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Water extractable (1→3,1→4)-β-D-glucans from barley and oats: An intervarietal study on their structural features and rheological behaviour

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Abstract

The fine structures and rheological behaviours of aqueous flour dispersions and of β-glucan, (1→3,1→4)-β-D-glucan isolates obtained from 18 registered varieties of normal covered barley seeds and four registered oat varieties, grown in the same location in Greece, were investigated. The β-glucan content of the barleys and oats varied between 2.5–5.4 and 2.1–3.9%, respectively (dry matter basis). Heat treatment of the barley and oat flour dispersions with 80% (v/v) ethanol, to inactivate endogenous β-glucanases, had a stabilizing effect on the viscosity profile of the flour slurries. The relationship between total β-glucan content and aqueous slurry viscosity (at $247\ \text{s}^{-1}$) of the heat-treated barley flours was weak ($r^2 = 0.45$, $p < 0.05$, $n = 18$). β-Glucans were isolated by water extraction at temperatures slightly below the gelatinization temperature of starch, enzymatic removal of starch and partial removal of contaminating proteins by adjustment to pH 4.0–4.5, and subsequent precipitation of the water-soluble β-glucans with 80% (v/v) ethanol. The cellulosic oligomers released by the action of a (1→3,1→4)-β-D-glucan hydrolase showed cellotriosyl and cellotetraosyl units, accounted for 91.1–92.1% for barley and between 92.4 and 94.1% for the oat preparations; the respective molar ratios of tri- to tetra-saccharides (DP3/DP4) ranged between 2.73–3.05 (barley) and 2.16–2.42 (oat). Steady shear measurements confirmed the random coil type behaviour of freshly prepared β-glucan solutions (5 and 7%, w/v). The rate at which shear thinning began was dependent on both concentration and molecular size of the polysaccharide. Most of the β-glucan dispersions followed the Cox–Merz rule, except Mucio, a variety with high M_w β-glucan (2.39×10^5). Viscoelastic characterization, at 8% (w/v), of three barley β-glucan aqueous dispersions differing in molecular size, indicated that the low molecular weight sample exhibited shorter gelation time and higher gelation rate ($I_E = [\text{dlog}\ G'/\text{d}t]_{\text{max}}$) than its higher molecular weight counterparts. Small deformation oscillatory measurements on gels of all barley β-glucan isolates (10% (w/v), 7 d storage, 25 °C) revealed a strong negative relationship ($r^2 = 0.88$, $p < 0.01$) between G' (1 Hz, strain 0.1%) and apparent M_w of the polysaccharide.
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1. Introduction

(1→3,1→4)-β-D-Glucans, referred to hereafter as β-glucans, are non-starchy polysaccharides found in walls of endosperm and aleurone cells of barley and oat grains (Bacic and Stone, 1981). These polysaccharides are

Abbreviations β-glucans, (1→3,1→4)-β-D-glucans.
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polymers of β-D-glucopyranose (Preece and Mackenzie, 1952), where about 30% of glucose residues are C(O)3-linked and 70% are C(O)4-linked. β-Glucan preparations generally contain small amounts of protein and arabinoxylans (Westerlund et al., 1993). About 90% of the 4-linked β-D-glucopyranosyl residues occur in groups of two or three consecutive residues (Aspinall and Carpenter, 1984; Woodward et al., 1983a) separated by single (1→3)-linkages (Wood, 1993). The resultant polysaccharide can therefore be considered to consist mainly of 3-linked cellotriosyl and cellotetraosyl units (Dais and Perlin, 1982; Parrish et al., 1960; Woodward et al., 1983a);

a smaller fraction of longer cellulosic sequences are also present (Cui, 2001; Woodward et al., 1983a).

Barley β-glucans constitute a relatively minor fraction (2–11%) of the weight of the total kernel carbohydrates, but usually fall between 4 and 7% (Fincher and Stone, 1986; Newman et al., 1989). The corresponding range for β-glucan in oats is 3.2–9.4% by weight of the grain (Cui, 2001). However, being high molecular weight, water-soluble polymers, they have unique properties with both nutritional and technological significance. They are not digested by monogastric animals which is one reason for the low use of barley as a poultry feed (Wood, 1984). In addition, when present in elevated levels, β-glucans of barley produce highly viscous solutions, which can lead to problems during post-malting operations in the brewing process (Bourne et al., 1982; Jadhav et al., 1998). Several studies have shown that β-glucans are effective hypoglycemic and hypocholesterolemic agents in human diets (McIntosh et al., 1995; Newman et al., 1989; Wood, 1994). Thus, a knowledge of the physicochemical properties of β-glucans in solution is of fundamental importance.

There is a general agreement that the genetic background of barley and oats is a major determinant of the final $(1\rightarrow3,1\rightarrow4)$-β-D-glucan content of these cereal grains (Henry, 1987; Lim et al., 1992; Miller et al., 1993; Molina-Cano and Conde, 1982; Perez-Vendrell et al., 1996; Peterson, 1991; Stuart et al., 1988). Moreover, environmental conditions seem to exert a significant effect on the β-glucan content of the cereal kernel (Aastrup, 1979a). Among them, the availability of water during grain maturation influences the $(1\rightarrow3,1\rightarrow4)$-β-D-glucan levels, i.e. dry conditions before harvest have been related with high β-glucan levels (Anderson et al., 1978; Bendelow, 1975).

The main objectives of the present study were to screen for β-glucan content among a large number of barley and oat cultivars grown in Greece, and to assess the structural and rheological properties in solution and in the gel state of aqueous flour dispersions and β-glucan preparations obtained from the respective flours by water extraction at temperatures slightly below the gelatinization temperature of starch.

2. Materials and methods

2.1. Materials

Eighteen registered varieties of normal covered barley seeds (Athinaida, Elassona, Grammos, Kos, Nicky, Thessaloniki, Persefoni, Krini, Kronos, Mucio, Spitha, Kirki, Sophia, Kriton, Thermi, Kypros, Plaisant, Igri) and four registered oat varieties (Kassandra, Pallini, Vermion, Flega) belonging to two different oat species (*Avena sativa* L and *A. byzantina* cv. Kassandra) were obtained from experimental crops grown in 2001 at the National Agricultural Research Foundation, Cereal Institute, Thessaloniki, Greece at the same location and with similar agronomic practice. The β-glucan assay kit, β-glucan molecular size standards $(40\times10^3, 123\times10^3, 183\times10^3, 245\times10^3)$ and the $(1\rightarrow3, 1\rightarrow4)$-β-D-glucan hydrolase were from Megazyme International Ltd, Bray, Ireland. Analytical grade reagents were either from Sigma-Aldrich Co. (Deisenhofen, Germany) or from Merck (Darmstadt, Germany). The microbial *alpha*-amylase preparation (Termamyl 120L) was from Novozyme A/S, Bagsvaerd, Denmark.

