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Structure and physicochemical properties of barley non-starch polysaccharides — I. Water-extractable β-glucans and arabinoxylans[1]

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Water-soluble non-starch polysaccharides were extracted from a Canadian malting barley (cv. Harrington) by sequential treatment with water at 40°C (WE40) and 65°C (WE65). The yields were 1.4 and 1.3% (w/w), respectively, of the dry barley grist. The WE40 extract was composed of 82.5% glucose, 8.9% xylose, and 7.0% arabiose residues, whereas WE65 contained 93.3% Glc, 3.3% Xyl, and 2.5% Ara. Only minute amounts of mannose and galactose residues were found in either fraction. Both extracts were further fractionated by stepwise $(NH_4)_2SO_4$ precipitation into several polysaccharide populations. Subfractions from both extracts, obtained up to 45% saturation with $(NH_4)_2SO_4$, contained mostly β-glucans, whereas subfractions precipitated at increasing saturation levels of $(NH_4)_2SO_4$ (45–100%) contained progressively more arabinoxylans and less β-glucans. Compared to WE40, the WE65 extract was enriched in β-glucan populations with higher molecular size, higher limiting viscosity values, and higher content of β-(1 → 4) linkages. The ratio of tri-/tetrasaccharide oligomers was also higher in β-glucans extracted at 65°C than those extracted at 40°C. Arabinoxylans in both extracts, WE40 and WE65, were highly substituted and contained large proportions of doubly substituted xylose residues.
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INTRODUCTION

The two major non-starch polysaccharides in barley are mixed linkage β-glucans and arabinoxylans (Henry, 1987). β-Glucans are present, predominantly, in endosperm cell walls (Fincher, 1975) with smaller amounts in aleurone cell walls (Bacic and Stone, 1981). These polysaccharides produce viscous solutions that may cause processing problems during brewing (Bamforth, 1982) and digestive problems when barley is fed to chickens (Hesselman et al., 1981). The general structural characteristics of β-glucans are well understood (MacGregor and Fincher, 1993), but fine structural variations that could induce poor solubility properties, increase the tendency of β-glucans to form gels, encourage β-glucan molecules to aggregate with one another or with molecules of other polysaccharides or could lead to the formation of β-glucan precipitates during enzyme hydrolysis have not been examined in detail.

Arabinoxylans are minor components of barley endosperm cell walls (Ballance and Manners, 1978), and major components in aleurone cell walls (Bacic and Stone, 1981), but are mainly located in the outer layers of the barley kernel and husk (Aspinall and Ferrier, 1957; Henry, 1987). Although such polysaccharides also have the potential to form viscous solutions (Medcalf et al., 1968) and have the potential to contribute to processing problems normally attributed to β-glucans, they have not been studied widely. Several studies have concentrated on characterizing arabinoxylan fractions from specific barley tissues such as endosperm (Fincher and Stone, 1986) or water-insoluble fractions (Vietor et al., 1993). There has not been a comprehensive study carried out on the total arabinoxylans in barley, and any fraction of these polysaccharides could

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contribute to processing problems during brewing. It was evident that much more detailed information was required on the structural/functional relationships of total barley β-glucans and arabinoxylans.

Minor structural features that could control important functional properties could be impossible to detect in global preparations of these polysaccharides. In the current study, therefore, relatively narrow fractions of barley β-glucans and arabinoxylans were prepared by stepwise extraction of barley, followed by subfractionation of the initial fractions by precipitation with ammonium sulphate. This report describes the detailed structures of the water-extractable β-glucans and arabinoxylans.

MATERIAL AND METHODS

Preparation of barley

Barley (cv. Harrington) was ground in a Wiley mill (0.5 mm screen). The grist was then boiled in 90% ethanol for 20 min to inactivate the endogenous enzymes, washed with fresh ethanol, and oven-dried (40°C).

Extraction and purification of non-starch polysaccharides

Non-starch barley polysaccharides were sequentially extracted as follows. Barley grist (500 g) was mixed with water (1.5 l) at 40°C for 30 min. The aqueous extract was separated from the insoluble residue by centrifugation (5000*g*, 20 min); the extraction procedure was repeated twice. The combined supernatants were brought to 95°C for 5 min, then cooled to room temperature (RT). The denatured proteins in the supernatant were removed by filtration using celite (20 g/l) as a filter aid. The residual proteins were removed by adsorption on Vega clay (Pembina Mountain Clay, Winnipeg, MB). The extract was stirred with the clay (10 g/l) for 20 min, then centrifuged (10 000*g*, 20 min). Porcine pancreas α-amylase (EC 3.2.1.1, type I-A, Sigma, St. Louis MO) was used to digest starch contaminants in the extract. The extract was incubated with the enzyme for 24 h (pH 6.5, 35°C), then dialysed against distilled water (12 000–14 000 mol. wt cut off, 4°C) until the dialysate was free of sugars. The enzyme was inactivated by heat (95°C, 20 min) and removed by centrifugation (10 000*g*, 20 min). The extract was free of starch as judged by the iodine test. Finally, the extract was freeze-dried, and the fraction was designated WE40.

The insoluble residue of barley grist was suspended in water (1 l) containing thermostable α-amylase (4500 U, Megazyme). The suspension was extracted (with stirring) at 65°C for 90 min and centrifuged (5000*g*, 20 min). The extraction was repeated once. The combined extracts were purified in the same manner as described above for the 40°C extract. The purified extract obtained at 65°C was designated WE65.

