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Structure and rheological properties of water soluble β-glucans from oat cultivars of *Avena sativa* and *Avena bysantina*

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Abstract

Oat β-glucans were extracted (water at 47 °C) from milled seeds of two Greek cultivars (*A. sativa cv.* Pallini and *A. bysantina cv.* Kassandra) and partially purified by pH adjustment of the β-glucan solutions to 4.5. Chemical analysis of the extracted gums revealed that they were composed mainly of β-glucans (>85% d.b.) together with some contaminating proteins (<9% d.b.). The fine structure of the β-glucan preparations was assessed by ^{13}C-NMR spectroscopy and high-performance anion-exchange chromatography of the cellulosic oligomers released by the action of lichenase. The tri- and tetra-saccharides accounted for 90.9–92.3% of the total oligomers analyzed and the calculated molar ratios of trimers/tetramers varied between 1.99–2.11. Molecular size characterization was carried out with high performance size exclusion chromatography combined with a multi-angle laser light scattering and a refractive index detector; for samples with weight average molecular weight (M_w) ranging between 0.27 and 0.78×10^6, the values of limiting viscosity ($[\eta]$), critical concentration (c^{**}) and coil overlap parameter ($c^{**}[\eta]$) were within 4.9–6.4 dl/g, 1.2–2.0 g/dl and 7.8–10.1, respectively. The shear thinning behavior was dependent on the molecular weight and concentration of the β-glucan preparations. All β-glucan samples were able to form gels, as revealed by dynamic rheometry; the low molecular weight samples exhibited shorter gelation times and higher gelation rates ($I_E = [\mathrm{dlog}\, G'/\mathrm{d}t]_{max}$) than their high molecular weight counterparts. The gelation rate increased with increasing concentration and gel curing temperatures reaching a maximum at 32 °C; for higher temperatures the I_E values decreased. For small molecular size β-glucans, a biphasic melting behavior was observed for gels at curing temperatures of 2–32 °C, whereas at higher temperatures melting of the gel network occurred as one-step process. Differential scanning calorimetry showed that gels cured at 24 °C exhibit a broad melting transition; Tm ~ 63 °C and ΔH ~ 5 mJ/mg. The mechanical properties of casted (dispersions) films from two β-glucan preparations, differing in molecular weight, with or without sorbitol, were examined by tensile measurements. The large deformation mechanical tests showed decreases in tensile (Young's) modulus (E) and strength (σ_{max}), and an increase in percentage elongation with increasing water content and/or addition of sorbitol. The relationships between tensile parameters (E and σ_{max}) and water content showed an increase in stiffness of the films from 2–7% moisture, and a strong softening effect at higher water contents.

Keywords: Oat (1 → 3)(1 → 4)-β-D-glucan; Molecular weight; Structure; Lichenase; Coil overlap parameter; Flow behavior; Gelation; Melting; Tensile strength; Edible films; Plasticizer

1. Introduction

Cereal (1 → 3)(1 → 4)-β-D-glucans (β-glucans) occur in the sub-aleurone and endosperm cell walls of the grains. The content of β-glucan in barley, oats, rye and wheat is generally in the range of 3–11%, 3–7%, 1–2%, and <1%, respectively. In allowing a health claim for an association between consumption of oatmeal, rolled oats and oat bran, and reduced risk of coronary heart disease, the Food and Drug Administration (FDA) in USA has accepted that oat β-glucan is a functional, bio-active ingredient (Cui and Wood, 2000). In clinical studies, β-glucans were shown to reduce serum cholesterol levels and attenuate postprandial blood glucose and insulin responses in a viscosity related fashion (Klopfenstein, 1988; Newman et al., 1989; Wood, 1991; Kahlon et al., 1993; Braaten et al., 1994; Wood et al.,

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1994b; Kalra and Jood, 2000). Increased gut viscosity by oat β-glucan may either impede the uptake of dietary cholesterol or inhibit bile salt reabsorption (Shinnick and Marlet, 1993). The potential application of β-glucans as food hydrocolloids has also been proposed based on their rheological characteristics. Thus, β-glucans, because of the high viscosity of their solutions, could be used as thickening agents in sauces, salad dressings, or in ice cream formulations (Wood, 1986). In addition to solution viscosity enhancement, β-glucans from oat (Cui and Wood, 2000; Doublier and Wood, 1995), barley (Cui and Wood, 2000; Gómez et al., 1997; Morgan and Ofman, 1998; Böhm and Kulicke, 1999b), and wheat (Cui and Wood, 2000; Cui et al., 2000) were shown to gel under certain conditions. Oatrim, a product containing oat β-glucans and amylodextrins, as well as hydrolyzed oat flour have been proposed as fat-mimetics; in a gel-form substituting for shortening in oatmeal-raisin cookies. This product is being used experimentally in various reduced-fat and soluble fiber-enriched foods, such as meats, muffins, cakes, frozen desserts, salad dressings, sauces, gravies, soups, mayonnaise, margarine, breakfast cereals and candy products (Inglett, 1990; Inglett and Grisamore, 1991; Inglett and Warner, 1992).

β-Glucans are linear homopolysaccharides composed of D-glucopyranosyl residues (Glc*p*) linked via a mixture of β-(1 → 3) and β-(1 → 4) linkages. Numerous structural studies (Dais and Perlin, 1982; Woodward et al., 1983; Varum and Smidsrod, 1988; Wood et al., 1994c) have established that (1 → 3)(1 → 4)-β-D-glucans are unbranched, and that the (1 → 3) linkages occur singly, whereas most of the (1 → 4) linkages occur in groups of two or three. The resultant structure is a polysaccharide built mainly from β-(1 → 3)-linked cellotriosyl (58–72%) and cellotetraosyl (20–34%) units, but there is evidence for a minor amount of sequences with consecutive (1 → 4) linkages longer than the tetraose type and up to 14 glucosyl units (Cui, 2001). Markov chain analysis of the distribution of cellotriosyl and cellotetraosyl segments in water-soluble barley β-glucans favors a random arrangement of the major structural building blocks (Staudte et al., 1983). The apparent molecular weight of isolated mixed-linkage β-glucan fractions vary from 2.0×10^4–40.0×10^6 (Fincher and Stone, 1986). The apparent discrepancies in the M_w estimates of β-glucans from different sources may be attributed to variation in cell wall structure (thicker cell walls show greater resistance to extraction of high molecular weight polymers), different extraction and isolation methods (solvent and temperature affect the solubilization), aggregation phenomena (dependent on the structural features and solvent quality) and depolymerization events (endogenous or microbial β-glucanases from contaminating microorganisms) taking place during the extraction step.(Izydorczyk and Biliaderis, 2000) The fine structure, molecular size and molecular weight distribution of these cell wall polysaccharides are important determinants of their physical properties and functionality, including their physiological responses when they are considered as ingredients in cereal-based foods and other formulated products. It is well known that the source (cereal species, cultivar), processing treatments (milling, temperature-pH-shear effects, etc.), and interactions with other constituents (polymers or small molecular weight solutes) in the primary source or in a composite food matrix are likely to influence the concentration, structural features, and dispersibility-solubility of β-glucans and thereby modulate their physiological action in the gastro-intestinal tract.

Cereal β-D-glucans can form gels under certain conditions. The gelling ability and insolubility of freeze-dried cereal β-D-glucans follow the order of wheat > barley > oat; this trend seems to correspond with the ratio of cellotriosyl to cellotetraosyl units in the cereal β-D-glucan (~4, 3 and 2 for wheat, barley and oat β-D-glucans, respectively). In addition to the structural features, molecular weight also plays an important role in the polysaccharide conformation (in aqueous systems) and hence, in the gelation potential of cereal β-D-glucans (Cui and Wood, 2000; Böhm and Kulicke, 1999b).

Over the last decade there has been a rapidly growing interest in the development and use of biobased packaging materials. The utility of edible films and coatings lies in their capacity to act as an adjunct for improving food quality, extending shelf life, and possibly improving economic efficient of packaging materials (Kester and Fennema, 1986). Moreover, edible films and biodegradable polymer films offer alternative packaging options with no contribution to the environmental pollution (Krochta and De Mulder-Johnston, 1997). The obvious appealing characteristics of edible films include the renewable nature of their ingredients, the film's ability to function as carriers of food additives (e.g. antioxidants, flavors, antimicrobial agents), and the potential use of such films in the interior of heterogeneous food systems as selective barrier to the transport of vapors, gases, and solutes (Cherian et al., 1995; Diab et al., 2001). Polysaccharides (cellulose derivatives, pectin, starch, alginates, chitosan, carrageenans, pullulan etc.) are well known for having good film—forming properties. Coatings made with these biopolymers or their blends are generally considered as effective gas barriers. In contrast, minimal moisture barrier properties can be expected, because of the hydrophilic nature of these materials (Kester and Fennema, 1986). The addition of plasticising agents (e.g. polyols) to edible films (added at concentrations ranging from 10 to 60 g/100 g dry matter depending upon polymer rigidity) is often required to overcome film brittleness caused by extensive intermolecular forces; the plasticizer must be compatible with the polymer matrix. Plasticizers reduce these forces and increase the mobility of polymer chains, thereby improving flexibility and extensibility of the film. This avoids chipping or cracking of the film during subsequent handling and storage.

The main objective of this study was to examine the molecular and structural features of several β-glucan

preparations from two Greek oat cultivars belonging to two different oat species in conjunction with their rheological properties (viscosity, gelation characteristics). The potential of using β-glucans as a raw material to form edible films was also explored; in this respect, the mechanical properties of β-glucan films as a function of polymer molecular size, water content and the presence of sorbitol as a plasticizing co-solute were examined.