2.2. Extraction and purification of β-glucan

Barley and oat grains were ground in a Retsch laboratory mill to pass a 0.5-mm screen and then refluxed with 80% (v/v) ethanol at a ratio of flour/ethanol solution of 1:10, for 2 h at 90 °C, to inactivate the endogenous β-glucanases and remove low molecular weight polar substances. The supernatant was removed by vacuum filtration and the residue washed with two volumes of ethanol (80%, v/v), and dried at 40 °C overnight. The pretreated barley and oat flours were suspended in distilled water (1:10 w/w flour to water ratio, using ∼100–120 g of flour), and heated at 50 °C for 2 h with continuous stirring. The insoluble residue was removed by centrifugation (3000*g*, 10 min). A thermostable *alpha*-amylase (Termamyl 120L) was added to the supernatant to hydrolyse contaminating starch (at 90 °C, 3 h, pH 4.5). Following hydrolysis, the suspension was cooled and sodium azide was added (0.02% w/v); the pH was adjusted to 4.0 (2 M HCl) and held at 25 °C overnight. The suspension was centrifuged and the pH of the clear viscous supernatant raised to 7.0 with 2 M NaOH. The supernatant was exhaustively dialyzed against distilled water for 3 d and any precipitate formed also removed by centrifugation. The volume of the supernatant was reduced to 100–200 ml, dialysed and concentrated to one third the initial volume. The β-glucan was precipitated by adding two volumes of 80% (v/v) ethanol to the concentrated liquor and holding it overnight at 4 °C with stirring. The fibrous precipitate was collected by filtration under vacuum, washed twice with 80% (v/v) ethanol, resuspended in 2-propanol and stirred overnight, filtered and dried in oven at 40 °C to remove excess propanol.

2.3. Chemical analysis

Total β-glucan content was determined (McCleary and Glennie-Holmes, 1985) by first hydrolysing with a $(1\rightarrow3,1\rightarrow4)$-β-D-glucan hydrolase and then a β-glucosidase. The results are means of triplicate measurements. Protein content of the β-glucan isolates was determined (Lowry et al., 1951) using bovine serum albumin as standard. The results are means of duplicate measurements. The purity of the β-glucan isolates, checked by β-glucan assay, varied between 85.8–91.3 and 90.4–93.7% for the barley and oat samples, respectively.

2.4. (1→3,1→4)-β-D-glucan hydrolase digestion

β-Glucan isolates (2 mg/ml) were dissolved in phosphate buffer (0.01 M, pH 6.5) and treated with (1→3,1→4)-β-4-glucanohydrolase [EC 3.2.1.73, (4 U/ml)] for 20 h at 40 °C. The digests were subsequently heated for 15 min at 95 °C to inactivate the enzyme and centrifuged at 10,000g for 10 min. Oligosaccharides released from the β-glucans by the (1→3,1→4)-β-D-glucan hydrolase were analysed using a high-performance-anion-exchange chromatography (HPAEC) system (Dionex) equipped with a pulsed ampero-metric detector (PAD-2), a CarboPac (PA1) column (4×250 mm) and a PA1 guard column (4×50 mm) as described by Wood et al. (1994).

2.5. Molecular weight determination

The molecular weight of the β-glucans was estimated by analysis of the main peaks using a high performance size exclusion chromatography (HPSEC) system which consisted of a single pump (Marathon IV, Rigas Labs, Thessaloniki, Greece), a sample injection valve (Model 7010, Rheodyne) with a 200-μl loop, a guard column (TSK PWH, TosoHass GmbH, Stuttgart, Germany), a SEC column (TSK G5000 PW-SEC, 7.8×600 mm, TossoHass GmbH), and a RI detector (ERC-7515A, ERC-Inc. Nishiaoki, Kawaguchi-City, Japan). The columns were kept at room temperature and the flow rate of the mobile phase (0.15 M NaNO$_3$, containing 0.02% (w/v) NaN$_3$) was set at 0.5 ml/min. Dried samples (15 mg) were dissolved in distilled water (5 ml), stirred and filtered through a 1-μm cellulose filter. The mobile phase was filtered through a 0.1-μm cellulose acetate membrane. β-Glucan samples and standards used were dissolved in 0.2 M NaOH with stirring overnight and then filtered through 3-μm polycarbonate membranes before analysis with the HPSEC system. Calculations of the apparent M_w for the eluted polysaccharides (peak fractions) were based on calibrations with β-glucan standards of known molecular weight.

2.6. Rheological measurements

Rheological measurements of flour dispersions and β-glucan solutions were performed using a rotational rheometer (Physica, MCR 300) equipped with a single gap-measuring cylinder CC 27 for flour dispersions (dimensions of cup and bob, 28.92 and 26.66 mm, respectively) or a double gap measuring cylinder DG 26 (internal and external gap 0.42 and 0.47 mm, respectively) for β-glucan solutions, a Paar Physica circulating water bath (Viscotherm VT2) and a controlled Peltier system (TEZ 150P/MCR) with an accuracy ±0.1 °C driven by US200 V2.21 rheometer software. Ground barley and oat samples with and without heating in 80% (v/v) ethanol in water, were suspended in distilled water (1:6 w/w flour to water

ratio) and the viscosity measured at different time intervals (0, 1, 2, 4, 6, and 24 h) at 25 °C at shear rates between 1 and 1200 s^{-1}.

Stock β-glucan dispersions were prepared by dissolving β-glucans in double distilled water with continuous stirring at 85 °C for several hours. The solutions were then cooled to room temperature and the final concentration adjusted with distilled water. The β-glucan concentration is reported as dry matter per volume (w/v). Flow curves were measured at shear rates between 0.01 and 1200 s^{-1}. Oscillatory measurements were performed with a strain of 0.1% over an angular frequency range of 0.1–100 Hz. Gelation and melting kinetics were probed at a fixed frequency, 1 Hz, and a constant strain, 0.1%. Melting was induced at a heating rate of 3 °C/min. In all rheological measurements, a few drops of paraffin oil were placed above the aqueous β-glucan dispersions, to prevent solvent evaporation.

For gelation, the isolated β-glucan samples were dispersed in distilled water (containing 0.02% NaN$_3$, to prevent microbial contamination) at 10% (w/v) concentration. Gel discs were prepared by pouring the β-glucan dispersions into cylindrical plastic moulds. The β-glucan dispersions were then covered with a thin layer of paraffin oil to prevent evaporation of the solvent, and held at room temperature until gelation was complete. Lack of flow after tilting the moulds was considered to be a sign of gel setting. After completion of gelation and removal of the surface oil (using a pasteur pipette and wiping off the residual oil with a soft tissue paper), a sinusoidal deformation was applied to the gel disc using a plate–plate (25-cm diameter) geometry (the plate had a serrated surface to minimize slippage during measurements) with a strain of 0.1% over a frequency range of 0.1–100 Hz.

