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Extraction, fractionation, structural and physical characterization of wheat β-D-glucans

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Abstract

A high purity wheat β-D-glucan (91.58%) was obtained from white wheat bran after alkali extraction and multi-precipitation with ammonium sulphate compared to previous reported purity of only 70%. The molecular weight (M_w) of the purified wheat β-D-glucan was 487,000 g/mol with a broad M_w distribution (polydispersity is $M_w/M_n = 1.65$). By applying an ammonium sulphate gradient precipitation method, the purified wheat β-D-glucan was fractionated into six fractions with M_w ranged from 43,000 to 758,000 g/mol and much narrower M_w distribution (polydispersity is $M_w/M_n = 1.03$ to 1.26). Structural analysis revealed that there were no significant differences between the six fractions and between the fractions and the original sample. This set of sample was used to investigate the effect of M_w on physical properties of wheat β-D-glucan. Dynamic rheometry and scanning calorimetric studies revealed that the gelation rate and the melting enthalpy (ΔH) of wheat β-D-glucan increased with the decrease of molecular weight indicating smaller wheat β-D-glucan molecules (must be above a minimum M_w) is easier to form junction zones and establish stronger three-dimensional network due to their high mobility and structural regularity (high ratio of tri/tetra). In contrast, the melting temperature of wheat β-D-glucan gels increased with the increase of molecular weight, suggesting a more extended structural network was formed for high M_w wheat β-D-glucans.

Keywords: Wheat (1 → 3) (1 → 4)-β-D-glucan; Purification; Fractionation; Molecular weight and molecular weight distribution; Structural characterization; Gelation

1. Introduction

Cereal (1 → 3) (1 → 4)-β-D-glucans are cell wall polysaccharides of cereal endosperm and aleurone cells. Cereal β-D-glucans are linear homoglucans of D-glucopyranose arranged as blocks of consecutive (1 → 4)-linked β-D-glucose residues separated by single (1 → 3)-linkages. The polysaccharide chain mainly consists of β-(1 → 3)-linked cellotriosyl (58–72%) and cellotetraosyl (20–34%) units; however, there is evidence for a minor amount of β-(1 → 4)-linked cellulosic blocks which have more than four residues and up to 14 (Cui, 2001). The presence of (1 → 3)-linkages prevents crystallization of otherwise long cellulosic chain and bestows apparent water solubility of the cereal β-glucan (Grimm, Kruger, & Burchard, 1995). Significant structural differences in cereal β-glucans are characterized by the trisaccharide-to-tetrasaccharide ratios, which follow the order of wheat (4.2–4.5), barley (2.8–3.3), and oat (2.0–2.4) (Cui & Wood, 2000). Cereal β-glucans show shear thinning behavior at low concentration. As the concentration increases to a certain level, they form gels and their gelling properties are influenced by molecular weights and structural properties (Cui, 2001; Lazaridou, Biliaderis, Micha-Screttas, & Steele, 2004; Lazaridou & Biliaderis, 2004; Lazaridou, Biliaderis, & Izydorczyk, 2003).

Increasing interests in cereal β-glucan are largely due to their beneficial physiological effects on human health and potential importance as an ingredient for the functional food industry. It has been reported that oat glucans may reduce serum cholesterol and glucose levels in humans (Bhatty, 1999; Klopfenstein, 1988; Yokoyama et al., 1997). Lower glycemic and insulin responses were also reported when wheat pasta enriched in barley β-glucan was consumed (Thacker, Campbell, & GrootWassink, 1988).

The variation in structural features such as tri/tetra ratios, the amount of long cellulose-like fragments, molecular weight and molecular weight distribution of cereal β-glucans are responsible for physical and functional properties including

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physiological responses when they are included in cereal-based foods and other formulated products (Izydorczyk & Biliaderis, 2000). Compared with other cereal β-glucan, wheat β-glucan has the higher tri/tetra ratio (Cui & Wood, 2000). This unique structural feature seemed responsible for its unique physical properties such as poor solubility and fast gelation (Cui, 2001; Cui & Wood, 2000). However, little attention has been given to wheat β-glucan, because of its relatively low concentration in whole-wheat and refined flour (Bacic & Stone, 1980). There are limited reports on the fine structural features and structure–function relationship of wheat β-glucans, due perhaps to the lack of efficient methods to obtain significant amounts of pure and structural homogeneous samples. The objectives of the present study were to: (1) improve the extraction and purification method to obtain highly purified wheat β-D-glucan and its fractions; (2) characterize their fine structures and molecular weight distributions of wheat β-D-glucan and its six fractions; (3) study the relationship between molecular weight (structural feature) and gelling properties of wheat β-D-glucan using dynamic rheological and calorimetric methodologies.

2. Materials and methods

2.1. Materials

White wheat bran 300, white wheat bran powder 50 and red wheat bran provided by Hayhoe Mills Ltd. (Woodbridge, Ont., Canada) were used for wheat bran composition analysis. Wheat β-glucan was extracted from white wheat bran powder 50 (50 mesh, Hayhoe Mills Ltd.). Thermal stable α-amylase, lichenase, β-glucosidase, and β-xylanase were purchased from Megazyme International (Bray, Co. Wicklow, Ireland). All chemicals were of reagent grade unless otherwise specified.