2. Materials and methods

2.1. Materials

Whole oat seeds from *Avena sativa* L., cultivar Pallini (P), and from *Avena bysantina*, cultivar Kassandra (two oat samples K-I, K-II obtained from crops of two consecutive years), were provided by the National Agricultural Research Foundation, Cereal Research Institute, Thessaloniki, Greece. Sorbitol and other chemicals of analytical grade were obtained either from Sigma-ALDRICH Co. (Gillingham-Dorset) or from Merck (Darmstadt, Germany).

2.2. Extraction and purification of β-glucan

Oat seeds were milled in a Camas mill to pass 0.8 mm screen. A schematic outline of the extraction and purification procedures employed for isolation/purification of β-glucans is presented in Fig. 1. The reflux of oat flour with alcohol at 85 °C removed most of the lipids and aimed at the inactivation of endogenous β-glucanases. The aqueous extraction of β-glucans at a temperature (47 °C) below the gelatinization temperature of starch resulted in very little starch solubilization (samples P_1, K-I_1, K-II_1). A step for further purification was used to remove some proteins by adjusting the pH of the β-glucan solutions to 4.5 (samples P_2, K-I_2, K-II_2).

The protein content of the β-glucan preparations was determined by the method of Lowry et al. (1951). The β-glucan content was determined by the method of McCleary and Glennie-Holmes (1985) using the Megazyme® mixed linkage β-glucan assay kit.

2.3. Molecular and structural characterization of β-glucan

Size exclusion chromatography for molecular characterization of the isolated/purified β-glucans was carried out with a high performance size exclusion chromatography (HPSEC) system which consisted of a pump (Waters 510), an injection valve (Model 7010, Rheodyne) with a 200 μL sample loop, a guard column (TSK PWH, TosoHaas GmbH, Stuttgart, Germany), a SEC column (TSK G5000 PW column, 7.8 × 600 mm, TSK PW, TosoHaas GmbH), a multiangle laser light scattering detector (Dawn DSP, Wyatt Technology Inc., Santa Barbara, CA) and a RI detector (Waters 410). The flow rate of the mobile phase (0.15 M $NaNO_3$ containing 0.02% NaN_3), which was filtered through 0.2 μm and then 0.1 μm of cellulose acetate membranes, was 0.4 ml min^{-1}; chromatography was performed at 25 °C. Calculations of weight-average molecular weight (M_w) and *z*-average root-mean-square (RMS) radii of gyration (R_g) were performed by the Astra 4.72 software (Wyatt Technology). Calculations were carried out with a $(dn/dc) = 0.147$ ml g^{-1}. Pullulan standards with known M_w values (P-50, M_w 47,300; P-400, M_w 404,000; P-800, M_w 788,000) were used to determine the proper experimental setup and calculations. Estimates of the polydispersity index (M_w/M_n) were also obtained.

The distribution of cellulosic oligomers in the chain of β-glucans was determined by lichenase treatment and chromatography. β-Glucan samples were dissolved in phosphate buffer (2 mg/mL, 0.01 M, pH 6.5) and incubated with lichenase [(1−3),(1−4)-β-glucan-4-glucanohydrolase, EC 3.2.1.73, 4 U/mL] from Megazyme International Ltd. (Bray, Ireland) for 20 h at 40 °C. The digests were heated to 95 °C (15 min) to inactivate the enzyme. High-performance anion-exchange chromatography (HPAEC) combined with pulsed amperometric detection was utilized for analysis of oligosaccharides released from β-glucan by lichenase degradation. A Dionex system equipped with a CarboPac PA1 column (4 × 250 mm) and a PA1 guard column (4 × 50 mm) was used and the running conditions of Wood et al. (1994a) were adopted. The purity and some structural features of the β-glucan samples were also assessed with ^{13}C-NMR spectroscopy (Bruker AM 300 FT spectrometer, 300 Hz). The proton-decoupled ^{13}C-NMR spectra were recorded at 70 °C, polysaccharide concentration 2% (w/v) in 50% (v/v) d6-DMSO/H_2O; 30,000 pulses with a pulse repetition time of 1.245 s, and an r.f. pulse angle 80.0° were employed. Chemical shifts are expressed in parts per million downfield from external Me_4Si but were actually measured by reference to internal 1,4-dioxane ($\delta = 67.4$ ppm).

2.4. Rheological measurements

Fresh solutions for all rheological measurements were made each time in hermetically sealed glass vials by gentle stirring of the β-glucan samples in double distilled water at 85 °C until complete solubilization of the material. The intrinsic or limiting viscosities $[\eta]$ of aqueous solutions of β-glucans were measured with Ubbelodhe No. 1 capillary viscometers at 20 ± 0.1 °C; calculations were according to the Huggins equation. The flow and viscoelastic behavior of the fresh solutions as well as the gel curing—melting events for the β-glucan preparations were performed on a rotational Physica MCR 300 rheometer (Physica Messtechnic GmbH, Stuttgart, Germany) using a concentric cylinder (diameter of cup and bob, 28.92 and 26.66, respectively) and a double gap cylindrical geometry; temperature was regulated by a Paar Physica circulating bath and a controlled peltier system (TEZ 150P/MCR) with an accuracy of

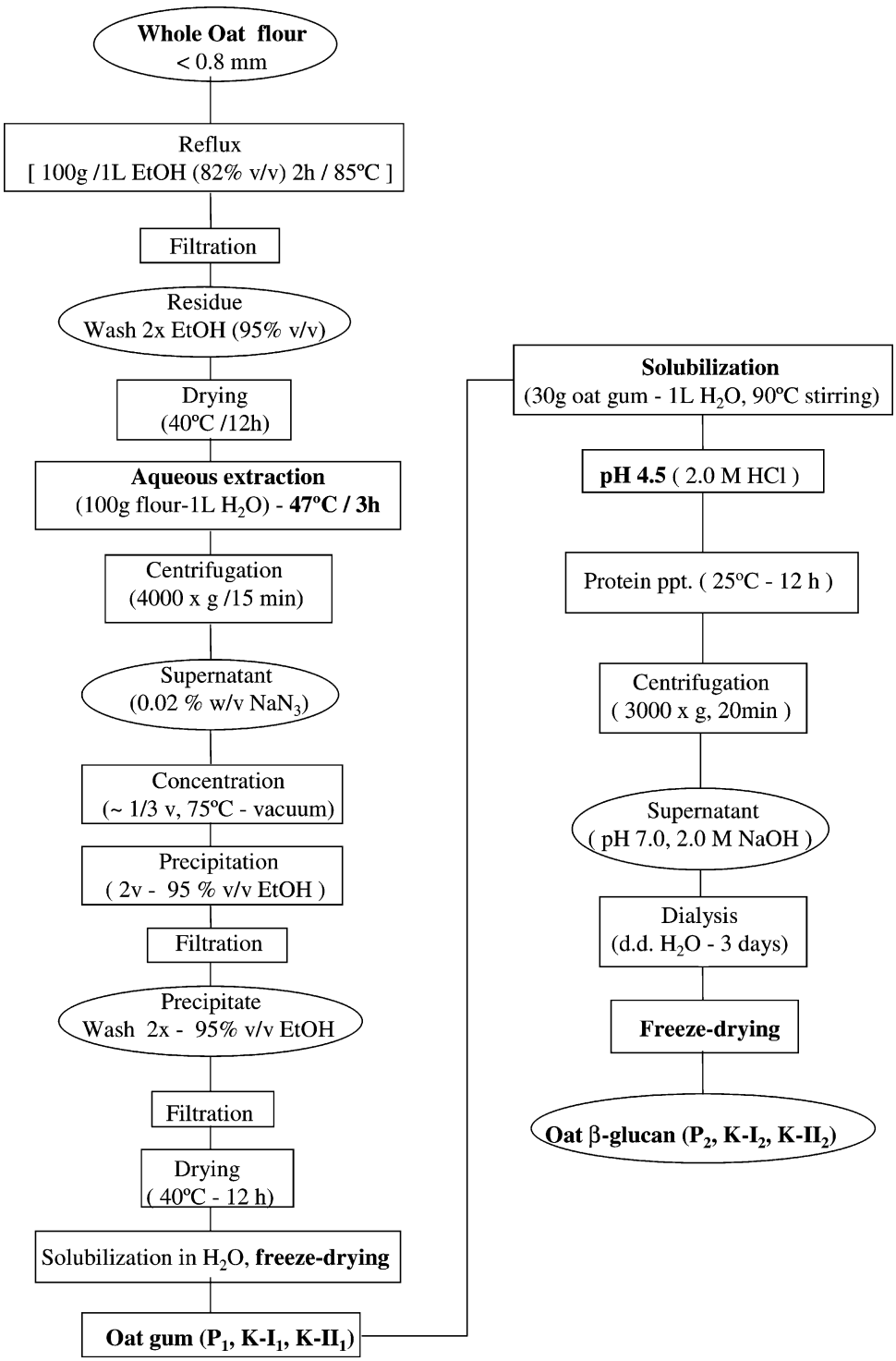

Fig. 1. Extraction and purification scheme of β-glucans from whole flours of two oat cultivars (*A. sativa cv.* Pallini, **P** and *A. bysantina cv.* Kassandra, **K**).

± 0.1 °C. Measurements were performed at different temperatures (5–45 °C). Three types of rheological measurements were performed and the data were analyzed with the supporting rheometer software US200 V2.21: (a) flow behavior by measuring steady shear viscosity (η) over a range of shear rates ($\dot{\gamma}$) of 0.05–1200 (s^{-1}); (b) oscillatory measurements of G' (storage modulus), G'' (loss modulus), η' (dynamic viscosity), η^* (complex viscosity) and tanδ (G''/G') were performed with a strain 0.1% and a range of angular frequencies (0.5–100 rad/s); (c) isothermal gel curing events and the melting behavior (heating rate at 3 °C/min) of the gels were probed at a strain level of 0.1%

and a frequency of 1 Hz. A thin layer of paraffin oil was added to cover the samples in order to prevent evaporation during measurements.