3. Results and discussion

3.1. β-Glucan content of barley and oat cultivars

The values of β-glucan and protein content for each variety are presented in Table 1. The β-glucan content of the Greek barleys studied was found to vary between 2.5 and 5.4% (dry matter basis). This variability is comparable with the results obtained by other researchers for European barleys. The mean β-glucan content of Greek barley varieties was 4.4%, similar to values reported for barleys from Denmark, Finland, England and Sweden (4.1, 4.1, 4.1 and 3.7%, respectively) (Aman, 1986; Jorgensen and Aastrup, 1988; Lehtonen and Aikasalo, 1987). Barleys from Canada and Australia had mean β-glucan contents of 5.2 and 4.7%, respectively (Aman and Graham, 1987; Anderson et al., 1978; McCleary and Glennie-Holmes, 1985). Differences in the total β-glucan content were observed also among the oat genotypes and ranged from 2.1 to 3.9%, similar to the results reported by

Table 1
β-Glucan content of cereal flours, and protein content and apparent M_w of β-glucan isolates

Cultivar	Flour	β-Glucan isolate	
	β-Glucan content (%, w/w)	Protein content (%, w/w)	Apparent $M_w \times 10^5$
Barley			
Athinaida	4.2	4.5	1.26
Elassona	2.5	7.8	2.15
Grammos	4.5	4.2	2.07
Kos	5.0	5.5	1.73
Nicky	4.9	6.3	2.00
Thessaloniki	4.9	7.8	1.87
Persefoni	5.0	4.5	1.61
Krini	4.0	4.3	1.88
Kronos	3.6	4.3	1.34
Mucio	4.3	5.0	2.39
Spitha	4.7	3.5	1.86
Kirki	4.3	6.7	1.46
Sophia	5.2	4.7	1.83
Kriton	5.4	5.6	1.88
Thermi	3.8	4.6	1.50
Kypros	4.9	4.9	1.91
Plaisant	3.9	5.0	1.82
Igri	4.2	4.1	2.01
Oats			
Kassandra	3.9	3.5	1.02
Pallini	3.0	5.6	0.76
Vermion	2.3	4.0	1.10
Flega	2.1	4.9	0.44

Peterson (1991). Table 1 shows that Kriton and Sophia, the barley cultivars, had the highest β-glucan content (5.4, 5.2%, respectively), whereas Elassona had the lowest (2.5%). There are several reports that the β-glucan contents of barley and oats are highly influenced by genotypic and environment factors (Lim et al., 1992; Molina-Cando and Conde, 1982; Perez-Vendrell et al., 1996; Peterson, 1991; Stuart et al., 1988). Since these cultivars were grown in the same region, the variability in β-glucan content among the samples examined could be attributed mostly to varietal factors and less to effects arising from the environment (Henry and Blakeney, 1986; Miller et al., 1993; Molina-Cano and Conde, 1982; Morgan et al., 1983).

In the extraction protocol, the pH was adjusted to 4.0–4.5 before hydrolysis with the bacterial *alpha*-amylase and before storage of the extracts overnight. Under these conditions, some precipitation of contaminating proteins is known to occur. The protein content of the β-glucan samples varied between 3.5 and 7.8%; and in the final barley and oat β-glucan isolates protein content was between 3.5–7.8 and 3.5–5.6%, respectively. Among the barley cultivars, the samples from Elassona and Thessaloniki had the highest protein content (7.8%), whereas the Spitha preparation had the lowest (3.5%). Genetic factors have been reported to greatly influence protein levels in cereal grain β-glucan preparations (Wood et al., 1978).

3.2. Viscosities of aqueous dispersions of barley and oat flour

Changes in viscosity of barley and oat flour slurries were monitored at shear rates ranging between 1 and 1200 s^{-1}, following different time intervals (0, 1, 2, 4, 6 and 24 h) Before each measurement, the samples were stirred to ensure a proper homogeneity of the preparation. A significant decrease in viscosity was noticed for almost all samples with increasing storage time (Fig. 1). This decrease in viscosity is attributed mainly to the degradation of β-glucans by endogenous β-glucanases, since the viscosities were determined on flour extracts that had not been heat-treated. In previous studies, where extract viscosity was measured under acidic conditions to prevent β-glucanase action, no changes in slurry viscosities were observed (Aastrup, 1979b; Bhatty, 1995; Perez-Vendrell et al., 1996).

Aastrup (1979b) reported that the viscosity of barley flour slurries were affected by the presence of endogenous enzymes. Two endogenous $(1 \rightarrow 3, 1 \rightarrow 4)$-β-D-glucan 4-glucanohydrolase isoenzymes are responsible for the degradation of barley β-glucans, as judged by their ability to rapidly depolymerise this polysaccharide (Woodward et al., 1983b). Heat-treatment of barley and oat flours with ethanol (80% (v/v), 2 h at 90 °C) to inactivate the β-glucanases prior to viscosity measurement had a pronounced stabilizing effect on the viscosity (Fig. 1). In fact, the dispersions showed an immediate increase in viscosity, due to initial solubilization of the β-glucans, and no detectable decline thereafter. Izydorczyk et al. (2000) previously showed that heat treatment stabilizes the viscosity profile of flour slurries.
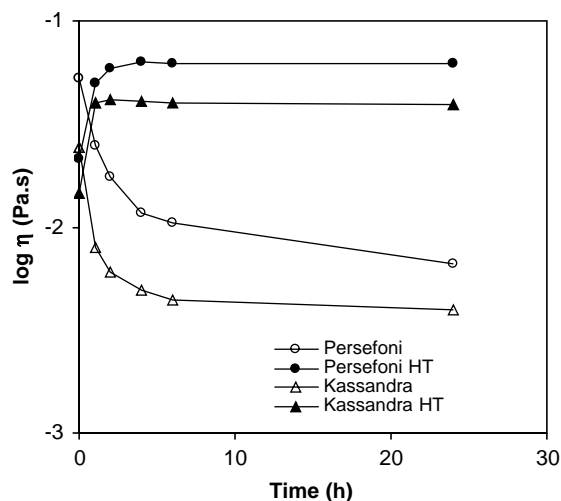

Fig. 1. Effect of heat treatment (HT) (i.e. boiling the flours in 80% (v/v) ethanol for 2 h) on viscosity at 247 s^{-1} of oat (Kassandra) and barley (Persefoni) aqueous flour slurries (1:6 w/w), as a function of stirring time.

3.3. Relationship between flour slurry viscosity and total β-glucan content

Several authors have investigated the relationship between slurry viscosities and total β-glucan content in an attempt to select barley grains for uses in brewing, animal feeding and human nutrition. Using acidic extraction conditions, Aastrup (1979b) found an $r^2 > 0.99$ ($n = 18$) for β-glucan content vs. viscosity of acid extracts, while Lance (1984) and Ulrich et al. (1986) reported an $r^2 = 0.54$ ($n = 30$) and 0.42 ($n = 50$), respectively. In the present study, using distilled water as the extractant and different barley samples (heat-treated), a weak relationship was found between total β-glucan content and slurry viscosity (at 247 s^{-1}), $r^2 = 0.45$ ($p < 0.05$, $n = 18$) (Fig. 2). Bourne and Pierce (1970) reported that differences in structural and physicochemical characteristics exist among different genotypes and that longer β-glucan polymers have a greater influence on viscosity than shorter polymers. Moreover, differences in their extractability from cell walls would also influence the viscosity of the aqueous flour slurries. It is not surprising, therefore, that variations occur in viscosity–concentration relationships and that viscosity, on its own, may not be a reliable indicator of the β-glucan content.