Fractionation of purified extracts

The purified extracts, WE40 and WE65, were fractionated by a graded ammonium sulphate precipitation technique (Izydorczyk et al., 1996). The freeze-dried extract of WE40 (3 g/l) was dissolved in phosphate buffer (0.1 M, pH 7), and $(NH_4)_2SO_4$ was added slowly to the solution to obtain 28% saturation; the solution was allowed to stand overnight at 5°C. The precipitated material was collected by centrifugation (5000*g*, 10 min), redissolved in H_2O, dialysed until free of $(NH_4)_2SO_4$ and freeze-dried; the subfraction was designated $WE40_{28}$. The saturation level of $(NH_4)_2SO_4$ was subsequently adjusted stepwise to 30, 35, 45, 60, 80, and 100%; the corresponding subfractions, obtained in a similar manner as described above, were designated $WE40_{30}$, $WE40_{35}$, $WE40_{45}$, $WE40_{60}$, $WE40_{80}$, and $WE40_{100}$, respectively. The WE65 extract was also fractionated with $(NH_4)_2SO_4$. Five subfractions were obtained: $WE65_{30}$; $WE65_{35}$; $WE65_{45}$; $WE65_{60}$; and $WE65_{100}$ (the subscripts refer to the saturation level of $(NH_4)_2SO_4$ at which the material was collected). Because of the structural similarity of subfractions $WE40_{28}$ and $WE40_{30}$, collection of WE65 subfraction at 28% of $(NH_4)_2SO_4$ was omitted. When the level of $(NH_4)_2SO_4$ saturation of WE65 extract was brought to 80%, no precipitate was observed, and the saturation level was increased directly to 100%.

Analytical methods

Protein content in water-extractable fractions was determined by the method of Lowry et al. (1951) with bovine serum albumin as a standard. Methylation analysis was conducted according to the method of Ciucanu and Kerek (1984). The partially methylated alditol acetates were quantified by capillary gas–liquid chromatography (GLC, fused silica column SP 2330, 60 m × 0.25 mm i.d., 0.20 μm film thickness). Qualitative analysis of the partially methylated acetates was performed by coupled GLC–mass spectrometry (MS). Identification of the methylated alditol derivatives was possible by comparing the resultant spectra with those published (Carpita and Shea, 1988). The effective carbon response factors, as given by Sweet et al. (1975), were used for calculation of the molar quantities of permethylated products determined by GLC. The detection of 2-*O*-methyl-xylitol and 3-*O*-methyl-xylitol, not resolved under the chromatographic conditions employed, was achieved from the mass spectra by the integration of signals of fragment ions characteristic for these two derivatives, i.e. *m/e* 118 (specific for 2-*O*-methyl-xylitol) and *m/e* 129 (specific for 3-*O*-methyl xylitol).

β-Glucan fractions (2 mg/ml) were dissolved in phosphate buffer (0.01 M, pH 6.5) and digested (in duplicate) with (1 → 3, 1 → 4)-β-D-glucan-4-glucanohydrolase (2 U/ml, lichenase, Megazyme Pty, Sydney, Australia) for

Table 1. Yields of water-extractable material in 40 and 65°C extracts and in their subfractions

Fraction/subfraction	Yield (%)	Fraction/subfraction	Yield (%)
WE40	1.4[a]	WE65	1.3[a]
$WE40_{28}$	13.8[b]	$WE65_{30}$	60.5[b]
$WE40_{30}$	14.4	$WE65_{35}$	23.4
$WE40_{35}$	37.2	$WE65_{45}$	9.3
$WE40_{45}$	19.2	$WE65_{80}$	3.8
$WE40_{60}$	7.6	$WE65_{100}$	2.9
$WE40_{80}$	6.5		
$WE40_{100}$	1.2		

[a]Yield of fractions (%, w/w) based on the total barley grist.
[b]Yield of subfractions (%, w/w), obtained by $(NH_4)_2SO_4$ fractional precipitation, based on the amount of material recovered after fractionation.

20 h at 40°C. Material that precipitated during hydrolysis was centrifuged (10 000*g*, 10 min), washed extensively with ethanol, and air-dried. The water-insoluble residue was then dissolved in DMSO (1–2 mg/ml) by stirring at 95°C for 2 h and at room temperature for 24 h, then diluted with H_2O to 20% DMSO (v/v). In the supernatants from the lichenase digestion, the enzyme was inactivated by heating solutions at 90°C for 15 min. Oligosaccharides released by lichenase from β-glucan fractions were analysed by high-performance anion-exchange chromatography (HPAEC) using a Waters 625 pump and 715 WISP sample injector (Waters Associates, Milford MA), and a Dionex CarboPac PA1 column (4 × 250 mm) with a PA1 guard column and pulsed amperometric detector (PAD-2) (Dionex Canada Ltd., Etobicoke ON). Samples were filtered before analysis (Whatman GF/A glass fiber filter). The running conditions were adapted from Wood et al. (1994a). Eluent A was 150 mM NaOH, eluent B was 150 mM NaOH containing 300 mM sodium acetate. Samples were eluted for 1 min with 65% A and 35% B, then with a first gradient to 50% B from 1–9 min and with a second gradient to 100% B from 9–22 min. The run-time was 35 min at a flow rate of 1 ml/min at ambient temperature. Pulse potential, E (volts) and duration, t (msec) were: $E_1 = 0.05$, $t_1 = 420$; $E_2 = 0.75$, $t_2 = 180$; $E_3 = -0.20$, $t_3 = 360$. The response time of the detector was 3 s, and the output range (sensitivity) was 10K nA. The instruments were controlled and the data were processed using Waters Millennium 2010 chromatography software.

The relative amounts of monosaccharides in polysaccharide fractions were determined (in duplicate) by HPLC after hydrolysis with 1 M H_2SO_4 for 2 h at 100°C and neutralization with barium hydroxide. The HPLC system was equipped with a Dionex CarboPac PA1 column (4 × 250 mm), a PA1 guard column, and a PAD cell. Hydrolysed samples were eluted at 1.0 ml/min with 10 mM NaOH for 4 min, then with nanopure water for a total run time of 35 min. Concentrated base (300 mM NaOH) was added to the post-column effluent at 0.5 ml/min (Dionex DQP-1 pump). Pulse potential, E (volts) and duration, t (msec) were: $E_1 = 0.05$, $t_1 = 300$; $E_2 = 0.75$, $t_2 = 120$; $E_3 = -0.20$, $t_3 = 300$. The response time of the detector was 3 s, and the output range (sensitivity) was 3K nA. Standard monosaccharides (Ara, Gal, Glc, Xyl, and Man) were obtained from Sigma (St Louis MO).