2.5. Calorimetry

Differential scanning calorimetry (DSC) measurements were carried out with a PL DSC—Gold calorimeter (Polymer Labs. Ltd, Epsom, UK). Samples of about 4 mg dry matter of the β-glucan dispersions (10% w/v) were sealed hermetically into DSC pans and stored at 24 °C for 92 h. The samples were then heated at a heating rate of 5 °C/min.

2.6. Tensile tests

For large deformation mechanical tests (tensile mode), β-glucan films (thickness 0.1 ± 0.02 mm) with or without added sorbitol (S) were prepared by casting aqueous solutions (3% w/v). Four different formulations, [K-I_2 (100), K-I_2-S (85:15 w/w), P_2 (100) and P_2-S (85:15 w/w)] were obtained by pouring slowly the β-glucan powder under continuous magnetic stirring in distilled water at 60 °C and adding sorbitol after complete dispersion of the β-glucan. The solutions were kept for about half an hour in a water bath before they were cast over plastic frames (10 × 10 × 1.5 cm). The K-I_2, and K-I_2-S solutions were allowed to dry at 37–42 °C for 2 days and after that at 30 °C until complete drying. The P_2, and P_2-S solutions were allowed to dry at 45–50 °C for 2 days and then at 30 °C until complete drying.

Before testing, rectangular film strips (5 × 1 cm) were cut and conditioned for 12 days at 25 °C at various moisture contents. Tensile testing was performed with a TA-XT2i instrument (Stable Micro systems, Godalming, Surrey, UK) according to the ASTM D828-88 (ASTM, 1989) method at 25 °C. The initial grip separation was set at 40 mm and the cross head speed at 0.5 mm/min. The thickness of each sample was measured at three different points with a micrometer and an average value was obtained. Calculations of tensile (Young's) modulus (E), tensile strength (σ_{max}) and percentage elongation were made as described elsewhere (Biliaderis et al., 1999). The Young's modulus (E) was calculated from the initial slope of the stress–strain curves, while σ_{max} and (%) elongation correspond to the tensile strength and percentage elongation at break, respectively. Each of the reported moduli and σ_{max} values represent an average of at least ten measurements of samples similarly conditioned to a certain moisture level in chambers of fixed relative humidity using saturated salt solutions. The moisture content of samples used for large deformation mechanical testing was determined by drying the samples at 105 °C to constant weight (Biliaderis et al., 1999).

3. Results and discussion

3.1. Purity and molecular characterization of β-glucans

The β-glucan and protein content of all samples are given in Table 1. The isolation/purification protocol adopted in the present study appears to provide β-glucan preparations with a low protein content (<9% d.b.) and a fairly high level of β-glucans (>85% d.b.). The data in Table 1 clearly show that the additional purification step by

Table 1
Compositional, molecular and structural features of β-glucan preparations isolated from whole oat flours of two Greek cultivars

Samples	K-I_1	K-I_2	K-II_1	K-II_2	P_1	P_2
β-glucans (% d.b.)	85.1	89.6	86.2	90.4	84.5	87.3
Protein (% d.b.)	7.2	3.8	6.6	3.1	9.0	6.5
M_w[a] × 10^{-6}	0.71[d] ± 0.04	0.30 ± 0.00	0.85 ± 0.03	0.78 ± 0.01	0.27 ± 0.00	0.18 ± 0.00
M_w/M_n	2.28	2.15	1.50	1.58	2.39	2.31
DP 3[b]	55.14 ± 1.20	55.27 ± 1.00	54.56 ± 1.00	54.66 ± 1.20	56.53 ± 1.00	56.79 ± 0.95
DP 4	36.28 ± 0.80	36.18 ± 0.70	36.34 ± 0.85	36.23 ± 0.70	35.28 ± 0.70	35.50 ± 0.80
DP 5	3.88 ± 0.01	3.78 ± 0.02	3.85 ± 0.03	3.67 ± 0.02	3.97 ± 0.04	4.05 ± 0.04
DP 6	2.43 ± 0.02	2.35 ± 0.02	2.50 ± 0.02	2.38 ± 0.03	2.33 ± 0.02	2.26 ± 0.01
DP 7	0.51 ± 0.01	0.48 ± 0.01	0.54 ± 0.01	0.53 ± 0.02	0.47 ± 0.01	0.41 ± 0.01
DP 8	0.68 ± 0.03	0.73 ± 0.02	0.79 ± 0.02	0.83 ± 0.03	0.58 ± 0.01	0.46 ± 0.01
DP 9	0.92 ± 0.02	0.97 ± 0.02	1.21 ± 0.01	1.27 ± 0.02	0.68 ± 0.01	0.47 ± 0.01
DP 10	0.12 ± 0.02	0.16 ± 0.01	0.20 ± 0.01	0.19 ± 0.01	0.08 ± 0.00	0.06 ± 0.00
DP 11	0.05 ± 0.01	0.07 ± 0.01	0.10 ± 0.00	0.21 ± 0.00	0.07 ± 0.00	0.01 ± 0.00
DP 12	tr.	tr.	tr.	tr.	tr.	tr.
MolarDP 3/DP 4	2.01	2.02	1.99	2.00	2.11	2.11
DP 3 + DP 4 (%)	91.42	91.45	90.90	90.89	91.80	92.29
(1 → 4)/(1 → 3)[c]	2.37	2.43	2.51	2.60	2.41	2.34

[a] From the 2nd peak (major peak) of the HPLC chromatograms.
[b] Weight percent from the chromatograms of the lichenase digests.
[c] From ^{13}c-NMR spectra (peak areas of C6 of (A + C + D)/B glucosyl-residues).
[d] Values are means (± S.D.) of triplicate measurements.

adjusting the pH of the β-glucan solutions to 4.5, reduced the protein content of the sample (compare preparations P_1, K-I_1, K-II_1 with their purified counterparts P_2, K-I_2, K-I_2).

Estimates of molecular characteristics (M_w, R_g, M_w/M_n) obtained from the HPSEC elution profiles (Fig. 2) of the β-glucan preparations are summarized in Table 1. The M_w values varied from 0.18 to 0.85×10^6 differing from those of $1-2 \times 10^6$ reported in the literature (Wood, 1991; Doublier and Wood, 1995; Johansson et al., 2000) for oat β-glucans. The large variation of the reported molecular weights of cereal β-glucan reflects the diversity of origin and/or the methodology used for the determination of the values. Also, the extraction protocol (solvents, conditions and sample history) has an impact on the M_w values (Cui and Wood, 2000). Zhang et al. (1999) have found that the molecular weight of β-glucan is increased with increasing extraction temperature. The low M_w values of β-glucan preparations in the present study can be explained by the relatively mild extraction conditions used (water extraction at 47 °C). Moreover, the type of cultivar seems to affect the molecular weight of β-glucans; β-glucans isolated from Kassandra exhibited higher molecular weights than those from Pallini. The data of Table 1 also suggest that any step taken to further purify the β-glucans result in a significant reduction of the molecular weight; comparing the preparations P_1, K-I_1, K-II_1 with their purified counterparts P_2, K-I_2, K-II_2, the most pronounced change was for the K-I. The polydispersity values, M_w/M_n, varied between 1.50–2.39.

Typical chromatograms of two oat β-glucans preparations before and after purification obtained from the HPSEC system are shown in Fig. 2. A relatively broad molar weight distribution was observed for all samples with a major peak. The purified samples gave a sharper peak, shifted towards lower hydrodynamic volumes. Interestingly, the exponent α of the R_g vs M_w relationship ($R_g \sim M_w^{\alpha}$) indicated different conformation for the high and low molecular weight populations within the same elution peak.

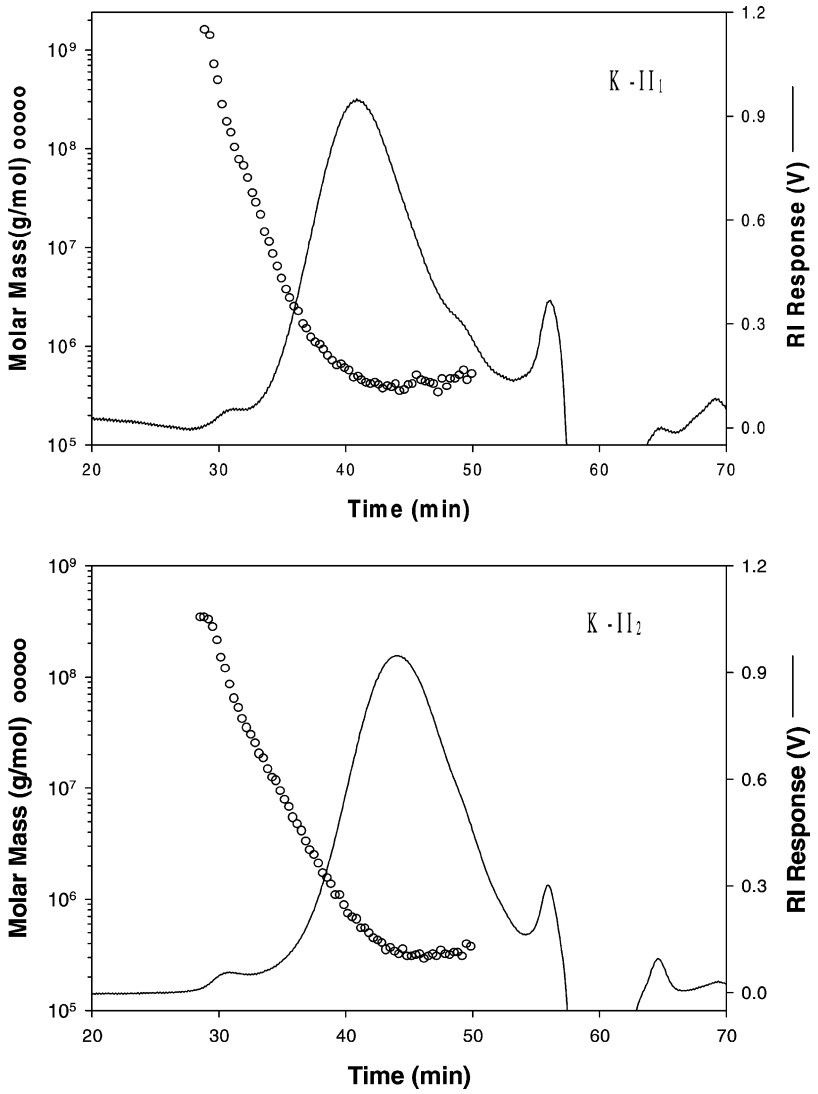

Fig. 2. Size exclusion chromatograms of two oat β-glucan preparations before (K-II_1) and after partial purification (K-II_2) of the sample.