3.4. Molecular characterization of β-glucan isolates

Molecular weight is a fundamental parameter for characterizing a polysaccharide and determines its rheological properties. Fig. 3 presents typical chromatograms of six barley β-glucan isolates. Table 1 summarizes the apparent M_w of barley β-glucans corresponding to the maximum of the main chromatographic peak recorded by the refractive index detector. The apparent molecular weight range of all barley samples was 1.26×10^5–2.39×10^5 Da and that of the oat preparations of 0.44×10^5–1.10×10^5. Of all the isolates, only Mucio showed a high molecular weight fraction eluting in the void volume, i.e. the M_w of this sample is much higher than
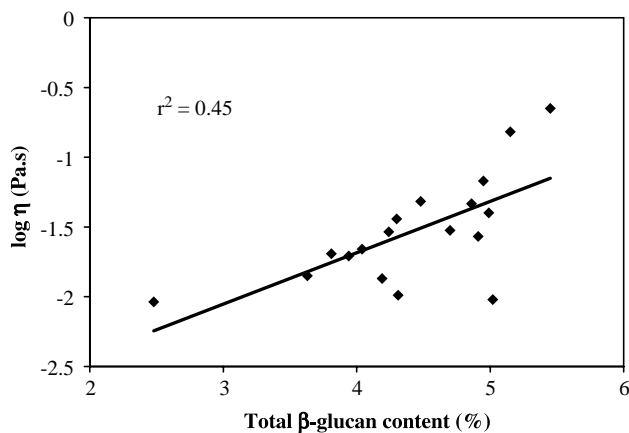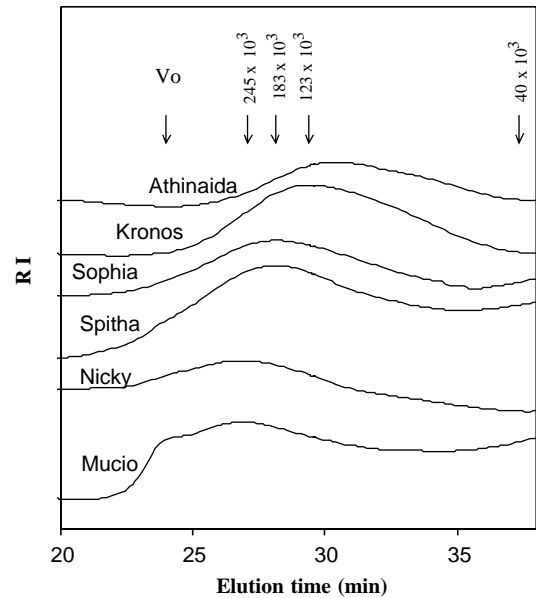

Fig. 3. HPSEC traces of six barley β-glucan samples detected by RI; the arrows indicate the elution time of the peak fraction of the four β-glucan M_w standards used for column calibration.

the estimate given in Table 1 based on the main eluting peak. The values reported in Table 1 agree reasonably well with those reported by Woodward et al. (1983a), 2×10^5 and 2.9×10^5 and Saulnier et al. (1994), 1.5×10^5. However, they were much smaller than those reported by Wood et al. (1994), 2–2.5×10^6 and Bhatty (1995) who suggested that the M_w of oat and barley β-glucans was greater than 2×10^6. Differences in the molecular weight values may reflect variations due to genetic factors (Autio et al., 1992). In addition, the mild conditions of extraction (50 °C) employed in the present study may solubilize only low molecular weight β-glucan fractions. In fact, McCleary (1988) reported that the M_w of the extracted cereal β-glucans increases as the extraction temperature is increased, e.g. the M_w of β-glucan extracted at 90 °C was up to 30% higher than at 37 °C.

The content of oligosaccharides produced by the action of the $(1 \rightarrow 3, 1 \rightarrow 4)$-β-D-glucan hydrolase on barley and oat β-glucans and the relative percentage of each oligosaccharide fraction as determined by the HPAEC-PAD analysis are shown in Table 2. All β-glucans were degraded with tri- and tetra-saccharides as the main products. In addition, oligomers with a high degree of polymerization, DP12 and 13, were observed. In agreement with Woodward et al. (1988) and Wood et al. (1994), the sum of the DP3 and DP4 oligosaccharides for the 18 barley β-glucans ranged from 91.1 to 92.1% and from 92.4 to 94.0% for the oat preparations, indicating that these oligosaccharides are the major building blocks of water-extractable barley and oat β-glucans. The percentages of oligosaccharides with DP5–DP13 were similar to those reported by Wood et al. (1994). For all barley varieties, oligosaccharide content declines through DP7, but the content of DP9

Fig. 2. Heat-treated flour slurry (1:6 w/w) viscosities (at 247 s^{-1} after 1 h stirring) vs. β-Glucan content of 18 barley cultivars.

