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

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Three fractions containing hemicellulosic material were obtained by sequential extraction of barley residue (left after removal of water-extractable polysaccharides) with saturated barium hydroxide [$Ba(OH)_2$ fraction], distilled water [$Ba(OH)_2/H_2O$ fraction], and 1 M sodium hydroxide [NaOH fraction]. The yields of the fractions were 1.6, 1.7, and 2.6% (w/w), respectively, of the dry barley grist. The $Ba(OH)_2$ fraction contained mainly arabinose and xylose, 35.8% and 60.9%, respectively. The $Ba(OH)_2/H_2O$ fraction in addition to 26.7% Ara and 36.6% Xyl contained also 34.8% Glc. The NaOH fraction was composed of 14.2% Ara, 44.0% Xyl, and 40.9% Glc. The $Ba(OH)_2/H_2O$ and NaOH extracts were further fractionated by stepwise $(NH_4)_2SO_4$ precipitation into several subfractions with varying amounts of β-glucans and arabinoxylans. β-Glucans in $Ba(OH)_2/H_2O$ and NaOH fractions were characterized by high ratios of β-(1→4)/β-(1→3) linkages, large amounts of contiguously linked β-(1→4) segments, and high ratios of cellotriosyl/cellotetraosyl units. The alkali-extractable arabinoxylans, especially those NaOH-extractable, were characterized by a very low degree of substitution, high xylose/arabinose ratio, and a small content of doubly substituted xylose residues. Some populations of arabinoxylans displayed structural features that would enable them to self-associate or to interact with β-glucans.

INTRODUCTION

Our earlier article described the isolation, fractionation and structural variation of water-extractable barley β-glucans and arabinoxylans (Izydorczyk *et al.*, 1997). However, the major portion of these polysaccharides in barley is not water-extractable. The reasons for their initial water insolubility have not yet been fully revealed. It has been speculated that ester linkages are responsible for holding the insoluble portion of β-glucans in the starchy endosperm cell walls of barley, but the nature of those linkages remains unknown (Bamforth, 1994). Difficulties in extracting arabinoxylans with water might be related to the formation of diferulic acid bridges between adjacent arabinoxylan chains (Geissmann & Neukom, 1973). Non-covalent interactions between β-glucans and cellulose or between β-glucans and arabinoxylans are also plausible explanations for the lack of extractability of these polysaccharides in aqueous media. However, neither the molecular characteristics required for such interactions (such as the abundance of longer blocks of adjacent (1→4) linkages in β-glucans or unsubstituted xylan backbone in arabinoxylans) nor actual interactions have yet been demonstrated experimentally.

In contrast to the extensively studied water-extractable β-glucans, β-glucans that are not readily extracted with water have received relatively little attention. So far, the only physicochemical parameter reported to distinguish water- and alkali-extractable β-glucans has been the molecular weight: the water-extractable β-glucans have been found to be smaller than those extracted by alkali (Forrest & Wainwright, 1977; Saulnier *et al.*, 1994). It should be noted that the initially insoluble β-glucans or arabinoxylans may

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become more soluble during malting or mashing, contributing to filtration problems or haze formation in beer. This potential solubility of β-glucans and arabinoxylans may be attributed to 'β-glucan solubilase', an enzyme which is postulated to open up cell walls by disrupting the feruloyl-linked arabinoxylan matrix and, therefore, releasing the two polymers (Bamforth *et al.*, 1996). The main objective of this study was to provide further insights into the structure of alkali-extractable β-glucans and arabinoxylans from barley and to compare them with water-extractable portions of these polysaccharides.