For the species eluted between the onset of the peak and the main peak volume, the values of α ranged from 0.13 to 0.42, whereas for the lower M_w species (species eluted after the peak volume) ranged from 0.66 to 0.91 (results not shown). The latter values suggest a more extended and a stiffer conformation for the low M_w β-glucans. This relation between the molecular weight and conformation of β-glucan chains should be explored further. In all chromatograms, the peak in the very low molecular weight range represents the contaminating protein fraction, as verified by a UV detector. A small peak was also evident in the high molecular weight region of the chromatogram of each sample and it may represent high molecular weight species, although the possibility of having aggregates of β-glucan under the HPSEC running conditions (0.15 M $NaNO_3$, 25 °C) cannot be excluded. In fact, aggregation phenomena in β-glucan solutions have been reported in some recent studies employing HPSEC systems (Zhang et al., 1999; Wang et al., 2002). According to Wang et al. (2002) a complete dispersion of the β-glucan solutions and disruption of their aggregates without polymer degradation can be achieved by microwave heating in a high-pressure vessel for 4–10 min at 100–121 °C and not by heating and stirring at 80 °C.

The molecular and structural features of β-D-glucans play an important role in the solubility and conformation, and hence in their rheological properties (Cui and Wood, 2000). β-Glucans containing blocks of adjacent β-(1 → 4) linkages may exhibit a tendency for interchain aggregation (and hence lower solubility) through strong hydrogen bonds along the cellulose-like regions; the β-(1 → 3) linkages break up the regularity of the β-(1 → 4) linkage sequence, making it more soluble and flexible. According to a popular model, a plausible cause for aggregation of (1 → 3)(1 → 4)-β-D-glucans would be the cellulose-like sequences of more than three contiguous β-(1 → 4)-linked glucosyl units which stick together leading to gels (Fincher and Stone, 1986). An alternative model for gelation has been proposed lately, according to which association of consecutive cellotriose units (linked via β-(1 → 3) bonds) may form extended junction zones and lead to the development of a gel network structure (Böhm and Kulicke, 1999b).

The enzyme lichenase, a (1 → 3)(1 → 4)-β-D-glucan-4-glucanohydrolase, specifically cleaves the (1 → 4)-glycosidic bond of the 3-substituted glucose residues in β-glucans yielding oligomers with different degree of polymerization (DP). The major products are 3-*O*-β-cellobiosyl-D-glucose (DP 3) and 3-*O*-β-cellotriosyl-D-glucose (DP 4), but cellodextrin-like oligosaccharides (DP ≥ 5) are also produced from the polymer regions containing more than three consecutive 4-linked glucose residues. The relative amounts of oligosaccharides released by lichenase constitute a fingerprint of the structure of β-glucans. The HPAEC chromatograms of the different β-glucan samples were similar and showed only minor differences in the contents of oligomers with a DP 3–11. The calculated weight percent of the oligosaccharides for all lichenase digests are summarized in Table 1. As expected, the major products were trimers and tetramers; the tri- and tetra-saccharides from all samples accounted for 90.9–92.3% of the total oligomers analyzed. These values do not differ from those of Doublier and Wood (1995), Cui et al. (2000) and Wood et al. (1991, 1994c) and who reported 91.9–92.6, 91.9, 91.0, and 89.9% and respectively, for the contents of DP3 and DP4 of lichenase hydrolyzates from different oat β-glucan preparations. The calculated molar ratios of trimers/tetramers for oat β-glucan preparations varied within the narrow range of 1.99–2.11. These findings are very close to those of 2.2, 2.1–2.2, and 1.8–2.3 that have been reported by Cui et al. (2000), Wood et al. (1991) and Miller et al. (1993), respectively. Significant structural differences in cereal β-glucans, as indicated by the trisaccharide-to-tetrasaccharide ratios, have been reported between different genera of the cereals (rye 2.7, barley 2.9–3.4, and wheat 3.0–4.5) and not within the same genera (Cui et al., 2000; Wood et al., 1991).

The purity of the isolated oat β-glucans was further confirmed by the ^{13}C-NMR spectra of the samples as showed in Fig. 3. The spectral features were all typical of a mixed linkage cereal β-glucan as assigned by Cui and Wood (2000), Cui et al. (2000), Dais and Perlin (1982) and Wood et al. (1994c). Considering the generalized β-glucan structure:

$$\underset{\rightarrow {}^4G_1}{\mathbf{A}}\ \underset{\rightarrow[\,{}^3G_1}{\mathbf{B}}\ \underset{\rightarrow {}^4G_1}{\mathbf{C}}\ \underset{\rightarrow {}^4G_1}{\mathbf{A}}\ \underset{\rightarrow]_m[\,{}^3G_1}{\mathbf{B}}\ \underset{\rightarrow {}^4G_1}{\mathbf{C}}\ \underset{\rightarrow {}^4G_1}{\mathbf{D}}$$

$$\underset{\rightarrow {}^4G_1 \rightarrow]_n}{\mathbf{A}}\ \underset{\rightarrow {}^3G_1}{\mathbf{B}}\ \underset{\rightarrow {}^4G_1 \rightarrow}{\mathbf{C}}$$

assignment of several resonances to carbons of individual β-glucose residues (**A**, **B**, **C**, **D**) is feasible based on literature data (Cui et al., 2000). All spectra showed single resonances at 86.3 ppm for each carbon of the O-3-linked glucose (residue **B**), indicating a single environment for this residue in the polymer. Similarly, there was a single resonance for the C-4 of O-3-linked glucose at 69.2 ppm (Varum and Smidsrod, 1988; Wood et al., 1994c). The expected three distinct resonances for the three different types of 4-O-substituted residues (**A**, **C**, and **D**) at about 79.3–79.9 ppm (Cui et al., 2000; Dais and Perlin, 1982) were not resolved, giving instead a single resonance. Among the other resolved resonances, one can distinguish the carbon anomeric region (C-1) at ~103 ppm; the resonance at 103.7 corresponds to C-1 of **A** residue and the 103.2 to C-1 of the **B**, **C**, and **D** residues. On the other hand, the doublet at ~61 ppm (C-6 region) corresponds to the C-6 of **B** residues (O-3-linked glucan) at 61.7 ppm and to the C-6 of **A**, **C**, and **D** residues at 61.1 ppm. The relative intensities of the latter two resonances can thus be used as an index of the ratio of (1 → 4)/(1 → 3) linkages on the β-glucan chain. The calculated ratios of the two types of linkages in the native

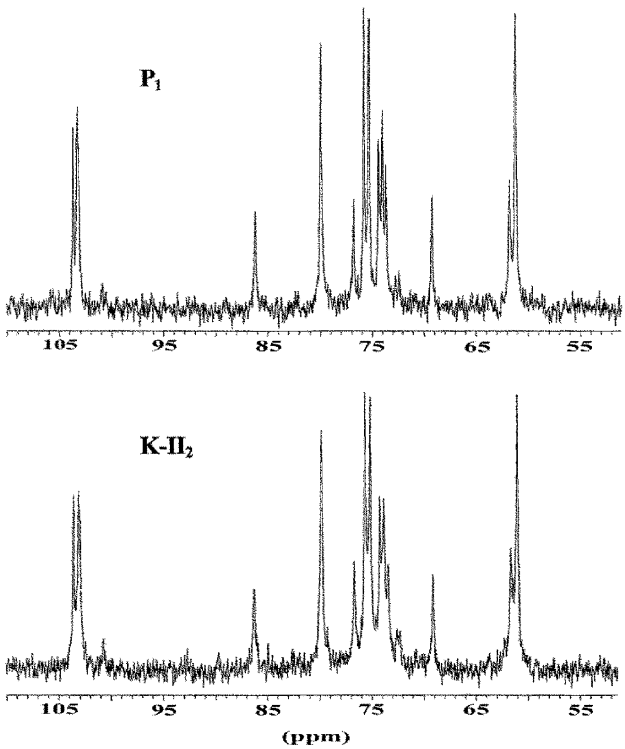

Fig. 3. ^{13}C NMR spectra of two representative samples of oat β-glucans.

β-glucan structures were within a range of 2.34–2.60 for all six samples analyzed (Table 1), which is in agreement with the values of 2.2–2.6 reported by Dais and Perlin (1982).

3.2. Solution rheology

The intrinsic viscosities, $[\eta]$, (Table 2) were obtained by extrapolation of viscometric data to zero concentration according to the Huggins equation:

$$\eta_{spec}/c = [\eta] + k_H[\eta]^2 c$$

where $\eta_{spec} = (\eta_{solution}/\eta_{solvent}) - 1$, and k_H is the Huggins constant.

As expected, the $[\eta]$ values of the samples increased with increasing M_w. The calculated values of 4.9–6.4 (dL/g) were in the same range of 2.58–9.63 (dL/g) and 2.0–7.4 (dL/g) found by others researchers for oat β-glucans preparations with M_w ranging between $100–1200 \times 10^3$ and M_n between $63–330 \times 10^3$, respectively (Doublier and Wood, 1995; Varum and Smidsrod, 1988).