Molecular weights of polysaccharide fractions were estimated by high-performance size exclusion chromatography (HPSEC). A Jordi Gel DVB sulphonated mixed bed column (10 × 250 mm, 5 μm particle size, Jordi Associates, Bellingham MA) and a Waters 510 pump, 712 WISP sample injector, and 410 differential refractometer were used. Samples were dissolved in nanopure water (3 mg/ml) by heating (95°C) and stirring, and filtered through a Whatman GF/A glass fiber filter (sodium azide was added after heating to 0.02%, w/v). Samples were eluted isocratically for 40 min with 0.7 ml/min nanopure water at 35°C. A Shodex standard P-82 kit containing pullulan standards with molecular weights ranging from 5.8×10^8 to 1660.0×10^3 was used to calibrate the column.

The apparent viscosities of aqueous solutions of β-glucans and arabinoxylans were determined with a modified Ubbelohde viscometer (International Research Glassware, Kenilworth NJ) at 20.0 ± 0.1°C. Limiting viscosities $[\eta]$ were calculated from the Huggins equation (Huggins, 1942).

RESULTS

Extraction and fractionation of barley polysaccharides

The purified water extracts obtained at 40 and 65°C constituted 1.4 and 1.3%, respectively, of the dry barley grist. Recently, Saulnier et al. (1994) reported a comparable yield of barley polysaccharides extracted at 40°C (1.65%), but considerably higher for polysaccharides extracted at 90°C (12.9%). The differences between yields at 60 and 90°C were to be expected, given that extraction conditions (temperature, solvent) and purification procedures affect the amounts of extracted barley polysaccharides (Wood et al., 1978).

The yields of subfractions, obtained by fractionation of WE40 and WE65 with ammonium sulphate, are given in Table 1. Up to 30% saturation with $(NH_4)_2SO_4$, substantially more material precipitated from WE65 than from WE40. In contrast, WE40 yielded more material than

Table 2. Monosaccharide composition (mol%) and limiting viscosity values of water-extractable non-starch polysaccharides from barley

Fraction/ subfraction	[η] (dl/g)	Protein[a] (%)	Ara	Xyl	Glc	Gal	Man	Total Ara + Xyl	Xyl/Ara ratio
WE40	2.9	0.50 ± 0.08	7.0	8.9	82.5	1.2	0.3	15.9	1.27
$WE40_{28}$	3.5		0.7	–	99.3	–	–		
$WE40_{30}$	3.2		0.3	–	99.7	–	–		
$WE40_{35}$	2.2		–	–	100.0	–	–		
$WE40_{45}$	1.1		1.3	1.7	97.0	–	–	3.0	1.30
$WE40_{60}$	1.9		21.3	31.5	47.1	–	–	52.8	1.48
$WE40_{80}$	1.6		35.3	51.1	10.8	0.5	2.4	86.4	1.45
$WE40_{100}$	1.6		36.7	44.8	5.0	2.8	10.7	81.5	1.22
WE65	3.7	0.92 ± 0.10	2.5	3.3	93.3	0.7	0.2	5.8	1.32
$WE65_{30}$	4.4		–	–	99.4	0.6	–		
$WE65_{35}$	2.5		–	–	99.8	0.2	–		
$WE65_{45}$	1.6		1.4	2.2	96.5	–	–	3.6	
$WE65_{60}$	1.9		21.3	34.9	43.6	0.2	–	56.2	1.63
$WE65_{100}$	2.1		28.8	40.0	11.9	0.9	18.4	68.8	1.38

[a]Lowry method; $n = 2 \pm$ SD.

WE65 at a higher saturation level (>60%) of $(NH_4)_2SO_4$. Monosaccharide analysis of WE40 and WE65 revealed that both extracts contained a mixture of primarily two polysaccharides, arabinoxylans and β-glucans (Table 2). Trace amounts of galactose and mannose in WE40 and WE65 may indicate that galactomannans and/or glucomannans were also present in barley polysaccharides in very small amounts. The relative proportions of arabinoxylans and β-glucans in the two extracts differed; WE40 contained approximately 16% arabinoxylans, while WE65 only about 6% (based on the amount of arabinose and xylose sugars). Stepwise precipitation with $(NH_4)_2SO_4$ was useful in separating β-glucans from arabinoxylans as well as in fractionation of these polysaccharides into several subpopulations. Subfractions of WE40 and WE65 obtained at up to 45% saturation with $(NH_4)_2SO_4$ were essentially free of arabinoxylans. Subfractions precipitated at higher saturation levels of $(NH_4)_2SO_4$ (>45%) contained progressively more arabinoxylans and less β-glucans. Water-soluble arabinoxylans in various subfractions differed in the relative proportions of xylose and arabinose residues; the Xyl/Ara ratios ranged between 1.22–1.48 and 1.38–1.67 for WE40 and WE65, respectively. In general, arabinoxylans obtained at higher ammonium sulphate saturation were more highly substituted, as indicated by the lower Xyl/Ara ratios. These observations are in agreement with similar trends reported for wheat and rye water-soluble arabinoxylans fractionated with $(NH_4)_2SO_4$ (Izydorczyk and Biliaderis, 1992; Vinkx et al., 1993).