Table 2
Structural features of barley and oat β-glucan isolates

Sample	DP3	DP4	DP5	DP6	DP7	DP8	DP9	DP10	DP11	DP12	DP13	Molar ratio, DP3/DP4	DP3+DP4
Barley													
Athinaida	63.80[a]±0.01	27.98±0.01	3.93±0.1	2.00±0.03	0.41±0.01	0.45±0.02	0.99±0.01	0.12±0.02	0.15±0.00	0.10±0.01	0.00±0.00	3.02	91.84
Elassona	63.34±0.27	28.16±0.06	3.65±0.08	1.97±0.07	0.43±0.02	0.49±0.03	1.31±0.07	0.17±0.03	0.23±0.01	0.18±0.02	0.07±0.00	2.97	91.50
Grammos	63.17±0.14	28.06±0.2	3.64±0.09	2.02±0.09	0.46±0.04	0.51±0.08	1.42±0.02	0.17±0.01	0.25±0.01	0.17±0.01	0.08±0.01	2.97	91.23
Kos	62.85±0.35	28.67±0.14	3.74±0.18	1.95±0.09	0.44±0.04	0.53±0.06	1.26±0.1	0.13±0.00	0.22±0.01	0.15±0.02	0.06±0.00	2.90	91.52
Nicky	63.03±0.24	28.46±0.1	3.70±0.14	1.92±0.06	0.42±0.01	0.49±0.05	1.29±0.03	0.16±0.01	0.24±0.01	0.16±0.01	0.08±0.00	2.93	91.50
Thessaloniki	61.71±0.32	29.37±0.18	3.76±0.19	1.98±0.11	0.47±0.02	0.52±0.03	1.39±0.09	0.17±0.02	0.28±0.02	0.2±0.01	0.09±0.01	2.78	91.08
Persefoni	62.03±0.44	29.22±0.25	3.55±0.18	1.92±0.14	0.44±0.03	0.51±0.07	1.51±0.16	0.18±0.02	0.28±0.04	0.2±0.04	0.09±0.01	2.81	91.24
Krini	61.44±0.65	29.79±0.01	3.64±0.29	1.90±0.15	0.46±0.03	0.57±0.00	1.38±0.11	0.19±0.04	0.29±0.02	0.18±0.02	0.10±0.01	2.73	91.24
Kronos	61.55±0.96	29.67±0.04	3.67±0.36	1.95±0.21	0.45±0.02	0.55±0.1	1.30±0.14	0.21±0.07	0.28±0.03	0.20±0.4	0.10±0.02	2.74	91.22
Mucio	64.08±0.66	27.8±0.21	3.55±0.37	1.79±0.19	0.36±0.01	0.50±0.08	1.26±0.14	0.17±0.01	0.24±0.01	0.14±0.02	0.07±0.01	3.05	91.87
Spitha	62.31±0.54	29.14±0.05	3.67±0.22	1.88±0.11	0.43±0.02	0.55±0.06	1.28±0.08	0.19±0.04	0.25±0.01	0.16±0.02	0.07±0.00	2.83	91.45
Kirki	61.90±0.71	29.55±0.04	3.62±0.25	1.93±0.15	0.44±0.03	0.46±0.02	1.34±0.11	0.18±0.04	0.28±0.05	0.17±0.02	0.08±0.01	2.77	91.46
Sophia	62.10±0.85	29.19±0.01	3.59±0.28	1.93±0.17	0.46±0.08	0.48±0.04	1.46±0.16	0.19±0.05	0.28±0.04	0.17±0.02	0.10±0.01	2.81	91.29
Kriton	63.81±0.76	28.15±0.05	3.67±0.28	1.86±0.14	0.41±0.03	0.41±0.05	1.15±0.14	0.13±0.02	0.21±0.03	0.13±0.02	0.06±0.01	3.00	91.96
Thermi	62.56±0.75	28.86±0.12	3.56±0.34	1.93±0.17	0.43±0.04	0.45±0.05	1.43±0.15	0.18±0.04	0.27±0.04	0.17±0.02	0.09±0.01	2.86	91.42
Kypros	61.62±0.63	29.68±0.04	3.48±0.26	1.92±0.13	0.42±0.02	0.47±0.01	1.54±0.16	0.19±0.03	0.31±0.03	0.20±0.02	0.10±0.01	2.74	91.31
Plaisant	64.2±0.43	27.90±0.01	3.49±0.17	1.80±0.07	0.39±0.05	0.46±0.07	1.19±0.05	0.15±0.02	0.21±0.01	0.40±0.01	0.06±0.01	3.04	92.11
Igri	63.04±0.5	28.63±0.08	3.58±0.28	1.91±0.08	0.40±0.02	0.49±0.01	1.29±0.09	0.16±0.03	0.24±0.03	0.16±0.02	0.09±0.03	2.91	91.67
Oats													
Kassandra	57.78±0.59	34.64±0.15	3.40±0.29	2.08±0.17	0.43±0.03	0.57±0.08	0.82±0.09	0.10±0.01	0.10±0.01	0.07±0.05	0.00±0.00	2.20	92.42
Pallini	57.56±0.61	35.29±0.04	3.45±0.27	2.06±0.16	0.39±0.03	0.44±0.03	0.63±0.1	0.08±0.02	0.06±0.01	0.03±0.02	0.00±0.00	2.16	92.85
Vermion	59.28±0.00	33.17±0.00	3.69±0.00	2.15±0.00	0.40±0.00	0.56±0.00	0.59±0.00	0.09±0.00	0.07±0.00	0.00±0.00	0.00±0.00	2.36	92.44
Flega	60.80±0.26	33.21±0.12	3.71±0.21	1.75±0.14	0.22±0.02	0.17±0.00	0.14±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.42	94.01

[a] Weight percent from the chromatograms of the $(1\rightarrow3,1\rightarrow4)$-β-D-glucan hydrolase digest of $(1\rightarrow3,1\rightarrow4)$-β-D-glucans.

oligosaccharide (1–1.54%) increased, and thereafter the content of oligomers with DP>9 was very low. The molar ratio of tri- to tetra-saccharides ranged between 2.73 and 3.05 for the barley and 2.16–2.42 for the oat β-glucans. Inspection of the data in Table 2 reveals that the molar ratio of tri- to tetra-saccharides (3.00–3.05) in β-glucans from Athinaida, Mucio, Kriton and Plaisant was somewhat higher than the other varieties. The corresponding ratios for the oat varieties (2.16–2.42) were the lowest amongst all grain cultivars examined.

Böhm and Kulicke (1999) assumed that there was a relationship between structural regularity and gelation rate. They suggested that a high tri- to tetra-saccharide ratio, giving a more regular conformation to the β-glucan chain, encourages rapid gelation. Among the cultivars investigated, the four varieties with the highest molar ratio of DP3–DP4 would be expected to have the most suitable structural features for rapid formation of extended junction zones and establishment of a gel network in an aqueous medium. However, as it will be seen in Section 3.6, Mucio β-glucan (with an apparent $M_w \sim 2.39 \times 10^5$) and a ratio of tri- to tetra-saccharide oligomers (3.02–3.05) almost equal to Athinaida ($M_w \sim 1.26 \times 10^5$), did not form a gel.

3.5. Shear rate and temperature dependence of β-glucan viscosity

Freshly prepared β-glucan solutions (5 and 7%, w/v) exhibited the well-characterized behaviour of polysaccharides in random coil conformations. Fig. 4 shows that the apparent viscosity decreased with increasing shear rate, with a Newtonian region in the low shear rate zone for all samples, except for Athinaida and the oat β-glucan dispersions. The shear thinning flow behaviour is caused by the disruption of molecular entanglements of the polysaccharide by the applied shear. It is believed that, at low shear rates, those entanglements that are disrupted by the imposed deformation are replaced by new interactions between different molecules, but lead to no change in the density of entanglements, and hence, no reduction in viscosity (Morris et al., 1981). This situation corresponds to the 'horizontal plateau' in viscosity–shear rate plots, such as shown in Fig. 4a. On the other hand, in the high shear rate zone, the rate of externally imposed movement is greater than the rate of formation of new entanglements. Thus, the overall content of junctions in the three-dimensional network decreases progressively and the resistance to flow correspondingly decreases. Moreover, the shear thinning shifts towards lower shear rates as the concentration and molecular weight of the samples increases. As expected, with increasing molecular weight there was an increase in viscosity and shear thinning properties, at equivalent polysaccharide concentrations.