Intrinsic viscosity provides a convenient measure of the hydrodynamic volume of individual polymer coils, and when multiplied by concentration gives an index of total degree of space-occupancy, the reduced concentration ($c[\eta]$). Double logarithmic plots of η_{sp} vs. $c[\eta]$ for most disordered polysaccharides superimpose closely, falling into distinct linear regions, regardless of the polymer primary structure and its molecular weight (Morris et al., 1981). As shown in Fig. 4, the results obtained for three oat β-glucan isolates comply reasonably well this generalization, showing three linear regions, with the slopes and intercepts listed in Table 2. The first transition (at concentration c^*) is attributed to initial contact between the individual coils when, in aggregate, their swept-out volume becomes equal to the total volume of the solution, and normally occurs when the degree of space-occupancy reaches $c[\eta] \sim 1$. The two regions of higher concentration-dependence correspond, respectively, to initial compression and subsequent interpenetration of the polymer coils in response to increasing space-occupancy, and the concentration at their point of intersection is denoted as c^{**}. The intermediate zone between the dilute ($c < c^*$) and concentrated ($c > c^{**}$) domains appears to be characteristic of polymers with rigid, rod-like conformation (Cuvelier and Launay, 1986). It seems that for rod polymers the transition between the dilute and concentrated regimes is smoother than for flexible polymers. The transition domain at intermediate concentrations has already been reported for some synthetic polymers as well as for xanthan, hydroxylmethylcellulose (Cuvelier and Launay, 1986), β-glucan (Doublier and Wood, 1995), wheat arabinoxylan (Izydorczyk and Biliaderis, 1992), okra and dika nut polysaccharides (Ndjouenkeu et al., 1996), and microbial levan (Kasapis et al., 1994). The observed rheological behavior of β-glucan is not surprising, since the chain of this polymer has been characterized as an expanded partially stiff worm-like cylinder (Gómez et al., 1997; Böhm and Kulicke, 1999a). The slope values of the double logarithmic plots of $(\eta_{sp})_0$ against $c[\eta]$ at the three regimes were 1.0 ($c < c^*$), 1.6 ($c^* < c < c^{**}$), and 3.8 ($c > c^{**}$). These values are in agreement with those reported by Doublier and Wood (1995) for other oat β-glucan preparations. Measured values of coil overlap parameter $c^{**}[\eta]$ varied widely (compared with the $c^*[\eta]$) between 7.8 and 10.1. The values for c^* and c^{**} (Table 2) also seem to increase with a decrease in molecular weight and intrinsic viscosity $[\eta]$ of the β-glucan

Table 2
Concentration-dependence of 'zero-shear' viscosity (slopes and critical concentration values) of three β-glucan isolates

Samples	Slope1	Slope2	Slope3	$c^*[\eta]$	$c^{**}[\eta]$	c^* (g/dL)	c^{**} (g/dL)	$[\eta]$ (dL/g)
K-II$_2$	1.00	1.51	3.79	1.22	7.75	0.192	1.219	6.4
K-I$_1$	0.95	1.67	3.73	1.25	9.90	0.224	1.770	5.6
P$_1$	0.87	1.48	3.69	1.11	10.05	0.225	2.038	4.9
All samples	0.97	1.57	3.76	1.20	9.10	-	-	-

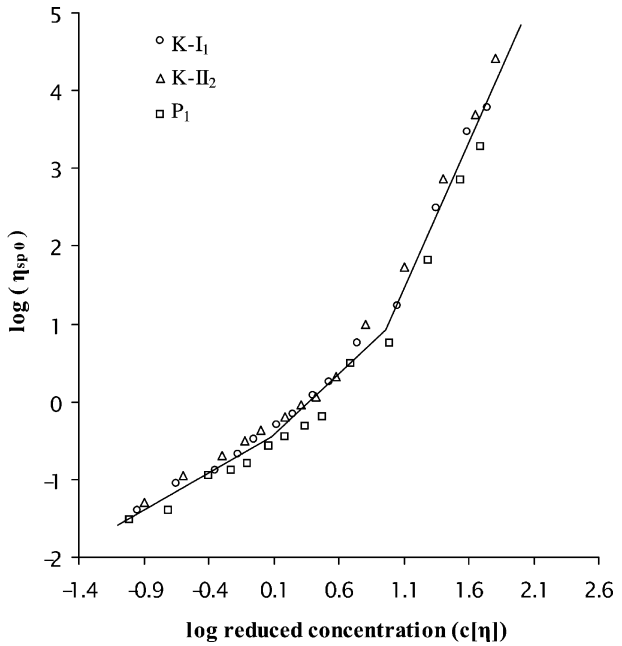

Fig. 4. Specific viscosity (η_{spo}) as a function of the reduced concentration ($c[\eta]$) for three oat β-glucan preparations.

samples, which means that coil overlap happens at lower concentrations in the case of high molecular weight samples. This explains why a slight variation in concentration or in molecular weight (i.e. $[\eta]$) can result in significant changes in zero-shear rate viscosity. Although one might argue that identification of three concentration regions in the double-logarithmic plots of η_{sp} vs. $c[\eta]$ and the resultant 'critical concentrations' is a bit arbitrary, the data plot in Fig. 4 do show the presence of an intermediate transition zone between the dilute and the concentrated regimes. In a recent report by Böhm and Kulicke (1999a), the authors did not attempt to identify discreet regions, giving a smooth curve to fit their data.

Rheologically, solutions of cereal β-D-glucans fall into the category of viscoelastic fluids behaving similarly to the well-characterized random coil type polysaccharides such as guar and locust bean gum (Cui and Wood, 2000; Doublier and Wood, 1995). Fig. 5 shows the dependence of apparent viscosity on shear rate for two β-glucan preparations, differing in molecular weight (K-II$_2$, P$_1$). For measurements taken at concentrations above c^{**} there was a reduction in viscosity (shear thinning) with increasing shear rate. This feature was shifted toward lower shear rates as the concentration and molecular weight of the samples increased. As expected, with increasing molecular weight, there was an increase in viscosity and the shear thinning properties of the oat β-glucan dispersions at equivalent polysaccharide concentrations. For concentrations below c^{**}, all samples exhibited a Newtonian-like behavior, whereas at concentrations above c^{**} there was a Newtonian-like region with constant viscosity (η_0, at low shear rates) which was followed by a power-law region at high

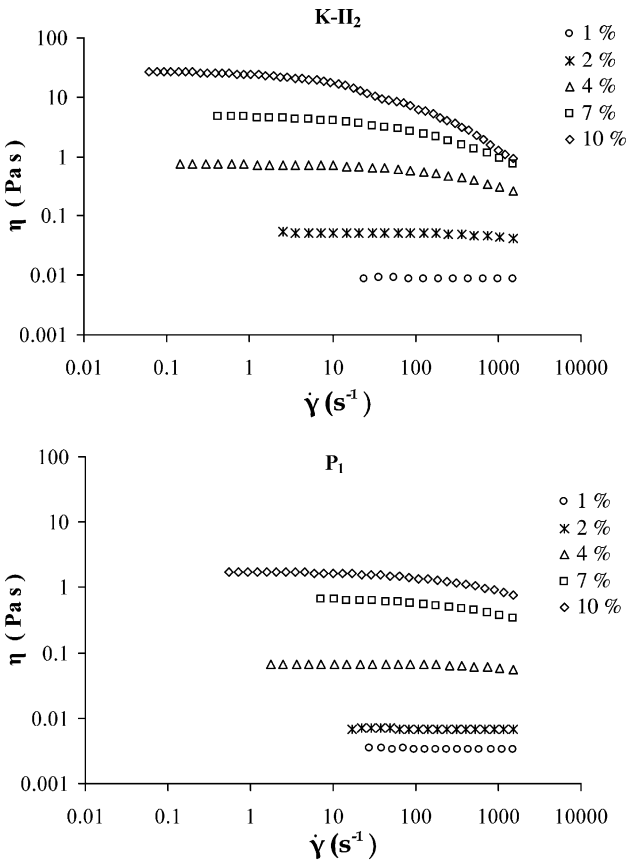

Fig. 5. Effect of polymer concentration (w/v) on viscosity for two oat β-glucan preparations at 20 °C.

shear rates (Fig. 5). The viscosity data of the β-glucan solutions at 10% (w/v) and 10 °C (K-II$_1$, K-II$_2$, K-I$_1$, P$_1$) followed a common curve of dimensionless shear rate (defined as $\dot{\gamma}/\dot{\gamma}_{0.6}$), against dimensionless viscosity (defined as η/η_0), where $\dot{\gamma}_{0.6}$ is the shear rate at which the magnitude of apparent viscosity equals $\eta_0/1.67$ (Fig. 6). Morris et al. (1981) presented similar plots of different gum solutions

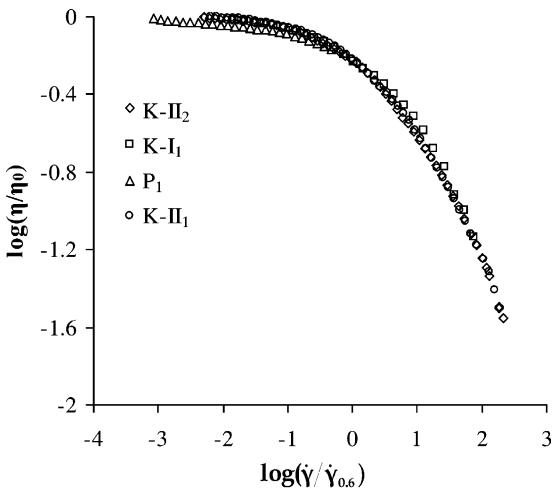

Fig. 6. Generalized shear-rate dependence of viscosity for four oat β-glucan preparations (10% w/v, 10 °C).

