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Structural characteristics and rheological properties of partially hydrolyzed oat β-glucan: the effects of molecular weight and hydrolysis method

Susan M. Tosh*, Peter J. Wood, Qi Wang, John Weisz

Food Research Branch, Agriculture and Agri-Food Canada, 93 Stone Road West, Guelph, Ont., Canada N1G 5C9

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Abstract

Partial hydrolysates of (1 → 3)(1 → 4)-β-D-glucan from oats were produced by three hydrolysis methods: acid, cellulase or lichenase. The molecular weights ranged from 31 000 to 237 000 g/mol. Six percent solutions of small molecular weight β-glucans formed elastic gels after 4 days at 4 °C whereas larger molecular weight β-glucans remained viscous liquids after 7 days. The melting temperature of the gels increased as they aged and the peak heat flow temperature, measured by differential scanning calorimetry, was 62 ± 2 °C. Partial hydrolysates produced with cellulase, which was shown to preferentially cleave regions of the molecule with longer contiguous β-(1 → 4)-linked D-glucopyranosyl units, tended to produce more elastic gels with stronger junction zones than partial hydrolysates produced with lichenase which cleaves the β-(1 → 4) glycosidic 3-*o*-substituted glucose links. This suggests that β-(1 → 3)-linked cellotriose sections of the polymer are probably the segments which form the junction zones in the gel network rather than cellulose-like segments.

Keywords: Oats; β-Glucan; Gelation; Molecular weight; Primary structure; Partial hydrolysis

1. Introduction

The polysaccharide (1 → 3)(1 → 4)-β-D-glucan, usually referred to as β-glucan, is a component of cell walls of Graminae and a major component of the endospermic cell walls in oats and barley (Wood, 1986). In recent years, the health benefits of adding oat bran and oat β-glucan to the diet have been widely studied. Oat products may reduce serum cholesterol levels (Braaten et al., 1994) and attenuate post-prandial blood glucose levels (Wood et al., 1994a), which is of benefit to people with diabetes. Oat bran has also been shown to enhance the growth of probiotic bacteria in the colon potentially improving colonic health (Malkki & Virtanen, 2001). In the upper gastrointestinal tract, the function of β-glucan is primarily related to its structure, molecular weight and interaction with other components of the diet. Therefore, it is important to understand structure–function relationships for this polymer.

The primary structure of β-glucan is a linear polymer of D-glucopyranosyl units linked by either isolated β-(1 → 3) linkages or groups of β-(1 → 4) linkages (Woodward, Fincher, & Stone, 1983). Unlike cellulose, which is quite stiff, the β-(1 → 3) linkages in β-glucan confer a conformation that is often described as a wormlike coil; unlike cellulose cereal β-glucans are water soluble.

Gelling of barley β-glucans has been reported (Letters, 1977; Morgan & Ofman, 1998). Böhm and Kulicke (1999a) reported typical gel behavior in 6% (w/w) barley β-glucan ($M_w = 165\,000$) after 3 days storage at 25 °C. Further investigation showed that the gelation rate declined with decreasing concentration or increasing molecular weight (Böhm & Kulicke, 1999b). Furthermore, β-glucans with broad polydispersities were found to gel faster than samples of narrow polydispersity (Pd). Although low molecular weight β-glucans gelled more quickly, the force required to rupture aged gels (in torsion) increased with increasing molecular weight. Static and dynamic light scattering revealed stable aggregates in dilute solutions of barley β-glucan isolated from beer (Grimm, Kruger, & Burchard, 1995), which was the depolymerized residual β-glucan from

* Corresponding author. Tel.: +1-519-829-2400x3040; fax: +1-519-829-2600. AAFC contribution #S139.
E-mail address: toshs@agr.gc.ca (S.M. Tosh).

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barley malt. The aggregates appeared to have a fringed micelle structure with an elongated, dense core and a flexible, voluminous outer layer. The same forces that cause aggregation in dilute solutions may be acting to form the gel network in concentrated solutions. Therefore, the interaction points in the gel network may be similar to the cores of the fringed micelles.

Two possible aggregation mechanisms have been proposed. The first suggestion was that the long runs of β-(1 → 4)-linked D-glucopyranosyl units would behave similarly to cellulose (Doublier & Wood, 1995; Woodward et al., 1983). Hydrogen bonds would form between these cellulose-like regions on two or more polymer chains and form stable junction zones. When polymer chains possess two or more of these cellulose-like regions a gel network could form in a solution of sufficient concentration. However, Doublier and Wood (1995) were unable to detect differences in amounts of cellulose-like regions between a partially hydrolyzed and gelling β-glucan and a high molecular weight non-gelling sample. The second proposal was that hydrogen bonds could form between consecutive cellotriosyl-units and form stable junction zones (Böhm & Kulicke, 1999b; Cui & Wood, 2000). X-ray fiber diffraction studies done on lichenan and barley β-glucan lend support to this idea. The crystalline structure of these β-glucans showed that three consecutive β-(1 → 3)-linked cellotriosyl units assumed a mono-helical shape and can form hydrogen bonds with similar runs of cellotriosyl units in an antiparallel conformation (Tvaroska, Ogawa, Deslandes, & Marchessault, 1983).

Although native (as extracted by mild reagents) oat β-glucan produces very viscous solutions, some partially hydrolyzed oat β-glucans formed gels (Doublier & Wood, 1995). Furthermore, some oat β-glucan solutions segregated into gelling and non-gelling fractions (Johansson et al., 2000). Although factors controlling gelation behavior of oat β-glucan are not well studied, clearly molecular weight influences not only flow viscosity but also gelation. Molecular weight is an important factor in physiological function as well, since the effectiveness of β-glucan in reducing plasma glucose levels was diminished when the molecular weight of the polymer was reduced (Wood, Beer, & Butler, 2000).

The objective of this study was to produce partially depolymerized oat β-glucan by controlling the molecular weight distribution and using different mechanisms of hydrolysis, which might be expected to influence structural characteristics and behavior of the polymers in solution. Since the ability of oat β-glucan to form a gel appears to depend on molecular weight (Doublier & Wood, 1995), the oat gum was hydrolyzed to different extents to produce fractions with target molecular weights of 200 000, 100 000 and 40 000 g/mol.

Because the D-glucopyranosyl units are connected with two types of linkages different hydrolysis methods will produce partial hydrolysates with different primary structures. Hydrolysis with lichenase (endo-(1 → 3)(1 → 4)-β-D-glucan-4-glucanohydrolase from *Bacillus subtilis*, EC 3.2.1.73) cleaves only β-(1 → 4) glucosidic linkages of a three-substituted unit. Cellulase (endo-(1 → 4)-β-D-glucan-4-glucanohydrolase from *Trichoderma* sp., EC 3.2.1.4) requires two adjacent β-(1 → 4) glucosidic linkages. Neither enzyme cleaves the β-(1 → 3) glucosidic linkage. Acid can cause hydrolysis of all of the linkages, but it is unknown whether it cleaves the β-(1 → 3) and β-(1 → 4) glucosidic linkages at the same rate.

2. Methods

2.1. Sample preparation

The initial unhydrolyzed oat β-glucan was prepared at the POS Pilot Plant (Saskatoon SK) by extraction of oat bran at pH 10, essentially as previously described (Wood, Weisz, Fedec, & Burrows, 1989). However, instead of using a decanting centrifuge to separate the bran from the extraction solvent, a Tolhurst basket centrifuge (Halpen Engineering, Weston, Ont.) was used. The oat gum was 92% (dwb) β-glucan and <1% (dwb) total starch and pentosans.