The influence of concentration on the apparent viscosity of β-glucan solutions (5 and 7%, w/v) at 20 °C with varying molecular weights is also seen in Fig. 4a. The shear rate at

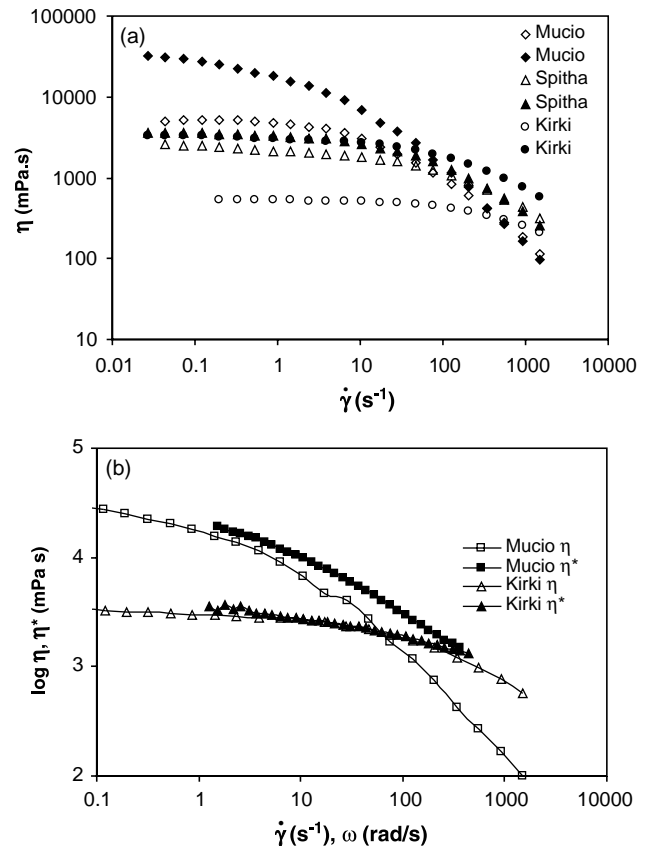Fig. 4. Effect of concentration (5% (w/v), open symbols, 7% (w/v), solid symbols) on viscosity of three barley β-glucan aqueous dispersions (a) and Cox–Merz plots for Kirki ($M_w = 1.46 \times 10^5$) and Mucio ($M_w = 2.39 \times 10^5$) β-glucan preparations (b), at 7% (w/v) at 20 °C.

which the apparent viscosity began to decrease was dependent on both concentration and molecular size; the apparent viscosity (η) increased with the increase in β-glucan concentration (from 5 to 7%, w/v) and molar mass of the polysaccharide. The power law equation, which has been used by several authors to characterize non-Newtonian flow, was found to be a useful tool in describing oat β-glucan rheological behaviour (Autio, 1988)

$$\sigma = K\dot{\gamma}^{n-1},$$

where σ is shear stress, $\dot{\gamma}$ is shear rate, K is the consistency index and n is the flow behaviour index. Deviation from the Newtonian flow ($n=1$ for Newtonian flow) is reflected by the value of the n parameter. In the present study, the power law equation was used in an attempt to describe and express the flow behaviour. When the logarithm of viscosity for all β-glucan aqueous dispersions of different M_w was plotted vs. logarithm of shear rate, within the range of 10–1200 s^{-1}, almost straight lines, with slopes equal to n and intercept equal to K, were obtained.

The effect of temperature on apparent viscosity was investigated for all barley and oat β-glucans dispersions (5%, w/v) at different temperatures (10, 20, 30, 40, 50 °C) in a range of shear rates between 0.01 and 1200 s^{-1} (Fig. 5a).
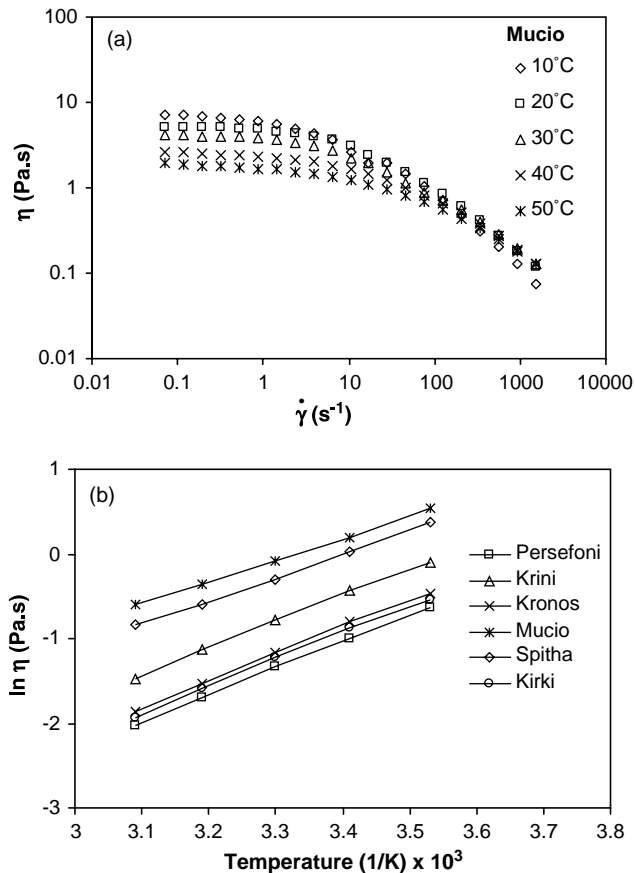
Fig. 5. Effect of temperature on the apparent viscosity of a 5% (w/v) barley β-glucan dispersion (a) and Arrhenius plots of viscosity vs. absolute temperature at 0.87 s^{-1} for six barley β-glucan dispersions, 5% (w/v) (b).

The apparent viscosity decreased with increasing temperature for all the samples studied. At high temperatures (40 and 50 °C) close to Newtonian behaviour was observed. The β-glucan preparations from barley cultivars Athinaida, Kronos, Kirki and Persefoni (M_w equal to 1.26×10^5; 1.34×10^5; 1.46×10^5; 1.61×10^5, respectively) and from oat cultivars Pallini and Vermion (M_w equal to 0.76×10^5; 1.10×10^5, respectively) showed n values equal to 0.99. On the other hand, Mucio, Elassona and Grammos ($M_w = 2.39 \times 10^5$, 2.15×10^5, 2.07×10^5, respectively) deviated from Newtonian behaviour even at elevated temperatures, exhibiting flow index (n) values between 0.54 and 0.8. Arrhenius plots were developed to model the temperature effects on viscosity for the aqueous β-glucan dispersions

$$\eta(T) = A\, e^{(E_a/RT)}$$

where η is the viscosity (Pa s), A is a constant, e is the natural logarithm base, E_a is the activation energy (kJ/mol), R is the universal gas constant and T is the absolute temperature (K). Three shear rates were used: 0.87, 10.5 and 125 s^{-1}. By plotting the natural logarithm of apparent viscosity (ln η) against (1/T), straight lines ($r^2 > 0.99$) were obtained and apparent activation energies (E_a) were calculated from the resulting slopes, which are equal to

E_a/R (where $R = 8.314$ J/mol per K), as shown in Fig. 5b. For the 5% (w/v) β-glucan dispersions, the apparent activation energies varied between 11.3 and 33.7 kJ/mol, and in general there was a decrease in E_a values with increasing shear rate. Fig. 6 shows the flow index (n) and consistency index (K) as a function of temperature for samples differing in molecular size. As can be seen, the K-values decreased and the n values increased with increasing temperature, indicating that at higher temperatures, the flow characteristics pass from a pseudoplastic to a more Newtonian-like behaviour. In contrast, as β-glucan molecular size increased, there was an increase in K and a decrease in n, implying that the β-glucan dispersions become more pseudoplastic as M_w increases. These findings are in agreement with those of Autio et al. (1992) who reported a wide range in values of n and K as a result of M_w variation.