using a shear rate of $\dot{\gamma}_{0.1}$, i.e. corresponding to the change in apparent viscosity of $\eta_0/10$. In the plots of Fig. 6, the ratio of $\eta_0/1.67$ was used instead, since it was not feasible to obtain a 10-fold decrease in viscosity for all samples studied (within the accessible range of shear rates). The data for all four β-glucan samples seemed to follow a common master curve using this transformation of viscosity values vs. shear rate. A number of rheological models can be used to describe the shear rate dependence of apparent viscosity data. Morris (1984) suggested the model:

$$\eta = \eta_0/[1 + m(\dot{\gamma}/\dot{\gamma}_{0.6})^p]$$

The data from the generalized shear thinning profiles (Fig. 6) can be fitted well ($r^2 = 0.996$) within experimental error ($p < 0.05$) by this equation:

$$\eta = \eta_0/[1 + 0.714(\dot{\gamma}/\dot{\gamma}_{0.6})^{0.66}]$$

A further generality of behavior observed for disordered polymer chains interacting solely by physical entanglements is that the complex viscosity (η^*) from small-deformation oscillation measurements and the steady-shear viscosity (η) from rotational measurements superimpose closely at equivalent numerical values of frequency (ω, rad s^{-1}) and shear rate ($\dot{\gamma}$, s^{-1}). This direct relationship between rheological response to destructive and non-destructive deformation is known as the Cox-Merz rule (Cox and Merz, 1958). This rule has been confirmed experimentally for several synthetic polymers (Ferry, 1960), as well as for most random coil biopolymer solutions (Morris et al., 1981; Andrade et al., 1999):

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

In contrast to disordered biopolymers, solutions of polysaccharides with a rigid, ordered chain conformation showing a weak-gel behavior (e.g. xanthan) violate the Cox-Merz rule; i.e. dynamic viscosity (η^*) from small deformation measurements is substantially higher than steady shear viscosity (η) at equivalent rates of deformation, indicating a tenuous network which remains intact under low-amplitude oscillation but is disrupted by continuous shear (Lapasin and Pricl, 1999).

The application of the Cox-Merz rule for two different oat β-glucan solutions at 7 and 10% (w/v) at 20 °C is presented in Fig. 7. There were no large departures from the empirical Cox-Merz correlation for these samples, except in the case of 10% dispersion of K-II$_2$, which was declined from the rule at high shear rates. The latter may indicate the presence of high-intensity entanglements and/or intermolecular aggregation for this system at rest. Similarly, other researchers have found that solutions of β-glucans (Böhm and Kulicke, 1999a; Autio, 1988) and konjac flour (Jacon et al., 1993) follow the Cox-Merz rule at low polymer concentrations. However, at high polymer concentrations deviations were observed in that η^* was higher than η, especially at high values of $\omega/\dot{\gamma}$ (Fig. 7). This may suggest associations and molecular clusters due to chain aggregation which are sensitive to shear forces (Böhm and Kulicke, 1999a).

3.3. Gelation properties

The most rigorous method of directly measuring the gelation time (t_c) employs the application of a low-frequency, small amplitude oscillation strain to the system. The gelation capacity of aqueous β-glucan dispersions was monitored isothermally at different temperatures and polymer concentrations for the different β-glucan preparations; i.e. the time-dependent evolution of G', G'' and $\tan\delta$ was monitored periodically at 1 Hz frequency and strain level of 0.1%. The mechanical spectra (are not shown) of the freshly prepared β-glucan solutions were typical of a viscoelastic liquid ($G'' > G'$) where the moduli are highly dependent on frequency. However, after an induction period the β-glucan solutions began to adopt gel properties; the mechanical spectra became typical of elastic gel networks ($G' \gg G''$, the moduli being less dependent on frequency, and the dynamic viscosity, η', decreased sharply). Two representative kinetic profiles of the gelation process (gel curing at 24 °C) for β-glucan preparations (10% w/v

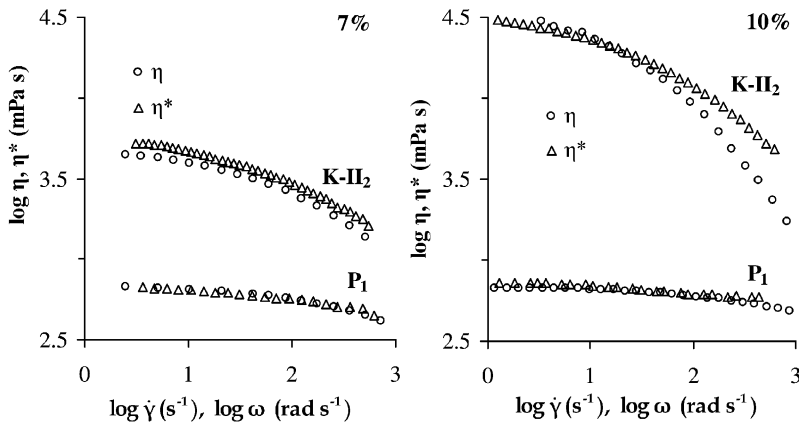

Fig. 7. Cox-Merz rule for two oat β-glucan preparations at 7 and 10% (w/v), 20 °C.

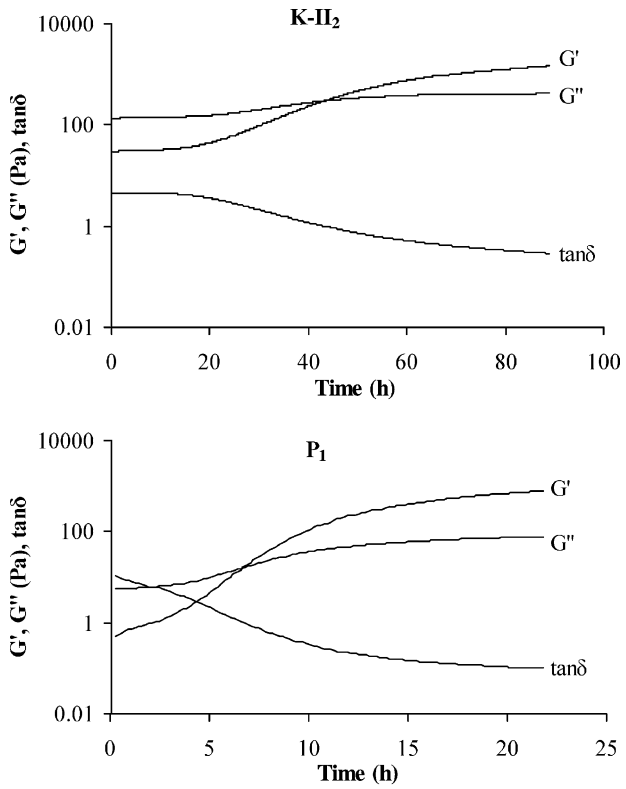

Fig. 8. Gelation kinetics for two oat β-glucan preparations at 10% (w/v) (frequency 1 Hz, strain 0.1%, 24 °C).

aqueous dispersions) differing in molecular weight are shown in Fig. 8. The induction period for gelation was longer for the sample with the higher molecular weight (K-II_2), compared to that with the lower M_w (P_1). After this induction period, there was a rapid rise in the G', whereas the G'' showed a much slower rate of increase. The increase in the rate of G' development was more pronounced for P_1 than the K-II_2. Böhm and Kulicke (1999b)) also found that the logarithm of G' as a function of time generally evolved as a sigmoidal curve for barley β-glucans, and it varied with the concentration and molecular weight of the polymer. The slope of log $G'(t)$ at the turning point (maximum slope) was chosen by these authors as a measure of the gelation rate; it was named as 'elasticity increment, I_E'', and calculated as $I_E = (\mathrm{dlog}\, G'/\mathrm{d}t)_{max}$. Its dimension is reciprocal time and indicates the number of decades G' increases at maximum per unit time. For the 10% (w/v), 24 °C systems, the I_E values were 0.328, 0.050 h^{-1} for P_1, and K-II_2, respectively. A high I_E value reflects rapid gelation. The gelation time, defined as the time where $G' = G''$ (i.e. $\tan\delta = 1$), seems to increase with an increase in the molecular weight of the sample; thus the gelation time for P_1 and K-II_2 were at 6.8 and 43.3 h, respectively (Fig. 8).

Doublier and Wood (1995) reported that partially hydrolyzed oat gums exhibited gel-like behavior and speculated that gelation in native oat gum solutions may be prevented because of high viscosity; in fact, none of the unhydrolyzed, very high molecular weight oat β-glucan solutions (M_w 1.2×10^6) showed any tendency to form gel. The influence of molecular weight on gelation kinetics can be explained by the higher mobility of shorter chains(Doublier and Wood, 1995; Böhm and Kulicke, 1999b); molecules with smaller molecular size are more mobile and diffuse more easily, exhibiting a greater probability of forming aggregates. Similarly, in a previous study on aggregation kinetics of amylose aqueous solutions it has been demonstrated that the initial rate of G' increase was faster, and the time taken to reach a pseudoplateau region was less with decreasing amylose chain length (Clark et al., 1989). A closer look at the fine structure data of all samples (Table 1), in relation to the observed gelation rates, did not reveal any major differences among the samples. It is interesting also to note that the P_1 sample with the greatest tendency to gel had the lowest proportion of long cellulosic-like chain segments. It would appear, therefore, that molecular weight rather than fine structure is the most important determinant of the gelling behavior for oat β-glucans.