Linkage composition

The heterogeneous nature of various populations of arabinoxylans and β-glucans present in the barley water extracts was revealed by methylation analysis (Table 3Table 4). Four subfractions of the 40°C extract precipitated between 28 and 45% saturation of $(NH_4)_2SO_4$ were pure β-glucans. These polymers contained mainly β-(1 → 3) and β-(1 → 4) linkages, although some glucose residues additionally substituted at 2-, 3-, and 6-positions were also detected in $WE40_{35}$ and $WE40_{45}$ subfractions. Very little difference in the linkage composition was observed between $WE40_{28}$ and $WE40_{30}$ subfractions, except for a slightly higher content of terminal glucose residues in the latter, possibly due to the lower molecular weight of this subfraction. The ratios of β-(1 → 4)/β-(1 → 3) linkages in $WE40_{28}$ and $WE40_{30}$ were higher than in the remaining subfractions. In general, subfractions obtained at progressively higher $(NH_4)_2SO_4$ saturation had progressively lower β-(1 → 4)/β-(1 → 3) ratios. Some minor populations of β-glucans required very high concentration of $(NH_4)_2SO_4$ (65–100%) to precipitate from solutions. These β-glucans contained many terminal glucose units and relatively lower amounts of β-(1 → 4) linked glucose residues.

Pure β-glucans were also recovered from subfractions of the 65°C water extract between 30 and 45% saturation of $(NH_4)_2SO_4$. β-Glucans in subfraction $WE65_{30}$ had the highest content of β-(1 → 4) linked glucose residues (78%), and as a result the highest ratio of β-(1 → 4)/β-(1 → 3) linkages among all water-extractable β-glucan populations. These results diverge from the findings of Woodward et al. (1988), who reported lower β-(1 → 4) linkage content in 65°C than in 40°C water-extractable barley β-glucans.

Water-soluble arabinoxylans present in WE40 and WE65 required relatively high saturation levels of $(NH_4)_2SO_4$ to precipitate from the aqueous extracts. All arabinoxylans contained terminal arabinose in the furanose form, as indicated by the presence of 2,3,5-Me_3-Ara; this residue progressively increased in arabinoxylan populations precipitating at higher $(NH_4)_2SO_4$ saturation levels. The presence of small quantities of 3,5-Me_2-Ara, 2,5-Me_2-Ara, and 2,3-Me_2-Ara in $WE40_{100}$ and $WE65_{100}$ subfractions indicates that short arabinan side chains might be present in some arabinoxylans. Most of the xylose residues in arabinoxylans were present as un-, mono-, and disubstituted residues, as evidenced by the occurrence of 2,3-Me_2-Xyl, 2-Me-Xyl/3-Me-Xyl, and

Table 3. Linkage composition (mol%) of WE40 subfractions

Component	Linkage type	$WE40_{28}$	$WE40_{30}$	$WE40_{35}$	$WE40_{45}$	$WE40_{60}$	$WE40_{80}$	$WE40_{100}$
2,3,5-Me_3-Ara	(Ara*f*)1 →	–	–	–	–	17.4(37.8)[a]	34.7(38.1)	36.0(39.0)
2,3,4-Me_3-Xyl	(Xyl*p*)1 →	–	–	–	–	0.5(1.1)	2.1(2.3)	4.0(4.3)
3,5-Me_2-Ara	→ 2(Ara*f*)1 →	–	–	–	–	–	–	1.0(1.1)
2,5-Me_2-Ara	→ 3(Ara*f*)1 →	–	–	–	–	–	–	0.6(0.6)
2,3-Me_2-Ara	→ 5(Ara*f*)1 →	–	–	–	–	–	–	1.0(1.1)
2,3-Me_2-Xyl	→ 4(Xyl*p*)1 →	–	–	–	–	15.7(34.1)	28.2(30.9)	26.0(28.1)
2-Me-Xyl + 3-Me-Xyl	→ 3,4(Xyl*p*)1 → , → 2,4(Xyl*p*)1 →	–	–	–	–	6.9(15.0)	10.7(11.7)	8.9(9.6)
Xyl	→ 2,3,4(Xyl*p*)1 →	–	–	–	–	5.5(12.0)	15.3(16.8)	14.8(16.0)
2,3,4,6-Me_4-Glc	(Glc*p*)1 →	0.5	0.9	4.7	5.0	2.7(5.1)[b]	1.1(12.3)	1.0(12.0)
2,4,6,-Me_3-Glc	→ 3(Glc*p*)1 →	27.2	27.1	26.7	27.9	15.5(28.8)	2.4(27.8)	2.1(27.2)
2,3,6-Me_3-Glc	→ 4(Glc*p*)1 →	72.2	72.0	64.8	64.2	35.0(64.9)	5.4(60.7)	4.6(59.7)
2,6-Me_2-Glc	→ 3,4(Glc*p*)1 →	tr	tr	1.4	1.4	0.5(0.9)	–	–
3,6-Me_2-Glc	→ 2,4(Glc*p*)1 →	–	–	0.2	0.2	0.1(0.2)	–	–
2,4-Me_2-Glc	→ 3,6(Glc*p*)1 →	–	–	0.8	0.7	–	–	–
2,3-Me_2-Glc	→ 4,6(Glc*p*)1 →	–	–	0.8	0.5	–	–	–
(1 → 4)/(1 → 3) Glc ratio		2.65	2.65	2.42	2.30	2.25	2.25	2.19
Unsub/Subs Xyl[c]						1.26	1.08	1.09
Doubl/Singl Xyl[d]						0.80	1.43	1.66

[a]Amounts in brackets are based on arabinoxylan content only.
[b]Amounts in brackets are based on β-glucan content only.
[c]Ratio of unsubstituted xylose residues [→ 4(Xyl)1 →] to the sum of singly and doubly substituted xylose residues [→ 3,4(Xyl)1 → + → 2,4(Xyl)1 → + → 2,3,4(Xyl)1 →].
[d]Ratio of doubly [→ 2,3,4(Xyl)1 →] to singly substituted xylose residues [→ 3,4(Xyl)1 → + → 2,4(Xyl)1 →].