2.2. Extraction and purification of wheat β-glucan

Extraction of wheat β-glucan from wheat bran was carried out using the method of Cui, Wood, Blackwell, and Nikiforuk (2000). Wheat bran powder was first treated with 70% ethanol at 70 °C in water bath for 3 h. The ethanol extract was separated from residue by vacuum filter. The residue (Residue A) was washed twice with 70% ethanol, and dried at 60 °C for 12 h. Residue A was then treated with thermal stable α-amylase at 90 °C in aqueous conditions (12.5% w/w, pH 6.5–7.0) for 30 min. The dispersion was cooled down to room temperature and then centrifuged at 8000×*g* for 25 min. The supernatant was discarded. The residue (Residue B) washed with water, re-centrifuged (8000×*g*, 25 min), and dried at 70 °C for 12 h. Residue B was treated with 1.0 M NaOH at 25 °C for 3 h (solid to liquid ratio = 1:20) and centrifuged at 10,000×*g* for 25 min. The alkaline extract was neutralized to pH 4.75 with 2 M HCl. Sodium acetate buffer (0.2 M, pH 4.75) and β-xylanase (∼100 unit/100 ml of extract) were added at 50 °C with constant stirring for 2 h. The enzyme was deactivated by heating in a boiling water bath for 15 min, and the solution was centrifuged at 10,000×*g*, 25 °C for 30 min. The supernatant solution was combined with ethanol and the final concentration was adjusted to 50% ethanol (v/v), and the precipitate was recovered by centrifugation (10,000× *g*, 25 min). The pellet was dissolved in distilled water (the same volume as alkali solution used for the extraction) by heating to 70 °C for 3 h with constant stirring, and cooled to room temperature. The solution was dialyzed against deionized water for 48 h. An equal volume of 100% propan-2-ol (IPA) was added to the solution for precipitation. The resulting precipitate was suspended in 100% IPA and kept overnight at 4 °C. After removal of the solvent, the precipitate was dried in the vacuum oven at 40 °C for 2 h.

The solubilities of β-glucans and arabinoxylans in ammonium sulphate solution are different. β-Glucans can be precipitated at a much lower saturation level of ammonium sulphate (20–55%) than arabinoxylans (55–95%). Based on this principle, purified wheat β-glucan was obtained by the method of ammonium sulphate precipitation. The wheat β-glucan extract was dissolved in water (0.3%, w/v), and centrifuged at 15,000×*g* for 40 min to remove a small amount of residue. Ammonium sulphate (50%, w/v) was added slowly to the supernatant, with stirring to obtain a final concentration of 25% (w/v) of ammonium sulphate. The precipitate was centrifuged (15,000×*g*, 25 min), and redissolved in water (0.3%, w/v). The ammonium sulphate precipitation procedure was repeated, and the pellet was dissolved again in water. An equal volume of IPA was added slowly with vigorous stirring, and the precipitate allowed to settle and collected by centrifugation (15,000×*g*, 25 min). The precipitate was dissolved in water, and dialyzed against deionized water for 24 h. The solution was then precipitated by equal volume of IPA, and the precipitate was washed with 100% IPA, then, dried in a stream of air with gentle warming.

2.3. Fractionation of wheat β-glucan

Six fractions of purified wheat β-glucan were obtained by using the gradual ammonium sulphate precipitation method as described previously (Wang, Wood, Huang, & Cui, 2003). Slight modification was made by adjusting the ammonium sulphate concentration due to the lower molecular weight of wheat β-glucan. Exactly 3.5 g β-glucan was dispersed in 10 ml 50% ethanol, and the dispersion dissolved in 1750 ml water to make a starting solution of 0.2% (w/w). The solution was heated at 90 °C for 2 h under constant stirring, cooled, then centrifuged at 25 °C at 20,000×*g* for 40 min. The supernatant was collected for further fractionation. Ammonium sulphate was added slowly to the supernatant under constant stirring in a water bath at 25 °C to bring the $(NH_4)2SO_4$ concentration to 16.26% (w/w). The solution was allowed to stand for 1 h at 25 °C, and the precipitate collected by centrifuging (20,000× *g*, 20 min, 25 °C). The precipitate was re-dissolved in 500 ml distilled water by heating at 80 °C for 1 h and 60 °C for 2 h with constant stirring, and dialyzed against deionized water for 24 h at room temperature. β-Glucan was recovered by adding an equal volume of 100% IPA and centrifugation (10000×*g*, 20 min, 25 °C). The pellet was dispersed in 100% IPA, left for