For lichenase digestion, 2.0 g oat gum were dissolved in 200 ml 20 mM sodium phosphate buffer, pH 6.5 (3 h at 70 °C) and incubated at 50 °C for 1–2 h with 0.08–0.8 U lichenase (EC 3.2.1.73) from *Bacillus subtilis* (Megazyme, Bray, Ireland. Lot 60502. Specific activity: 118 U/mg, using 5 mg/ml barley β-glucan as substrate. Contaminants: endo-(1 → 3)-β-glucanase, cellulase and β-glucosidase <0.001%). Digests were then heated to 90 °C for 30 min to inactivate the enzyme.

For cellulase digestion, 2.0 g of oat gum was dissolved in 200 ml of 50 mM sodium acetate buffer, pH 4.7 and incubated at 50 °C for 60–75 min cellulase with 0.4–2 U of endo-cellulase (EC 3.2.1.4) from *Trichoderma reesei* (Megazyme, Bray, Ireland. Lot 50201. Specific activity: 118 U/mg at 40 °C, pH 4.5, CM-cellulose 4 M as substrate. Contaminants: endo-(1 → 3)-β-glucanase, and β-glucosidase <0.001%.). Digests were then heated to 90 °C for 30 min to inactivate the enzyme.

For acid digestion, 2.0 g oat gum was dissolved in 200 ml filtered water. Concentrated hydrochloric acid was added to bring acid concentration to 0.1 M. Samples were digested at 70 °C for 30–90 min, quickly cooled to room temperature and neutralized (pH 6–7) with 2 M NaOH.

To all digests, an equal volume of isopropyl alcohol (IPA) was added to precipitate the partially hydrolyzed β-glucan. Suspensions were centrifuged at 9700*g* for 10 min, the pellet was cut into small pieces and dewatered overnight in 100% IPA. After removal of the IPA, the samples were dried at 80 °C and ground into powder.

The samples were named according to their source (OG for oat gum), weight average molecular weight, M_w,

and the digestion method (A for acid, C for cellulase and L for lichenase).

2.2. Characterization

The purity of the samples was determined with an enzymatic analysis kit (Megazyme, Bray, Co., Wicklow, Ireland) using lichenase, β-glucosidase and glucose oxidase enzymes (McCleary & Glennie-Holmes, 1985).

Molecular weight, intrinsic viscosity, $[\eta]$, and radius of gyration, R_g, of the samples were determined by size exclusion chromatography. The chromatographic system was a Shimadzu SCL-10Avp control unit (Shimadzu Scientific Instruments, Inc., Columbia, MD) using Shodex Ohpak Kb-806M column (Showa Denko K.K., Tokyo, Japan) followed by an Ultrahydrogel linear column (Waters, Milford, CT) maintained at 40 °C and run at a flow rate of 0.6 ml/min with a buffer of 100 mM $NaNO_3$ containing 5 mM NaN_3. Samples were dissolved in water at a concentration of 1 mg/ml. Molecular weight and intrinsic viscosity distributions were determined using a Viscotek triple detector (Viscotek, Houston, TX), which had a refractive index detector, a viscometer (model 250) and a right angle laser light scattering detector. Values were calculated using TriSEC3.0 software (Viscotek, Houston, TX). A refractive index increment of 0.146 ml/g was used for the calculations. Pullulan (Shodex Std. P-82) was used as a molecular weight standard to calculate the instrument constants.

The primary structure of the polysaccharides was determined by high-performance anion exchange chromatography (Dionex, Sunnyvale, CA), using a Carbopac PA1 (4×250 mm^2) column and guard (3×25 mm^2). Detection was by pulsed amperometry with a gold electrode. Ten milligrams of β-glucan was dissolved in 5.0 ml 20 mM sodium phosphate buffer (pH 6.5) at 60 °C for 3 h. The solutions were cooled to 50 °C and incubated with 0.5 units of lichenase for 90 min. The resulting oligosaccharide solutions were filtered through a 0.45 μm filter before analysis. The eluents used were A: 150 mM sodium acetate in 150 mM NaOH and B: 150 mM NaOH with a flow rate of 1 ml/min and the column was preconditioned with 30% A:70% B for 5 min before the oligosaccharide mixture was applied. After injection, the eluent ratio was maintained at 30% A:70% B for 5 min and then a linear gradient was applied to change the ratio to 100% A over the next 8 min. The column was then cleaned with 100% eluent A for 12 min before preparing the column for the next sample. The pulse potentials, E, and durations, T, were $E_1 = 0.05$ V and $T_1 = 480$ ms during the measuring steps and $E_2 = 0.60$ V, $T_2 = 180$ ms, $E_3 = -0.60$ V, $T_3 = 60$ ms during the cleaning step. The response time was 1 s. The peaks were identified using glucose laminaribiose, cellodextrins (Seikagaku Corporation, Tokyo, Japan) and lichenase digests of standard β-glucans. The peak areas and retention times were calculated using Dionex software (ACI-BioLC, Sunnyvale, CA).

2.3. Rheology

Rheological measurements were made on 6% (w/v) solutions of partially hydrolyzed β-glucan. The concentration was more than $4/[\eta]$ and therefore, above the coil overlap concentration for the nine solutions (Robinson, Ross-Murphy, & Morris, 1982). Solutions were prepared by heating in 10 mM sodium phosphate buffer containing 1 mM sodium azide at 80 °C for 3 h and cooled to 25 °C. Using a controlled stress rheometer (CVO, Bohlin Instruments USA, NJ) fitted with a Peltier plate to control the temperature, the apparent viscosity was measured using a steel cone and plate (4°, 4 cm) from rest to a shear rate of 500 s^{-1} and back to 0.05 s^{-1}. Portions of the solutions were poured into Teflon lined moulds 1 cm in diameter and 3 mm deep. The moulds were sealed and stored at 5 °C for 1, 4 or 7 days. Aged solutions or gels were transferred to the Peltier plate of the rheometer (precooled to 5 °C) and the mechanical spectra were measured using a 1 cm flat plate (gap 2.5 mm) in control strain mode at 0.5% strain, which was determined to be within the linear viscoelastic range. Then, measurements were taken at constant frequency of 1 Hz as the gels were heated at 5 °C/min until the storage modulus, G', was equal to the loss modulus, G'', which was considered to be the melting temperature. A heating rate of 5 °C/min was shown to give melting temperatures that were not different from those observed at heating rates of 1 and 2 °C/min. All samples were run in quadruplicate.

2.4. Differential scanning calorimetry

Solutions were prepared in the same manner as for the rheological measurements and held at 5 °C for 4 or 7 days. Eighty to 90 mg of solution or gel (4.8–5.4 mg of β-glucan) was weighed into a high volume stainless steel pan and sealed. Samples were heated from 5 to 120 °C in a Differential Scanning Calorimeter (Model 2920, TA Instruments Inc., New Castle, DE) using 10 mM sodium phosphate/1 mM NaN_3 buffer in the reference pan. The endotherms were analyzed using the software (Universal Analysis, TA Instruments, Inc., New Castle, DE) provided with the instrument. Integration limits for the enthalpy, ΔH, determination were set at 50 and 80 °C. The onset temperature, T_o, and the peak temperature, at which heat flow was minimum, T_p, were also determined. Six replicates of each sample were run.

