing cycles of relatively dilute (>1% w/v) polysaccharide solutions, and their rheological properties and thermostability have been extensively examined using small strain dynamic rheometry, differential scanning calorimetry (DSC) and large deformation mechanical tests (Bohm & Kulicke, 1999; Lazaridou & Biliaderis, 2004, 2007; Lazaridou et al., 2003; Lazaridou et al., 2004; Tosh et al., 2004; Vaikousi et al., 2004). It has been well established by bulk conventional rheometry, that the gelation capacity of the polysaccharide is enhanced with decreasing Mw and an increasing ratio of tri- to tetra-cellulosic units in the β-glucan chains. In a previous study in our lab, particle tracking microrheology has been employed for the first time to study the sol–gel transition of barley β-glucan aqueous dispersions. With this technique aggregation of the polysaccharide chains was noted at earlier temporal stages of the experiment, compared to those observed by bulk rheology. Moreover, microheterogeneity in the network structure was evident by varying the polysaccharide concentration (Moschakis, Lazaridou, & Biliaderis, 2012). However, structural heterogeneity of the cereal β-glucan gels and chain aggregation events at a micro-level, as can be affected by the molecular/structural characteristics of these polysaccharides, have not yet been examined. Therefore the aim of the present study was to explore the dynamics of gel network formation of cereal β-glucans varying in their molecular structure by employing confocal microscopy, bulk rheometry and particle tracking microrheology.

2. Materials and methods

2.1. Isolation and molecular characterization of cereal β-glucans

Six mixed-linkage (1 → 3), (1 → 4) β-D-glucan isolates, varying in their molecular and structural features, were used in this study. Four of the β-glucan preparations were isolated from whole oat (oat100–2.1) and barley (bar100–2.8 and bar20–2.8) flours, as well as wheat bran (whe100–3.7), all derived from Greek cereal cultivars which were provided by the National Agricultural Research Foundation (NAGREF), Cereal Institute (Thessaloniki, Greece). The isolation–purification protocol for these preparations is described in detail elsewhere (Lazaridou et al., 2004; Vaikousi et al., 2004). Briefly, the oat100–2.1 and bar100–2.8 isolates were obtained from the respective oat and barley flours by an aqueous extraction, a dual-enzyme digestion by a thermostable bacterial α-amylase and pancreatin, followed by exhaustive dialysis using cellulose membranes (Sigma–Aldrich, St. Louis, MO, USA; molecular weight cut-off = 14,000) and precipitation of the polysaccharide component by ethanol. The bar20–2.8 sample was prepared using the same protocol, but following an acid hydrolysis of a 2% (w/v) solution of the isolated polysaccharide with H_3PO_4 (pH 2.0) at 95 °C for 8 h to obtain a low molecular weight β-glucan preparation (Vaikousi et al., 2004). The whe100–3.7 preparation was extracted from wheat bran according to Lazaridou et al. (2004) using a procedure of three key steps that includes α-amylase digestion, alkaline extraction and xylanase treatment, and finally formation of a gelled lower phase (containing >80% β-glucans) in a phase separated system originating from the aqueous dispersion of the crude isolated polysaccharide; this purification procedure was followed by the acid hydrolysis step with H_3PO_4 (pH 2.0) at 95 °C for 55 min (Vaikousi et al., 2004) to obtain a sample with a lower molecular weight. The oat20–2.1 and bar20–3.5 isolates were obtained from oat and barley concentrates (CEBA, Lund, Sweden), respectively, using a purification protocol described by Lazaridou et al. (2003) which involves digestion by α-amylase and pancreatin, dialysis and precipitation of the polysaccharide by ethanol. The β-glucan content of the initial concentrates (24.0 and 41.3% for the oat and barley preparations, respectively) as well as of the six β-glucan isolates were determined by the mixed-linkage (1 → 3), (1 → 4) β-D-glucan assay kit, purchased from Megazyme International Ltd (Bray, Ireland).

The apparent peak molecular weight (Mp) of the β-glucan samples was obtained with a high performance size exclusion chromatography (HPSEC) system combined with a refractive index (RI) detector from the peak fraction of the main eluting peak of the chromatograph and using a standard curve. The six β-glucan standards used for plotting the standard curve were isolated in our laboratory (Lazaridou et al., 2004) and characterized with a HPSEC-RI-MALLS (multi-angle laser light scattering detector) system (Skendi, Biliaderis, Lazaridou, & Izydorczyk, 2003); these standards had Mp 15, 33, 83, 186, 300 and 466×10^3, and polydispersity index (Mw/Mn), 1.9, 2.2, 1.7, 1.5, 1.9 and 2.4, respectively. The distribution of cellulosic oligomers in the chains of the β-glucan isolates was determined by treatment with lichenase [(1 → 3), (1 → 4)-β-glucan-4-glucanohydrolase, EC 3.2.1.73] and liquid chromatography using a high-performance anion-exchange chromatography (HPAEC), combined with a pulsed amperometric detector (PAD); the latter system was suitable for analysis of oligosaccharides released from β-glucan by the lichenase action. Details of both chromatographic systems and the conditions employed for these analyses are given in detail, elsewhere (Lazaridou et al., 2004).

2.2. Bulk rheology

For all rheological measurements, fresh aqueous solutions were prepared in hermetically sealed glass vials by stirring of the β-glucan samples at 5% (w/w) polysaccharide concentration in double-distilled water at 85 °C for approximately 2 h until a complete solubilization of the material was observed. The fresh aqueous solutions were subsequently subjected to an isothermal (25 °C) gel curing, and followed by melting behaviour evaluation of the formed gels by conventional bulk rheometry using a rotational Physica MCR 300 rheometer (Physica Messtechnic GmbH, Stuttgart, Germany) equipped with a double gap cylindrical geometry; the experimental details for probing gelation by bulk shear rheometry were as described by Lazaridou et al. (2003).

2.3. Confocal microscopy

The β-glucan aqueous solutions (5% w/w) were prepared, as previously described, and were stained with a Congo red dye