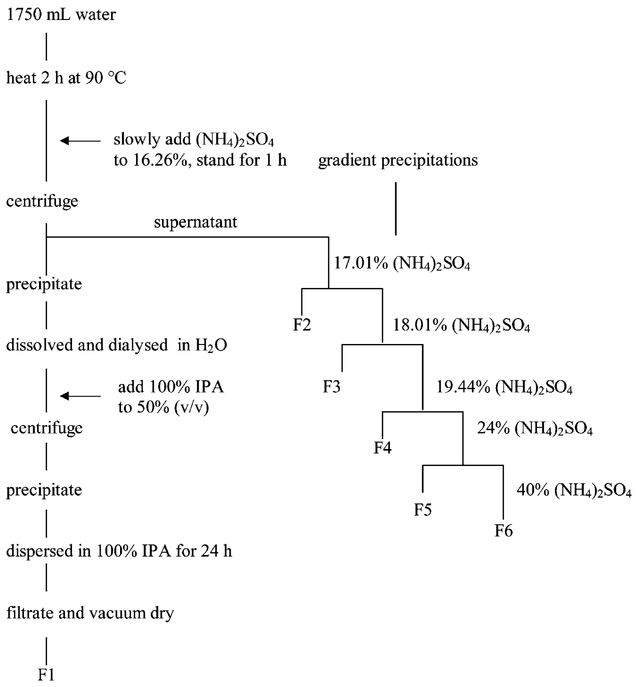

Fig. 1. Procedure for wheat β-glucan fractionation by the method of ammonium sulphate precipitation.

overnight at 4 °C, then recovered by filtration on a glass filter and dried in a vacuum oven at 80 °C for 3 h. This fraction was designated fraction 1 (F1).

The concentration of $(NH_4)_2SO_4$ was increased stepwise (F2:17.01%, F3: 18.01%, F4: 19.44%, F5: 24%) to a final concentration of 40%. The corresponding fractions at each step were obtained in a similar manner as described above, designated F2, F3, F4, F5 and F6, respectively. The fractionation procedure is summarized in Fig. 1.

2.4. Analytical methods

2.4.1. Chemical and monosaccharide analyses

The β-glucan content was determined by the specific enzyme method of McCleary & Glennie-Holmes (1985) using reagents supplied by Megazyme International (Bray, Co. Wicklow, Ireland). The water soluble β-glucan content of wheat bran was determined by the calcofluor flow injection analysis (Wood, Weisz, & Blackwell, 1991). Protein was analyzed by NA2100 Nitrogen and Protein Analyzer using the factor of 5.7 to convert nitrogen to protein. Starch was determined using the method described by Wood et al. (1991). Ash and moisture were determined according to methods described by AOAC (2000).

Monosaccharide composition was determined by hydrolyzing samples in 1 M H_2SO_4 at 100 °C for 2 h followed by high-performance anion-exchange chromatography (HPAEC) as described by Wood, Weisz, and Blackwell (1994).

2.4.2. Molecular and structural characterization

Molecular weight, gyration radius and intrinsic viscosity were determined by high performance size exclusion chromatography (HPSEC) equipped with three detectors; a right angle laser light scattering detector (RALLS), a differential viscometer (DP) and a refractive index detector (RI) (Triple Detector System, Model Dual 250,Viscotek, Houston, TX). The detectors were calibrated with pullulan standards (P-82, JM Science, Inc., NY, USA). A value of 0.146 ml/g was used as refractive index increment (d*n*/d*c*) for molecular weight calculation. (Wang, Wood, Huang, & Cui, 2003). The chromatographic system comprised of a Shimadzu SCL-10Avp pump and automatic injector (Shimadzu Scientific Instruments, Inc., Columbia, Maryland 21046, USA), two columns in series: a Shodex Ohpak KB-806M (Showa Denko K.K., Tokyo, Japan), and an Ultrahydrogel linear (Waters, Milford, CT, USA). The columns, viscometer and RI detector were maintained at 40 °C. The eluent was 0.1 M $NaNO_3$ containing 0.03% (w/w) NaN_3 at a flow rate of 0.6 ml/min.

The distribution of cellulosic oligomers in the chain of β-glucans was determined by lichenase treatment and chromatography. The released oligosaccharides of different fractions were analyzed by HPAEC. Accurately weighed samples were dissolved in 20 mM sodium phosphate buffer, pH 6.5, (1→3) (1→4)-D-β-glucan-4-glucanohydrolase (E.C. 3.2.1.73, lichenase, Megazyme International) was added and the mixture incubated at 50 °C for 1.5 h with constant stirring. The samples were centrifuged (25 °C, 15,000×*g* for 20 min) and the supernatant analyzed by HPAEC combined with a Pulsed Amperometric Detector with a gold electrode. A Dionex system (Dionex, Sunnyvale, CA) equipped with Carbopac PA1 column ($4\times250\ mm^2$) and guard ($3\times25\ mm^2$) was used. The oligosaccharides were separated by gradient elution using 150 mM sodium acetate in 150 mM NaOH (eluent A) and 150 mM NaOH (eluent B). The instrument was controlled and data were processed using Dionex AI 450 software. The weight ratio of tri/tetra oligosaccharide was calculated as the peak area ratio and converted to molar ratio by a factor 1.321, the ratio of tri/tetra molecular weight.