2.5. Data analysis

Significant differences between measurements were determined using SAS V. 7.0. (SAS Institute, Cary, NC). A completely randomized block design was used with

Table 1
Molecular weight (g/mol), polydispersity index, intrinsic viscosity (dl/g) and radius of gyration (nm) of original oat gum and partial hydrolysates

Sample	M_w [a]	M_p [b]	Pd[c]	$[\eta]_w$ [d]	R_{g_w} [e]
Oat gum	1,190,500	1,201,000	1.20	8.29	69.22
OG168A	167,900	154,700	1.72	2.43	23.31
OG88A	88,200	86,800	1.53	1.73	16.84
OG31A	30,800	25,900	1.24	0.73	9.07
OG205C	204,500	177,400	1.73	2.65	25.45
OG89C	89,100	85,500	1.50	1.78	16.94
OG40C	39,900	36,200	1.34	1.01	10.96
OG237L	236,900	206,900	1.72	3.12	28.40
OG99L	98,500	87,000	1.60	1.48	16.51
OG38L	37,500	32,200	1.40	0.88	10.16

[a] M_w : weight average molecular weight.
[b] M_p : peak molecular weight.
[c] Pd: polydispersity, the ratio of the weight average to number average molecular weights.
[d] $[\eta]_w$: weight average intrinsic viscosity.
[e] R_{g_w} : weight average radius of gyration.

analysis of covariates. A confidence level of 95% was required for differentiation of samples.

3. Results

3.1. Characterization

The purity of the oat β-glucan partial hydrolysis products was not affected by the hydrolysis process. Like the unhydrolyzed oat gum, the purity was 92 ± 2% (dwb).

The oat β-glucans were divided into three groups, with molecular weights about 200 000, 100 000 and 40 000 g/mol. The molecular weights, Pd index, intrinsic viscosity and radius of gyration are given in Table 1. The Pd of the β-glucans decreased as the molecular weight decreased ($p < 0.05$) but was not significantly different between samples of the same molecular weight ($p > 0.1$). This indicates that the enzymatic depolymerization methods produced polymers with a similar size distribution to the hydrolysis caused by acid treatment. All of the methods produced a size distribution that was wider than the original oat gum (Pd = 1.2).

The primary structure of the original oat gum and the hydrolysates represented by the lichenase-released oligosaccharides analyzed is shown in Table 2. As expected, the oligosaccharide fingerprint of the partial hydrolysis products made with lichenase was the same as the fingerprint of the original oat gum because the oligosaccharides were produced using exhaustive lichenase digestion. Both the acid and cellulase partial hydrolysis products yielded more cellobiose and laminaribiose fragments than the original gum or the partially lichenase digested products. Additionally, acid and cellulase digestion produced cellotriose oligosaccharides not present in the original oat gum and the most extensive cellulase digestion also produced some cellotetraose.

There were no peaks in the controls without lichenase treatment for the original oat gum but the hydrolysis product controls contained small but detectable amounts of material with the same retention time as the DP3 and DP4 lichenase products. The controls from the enzyme hydrolysates also contained cellobiose, but the acid hydrolyzed material did not. None of the controls contained cellotriose or cellotetraose fragments.

Extensive digestion with cellulase (OG40C) or acid (OG31A) significantly decrease the levels of nonasaccharides released from the cellulose-like regions found in (1 → 3)(1 → 4)-β-D-glucans. This DP9 material is cellodextrin like in behavior and essentially water insoluble (Wood, Weisz, & Blackwell, 1994b), so the peak probably represents just a portion of the oligosaccharide of DP9 or greater. The acid hydrolysates yield more glucose than the other digests.

The sum of the DP3 and DP4 remained unchanged at 92.3 ± 0.60% of the total area. The molar ratio of DP3:DP4 was also unaffected by the hydrolysis method and remained 2.36 ± 0.02 for the oat gum and the partial hydrolysates. These values are not corrected for the different response factors of the oligosaccharides (Wood et al., 1994b).

Table 2
Oligosaccharides derived from oat gum and partial hydrolysates by complete digestion with lichenase

Sample	Glucose	Cellobiose	Laminaribiose	Cellotriose	Cellotetraose	DP3	DP4	DP5	DP6	DP7	DP8	DP9
Oat gum	0.06	0.03	0.13	0.00	0.00	59.84	33.21	2.65	1.86	0.56	0.62	1.05
OG168A	0.15	0.12	0.24	0.00	0.00	58.97	32.92	2.99	2.05	0.63	0.76	1.18
OG88A	0.19	0.19	0.27	0.00	0.00	59.07	33.13	2.83	1.91	0.61	0.72	1.08
OG31A	0.43	0.53	0.44	0.37	0.00	58.66	33.69	2.46	1.50	0.53	0.58	0.81
OG205C	0.03	0.11	0.22	0.12	0.00	58.63	33.01	3.06	1.98	0.75	0.85	1.22
OG89C	0.02	0.30	0.28	0.45	0.00	58.57	32.76	3.05	1.87	0.84	0.87	0.98
OG40C	0.00	0.70	0.38	1.00	0.20	59.30	32.67	2.31	1.42	0.91	0.54	0.59
OG237L	0.04	0.05	0.14	0.00	0.00	59.80	33.43	2.58	1.79	0.53	0.64	0.99
OG99L	0.03	0.05	0.14	0.00	0.00	59.47	33.19	3.04	1.87	0.59	0.62	1.01
OG38L	0.02	0.07	0.11	0.00	0.00	58.66	33.40	3.13	1.96	0.66	0.80	1.20

Quantities are expressed as relative percentage of total area under oligosaccharide peaks. DP3: 3-*o*-β-cellobiosyl-D-glucose, DP4: 3-*o*-β-cellotriosyl-D-glucose and longer oligosaccharides follow the same pattern.

3.2. Rheology

The apparent viscosity was similar for samples of comparable molecular weight (Fig. 1). The largest hydolysates showed shear thinning behavior at shear rates greater than 1 s^{-1}. The largest molecular weight β-glucan, OG237L, exhibited a large decrease in apparent viscosity at high shear rates. The partial hydrolysates with molecular weights near 100 000 g/mol showed little shear thinning below shear rates of 100 s^{-1}. The low molecular weight β-glucans exhibited hysteresis, with the apparent viscosity higher as the shear rate was increasing than when it was decreasing.

There is a power-law relationship between the apparent viscosity at 10 s^{-1} (increasing) and M_w (Fig. 2) demonstrating that the differences in solution properties result from the differences in molecular weight rather than the hydrolysis method. The slope of the graph is close to what might be expected for random coil polysaccharides above the critical (entanglement) concentration where zero shear rate specific viscosity is approximately proportional to $c[\eta]^{3.9}$, where c is concentration and $[\eta]$ is intrinsic viscosity. The exponent of the relationship with molecular weight is modified according to the Mark–Houwink relationship between intrinsic viscosity and molecular weight, and by the fact that we did not use zero shear rate viscosity.

The strength of the gels was characterized by the storage modulus, G', and the viscosity of the sol was characterized by the loss modulus, G''. For the purposes of this experiment, a gel was defined as a material where G' was fairly insensitive to frequency (small positive slope) and $G' > G''$ at 1 Hz (Normand, Muller, Ravey, & Parker, 2000).