xylose derivatives, respectively. In addition to C-3 monosubstituted xylose residues (2-Me-Xyl), the mass spectra of the alditol derivatives also indicated the presence of C-2 monosubstituted residues (3-Me-Xyl). This is in agreement with a previous report by Vietor (1992), who also found C-2 monosubstituted xylose residues in arabinoxylans from barley. The increase in ammonium sulphate saturation produced arabinoxylan fractions with decreasing content of un- and monosubstituted xylose residues but increasing amounts of disubstituted units. These trends were observed for arabinoxylans in both extracts WE40 and WE65. In general, water-extractable barley arabinoxylans were very highly substituted. With the exception of arabinoxylans in $WE65_{60}$, the ratios of unsubstituted to substituted xylose residues in other arabinoxylan subfractions were quite low. Except in $WE40_{60}$ and $WE65_{60}$, doubly substituted

Table 4. Linkage composition (mol%) of WE65 subfractions

Component	Linkage type	$WE65_{30}$	$WE65_{35}$	$WE65_{45}$	$WE65_{60}$	$WE65_{100}$
2,3,5-Me_3-Ara	(Ara*f*)1 →	–	–	–	15.6(32.5)[a]	30.1(36.6)
2,3,4-Me_3-Xyl	(Xyl*p*)1 →	–	–	–	0.1(0.3)	4.9(5.0)
3,5-Me_2-Ara	→ 2(Ara*f*)1 →	–	–	–	tr	1.0(1.2)
2,5-Me_2-Ara	→ 3(Ara*f*)1 →	–	–	–	–	0.5(0.6)
2,3-Me_2-Ara	→ 5(Ara*f*)1 →	–	–	–	–	0.5(0.6)
2,3-Me_2-Xyl	→ 4(Xyl*p*)1 →	–	–	–	19.0(39.5)	21.2(25.8)
2-Me-Xyl + 3-Me-Xyl	→ 3,4(Xyl*p*)1 → , → 2,4(Xyl*p*)1 →	–	–	–	6.8(14.2)	10.9(13.2)
Xyl	→ 2,3,4(Xyl*p*)1 →	–	–	–	6.3(13.1)	13.1(16.0)
2,3,4,6-Me_4-Glc	(Glc*p*)1 →	1.1	1.2	1.3	1.2(2.4)[b]	1.2(6.8)
2,4,6,-Me_3-Glc	→ 3(Glc*p*)1 →	20.3	28.2	28.1	14.4(28.3)	4.5(25.4)
2,3,6-Me_3-Glc	→ 4(Glc*p*)1 →	78.0	70.2	70.1	35.3(69.3)	12.0(69.8)
2,6-Me_2-Glc	→ 3,4(Glc*p*)1 →	0.3	0.2	0.2	–	–
3,6-Me_2-Glc	→ 2,4(Glc*p*)1 →	0.2	0.1	0.1	–	–
2,4-Me_2-Glc	→ 3,6(Glc*p*)1 →	–	tr	0.1	–	–
2,3-Me_2-Glc	→ 4,6(Glc*p*)1 →	–	0.1	0.1	–	–
(1 → 4)/(1 → 3) Glc ratio		3.84	2.49	2.49	2.45	
Unsub/subs Xyl[c]					1.44	0.88
Doubl/Singl Xyl[d]					0.92	1.21

[a]Amounts in brackets are based on arabinoxylan content only.
[b]Amounts in brackets are based on β-glucan content only.
[c]Ratio of unsubstituted xylose residues [→ 4(Xyl)1 →] to the sum of singly and doubly substituted xylose residues [→ 3,4(Xyl)1 → + → 2,4(Xyl)1 → + → 2,3,4(Xyl)1 →].
[d]Ratio of doubly [→ 2,3,4(Xyl)1 →] to singly substituted xylose residues [→ 3,4(Xyl)1 → + → 2,4(Xyl)1 →].

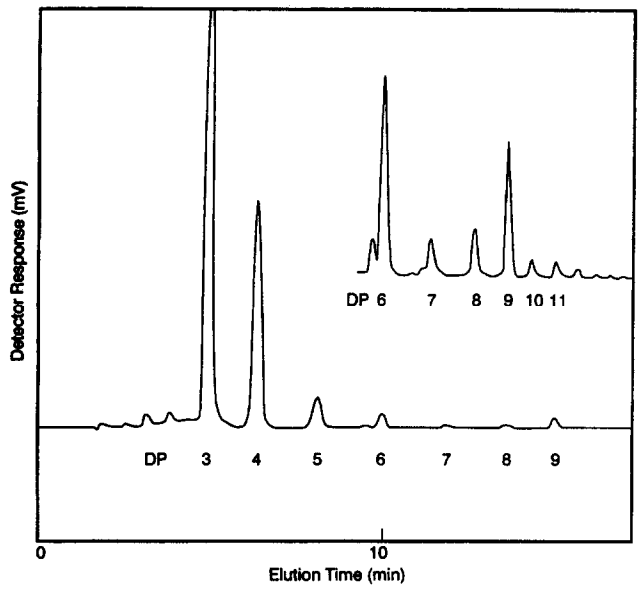

Fig. 1. High-performance anion exchange chromatography with pulsed amperometric detection of water soluble oligosaccharides released during lichenase digestion of the $WE40_{28}$ fraction. Inset shows elution pattern of oligosaccharides with higher DP.

xylose residues were present in very high amounts, resulting in exceptionally high ratios of doubly to singly substituted xylose residues. Such a marked presence of doubly substituted xylose has been reported only for wheat beeswing bran arabinoxylans (Brillouet and Joseleau, 1987).