2.4.3. Rheological measurements

The oscillatory measurements of viscoelastic behavior as well as the gel curing–melting events for the β-glucan dispersions were performed on a Bohlin CVO Rheometer (Bohlin Instruments, East Brunswick, NJ) using concentric cylinder (SSC2.3/26, 70 mm gap). Solutions were made in hermetically sealed glass vials by gentle stirring of the β-glucan samples in distilled water at 85 °C until complete solubilization of the material. Viscoelastic behavior, i.e. G' (storage modulus), G'' (loss modulus), and tan d(G''/G') were monitored at a strain 0.1% and a range of frequencies (0.1–10 Hz). Isothermal gel curing events and the melting behavior (heating rate at 1 °C/min) of the gels were probed at a strain level of 0.1% and a frequency of 1 Hz. A thin layer of low viscosity paraffin oil was added to cover the samples in order to prevent evaporation during analysis. All the measurements were duplicated.

2.4.4. Differential scanning calorimetry (DSC)

Thermal analyses were performed on a 2920 modulated DSC (TA Instruments, New Castle, DE, USA). Sample dispersions containing about 2.4–3.6 mg dry matter of the β-D-glucan (4%, w/w) were sealed hermetically into high volume stainless steel DSC pans and stored at 25 °C for 72 h. The samples were then heated at a heating rate of 5 °C/min. The reported values are means of duplicate measurements.

3. Results and discussions

3.1. Extraction and purification of wheat β-glucan

Table 1 shows the composition of different wheat brans. The two white wheat bran samples had similar composition, as they were produced from the same wheat cultivars and underwent similar milling process. The small differences in composition between the two samples may be caused by different processing batches or loss of some minor components during the further processing to obtain smaller particle sizes. Red wheat bran has a similar starch and ash content as the white wheat bran, but a higher protein and lower β-glucan contents. Considering the low β-glucan content in the red wheat bran, white bran was chosen for extraction of β-D-glucan. Table 1 also shows that the water soluble β-D-glucan content is much lower than the total β-glucan content, because β-glucans are known to be physically entrapped in the matrix of a cross-linked ferulic acid–arabinoxylan complex (Beer, Wood, Weisz, & Fillion, 1997; Cui et al., 2000). Some intermolecular interactions between the unsubstituted regions of arabinoxylan backbones and the cellulose like fragments from the β-glucan chains may also affect the extractability of wheat β-glucans by water (Izydorczyk & MacGragor, 2000). It was evident that there is less substitution in the linear xylan backbone of arabinoxylans from wheat brans compared to arabinoxylans from wheat endosperms (Cui, Wood, Weisz, & Beer, 1999; Izydorczyk, Biliaderis, & Bushuk, 1992). Therefore, alkaline extraction was necessary for extracting and isolating wheat β-D-glucans.

After extraction, crude wheat β-glucan was obtained at a yield of 1.36%. The low yield of extraction was mainly caused by the low content of β-glucan in wheat bran and the high mass loss during the long extraction procedure. The loss of water extractable β-glucans during α-amylase treatment may also be the cause of the low yield. The wheat bran extract contained 57.13% β-glucan together with 6.84% starch, 11.66% ash, and other types of carbohydrates such as arabinoxylans (Table 2). The low β-glucan content was not suitable for studying its structural and functional properties. Purification is, therefore, conducted to obtain a more desirable level of purity of wheat β-glucan.

Table 1
The composition of different wheat brans

	White wheat bran 300 (%)	White wheat bran powder 50 (%)	Red wheat bran (%)
Moisture	10.64	10.16	8.84
Protein	15.68	15.70	17.19
Ash	5.43	5.38	5.81
Starch	23.34	25.21	21.08
Total dietary fiber	33.49	27.12	34.70
Total β-glucan	2.51	2.38	2.15
Water extractable β-glucan	0.30	0.28	0.22

Table 2
Wheat β-glucan compositions

	Before purification	After purification
β-glucan	57.13%	91.58%
Moisture	6.38%	3.81%
Starch	6.84%	Trace
Protein	Trace	Trace
Ash	11.66%	–
Monosaccharide composition		
Glucose	89.34%	100%
Galactose	1.38%	Trace
Arabinose	3.58%	Trace
Xylose	5.70%	Trace

Due to the solubility differences of polysaccharides in different concentrations of ammonium sulphate, the ammonium sulphate precipitation method was used for further purification to get rid of undesired carbohydrates such as starch and arabinoxylan. After purification, the purity of β-glucan was increased to 91.58%, with other components present at only trace levels (Table 2). The efficiency of the purification method was also evaluated by monosaccharide composition analysis. HPAEC profiles showed three small peaks representing arabinose, galactose, and xylose with the main peak of glucose before purification, and after purification, only the glucose peak was observed (Fig. 2). These results indicated that