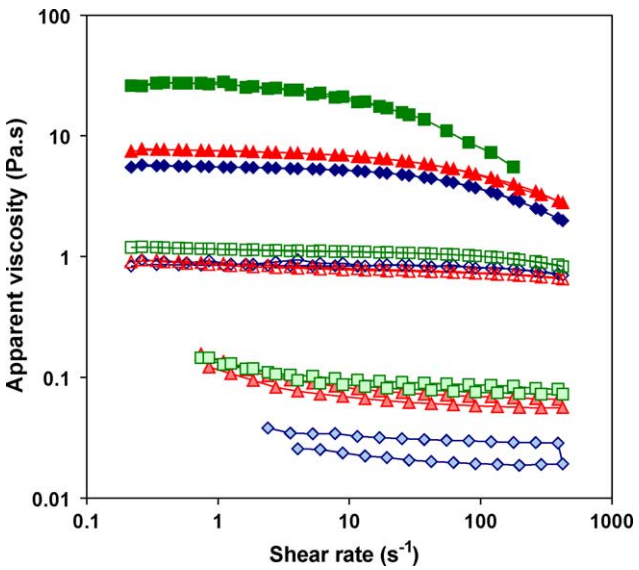

Fig. 1. Apparent viscosity of freshly dissolved 6% β-glucan partial hydrolysate solutions at 25 °C, showing ascending and descending shear rate values. Symbols: ◆—OG168A, ◇—OG88A, —◆—OG31A, ▲—OG205C, △—OG89C, —◇—OG40C, ■—OG237L, □—OG99L, and —◇—OG38L.

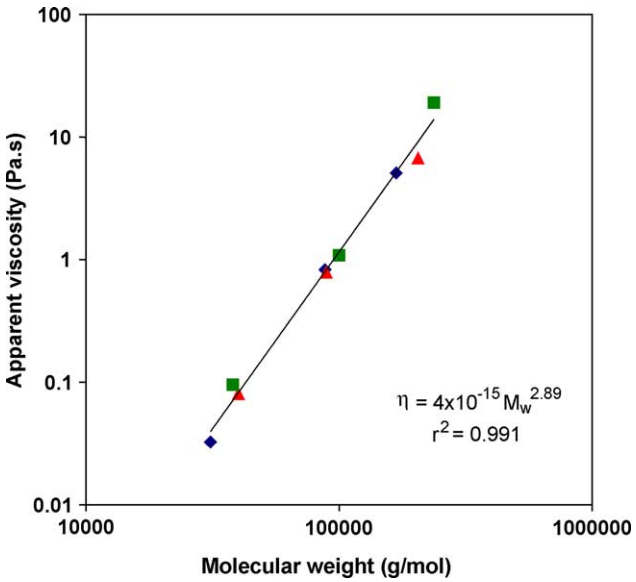

Fig. 2. Apparent viscosity of 6% β-glucan partial hydrolysate solutions at 10 s^{-1}. ◆—acid hydrolyzed, ▲—cellulase hydrolyzed, and ■—lichenase hydrolyzed.

Partially hydrolyzed oat β-glucan solutions gel slowly, requiring more than 6 h at 5 °C for even the fastest gelling product. Molecular weight was the most influential factor in determining the gelling time and gel strength. Two of the low molecular weight β-glucans (OG31A and OG40C, ~40 000 g/mol) gelled within 24 h. After 4 days aging at 5 °C, two of the four replicates of the medium molecular weight β-glucans (~100 000 g/mol) had gelled. The highest molecular weight β-glucans (~200 000 g/mol) remained liquid after 7 days.

The storage modulus at 1 Hz is shown in Fig. 3. The gel elasticity, as indicated by G', increased as the molecular

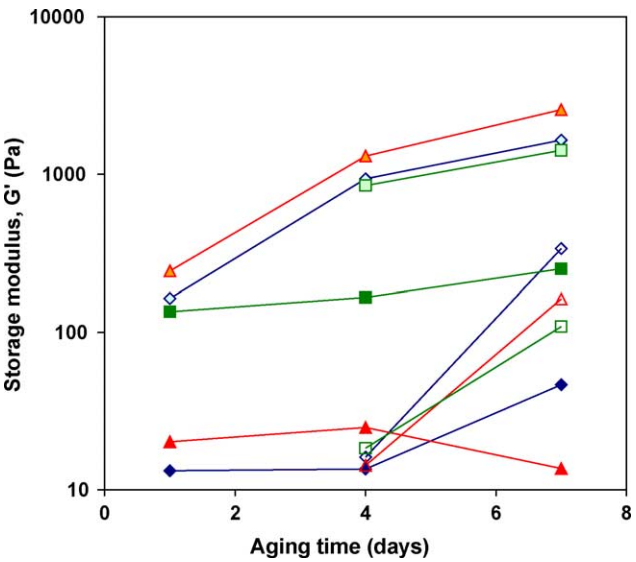

Fig. 3. Change in storage modulus, G', at 1 Hz of β-glucan solutions/gels over 7 days storage at 5 °C. Symbols: ◆—OG168A, ◇—OG88A, —◆—OG31A, ▲—OG205C, △—OG89C, —◇—OG40C, ■—OG237L, □—OG99L, and —◇—OG38L.

weight decreased. After 7 days storage at 5 °C, the lowest molecular weight cellulase product (OG40C) had the highest G', 2570 ± 100 Pa at 1 Hz. The lowest molecular weight β-glucans produced using acid and lichenase produced less elastic gels with $G' = 1618 \pm 260$ and $G' = 1420 \pm 390$ Pa, respectively. The medium molecular weight samples had formed weak gels by day 7; the elasticity of these gels appeared independent of the hydrolysis method. The average G' was 270 ± 90 Pa. The moduli of the largest molecular weight partial hydrolysates did not vary significantly over 7 days. The largest molecular weight hydrolysate, OG237L, had a G' of 180 ± 50 Pa which was much higher than the other liquid samples. This apparent elasticity possibly relates to the greater entanglements of this largest molecular weight sample.

We were unable to compare plateau values of G' in this study since it is unlikely that the medium or large partial hydrolysates have reached plateau values after 7 days.

Average frequency sweeps for the β-glucans on day 7 are shown in Figs. 4–6. For all three hydrolysis methods, the G' for the lowest MW partial hydrolysates was significantly larger than the other β-glucans and quite frequency independent. The G'' for these β-glucans was nearly an order of magnitude lower and had a small positive slope. This behavior is typical of elastic gels. The medium molecular weight β-glucans, OG88A, OG89C and OG99L, had frequency independent storage moduli which were much lower than the lowest molecular weight β-glucans but the loss moduli were fairly similar. This indicates that the solutions have gelled but are not as elastic. The storage moduli of the highest molecular weight β-glucans were less than the loss moduli over the range of frequencies tested and there were large positive slopes on both moduli, which is behavior typical of entangled semi-dilute polymer solutions. Thus a wide range of behaviors, from elastic gel to viscous liquid, was observed in the molecular weight range examined, 31 000–237 000 g/mol, after 7 days aging.