Hydrolysis of β-glucans with lichenase

Profiles of the oligomeric products produced by enzymic hydrolysis of barley β-glucans can be seen in Fig. 1, and the proportions of the soluble oligomers released by lichenase from all subfractions containing β-glucans are compiled in Table 5. All β-glucans were degraded mainly to tri- and tetrasaccharides. On the basis of the studies by Wood et al. (1994b) and Woodward et al. (1988) and of the fact that lichenase breaks specifically β-(1 → 4) linkages of glucose residues which are linked at C-O-3, the tri- and tetrasaccharides were assumed to originate from 3-*O*-β-D-cellobiosyl-D-glucose and 3-*O*-β-D-cellotriosyl-D-glucose, respectively. In agreement with previous reports (Woodward et al., 1988; Wood et al., 1994b), larger oligomers were also observed, but in our studies detectable peaks were identified up to DP13, with DP5, 6, and 9 being most abundant. β-Glucans extracted with water at 40°C had a slightly lower ratio of tri-/tetrasaccharide than those extracted at 65°C, which corroborates the findings of Woodward et al. (1988), who reported the DP3/DP4 ratios of 2.0 and 1.7 for 65 and 40°C water soluble β-glucans, respectively.

The general profiles of the oligosaccharides released from the subfractions of WE40 were relatively consistent, although the proportions of individual oligomers differed slightly. There was a small but progressive decrease in the amount of tri- and tetrasaccharides from the less soluble to the more soluble β-glucans (i.e. from $WE40_{28}$ to $WE40_{60}$). Also, the ratios of DP3/DP4 oligomers decreased in the same order. Similar trends were observed for the subfractions of WE65.

Both aqueous extracts of barley polysaccharides, WE40 and WE65, released, upon digestion with lichenase, some water-insoluble material which precipitated during hydrolysis. This material originated mainly from the least soluble fractions of β-glucans. $WE40_{28}$ and $WE65_{30}$ yielded 3.0 and 5.5% of this precipitate, respectively (based on the amount

Table 5.
Content of water-soluble oligosaccharides (mol%) released by the action of lichenase on β-glucans

DP	WE40	$WE40_{28}$	$WE40_{30}$	$WE40_{35}$	$WE40_{45}$	$WE40_{60}$
3	56.8	61.0	61.0	60.4	59.6	57.4
4	32.3	29.5	29.7	29.9	30.3	30.8
5	4.5	4.2	4.1	4.2	4.4	5.0
6	2.2	2.0	2.0	2.0	2.1	2.4
7	0.7	0.7	0.6	0.6	0.7	0.9
8	0.8	0.7	0.7	0.7	0.7	0.9
9	1.5	1.2	1.3	1.3	1.3	1.3
10–13	1.1	0.6	0.6	0.8	1.2	1.2
3 + 4 total	89.1	90.5	90.7	90.3	89.9	88.2
3:4 ratio	1.76	2.07	2.06	2.02	1.97	1.86
DP	WE65	$WE65_{30}$	$WE65_{35}$	$WE65_{45}$	$WE65_{60}$	
3	60.9	61.9	60.8	59.9	57.8	
4	28.5	28.8	29.2	29.4	29.3	
5	4.0	4.1	4.1	4.4	4.8	
6	1.9	2.0	2.0	2.2	2.3	
7	0.7	0.6	0.8	0.8	1.0	
8	0.7	0.7	0.8	0.8	1.0	
9	1.6	1.2	1.4	1.5	1.4	
10–13	1.7	0.7	0.9	1.1	2.4	
3 + 4 total	89.4	90.7	90.0	89.3	87.1	
3:4 ratio	2.13	2.15	2.08	2.03	1.97	

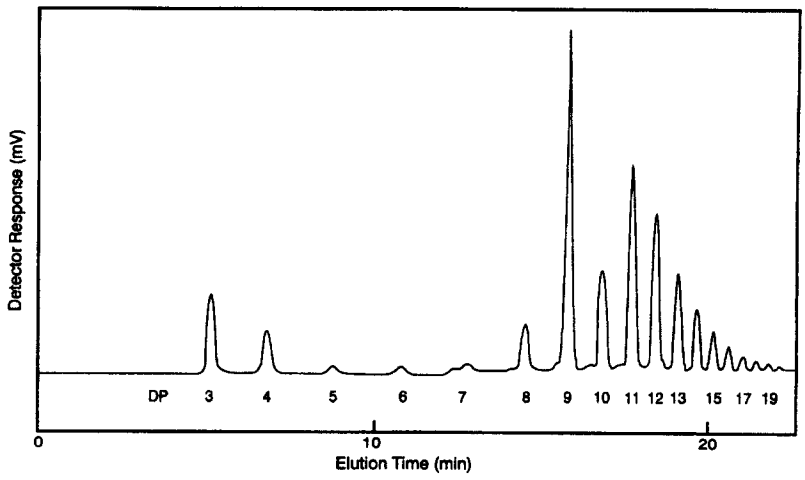

Fig. 2. High-performance anion exchange chromatography with pulsed amperometric detection of oligosaccharides in the water insoluble material released during lichenase digestion of the $WE40_{28}$ fraction.

of the unhydrolysed fractions), whereas the more soluble fractions produced only some traces of it. Such precipitates have also been observed by other researchers, who used lichenase to hydrolyse β-glucans. Wood et al. (1994b) reported that the insoluble material (3–4%) resulting from the action of lichenase on oat and barley β-glucans was composed of β-(1 → 4) linked oligosaccharides with DP9–15. In contrast to our results, Woodward et al. (1988) observed higher amounts of insoluble oligosaccharides released by 40°C than by 65°C water soluble β-glucans; that study, however, did not analyse the precipitated material in detail.