The gelation time and the gelation rate of P_1 as a function of concentration are shown in Fig. 9a. The gelation rate increased with polymer concentration, whereas an inverse relationship between concentration and gelation time was observed; the gelation time of P_1 decreased continuously from 8.3 h at 9% (w/v) to 1.8 h at 14% (w/v). The concentration determines the chain

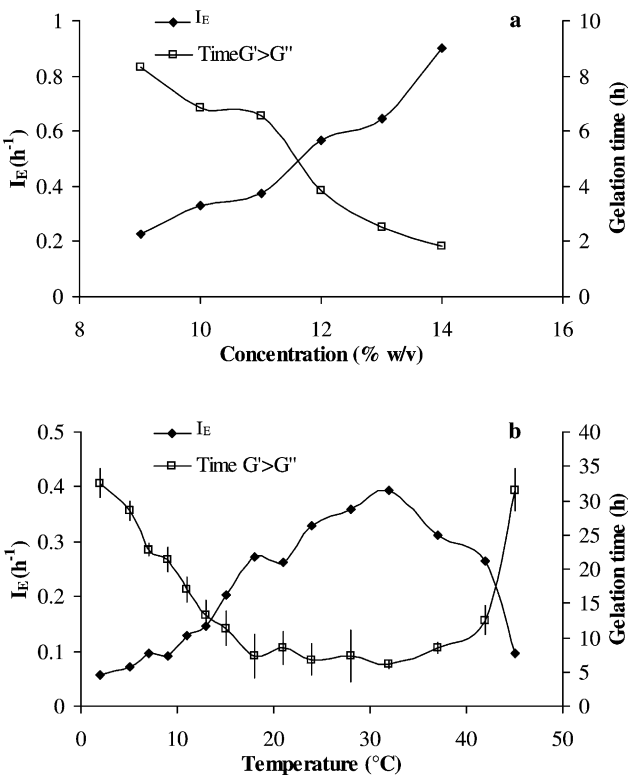

Fig. 9. Concentration- (at 24 °C, **a**) and temperature- (at 10% w/v, **b**) dependence of gelation kinetics for P_1; frequency 1 Hz, strain 0.1%, $I_E = (\mathrm{dlog}\, G'/\mathrm{d}t)_{max}$, gelation time is defined as the time when $G' > G''$.

segment density in polymer solutions, and hence the probability of contact between the coils, which is a basic requirement for establishment of a three-dimensional network. This explains why faster gelation happens when the polymer concentration increases. Similar observations have been made by Böhm and Kulicke (1999b) and Clark et al. (1989) for barley β-glucans and amylose gels, respectively.

It has been proposed that the gelation or setting time is inversely proportional to the initial gelation rate (Oakenfull and Scott, 1986, 1988). A gel is typified by its nearly frequency-independent storage modulus (plateau modulus) G'_{max} that is related to the number of cross-links in the network structure:

$$G'_{max} = cRT/M_e$$

where c, concentration; R, gas constant; T, absolute temperature, M_e, molar mass between two cross-links. According to this relationship, the larger the G'_{max}, the smaller the entanglement molar mass; i.e., the higher the cross-link density. When the experimental data of G'_{max} vs. concentration were fitted into an empirical quadratic equation ($r^2 = 0.96$), an estimate of 8.4% (w/v) for the 'critical gelling concentration' was obtained by extrapolation of this function.

The effect of temperature on the gelation time and rate for P_1 β-glucan dispersions (10% w/v) is illustrated in Fig. 9b. Increasing storage temperatures from 2 to 45 °C first decreased the gelation time and then increased it; a minimum plateau was observed in the range of 18–37 °C. In contrast, the I_E increased with increasing temperature, and reached a maximum at 32 °C with a value of 0.39 h^{-1}. Further increases in temperature from 32 to 45 °C resulted in a decline of I_E. A similar behavior for barley β-glucans gelation was observed by Böhm and Kulicke (1999b). These researchers compared the temperature dependence of crystallite growth rate from polymer melts with that from the elasticity increment, I_E, of the β-glucan gels and concluded that the gelation of (1 → 3)(1 → 4)-β-glucans could be described in terms of a sporadic nucleation mechanism similar to crystallization kinetics from polymer melts. In polymer melts, sporadic nucleus formation is zero above the melting point and it starts to increase with supercooling. Further lowering of the temperature diminishes molecular motion and, at the glass transition temperature, nucleus formation becomes again zero.

Fig. 10 shows the evolution of storage (G') modulus of K-II_2 and P_1 β-glucan gels (10% w/v) during heating at a constant heating rate (3 °C/min), measured with a strain of 0.1% and a frequency of 1 Hz. Compared to the one-step transition for the K-II_2, a two-step drop in the storage modulus (G') was observed for the low molecular weight sample, possibly reflecting different events that take place upon heating of the gels. The temperature at which $G' = G''$ was defined as the melting temperature of the network. The melting temperature was higher for the high molecular

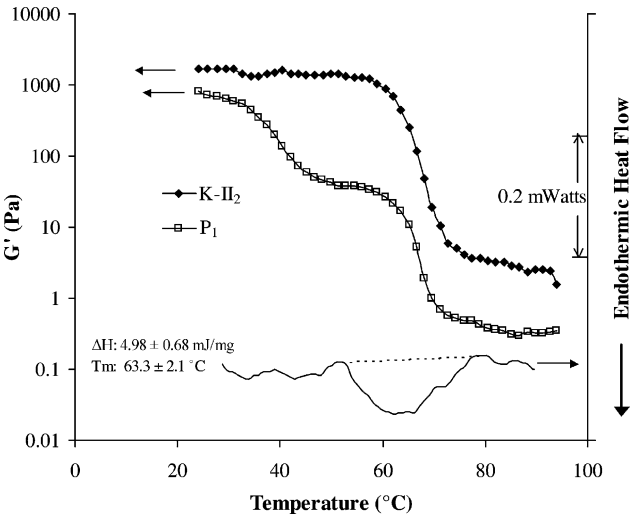

Fig. 10. Storage modulus (G') and heat flow as a function of temperature during melting of oat β-glucan gels (10% w/v) cured at 24 °C for 92 h. Conditions for dynamic rheometry: samples P_1 and K-II_2, strain 0.1%, frequency 1 Hz, and heating rate of 3 °C/min (rheometer); differential scanning calorimetry (DSC) of P_1 sample with a heating rate of 5 °C/min.

weight sample (68.2 °C, K-II_2) compared to that for the low molecular weight sample (62.1 °C, P_1). A higher melting point of structurally similar gels points to a larger extension of the junction zones and/or a better organization of the ordered domains in the network structure (Böhm and Kulicke, 1999b; Flory, 1956).

A direct comparison between the rheological and DSC data is also made in Fig. 10, although a slightly higher heating rate for the DSC measurements was employed (5 °C/min vs. 3 °C/min). The interacting polysaccharide chain segments at the junction zones make an enthalpic contribution to the development of the network structure; i.e. following storage of the β-glucan dispersions, a broad endothermic gel → sol transition occurs at 55–75 °C. Thus, calorimetry provides evidence for the presence of structural domains in the aging network; estimates of the melting point and melting enthalpy for P_1 gel were 63.3 °C and 4.98 ± 0.68 mJ/mg, respectively. These findings are in agreement with the data of Morgan and Ofman (1998), who observed an endothermic peak at around 58 °C for Glugagel, a gelling barley β-glucan preparation. Also, the DSC thermal events match (temperature range) with the second, high-temperature drop in G', suggesting that the first drop in modulus of the P_1 sample simply reflects a loosening of the network structure (aggregates) without a significant change in structural order (conformation) of the chain segments involved in the junction zones.

The melting temperature of β-glucan preparations was independent of the polysaccharide concentration (10–14% w/v). On the other hand, the trend in melting temperature for the P_1 gels (10% w/v) obtained at different gel curing temperatures (between 2 to 45 °C), was an inverse bell-shape relation, as for the gelation time presented in Fig. 9b; i.e., low melting temperatures were seen at

intermediate storage temperatures (12–32 °C). This implies that conditions favoring a quick gelation result in less organized gel network structures. Fig. 11 shows the storage modulus (G') profile upon heating for P_1 gels (10% w/v) prepared at three different storage temperatures. For systems obtained at gel curing temperatures less than 32 °C, a two-step melting was observed, whereas with gel curing at temperatures above 32 °C the networks exhibited a single melting transition at higher temperatures.

3.4. Tensile properties of β-glucans films

Fig. 12 illustrates the effect of moisture and sorbitol on the load-deformation curves of oat β-glucan (K-I_2) films, obtained by tensile tests at 25 °C. With increase of the moisture and sorbitol (plasticizer) content, the maximum force at break and the slope of the curves decreased, whereas the elastic deformation increased, reflecting the gradual transition from brittle to ductile failure of the material.

Fig. 13 shows the effect of varying moisture content, and addition of sorbitol on the mechanical properties of the films, as calculated from the force–distance curves and described by the tensile modulus (E), tensile strength (σ_{max}) and percentage elongation. These films were prepared using two oat β-glucan preparations differing in molecular size. In general, at low moisture contents, films exhibited high tensile modulus, high tensile strength, and low elongation values, at room temperature, typical of glassy materials. With increasing water content there were gradual decreases in modulus and tensile strength, and an increase in elongation for all films. These changes are typical of polymers going through their glass transition. Also, as showed in Fig. 13, the addition of sorbitol at 15% (d.b.) resulted in decreases of E and σ_{max} and in a significant

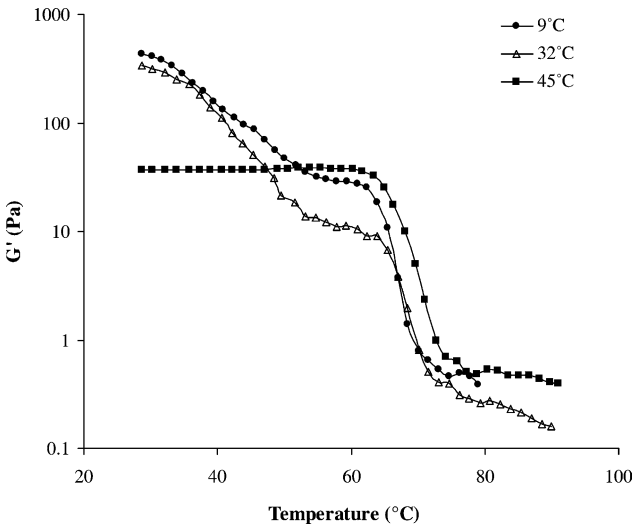

Fig. 11. Storage modulus (G') as a function of temperature during melting of P_1 gels obtained at different gel curing temperatures; β-glucans at 10% w/v, strain 0.1%, frequency 1 Hz, heating rate 3 °C/min).