The tan delta values for the β-glucans measured at 1 Hz (Fig. 7) confirm that small molecular weight partial hydrolysates gelled more rapidly, and after 7 days aging, had formed more elastic gels. The tan δ of the largest molecular weight hydrolysate, OG237L, remained above 1

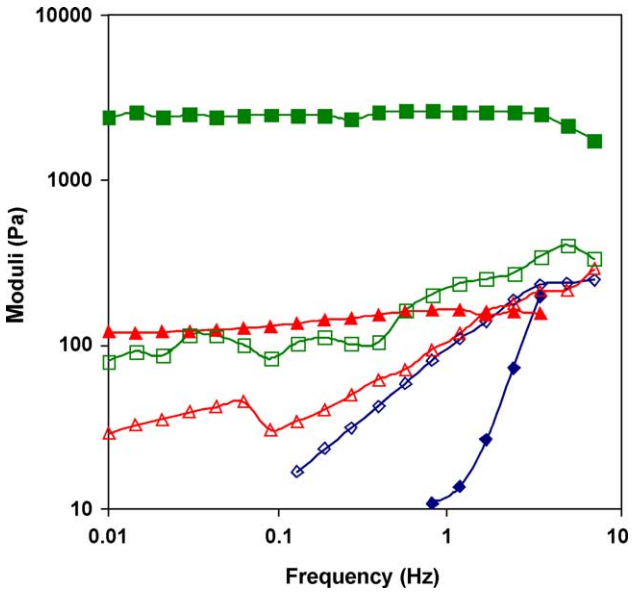

Fig. 5. Frequency sweeps of oat β-glucan solutions/gels partially hydrolyzed using cellulase. Aged 7 days at 5 °C. Symbols: OG205C: ◆—G'. ◇—G''; OG89C: ▲—G', △—G''; OG40C: ■—G', □—G''.

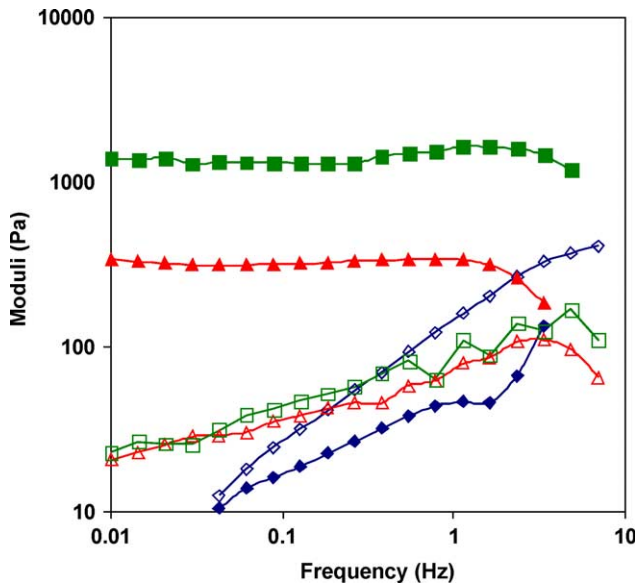

Fig. 4. Frequency sweeps of oat β-glucan solutions/gels partially hydrolyzed using acid. Aged 7 days at 5 °C. Symbols: OG168A: ◆—G'. ◇—G''; OG88A: ▲—G', △—G''; OG31A: ■—G', □—G''.

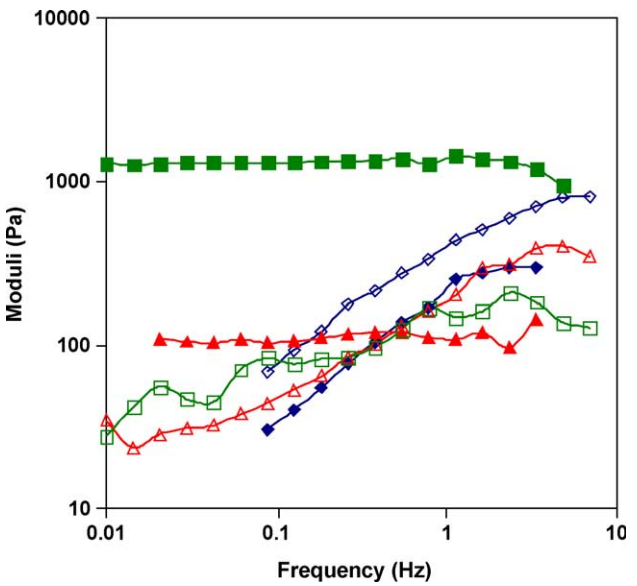

Fig. 6. Frequency sweeps of oat β-glucan solutions/gels partially hydrolyzed using lichenase. Aged 7 days at 5 °C. Symbols: OG237L: ◆—G'. ◇—G''; OG99L: ▲—G', △—G''; OG38L: ■—G', □—G''.

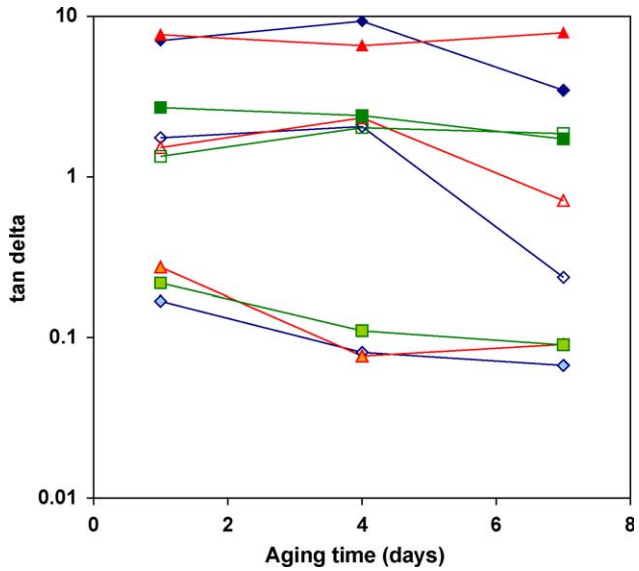

Fig. 7. Changes in tan delta of oat β-glucan partial hydrolysate solutions/gels at 1 Hz aged 7 days at 5 °C. Symbols: ◆—OG168A, ◇—OG88A, —◆—OG31A, ▲—OG205C, △—OG89C, —◇—OG40C, ■—OG237L, □—OG99L, and —◇—OG38L.

throughout the investigation, verifying that the high numerical value of G' is a result of entanglements rather than network formation. Because of the low viscosity of the ungelled β-glucans on day 1 it was not possible to accurately measure the phase angle of the solutions OG38L, OG88A, OG89C and OG99L.

Melting temperatures of the gels were measured using both small amplitude rheometry and differential scanning calorimetry. The rheometric measurements showed an increase in melting temperature over time (Fig. 8). After 1 day at 5 °C, the lowest molecular weight β-glucans had melting points of 57.7 ± 0.9 °C for cellulase digested β-glucan and 55.8 ± 0.6 °C for acid hydrolyzed β-glucan. Melting points for the other samples could not be measured on day 1 because they had not gelled. After 4 days aging, two replicates of each of the medium molecular weight β-glucans had gelled and two had not. The average melting temperature of the two replicates which had gelled are shown on Fig. 8. On day 7, the melting temperatures ranged from 57.5 ± 0.6 °C for OG31A to 59.5 ± 0.8 °C for OG89C. Overall, the cellulase treated β-glucans had significantly higher melting points than acid or lichenase treated β-glucans ($p = 0.04$).