The insoluble precipitate released from $WE40_{28}$ and $WE65_{30}$ was collected by centrifugation, air-dried, and partly dissolved in dimethyl sulphoxide. A portion of the precipitate remained insoluble even after 12 h solubilization in DMSO. Chromatography of the DMSO-soluble material revealed a mixture of oligosaccharides with DP3–20 (Fig. 2), and the methylation analysis confirmed the presence of cellulose-like fragments with β-(1 → 4) linkages. Oligomers with DP9, 11 and 12 predominated in the profiles. No substantial differences, except for the total amount, were observed between oligomers released from $WE40_{28}$ and $WE65_{30}$ fractions (Table 6). However, since unquantified amounts of the precipitates were insoluble in DMSO, our results allow only partial understanding of the nature of insoluble material formed during hydrolysis of β-glucans.

Table 6.
Content of oligosaccharides (mol%) in water-insoluble precipitate released during lichenase digestion of β-glucan fractions

DP	$WE40_{28}$ ppt	$WE65_{30}$ ppt
3	8.6	9.8
4	4.9	5.1
5	1.2	1.4
6	0.9	1.4
7	1.5	1.9
8	4.8	5.1
9	25.6	22.7
10	7.8	7.4
11	14.6	12.8
12	10.9	10.5
13	6.8	7.0
14	4.4	4.8
15	2.9	3.3
16	1.8	2.3
17	1.3	1.6
18	0.8	1.2
19	0.7	1.0
20	0.5	0.8

Molecular size distribution

Molecular weights of water-extractable barley β-glucans and arabinoxylans were estimated in this study by gel

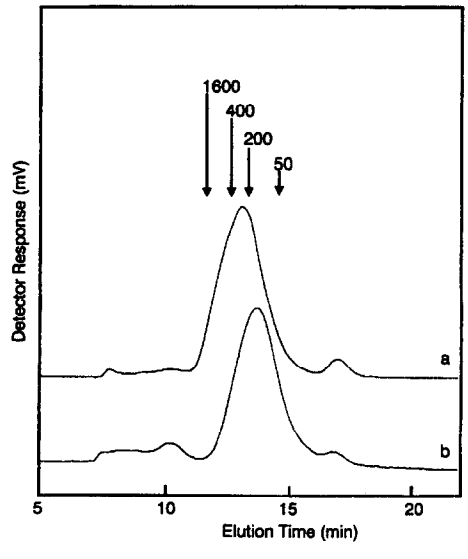

Fig. 3. High-performance size exclusion chromatography of the WE65 (a) and WE40 (b) fractions with refractive index detection. Arrows (↓) indicate elution time of pullulan standards with various molecular weights (MW): 1600, MW = 1660×10^3, 400, MW = 380×10^3; 200, MW = 186×10^3; 50, MW = 48×10^3.

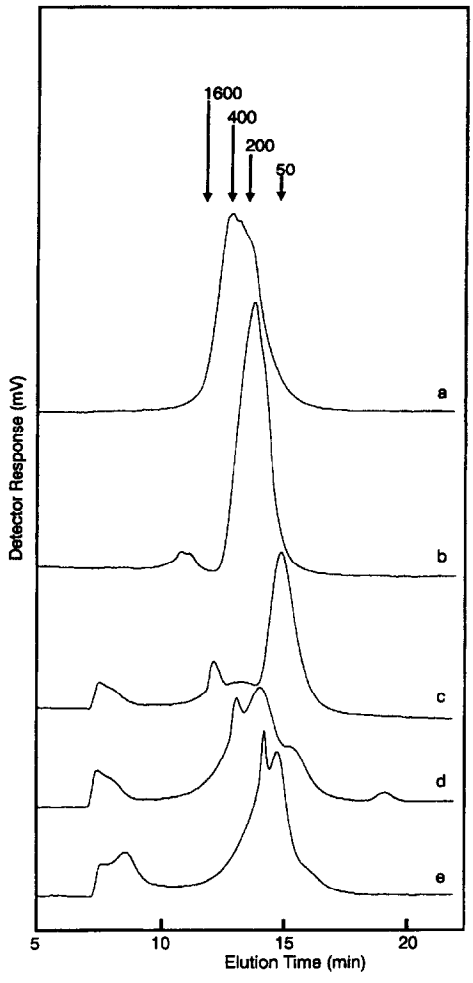

Fig. 4. High-performance size exclusion chromatography of subfractions of the WE40 fraction with refractive index detection: (a) $WE40_{28}$; (b) $WE40_{45}$; (c) $WE40_{60}$; (d) $WE40_{80}$; (e) $WE40_{100}$. Arrows (↓) indicate elution time of pullulan standards, see caption in Fig. 3.

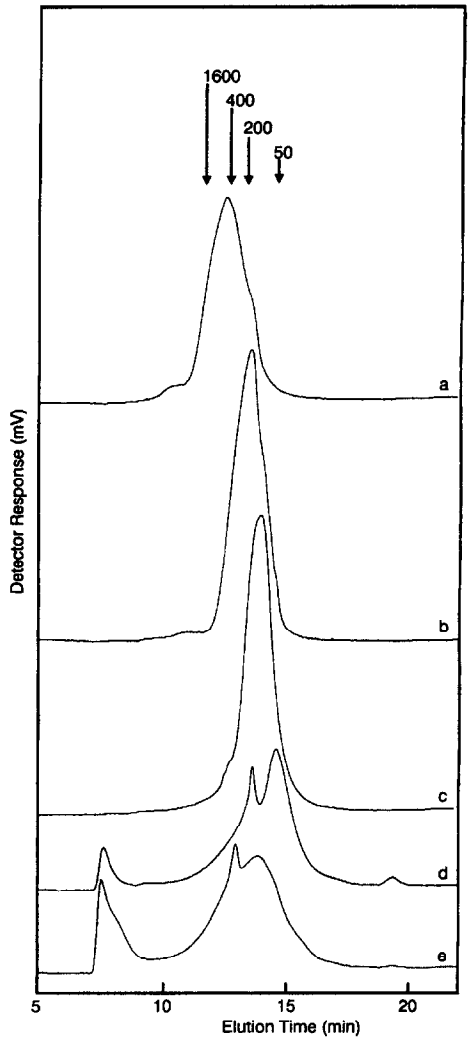

Fig. 5. High-performance size exclusion chromatography of subfractions of the WE65 fraction with refractive index detection: (a) $WE65_{30}$; (b) $WE65_{35}$; (c) $WE65_{45}$; (d) $WE65_{60}$; (e) $WE65_{100}$. Arrows (↓) indicate elution time of pullulan standards, see caption in Fig. 3.