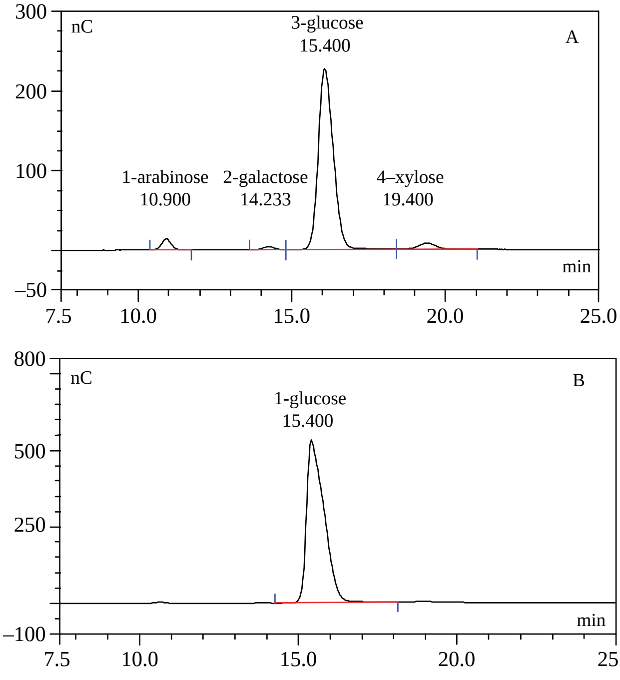

Fig. 2. High performance anion exchange chromatograms with pulsed amperometric detection of wheat β-glucan (A: before purification, B: after purification).

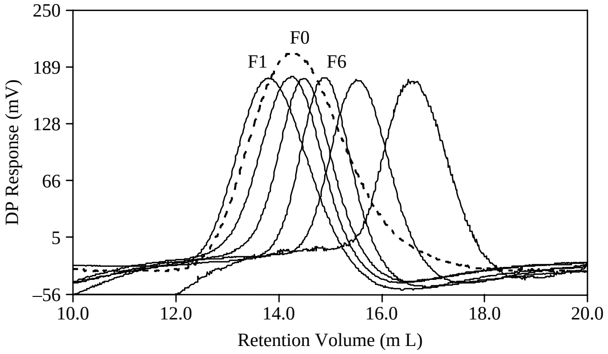

Fig. 3. High performance size exclusion chromatography DP response profiles of wheat β-glucan (F0) and the six fractions (F1–F6).

the ammonium sulphate precipitation method was effective for purifying wheat β-glucan. Instead of the ammonium sulphate precipitation method, Lazaridou et al. (2004) used gel precipitation method to achieve a purity of 82.9% for wheat β-glucan. They suggested that the strong β-glucan-arabinoxylan interaction might contribute to indigestibility by enzymes and the presence of ammonium sulphate enhanced this intermolecular interaction (Izydorczyk & MacGragor, 2000). In contrast, the data presented in the present paper did not show any influence of the β-glucan-arabinoxylan interaction. The possible reason could be that the β-glucan-arabinoxylan interaction was eliminated by the strong alkaline extraction, perhaps more effectively by the following xylanase digestion used in the procedure.

3.2. Fractionation and molecular weight distribution of wheat β-glucan

Purified wheat β-glucan was fractionated into six fractions (F1–F6) using gradient ammonium sulphate concentrations of 16.26, 17.01, 18.01, 19.44, 24 and 40.0% (w/v), respectively (Fig. 1). The difference in molecular weight distributions of these fractions compared to the original unfractionated β-glucan is shown by the HPSEC profiles (Fig. 3). The molecular weight, radius of gyration, polydispersity, and intrinsic viscosity of each fraction are summarized in Table 3. The original β-glucan had a weight average molecular weight (M_w) of 4.87×10^5, which was in a good agreement with the result of Cui (2001). The polydispersity index (M_w/M_n where M_n is the number average molecular weight) of 1.65 indicated a fairly broad M_w distribution of the original β-glucan. The molecular weight distribution of each fraction (F1–F6) was considerably narrower than that of the parent β-glucan (Table 3), suggesting the homogeneous molecular size distribution in each fraction, hence exhibit similar physical properties. Molecular weight of the fractions ranged from 7.58×10^5 (F1) to 0.43×10^5 (F6). The significant differences in molecular size between these fractions would allow the examination of molecular weight–function relationship. The total recovery of β-glucan from this fractionation procedure was 76%.

3.3. Structural characterization—oligosaccharide compositions

The relative amounts of oligosaccharides produced by lichenase treatment provide a fingerprint of the structure features of wheat β-glucan. These structural features play an important role on solubility and chain conformation, hence affect their rheological properties in solution. Consecutive β-(1→4) linked blocks (cellulose-like segments) in the β-glucan chain exhibit a tendency for interchain aggregation via strong hydrogen bonds, which corresponds to low solubility. The β-(1→3) linkages break up the regularity of the β-(1→4) linkage sequence, making the chain more flexible (Buliga, Brant, & Fincher, 1986). This conformational irregularity may prevent the chains to align closely over extended regions, hence making the β-glucan more soluble (Woodward, Phillips, & Fincher, 1988). On the other hand, the tri/tetra oligosaccharide ratio of β-glucans may also have significant influence on their rheological properties, because the sum of the two oligosaccharides is over 90% in all cereal β-D-glucans. Higher tri/tetra ratio leads to a higher probability of forming ordered consecutive cellotriosyl segments (higher conformational regularity), which may favor the formation of junction zones in solution, and essentially leads to the formation of three-dimensional gel network.