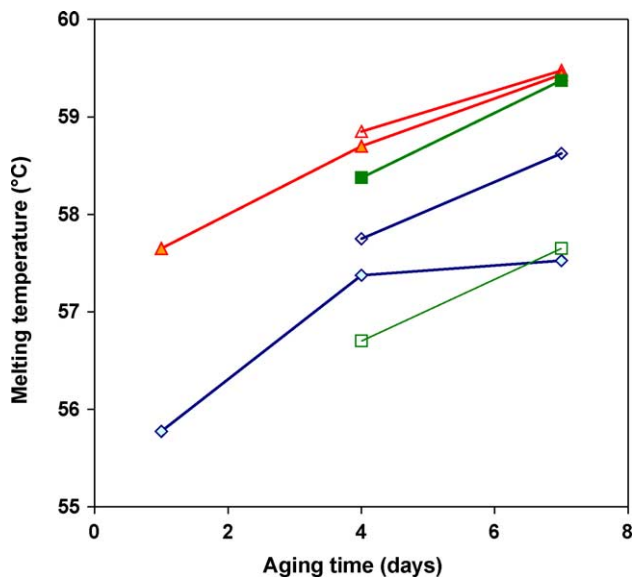

Fig. 8. Melting temperatures of oat β-glucan partial hydrolysate gels as measured by small amplitude oscillatory rheology (tan $\delta = 1$ at 1 Hz) aged 7 days at 5 °C. Symbols: ◇—OG88A, —◆—OG31A, △—OG89C, —◇—OG40C, □—OG99L, and —◇—OG38L.

3.3. Differential scanning calorimetry

The DSC method had the advantage of giving information about liquid as well as gelled samples. The endotherms were very broad, with melting occurring over a 30 °C range (Fig. 9), which is consistent with the findings of Morgan and Ofman (1998). Many of the endotherms had shoulders either side of the peak heat flow value indicating that there is more than one type of interaction. The onset temperatures, T_o, shown in Fig. 10, were between 52.8 and 56.1 °C after 4 days of aging at 4 °C and increased to between 55.2 and 58.8 °C after 7 days aging. The medium molecular weight β-glucans exhibited the highest T_o on both days ($p = 0.0006$). T_p did not change between 4 and 7 days aging time (Fig. 11). T_p for the lowest molecular weight partial hydrolysates was the lowest indicating that they had the least stable junction zones. The rheological measurements of the bulk properties of the gels as they melted appeared to reflect the behavior of T_o rather than T_p.

The enthalpy, ΔH, values (Fig. 12) were rather variable even within a single sample, suggesting that there may be some segregation of the polymers within the gel matrix similar to the segregation found by Johansson et al. (2000).

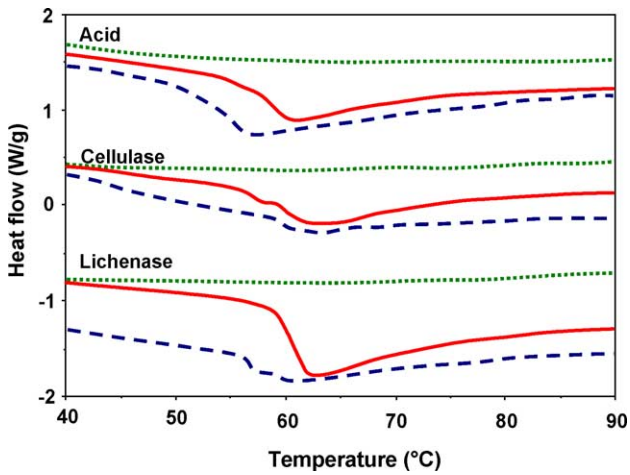

Fig. 9. Typical endotherms measuring melting of interactions in solutions/gels of partially hydrolyzed oat β-glucan held at 5 °C for 7 days. Symbols: … Large, — Medium, – – – Small.

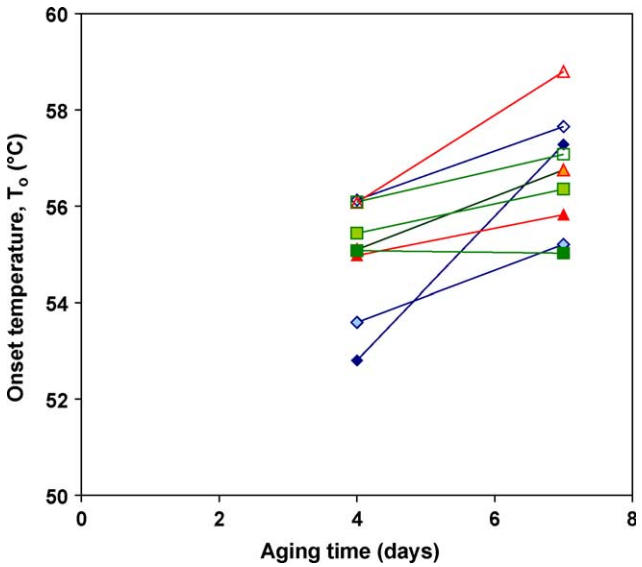

Fig. 10. Onset of melting temperature as measured by differential scanning calorimetry. Symbols: ◆—OG168A, ◇—OG88A, —◆— OG31A, ▲—OG205C, △—OG89C, —◇—OG40C, ■—OG237L, □—OG99L, and —◇—OG38L.

Nevertheless, there was a significant difference between the ΔH in gelled and liquid samples, with liquid samples having an ΔH of 1–5 J/g and gels having an ΔH of >10 J/g. The fact that liquid samples have a measurable ΔH shows that junction zones were forming between the polymers but not in sufficient number to form a gel network.

The breadth of the endotherm provides further clarification to the results of a previous paper where solubilization temperature was found to affect the rheological properties of 6% oat β-glucan solutions and gels (Tosh, Wood, & Wang, 2003). When partially hydrolyzed oat β-glucans were solubilized at 60 °C, stable aggregates caused shear thickening behavior at low shear rates. When the solubilization temperature was raised to 90 °C, the storage modulus of the gels produced were significantly lower than when the β-glucans were solubilized at 60–80 °C. The endotherms show that there are intermolecular interactions which remain stable up to 80 °C, these interactions might act as junction points in the developing gel network and facilitate gel development.

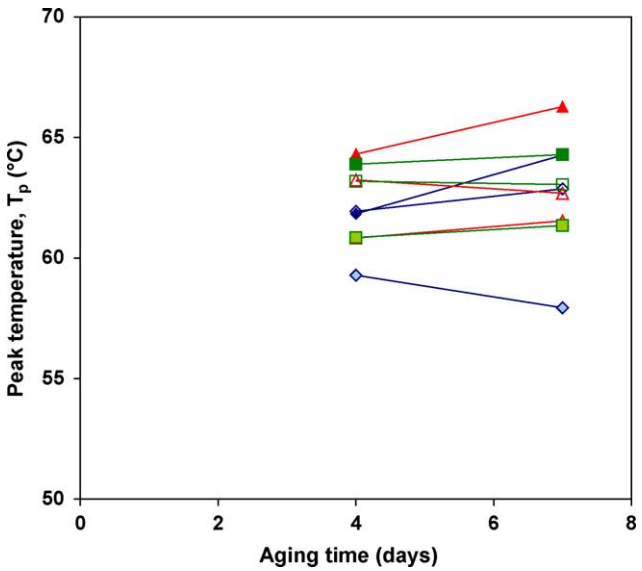

Fig. 11. Temperature at which peak heat flow is observed during melting in differential scanning calorimetry. Symbols: ◆—OG168A, ◇—OG88A, —◆—OG31A, ▲—OG205C, △—OG89C, —◇—OG40C, ■—OG237L, □—OG99L, and —◇—OG38L.