permeation chromatography and limiting viscosity measurements. Fig. 3 shows the gel permeation patterns of unfractionated WE40 and WE65 extracts. A slight shift towards higher hydrodynamic volumes of WE65 suggests a higher molecular size of polysaccharides in this extract compared to WE40. WE65 also exhibited a higher limiting viscosity value than WE40 (Table 2). Subfractions of WE40 and WE65 containing relatively pure β-glucans (Fig. 4*a–c* and Fig. 5*a–c*) eluted as broad but symmetric peaks. β-Glucans obtained with increasing ammonium sulphate saturation exhibited progressively lower peak molecular weights. This shift in molecular size was also reflected in decreasing limiting viscosity values for pure β-glucan subfractions, $WE40_{28-45}$ and $WE65_{30-45}$ (Table 2). A slight increase in $[\eta]$ values was observed in subfractions containing arabinoxylans, $WE65_{60}$ and $WE65_{100}$ as well as $WE40_{60-100}$ (Table 2). The presence of arabinoxylans was also observed in the elution patterns of the appropriate subfractions (Fig. 4*d* and *e*, Fig. 5*d* and *e*). The major, broad asymmetric peak, with retention time between 13 and 16 minutes, probably reflects the bimodal content of these subfractions; $WE40_{60}$ and $WE65_{60}$ contained almost equal amounts of β-glucans and arabinoxylans. The appearance of another component at very high hydrodynamic volumes might suggest that a certain portion of arabinoxylans exhibited either very high molecular size distribution or, more likely, some aggregation properties (with other arabinoxylans or with β-glucans). The exact identity of this component remains to be proven.

DISCUSSION

The present study was aimed at obtaining detailed structural characteristics of non-starch polysaccharides extracted sequentially with water at 40 and 65°C from a Canadian malting barley (cv. Harrington). Fractionation with ammonium sulphate revealed considerable heterogeneity of barley

β-glucans and arabinoxylans as several populations of both polymers were identified, varying in structural features and molecular weights.

Substantial differences in molecular characteristics were found between polysaccharides extracted at 40 and 65°C. Compared to WE40, the WE65 extract was enriched with β-glucan populations of higher molecular size, higher limiting viscosity value, and higher content of β-(1 → 4) linkages. The last characteristic was also reflected in a higher abundance of cellulose-like fragments released from $WE65_{30}$ upon enzymic hydrolysis. The ratios of tri-/tetrasaccharide (DP3/DP4) oligomers were also slightly higher in β-glucans extracted at 65°C than in those extracted at 40°C. The results of our studies suggest, therefore, that the lower solubility of 65°C water-extracted β-glucans from a Canadian malting barley might be associated with their higher molecular size and higher content of β-(1 → 4) linkages. β-Glucans containing blocks of adjacent β-(1 → 4) linkages will most likely exhibit a tendency for interchain aggregation through strong hydrogen bonds along the cellulose-like regions; this property would make the polymers potentially less soluble in water. It is reasonable to expect that the tendency for aggregation (and hence lower solubility) will be proportional to the frequency and length of the cellulosic stretches in the polymer chains. The propensity of β-glucans with higher β-(1 → 4) linkages to precipitate readily from solutions containing a relatively low saturation level of ammonium sulphate is also associated with their structural potential to form topological aggregates and consequently precipitate under reduced solvent conditions. Although the cellulosic stretches in β-glucans most likely affect their solubility, the conformation of the remaining portion of β-glucan chains might also influence their overall physical properties. Slightly higher ratios of DP3/DP4 oligosaccharides were consistently observed in β-glucans with lower solubility. It was suggested previously that long blocks of contiguous cellotriosyl residues might be responsible for the insolubility of barley β-glucans (Woodward et al., 1988; Izawa et al., 1993). Since a helix made up of three cellotriosyl residues would constitute a stable crystalline structure in β-glucan molecules (Tvaroska et al., 1983), it is possible, therefore, that a higher content of cellotriosyl fragments might impose some conformational regularity in β-glucans, and consequently a higher degree of organization of these polymers (i.e. lower solubility) in the cell walls. Whether the differences in the DP3/DP4 ratios between 40 and 65°C water extractable β-glucans are large enough to significantly affect their conformation/solubility, however, remains to be proven.

Arabinoxylans constituted a rather minor portion of water-extractable barley polysaccharides. Generally, all arabinoxylan populations exhibited a highly substituted xylan backbone with a large proportion of doubly substituted xylose residues. Compared to most β-glucans, arabinoxylans required a much higher concentration of $(NH_4)_2SO_4$ to effect their precipitation. Salting out of arabinoxylans and β-glucans is believed to be affected by formation of interchain aggregates along structurally regular segments in the polymeric chains such as unsubstituted xylan blocks in arabinoxylans and cellulosic stretches in β-glucans (Izydorczyk and Biliaderis, 1992; Izydorczyk et al., 1996). The topological entanglements in β-glucans must, therefore, be much stronger (more strongly stabilized by hydrogen bonds between glucose residues) and more abundant than in arabinoxylans, and hence salting out of β-glucans occurs at relatively low concentrations of $(NH_4)_2SO_4$.

In conclusion, both barley polysaccharides, arabinoxylans and β-glucans, exhibited a high degree of structural heterogeneity. Among the β-glucan populations, those with the highest molecular size and the highest content of β-(1 → 4) linkages (originating mainly from the 65°C water extract) might potentially cause problems in filtration or haze formation due to their tendency for interchain aggregation.

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