HPAEC results of released oligosaccharides of different fractions by lichenase treatment are summarized in Table 4. Similar composition of oligosaccharide was found between wheat β-glucan and its fractions. This suggests that the original β-glucan and its fractions had homogeneous structural features, with the only difference being the length of the molecular

Table 3
Molecular weight (M_w), radius gyration (R_g), intrinsic viscosity (h), and polydispersity (P_d) of unfractionated wheat β-glucan (F0) and its six fractions (F1–F6) obtained by stepwise precipitation with ammonium sulphate. Last column lists the yield of each fraction

	$M_w(\times10^5)$	R_g (nm)	$[\eta]$ (dl/g)	P_d	Yield (%)
F0	4.87	42.40	4.96	1.65	
F1	7.58	57.93	7.80	1.26	19.27
F2	4.80	45.09	5.75	1.19	14.70
F3	3.72	39.62	4.97	1.07	8.46
F4	2.50	32.67	4.01	1.03	8.89
F5	1.32	22.69	2.62	1.09	15.74
F6	0.43	11.88	1.16	1.09	8.17

Table 4
Oligosaccharide compositions of wheat β-glucan fractions

DP	3	4	5	6	7	8	9	10	10–14	3+4	¾ ratio
F0(%)	70.4	21.3	4.0	2.1	0.4	0.5	1.0	0.1	0.4	91.7	4.38
F1(%)	69.5	20.5	4.3	2.1	0.3	0.5	2.1	0.3	0.4	90.0	4.47
F2(%)	69.8	20.7	4.4	2.1	0.4	0.6	0.9	0.4	0.7	90.4	4.46
F3(%)	69.5	21.3	4.5	2.3	0.4	0.5	0.9	0.2	0.4	90.8	4.31
F4(%)	69.5	21.4	4.4	2.0	0.3	0.6	1.2	0.4	0.4	90.8	4.30
F5(%)	69.2	21.2	4.1	2.1	0.4	0.5	1.5	0.4	0.6	90.4	4.32
F6(%)	68.3	21.7	4.0	2.1	0.2	0.7	1.6	0.4	1.0	90.0	4.16

chains. Oligosaccharides with a DP 5–14 were also present (less than 10% by weight) in a similar amount in each fraction. The tri/tetra ratio was in the range of 4.16–4.47, essentially undistinguishable between the samples examined. The value of the tri/tetra ratio for wheat β-glucans was the highest found among other cereal β-glucans (oat: 2.0–2.4; barley 2.8–3.3, respectively), which probably is responsible for its low solubility and high gelation ability observed previously (Cui, 2001; Cui & Wood, 2000; Cui et al., 2000).

3.4. Gelation properties

Wheat β-D-glucan was first reported to form gels upon cooling at 4 °C over night (Cui, 2001; Cui et al., 2000), and its gelation rate is the highest among cereal β-D-glucans (Lazaridou & Biliaderis, 2004). There are two models to explain the gelation mechanism of cereal β-glucans. The first involves the interactions via hydrogen bonding between the longer cellulose-like sequences of more than three contiguous β-(1→4) linked monomers, which forms cellulose-like junction zone (Fincher & Stone, 1986; Woodward, Fincher, & Stone, 1983). Alternatively, the polymers may associate through consecutive cellotriosyl units called cellotriosyl sequence junction zones (Böhm & Kulicke, 1999; Cui et al., 2000). The oligosaccharide composition of wheat β-glucans (Table 4) and the reported data of other cereal β-glucans revealed that the small amount of cellulose-like segments (< 10%) were silimar in all cereal β-glucans. However, the amount of cellotriosyl units in the chain of cereal β-glucans is predominant (60–75%) and exhibit finger printing information of samples from different sources. Higher tri/tetra ratio leads to the higher probability of the presence of consecutive cellotriosyl units; this higher regularity in structure might be responsible for a greater gelling ability (Böhm & Kulicke, 1999; Cui, et al., 2000). Thus, the interactions between the consecutive cellotriosyl units could be the main factor responsible for the gel formations of cereal β-glucans. It was reported that sequences of three consecutive cellotriosyl units in β-glucan chains may form a stable, antiparallel complex stabilized by intermolecular hydrogen bonds (Tvaroska, Ogawa, Deslandes, & Marchessault, 1983), however, further evidence is still required to verify this conclusion.