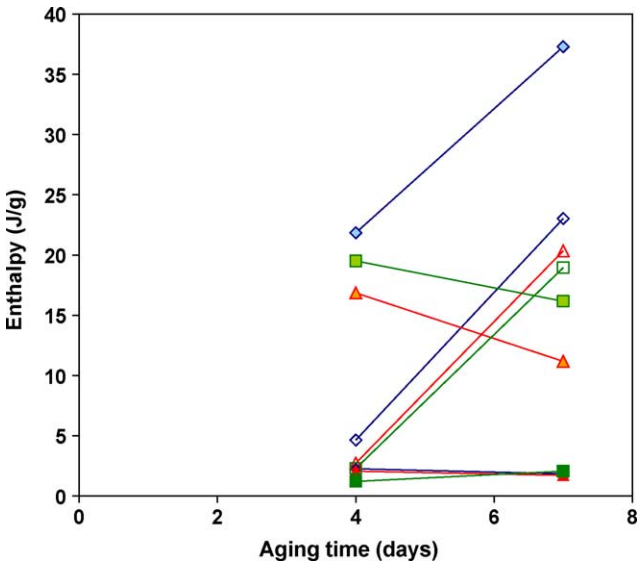

Fig. 12. Melting enthalpy, ΔH measured by differential scanning calorimetry. Symbols: ◆—OG168A, ◇—OG88A, —◆—OG31A, ▲—OG205C, —◇— OG89C, △—OG40C, ■—OG237L, □—OG99L, and —◇—OG38L.

4. Discussion

In evaluation of the oligosaccharides produced by exhaustive lichenase treatment of the different β-glucans, it is assumed that this endo enzyme is able to act on linkages at or close to the chain ends with the same specificity as elsewhere in the chain.

Partial lichenase treatment should result in partially hydrolyzed β-glucans with three-linked glucose at the reducing end and the non-reducing end glucose attached to the four-position of the penultimate glucose. However, chains that initially had one four-linked glucose at the reducing end and the non-reducing end glucose attached to the three-position of the penultimate glucose, unless the partial lichenase hydrolysis had taken place at or close to either or both of these chain ends, would mostly survive with at least one of these features. Subsequent exhaustive lichenase treatment of these latter chains would release glucose from the reducing end and laminaribiose from the non-reducing end. Cellobiose or higher cellodextrins would arise from chains that initially had more than one four-linked glucose on the reducing end. These are chain end features and so will be present in only small amounts and

would not increase as the partial hydrolysis with lichenase proceeds further. Therefore, the pattern of these oligosaccharides from the initial 'native' β-glucan and the partial hydrolysates from lichenase would not be expected to change much. This is what was observed (Table 2). Additionally, the higher oligosaccharides terminated by a three-linked glucose should be essentially preserved in the partial hydrolysates, again as observed.

The cellulase used requires two consecutive β-(1 → 4)-linked glucose units to act and does not cleave the β-(1 → 3)-glucosidic linkage (McCleary & Matheson, 1986). Whenever a (1 → 4)-glycosidic linkage of a glucose attached through the four-position to a three-substituted glucose is cleaved by cellulase, a reducing end glucose attached through the four-position to a three-substituted penultimate glucose is produced, and this chain end then yields glucose on exhaustive lichenase treatment. The non-reducing ends of cellulase-released chains give the usual oligosaccharides on lichenase treatment unless the non-reducing end glucose is linked to the three-position of the penultimate unit; this yields laminaribiose on exhaustive lichenase treatment. Reducing ends produced from cleavage of the (1 → 4)-glycosidic linkage of a glucose attached through the four-position to more than one additional four-linked glucose will yield cellobiose (from the β-(1 → 3)-linked cellotetraosyl units) and cellotriose, cellotetraose and higher cellodextrins from the areas with the longer runs of contiguous β-(1 → 4)-linked glucose units. Thus glucose, laminaribiose, cellobiose, cellotriose and cellotetraose would all be expected to increase with degree of partial hydrolysis, as observed in Table 2. Thus, cellotriose arises mainly from the β-(1 → 3)-linked cellopentaosyl units which yield the DP5 lichenase product, since of the DP5–9 products, this is present in the greatest amount. Cellotetraose can only arise from the (1 → 3)-linked cellohexaosyl (or higher) structures, which are the structures, which yield DP6 or higher lichenase products, present in much lesser amounts. In other words, cellodextrins released by lichenase from reducing chain ends formed by partial cellulase hydrolysis in this fashion always are a DP2 less than the oligosaccharide that would have been produced by lichenase from that point in the intact chain. Cellulase can, of course, act at any point along the runs of (1 → 4) linkages, allowing production of lower DP cellodextrin like structures. In summary, the β-(1 → 3)-linked cellodextrin structures of the intact chain can be cleaved to give reducing chain ends with single or multiple (1 → 4)-linked glucose units which, on lichenase treatment, yield glucose, cellobiose and the higher cellodextrins. However, cellodextrins of DP7 or higher are water insoluble and would likely not be detected.

Finally, it would be expected that the cellulase might have a somewhat greater affinity for the more cellulose-like parts of the β-glucan, in which case the areas of >3 contiguous (1 → 4)-linked glucose units would be attacked preferentially. There is some indication that this was so, with the most cellulase hydrolyzed material.

β-Glucans from partial acid hydrolysis would be expected to give much the same products as the partial cellulase hydrolysates. However, the acid can also cleave the (1 → 3) linkages, and this would give chains with reducing ends that yield cellobiose and higher cellodextrins, in this case the cellodextrins possible would be just one unit less in DP than the oligosaccharide that would have been produced by lichenase from the intact chain. This should lead to more of the higher cellodextrins in the lichenase digest of the partially acid hydrolyzed β-glucans than the digests from the cellulase treatments. This was not observed, and suggests that the acid hydrolysis did not cleave the (1 → 3) and (1 → 4) linkages in random fashion according to the percentage of each (~30 and ~70%, respectively).

At the non-reducing end, from a glucose attached to the three-position of the penultimate glucose, laminaribiose will be produced in increasing amount as the acid hydrolysis proceeds, similarly to cellulase hydrolyzed products. This was observed.

Although the occurrence of laminaribiose in exhaustive lichenase hydrolysis products can be explained by the presence of a (1 → 3)-linked glucose at the non-reducing end, this does not preclude the possibility of this product arising from an internal feature in which there are some alternating (1 → 3)- and (1 → 4)-linked glucose units (Roubreks, Mastromauro, Andersson, Christensen, & Aman, 2000), although the increase observed following cellulase (Wilhelmi & Morgan, 2001) and acid hydrolysis must be from the end units.

The data given in Table 2 is uncorrected for response factors (which have not been accurately determined because of lack of crystalline standards). However, it is known that the response factors decline rapidly from glucose to disaccharides, significantly again to trisaccharide but then decline less with each further increase in number of units (Wood et al., 1994b). Thus compared to the major tri- and tetrasaccharide products, the relative percentages of glucose, cellobiose and laminaribiose are less than the area proportions reported in Table 2, and the percentages of the higher oligosaccharides somewhat more.