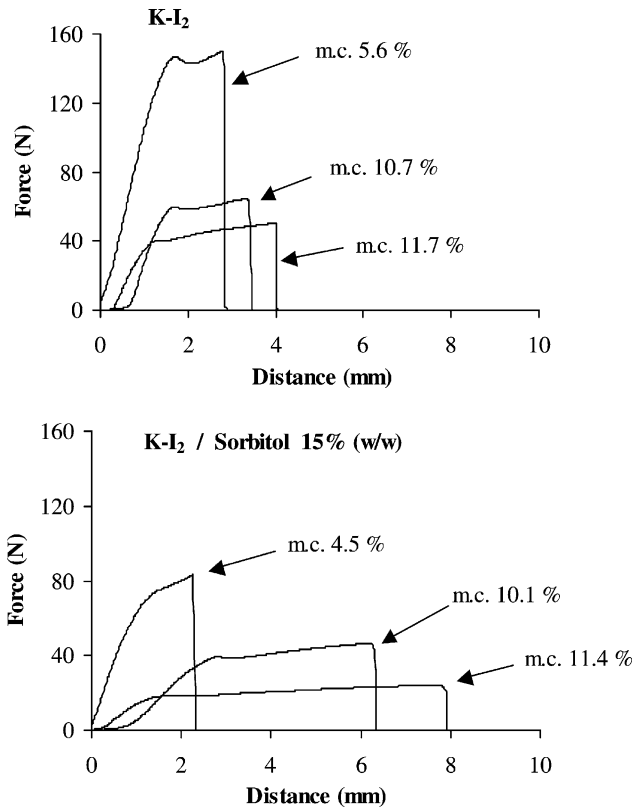

Fig. 12. Effect of moisture content on load-deformation curves (tensile test) of K-I_2 films: with 15% (w/w) (a) and without added sorbitol (b).

increase of elongation values, especially at high moisture contents. The effects of water and sorbitol on the mechanical properties of β-glucan films can be attributed to their plasticizing properties, as has been shown in previous studies for many biopolymer systems (Diab et al., 2001; Biliaderis et al., 1999; Slade and Levine, 1991; Kirby et al., 1993; Park et al., 1993; Bader and Goritz, 1994; Lawton, 1996; Le Meste et al., 1996; Van Soest et al., 1996a,b; Fontanet et al., 1997; Park and Ruckenstein, 2001; Lazaridou and Biliaderis, 2002). The observed range of σ_{max} values (20–80 MPa) for oat β-glucan films is comparable to many medium-strength commercial films, e.g. HPDE and LDPE films (Juran, 1988).

For most of the β-glucan films a bell-shape curve, describing the relationship between the tensile parameters (E, σ_{max}) and moisture was found (Fig. 13). Such behavior has been previously reported for mechanical parameters of foods and their components (Diab et al., 2001; Biliaderis et al., 1999; Fontanet et al., 1997; Lazaridou and Biliaderis, 2002; Nicholls et al., 1995; Harris and Peleg, 1996). The most striking feature of these plots is the apparent increase in stiffness as the moisture rises from 2 to 7%, whereas the softening/plasticizing effect of water becomes dominant above this level. Several suggestions have been made to explain such material toughening on partial plasticization with water. According to Harris and Peleg (1996), glassy biopolymers at low moisture are extremely

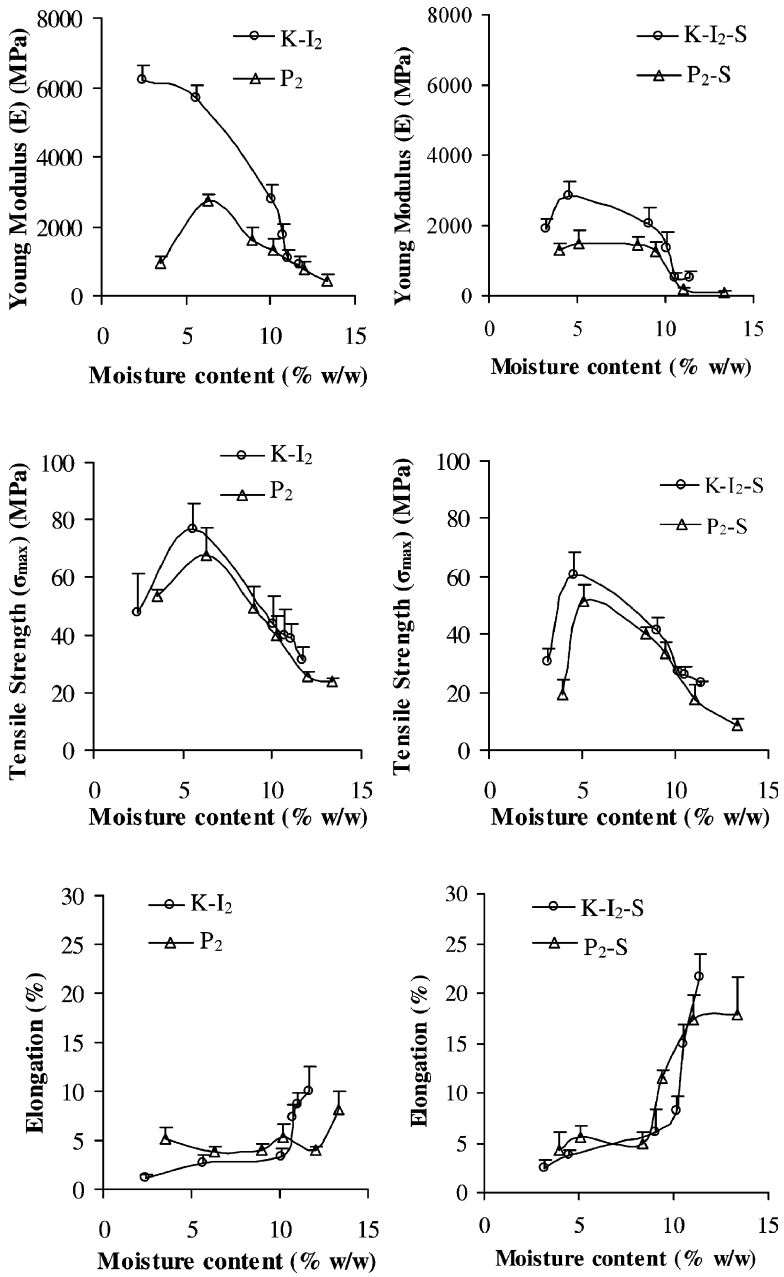

Fig. 13. Effect of moisture content and sorbitol at 15% (w/w) on Young modulus, tensile strength and elongation of edible films made from two oat β-glucan preparations (K-I_2, P_2).

brittle and very fragile, offering no resistant to applied load. With low levels of hydration, the plasticized matrix becomes more cohesive, more structural elements remain intact (offering more resistance to fracture), and the material would deform rather than disintegrate on compression. For extruded flat bread, Fontanet et al. (1997) have ascribed the hardening phenomenon to short range reorganization of the material as a result of increased molecular mobility by adding small amounts of water.

Moreover, the examination of the data for the tensile parameters of Fig. 13 revealed that E, σ_{max}, and elongation values (the latter at high moisture levels) are higher for the high molecular weight sample (K-I_2) compared to those of the low molecular weight sample (P_2) at certain moisture and sorbitol levels. Similar observations have been made for other biopolymers. For methyl cellulose and hydroxypropyl cellulose, Park et al. (1993) have shown an increase of tensile strength and elongation as the molecular weight of cellulose increased, whereas for thermoplastic starch Van Soest et al. (1996b) have reported an increase of elongation with molecular weight, but no significant influence on tensile modulus and tensile strength. In previous studies on synthetic polymers, a possible mechanism for increasing the apparent modulus with molecular weight has been suggested by Kennedy et al. (1994, 1995). Focusing on the interlamellar (amorphous)

region of linear polymers, these researchers ascribed this behavior to an increased number of chain entanglements per molecule with increasing chain length; as a disordered chain moves or slips through the impediment of entanglements, its apparent modulus will increase with molecular weight. Van Soest et al. (1996b) examined two different molecular weight grades of extruded thermoplastic starch and found higher elongation values in the rubbery state for the sample with the higher molecular weight.

4. Conclusions

Aqueous extractions of whole oat flours at relatively low temperatures yielded low molecular weight oat β-glucans. Further purification of the preparations by adjusting the pH of the β-glucans solutions to 4.5 resulted in materials of lower protein content and with even lower molecular weight. The present study revealed that cultivar type had an impact on the molecular characteristics of the isolated oat β-glucans. β-Glucans from Pallini cultivar (*A. sativa*) showed smaller molecular weight and limiting viscosity values, compared to the preparations from Kassandra cultivar (*A. bysantina*). For the isolated β-glucans, the general structural features, as revealed by ^{13}C-NMR and lichenase treatment-HPAEC analyses, were typical of mixed linkage cereal β-glucans and did not show great variations among the two cultivars and the different preparations examined. Differences in the critical concentrations (c* and c**), viscosity and shear thinning properties as well as gelling potential among the samples can be mainly explained in terms of differences in molecular size of the polymeric preparations. Apart from the dynamic rheological measurements, melting of the gel network was also detectable by calorimetry. The β-glucans seemed to be a promising film-forming hydrocolloid, which could be potentially useful as a biodegradable edible food packaging material. The physicochemical properties of β-glucan films under tensile deformation were found to be dependent on the molecular weight of the polymer and the plasticizer (water and sorbitol) content. Sorbitol, added as a co-plasticizer, improved the extensibility, but decreased the mechanical strength of the film. Further studies are needed to explore in detail the relationships among the molecular/structural features, species-cultivar effects and the rheological properties of oat β-glucans.

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