The gelation kinetics of three wheat β-glucan fractions (F1, F3, and F5) was monitored by oscillatory experimental measurements at 1 Hz, 0.1% strain and 10 °C as shown in Fig. 4. The freshly prepared cereal β-glucan solutions are typical of a viscoelastic liquid ($G'' > G'$), where the moduli are highly dependent on frequency. However, after an induction period, the β-glucan solutions begin to adopt gel-like structures where the G' overtakes G''. After a certain curing time, G' reaches the plateau, where the mechanical spectra become typical of elastic gel networks ($G' \gg G''$, the G' being independent and G' slightly dependent on frequency) as shown in Fig. 5. The gelation rate of β-glucans was determined using the elasticity increment, I_E, a parameter first introduced by Böhm & Kulicke (1999). The logarithm of G' as a function of time generally proved to be sigmoidally shaped for β-glucans. The slope of $\log G'(t)$ at the turning point (maximum slope) can be taken as a measure of the gelation rate, which is defined as 'elasticity increment, I_E', and calculated as $I_E = (\mathrm{d} \log G'/\mathrm{d}t)\mathrm{max}$. Its dimension is reciprocal

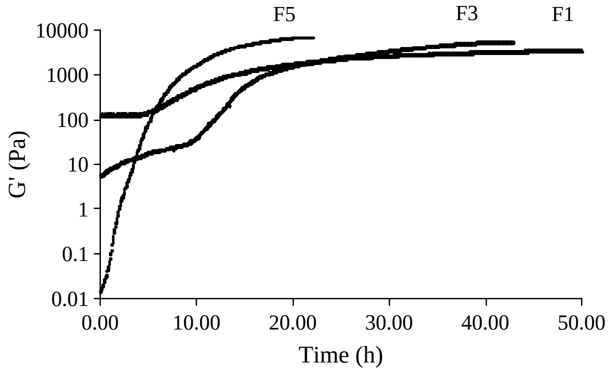

Fig. 4. Time dependence of G' for wheat β-glucan fractions (at 4% w/w, frequency 1 Hz, strain 0.1%, 25 °C).

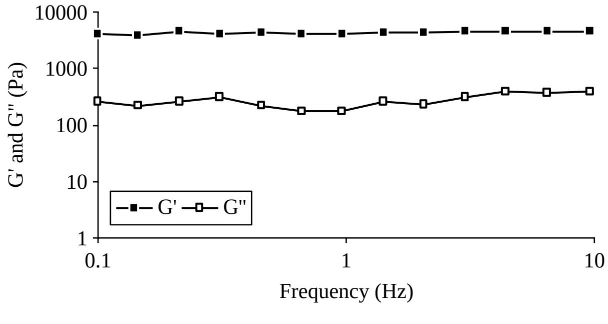

Fig. 5. Frequency dependence of storage (G') and loss (G'') modulus of 4% (w/w) wheat β-glucan fraction (F1).

Table 5
Gelation kinetics of wheat β-glucan fractions (at 4% w/w, frequency 1 Hz, strain 0.1%, 10 °C)

Samples	Gelation time (h)	I_E (h^{-1})	G' max (Pa)	Melting temperature (°C)
F1	6.84(±0.26)	0.068(±0.005)	3710(±150)	72.2(±0.5)
F3	4.97(±0.56)	0.081(±0.002)	5290(±270)	71.1(±0.6)
F5	3.49(±0.21)	0.444(±0.012)	6490(±110)	68.3(±0.2)

time and indicates the number of decades of G' increases at maximum per unit time; a high I_E value reflects rapid gelation.

Fig. 4 shows the different shapes of log $G'(t)$ curves for wheat β-D-glucan samples with different molecular sizes in 4% (w/w) concentration at 10 °C. The gelation time, elasticity increment, and maximum storage modulus are summarized in Table 5. With increase in molecular size, the gelation rate (I_E) decreased, while the gelation time and the induction period of gel increased. The influence of molecular size can be explained by the combination of high mobility and segment density of shorter chains in solution (Böhm & Kulicke, 1999; Doublier & Wood, 1995). At the same concentration (w/w), the smaller size molecules have shorter chains and higher molar concentration. The molecules have higher mobility and higher opportunity to form ordered structures or junction zones, which lead to faster formation of three-dimensional gel network. The plateau values of G' for wheat β-glucan gels decreased with increasing molecular size indicating the formation of higher density of gel networks of the small size molecules. Similar results have been reported by Böhm & Kulicke (1999) for barley β-glucan samples and by Lazaridou et al (2003) for oat β-glucan samples.

Fig. 6 shows the melting profiles of G' for three fractions of wheat β-D-glucans from 20–90 °C at a heating rate of 1 °C / min. A sharp decrease was observed in storage modulus values not in a single melting point but within a range of temperatures upon heating (50–75 °C). The melting profiles revealed losses in network strength, which confirmed that at high temperatures, intermolecular associations become thermoreversible (Vaikousi, Biliaderis, & Izydorczyk, 2004). The melting point (temperature at which $G'=G''$) shifts slightly towards higher temperatures with increasing molecular size (Table 5).