The general shape of the flow curves in Fig. 1 is similar to those found for lower concentrations of unhydrolyzed and hydrolyzed oat β-glucans (Doublier & Wood, 1995). The differences in response to shear between the different sizes of polymers must result from very transitory entanglements, less transitory hydrogen bonding of the molecules, more stable regions of multiple hydrogen bonded polymer sections and also aggregated gel particles. The time scale of breakage and reformation of these different types of associations will differ. For longer polymers, diffusion rates slow, limiting the build-up of long sections of associated units, but because of significant coil overlap there is considerable entanglement involving

perhaps several molecules. When stress is applied, the molecules must move past each other for flow to occur and the entanglements and transitory associations may be broken and reform in this time scale. As the strain rate is increased, entanglements and transitory associations are unable to reform, and the apparent viscosity decreases. For the medium length molecules, there is less entanglement because of the lower volume occupancy and degree of coil overlap. Consequently, the apparent viscosity is lower than for the same concentration of longer polymers at the same shear rate. Also, since there are fewer entanglements to be broken and reform there is less shear thinning over the range of shear rates used. The behavior of the solutions of smaller polymers was more like that of a weak gel. The apparent viscosity dropped rapidly with increasing shear, at low shear rates, as transitory molecular associations were disrupted by shear stress. Fully formed junction zones leading to gel characteristics have not yet formed, but there are more transitory associations of partially formed junction zones, which behave differently from simple entanglements and will not reform in the time scale. Aggregates, or microgel particles, detectable by DSC, were subject to deformation and breakdown with shear. As the shear strain rate increased, simple entanglements will also not reform and any remaining transitory network would be broken. When strain rate was decreased, the transitory associations would not reform immediately so the apparent viscosity was lower than when the strain rate was increasing (Rodd, Davis, Dunstan, Forrest, & Boger, 2000). The hysteresis loop resulted from the time required to rebuild the structure and possibly also because structures formed in a shear field were different from those formed in a quiescent system. It is interesting to note that as the shear rates decreased below 10 s^{-1}, structures appeared to be forming (or reforming) as indicated by an increase in the apparent viscosity.

Molecular weight of oat β-glucan had a strong effect on the gel elasticity. Unlike other gelling polysaccharides, such as carrageenan (Rochas, Rinaudo, & Landry, 1990) and agar (Suzuki, Sawai, & Takada, 2001), lower molecular weight preparations of oat β-glucan produced more elastic gels than those with higher molecular weight. Lower molecular weight amylose also exhibits increased gel elasticity. The storage modulus of 2% amylose decreases as the molecular weight was increased from 18 000 to 405 000 g/mol (Clark, Gidley, Richardson, & Ross-Murphy, 1989). These authors suggested, "For such long chains, relatively few cross-links are required before aggregate diffusion becomes significantly retarded, thereby slowing down subsequent cross-linking and modulus increase". It would certainly appear that the development of a gel network in oat β-glucan solutions is diffusion limited. Time is also an important consideration in the development of gel structure and with higher molecular weight samples the time to reach a maximum G' may become considerable. We did not determine plateau values.

The formation of intermolecular associations leading to junction zones, and a gel network in polysaccharide solutions, generally requires areas of structural regularity. In cereal β-glucans the most obvious candidate for this has been the cellulosic-like areas of the molecule, which when released by lichenase from the polymer are observed to form precipitates, as would cellodextrins of similar DP. However these regions are present in small amounts by weight (less on a molar basis). The dominant structural feature is β-(1 → 3)-linked cellotriosyl units, which are present in about twice the molar proportion of the β-(1 → 3)-linked cellotetraosyl units, and although the distribution of these two structural features has been determined to be random (Staudte, Woodward, Fincher, & Stone, 1983), statistically there must be a strong likelihood of areas of regularity, primarily of the β-(1 → 3)-linked cellotriosyl units, and less frequently also of the β-(1 → 3)-linked cellotetraosyl units. The formation of new junction zones can only occur when two reactive regions diffuse into positions where they can interact. Since the diffusion rate of small molecules is faster than large ones, and entanglement of longer chains further retards motion, the probability of two reactive sites coming into close proximity is greater in lower molecular weight β-glucans, and the greatest likelihood is that these are sites of consecutive β-(1 → 3)-linked cellotriosyl units. X-ray fiber and conformational analysis of lichenan indicated a 3-fold helix of β-(1 → 3)-linked cellotriosyl units with 6 units per cell in antiparallel association. The less regular barley β-glucan was similar but the pattern indicated a lower crystallinity. No pattern was observed with a sample of oat β-glucan (Chandrasekaran, personal communication), presumably because the increased proportion of β-(1 → 3)-linked cellotetraosyl units was lessening the statistical occurrence of consecutive β-(1 → 3)-linked cellotriosyl units.

There is some indication that partial hydrolysis with cellulase enhanced gelation more than partial hydrolysis with lichenase. OG38L had not gelled after 1 day of aging at 4 °C while, OG40C and OG31A did and OG38L consistently had lower G' values than OG40C. Also, partial hydrolysates produced with cellulase had the highest onset temperatures indicating junction zones with higher activation energies.

The endo-(1 → 4)-β-D-glucanase used in this study requires adjacent β-(1 → 4)-linked glucose units and may have a preference for longer runs of contiguous β-(1 → 4)-linked glucose units (Roubroeks, Andersson, Mastromauro, Christensen, & Aman, 2001; Wilhelmi & Morgan, 2001). The significant quantities of cellotriose and cellotetraose end units in the cellulase partial hydrolysates would tend to confirm this observation since cellotetraose units can only be produced from blocks with DP ≥ 5. Thus, the cellulase preferentially cleaves the cellulose-like blocks and leaves more of the DP3 units intact. Because the lichenase can cleave only β-(1 → 4) linkages adjacent to a β-(1 → 3) linkage it would disrupt more DP3 units. The stronger

propensity of partial hydrolysates produced with cellulase to gel would tend to support the model presented by Böhm and Kulicke (1999b), where the junction zones are consecutive cellotriose units rather than the alternate model where the long cellulose-like sequences form the junction zones.

The increase in melting temperature and endothermic onset temperature over time would indicate that the junction zones of the gel network were becoming more stable. Junction zones with low activation energies may have been strengthened by the formation of more hydrogen bonds or disrupted to allow for the reinforcement of other interaction points. The breadth of the endotherm and the shoulders on the peaks imply that junction zones with varying numbers of hydrogen bonds (varying lengths of interacting polymer) are possible. However, the constancy of T_p shows that there is one dominant structure in the network.

5. Conclusion

It would appear that 6% oat β-glucan solutions with $M_w < 150\,000$ g/mol will form gels at 5 °C in less than a week. Although some associations appear to form in solutions with higher molecular weight, restriction of diffusion of the polymers in the solution prevents the formation of a complete gel network in an experimentally reasonable period of time. The time required for a gel to form decreased as the molecular weight was reduced. The hydrolysis method had less effect on the gelation behavior than the molecular weight. Partial hydrolysates produced using cellulase appeared to have more stable junction zones and the small molecular weight polymers gelled more quickly than similar molecular weight products from lichenase hydrolysis. As one might expect, the non-specific acid hydrolysis produced β-glucans showed behavior intermediate between the two enzymatic treatments and were not significantly different from them. The higher tendency of the cellulase treated β-glucans to gel would support the consecutive cellotriose junction zone model as the major underlying mechanism involved in gel formation.

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