Fig. 4b shows the application of the Cox–Merz rule for the barley β-glucan dispersions (7%, w/v) by a comparison of the $\eta^*(\omega)$ with $\eta(\gamma)$. According to the Cox–Merz rule, the shear rate dependence of viscosity (η) is closely superimposable upon the dependence of complex viscosity η^* over the entire frequency range (ω), i.e.

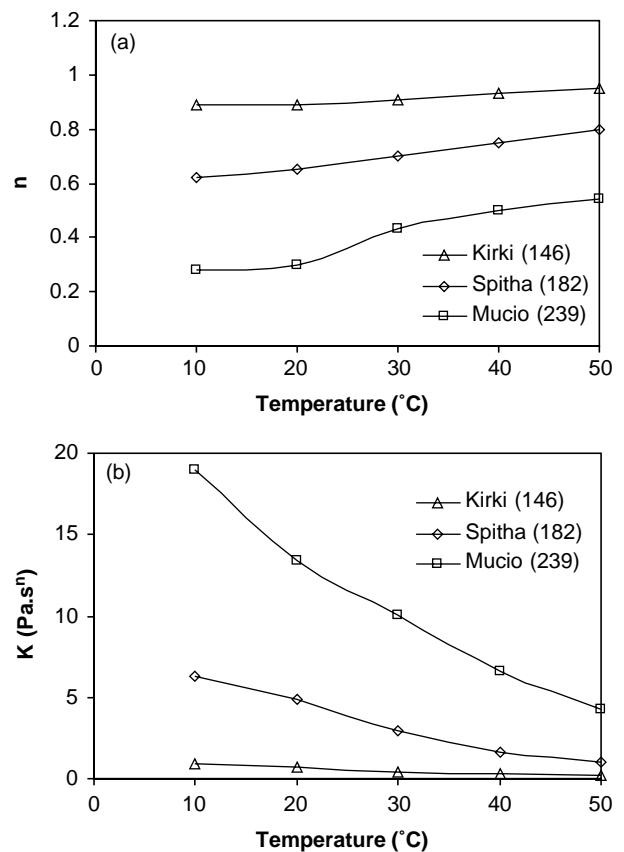$$\eta^*(\omega) = |\eta(\dot{\gamma})|_{\omega = \dot{\gamma}}$$

Fig. 6. Flow index, n, (a) and consistency index, K, (b) as a function of temperature for three β-glucan isolates of different M_w (numbers in parenthesis in kDa).

Most of the β-glucan dispersions studied followed this generality as shown in Fig. 4b for Kirki. However, Mucio, the variety with the highest M_w failed to obey the Cox–Merz rule, i.e. the shear viscosity (η) decreased more rapidly than the complex viscosity (η^*) in the non-Newtonian region, whereas in the low shear rate and frequency range, the dynamic and apparent viscosities were identical. The observations made for this variety may suggest the presence of associations and molecular clusters, which are more sensitive to shear forces than to small oscillatory movements.

3.6. Gelation of aqueous β-glucan dispersions

Fig. 7 shows the mechanical spectra of an 8% (w/v) aqueous β-glucan dispersion before and after storage, and the evolution of the viscoelastic parameters (G', G'', tan δ) on ageing at 25 °C. All the mechanical features indicate the existence a viscoelastic liquid state before gelation occurs, i.e. $G'' > G'$, high frequency dependence of both moduli G', G'' together with tan δ. The time dependence of storage and loss moduli (G',G'') for some representative β-glucan preparations revealed an induction period that increased with increasing M_w of the sample. Following this period, a remarkable increase in G' was recorded and the β-glucans began to adopt gel-like properties. At the end of storage, the materials behaved like typical gels, where G' was much greater than G'' and the two moduli became less dependent on the frequency (ω). The gelation time, at which $G' = G''$, was 11.3 and 45.7 h for Athinaida and Thermi, respectively (Table 3). On the other hand, the Mucio preparation with much higher M_w (2.39×10^5) did not show any tendency to gel even after 200 h storage (data not shown). These results suggest that the gelation time is inversely related to the M_w
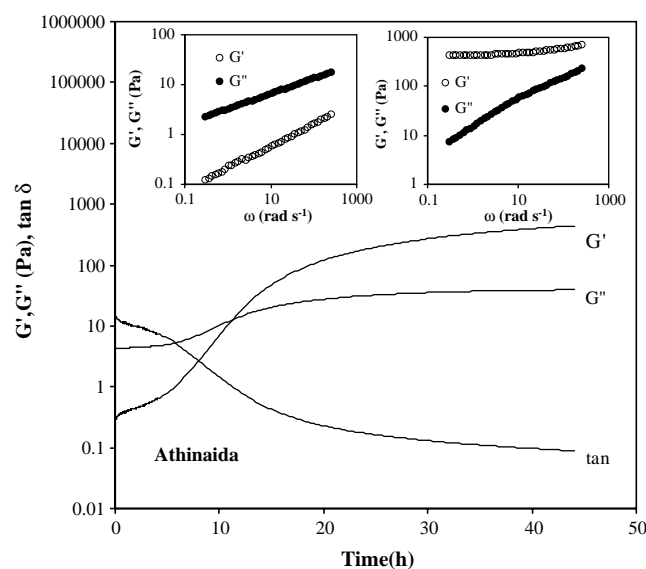

Fig. 7. Typical gelation kinetics of a barley β-glucan isolate (8% (w/v), frequency 1 Hz, strain 0.1%, 25 °C) and the mechanical spectra before and after storage for the specified period.

Table 3
Gelation kinetics (storage time 200 h) and melting temperature of three barley β-glucans preparations (8% (w/v), frequency 1 Hz, strain 0.1%, heating rate 3 °C/min)

Samples	I_E (h^{-1})	Gelation time (h)	G'_{max} (Pa)	Tan δ	Melting temp. (°C)
Athinaida	0.180	11.3	442	8.89×10^{-2}	69.3
Thermi	0.011	45.7	127	4.95×10^{-1}	67.7

of the polysaccharide and are in agreement with previous reports (Böhm and Kulicke, 1999; Lazaridou et al., 2003; Vaikousi et al., 2004). This effect can be attributed to the higher mobility shown by short chains; low M_w β-glucans diffuse more readily, and hence, have a greater possibility of forming junctions with neighboring chains (Doublier and Wood, 1995). Elasticity increment, I_E, a parameter introduced by Böhm and Kulicke (1999) to measure the gelation rate, indicates the maximum number of decades G' increases per unit time, i.e.

$$I_E = (\text{dlog } G'/\text{d}t)_{max}$$

Table 3 shows I_E-values for two barley β-glucan preparations (8%, w/v) differing in molecular size. The elasticity increments were 0.180 and 0.011 h^{-1} for Athinaida (apparent $M_w = 1.26 \times 10^5$) and Thermi (apparent $M_w = 1.50 \times 10^5$), respectively. Böhm and Kulicke (1999) claimed that for polymers with relatively small M_w, a higher mobility of chains is expected, allowing the three-dimensional gel network to form more rapidly. They also suggested that the aggregation of chain segments composed of consecutive trisaccharide units constitute ordered junctions in the β-glucan network. Table 3 also shows the melting temperatures (the point at which $G' = G''$) of the two β-glucan preparations upon heating at a constant rate (3 °C/min). The thermal profiles of both samples demonstrated a one-step drop in the storage modulus (Fig. 8). High melting temperatures reflect a better organization of the ordered domains in the network structure (Wood et al., 1994). The melting temperature was slightly lower for Athinaida, the preparation with the lower molecular weight. This observation might originate from minor structural differences between these two barley β-glucan isolates. Fine structure analysis showed that Athinaida had more trimers than tetramers (DP3/DP4 = 3.02) in comparison with Thermi (DP3/DP4 = 2.86). According to Cui and Wood (2000), the melting temperature of β-glucan gels follows the order: lichenin, barley and oat, which coincides with the order of their tri- to tetra-saccharide ratios.