3.5. Differential scanning calorimetry

DSC is a useful technique for obtaining thermodynamic information on the gelation process of polysaccharides. Gelation of β-glucans involves the formation of a network structure through inter-chain segment association and aggregated junction zones (Lazaridou et al., 2003; Morgan & Ofman, 1998). Endothermic peaks during the gel→sol transition from DSC measurement indicate the melting of structural domains. The apparent melting enthalpy values (ΔH) calculated from the DSC curves is a measure of energy required for disrupting the H-bonding within the junction zones (Lazaridou et al., 2004). The endothermic enthalpy is most likely proportional to the cross-link and junction zone density (Vaikousi et al., 2004). The onset melting temperature and the broadness of the endothermic peaks also provide information on gel properties.

Freshly prepared solutions of wheat β-glucan exhibited no thermal transition, but gel network structure developed progressively upon aging (Cui, 2001). When the melted gels in DSC pans are stored for the same period of time, similar DSC thermal curves are obtained, implying a time dependent thermoreversibility of the gel networks. Fig. 7 shows the DSC melting curve of three fractions (F1, F3 and F5) of wheat β-glucan gels prepared at 4% (w/w) and stored at 4 °C for a long enough period to allow the completion of gel formation (72 h). The ΔH values increased with decrease in molecular size. This can be explained by the theory that shorter chains of the small size molecules, which have less diffusion constraint, have higher mobility in solution compared with the large size molecules. Thus, it increases the opportunities of the regular segments in the chains to associate with each other and form high density junction zones. This phenomenon was also observed in oat

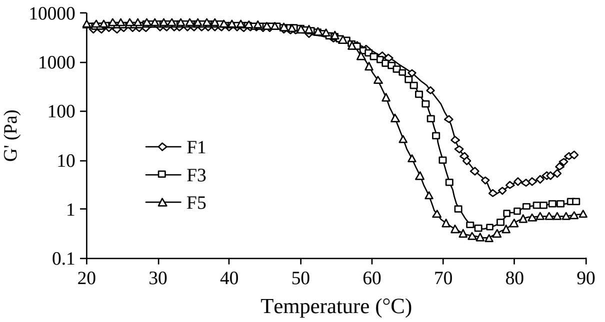

Fig. 6. Temperature dependence of storage modulus (G') during heating from 20 to 90 °C at a rate of 1 °C/min for wheat β-glucan fractions (4%, w/w).

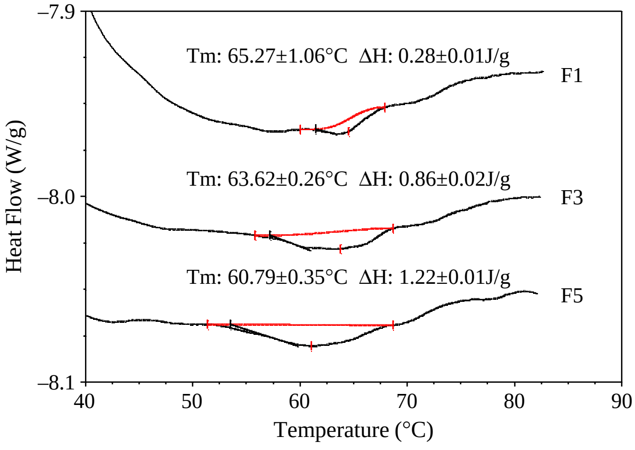

Fig. 7. DSC thermograms of gels obtained from wheat β-D-glucan fractions (4% w/w). T_m, peak temperature of melting (°C); ΔH, enthalpy (J/g). Each result represents the average of duplicate values±SD.

and barley β-glucans (Lazaridou et al., 2003; Morgan & Ofman, 1998; Vaikousi et al., 2004). The peak temperature of melting (T_m) increases with increasing β-glucan molecular size, indicating that the gel network of high M_w gels consisted of extended structural elements (microaggregates) although their gel formation cross-link density was lower (lower ΔH) (Lazaridou & Biliaderis, 2004). Furthermore, the endothermic peak broadness shows the reverse trend with the melting temperature. The broader gel → sol transition peak of the small size molecules suggests the diversity of their gel structure indicating a non-uniform network of the small molecules.

4. Conclusions

Wheat β-glucan with a purity of 91.58% after alkali extraction and purification was achieved. Six fractions with low polydispersity but significantly different M_w were obtained by gradient precipitation with ammonium sulphate. The M_w distribution of each fraction was much narrower compared to the parent sample. The HPAEC analysis revealed that there was no significant structural difference between the fractions and the parent sample (e.g. oligosaccharide pattern, tri/tetra oligosaccharide ratio), indicating no fractionation based on structural features had taken place. The gelation kinetic profiles measured by dynamic rheometry showed an increase in gelation rate with decrease in molecular size. However, the increase in melting temperature with the molecular size indicated more extended junction zones in the gel network formed by large size molecules. DSC and dynamic rheometry also revealed that the value of enthalpy (ΔH) and the apparent storage modulus (G') decreased with the increase of molecular size indicating the stronger gelling ability of smaller molecules of wheat β-glucan. It is assumed that there is a minimum M_w below which the β-D-glucan cannot form gels; however, this theoretical minimum gelation M_w has yet to be established.

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