The relationship between apparent M_w and the rheological properties of barley β-glucans was also explored by testing their ability to form gels from 10% (w/v) aqueous dispersions. Gelation of most β-glucan samples started after 3 days, depending on their M_w. All preparations were stored for 1 week at 25 °C before dynamic measurements were performed on the gels over an angular frequency range from
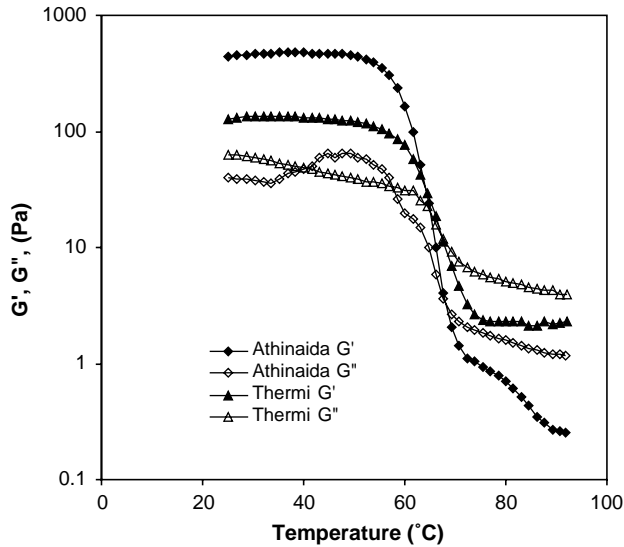
Fig. 8. Melting profiles of gels (8%, w/v) for two different barley β-glucan preparations following gelation at 25 °C. Heating rate 3 °C/min, frequency 1 Hz, strain 0.1%.

0.1 to 100 Hz and a strain equal to 0.1%. The mechanical spectra (10%, w/v concentrations) were all typical of elastic gels where $G' > G''$ and both moduli were frequency independent. The G' values (at 1 Hz) were found to decrease linearly with increasing molecular size of the β-glucan preparation (Fig. 9, $r^2 = 0.88$, $p < 0.01$). This may suggest that annealing processes and structural rearrangements in the gel network are more prevalent in samples with shorter chain lengths. For long chains, relatively few cross-links are required before diffusion and aggregation become significantly retarded, thereby slowing subsequent
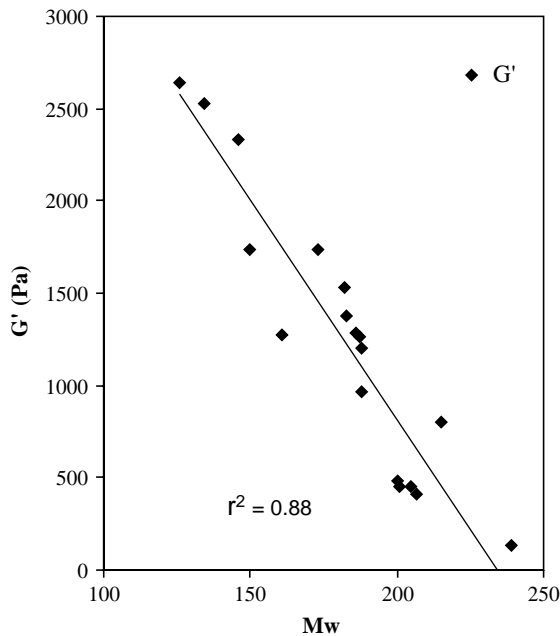

Fig. 9. The G' dependence (at 1 Hz, after 7 days of storage at 25 °C) on apparent M_w (kDa) for 18 barley β-glucan gel preparations (10%, w/v).

cross-linking and modulus increases. The scatter of data in Fig. 9 clearly indicates that, in addition to M_w, other factors may contribute to the mechanical properties of these hydrogels. The β-glucans exhibited minor differences in the molar ratios of tri- to tetramer, which are reported to affect their gelation properties (Böhm and Kulicke, 1999). Moreover, all β-glucan isolates examined in the present study contained an appreciable amount of contaminating protein which may influence the gelation process and physical characteristics of the resultant network structure.

4. Conclusions

In this study significant variations were shown in the β-glucan content of flours from several oat and barley cultivars as well as in the structural and physical characteristics among the respective β-glucan isolates. Differences in flour slurry viscosities were also observed among the samples. The effect of endogenous β-glucanase activity on slurry viscosity was confirmed by heat treatment of the flour in ethanol (80% (v/v), 90 °C) prior to viscosity measurement. Nevertheless, large variations existed between flour slurry viscosity and total β-glucan content of whole barley flours, implying the influence of other factors (solubility of β-glucans, M_w of the polysaccharide, etc). The apparent M_w of β-glucan preparations from the barley cultivars was between 1.26×10^5 and 2.39×10^5, and greatly influenced the rheology of the β-glucan solutions. The fine structure of all β-glucan isolates, analysed by $(1 \rightarrow 3,1 \rightarrow 4)$-β-D-glucan hydrolase digestion and HPAEC analysis, gave weight proportions of the DP3–DP14 oligomers comparable to those reported by other authors. The sum of tri- and tetra-saccharides reached between 91.1 and 92.1% of the total oligomers in the barley hydrolysates. The molar ratio of tri- to tetra-saccharides varied between 2.73–3.05 for barley and 2.16–2.42 for oat preparations. When aqueous β-glucan dispersions were subjected to steady shear measurements, between 0.1 and 1200 s^{-1}, a Newtonian plateau viscosity region was found at low shear rates and shear thinning behaviour in the high shear rate zone. Also, the apparent viscosity decreased with increasing temperature (10–50 °C). When the viscoelastic character of fresh β-glucan aqueous dispersions was examined at 8% (w/v); G' was below G'' at low frequencies, whereas at high frequencies the G' approached G'' for all β-glucan dispersions. The gelation kinetics of three barley β-glucan preparations (8% w/w, 25 °C) differing in M_w showed a large variation in the rate and time of gelation; the gelation rate increased and the gelation time decreased with decreasing molecular size of the polysaccharide. Aqueous dispersions of all barley β-glucan isolates at 10% (w/v) formed gels after 7 days storage at 25 °C. There was a strong negative relationship between G' and apparent M_w of the polysaccharide.

Acknowledgements

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