MATERIALS AND METHODS

Extraction and purification

The procedure used to isolate alkali-extractable polysaccharides from barley was based partially on a method for isolation of alkali-extractable cell-wall material from wheat flour, as described by Gruppen *et al.* (1992). Insoluble residue, left from barley grist (450 g, cv. Harrington) after sequential extraction with water at 40 and 65°C (Izydorczyk *et al.*, 1997), was suspended in a saturated $Ba(OH)_2$ solution (1 l) containing 1% (w/v) $NaBH_4$ to prevent alkaline degradation. The suspension was stirred for 15 h at room temperature (RT), then centrifuged (5000×*g*, 30 min). The residue was re-extracted with the same solvent (500 ml) for 3 h. The extracts were combined and neutralized with acetic acid. Porcine pancreatic α-amylase (Sigma Chemical Co., St. Louis, MO) and pronase (*Streptococcus griseus*, Boehringer Mannheim, Laval, PQ) were added to the solution and left overnight. The extract was free of starch as judged by the iodine test. The enzymes were inactivated by heat (95°C, 10 min), and the solution was dialysed extensively against distilled water and freeze-dried. The resultant extract is referred to as the $Ba(OH)_2$ fraction. The remaining residue was suspended in distilled water (1 l) and neutralized with acetic acid. After stirring for 15 h at RT, the suspension was centrifuged (5000×*g*, 30 min) and re-extracted with water (1 l) for 3 h at RT. The extracts were combined and treated with α-amylase and pronase as described above; this extract is referred to as the $Ba(OH)_2/H_2O$ fraction. The last fraction, referred to as the NaOH fraction, was obtained by suspending the insoluble residue remaining after $Ba(OH)_2$ and H_2O extractions, in 1 M NaOH (1 l) containing 1% (w/v) $NaBH_4$. The suspension was extracted for 15 h at RT and centrifuged (5000×g, 30 min). All purification steps were conducted as described above.

The $Ba(OH)_2/H_2O$ and NaOH extracts were further fractionated by ammonium sulphate precipitation as described earlier (Izydorczyk *et al.*, 1997). Four subfractions were obtained from each extract: $Ba(OH)_2/H_2O_{30}$, $Ba(OH)_2/H_2O_{50}$, $Ba(OH)_2/H_2O_{65}$, $Ba(OH)_2/H_2O_{100}$ and $NaOH_{30}$, $NaOH_{50}$, $NaOH_{65}$, $NaOH_{100}$ (the subscripts refer to saturation levels of ammonium sulphate at which the subfractions were collected).

Analytical methods

Protein content in alkali-extractable fractions was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as a standard. The monosaccharide composition of these fractions was determined (in duplicate) by HPLC after hydrolysis with 1 M H_2SO_4 for 2 h at 100°C and neutralization with barium hydroxide (Izydorczyk *et al.*, 1997). Methylation analysis was conducted according to the method of Ciucanu and Kerek (1984) as described earlier (Izydorczyk *et al.*, 1997).

Digestion of β-glucan fractions (2 mg/ml) with lichenase (4 U/ml, 0.01 M phosphate buffer, pH 6.5) and detection of the oligomers in soluble and insoluble digest products by HPAEC with PAD was conducted as described previously (Izydorczyk *et al.*, 1997).

Molecular weights of alkali-extractable fractions and subfractions were estimated by aqueous high-performance size exclusion chromatography using a Jordi Gel DVB sulfonated mixed bed column (10×250 mm, 5 μm particle size) and a Waters 410 differential refractometer (Izydorczyk *et al.*, 1997).

The apparent viscosities of aqueous solutions of alkali-extractable fractions and subfractions were determined with a modified Ubbelohde viscometer (International Research Glassware, Kenilworth, NJ) at 20.0±0.1°C. Limiting viscosities [η] were calculated from the Huggins equation (Huggins, 1942).

RESULTS AND DISCUSSION

Sequential extraction

Three distinct fractions containing hemicellulosic material were obtained by sequential extraction of barley residue (left after removal of water-extractable polysaccharides) with saturated barium hydroxide [$Ba(OH)_2$ fraction], distilled water [$Ba(OH)_2/H_2O$ fraction], and 1 M sodium hydroxide (NaOH fraction). The yields of the fractions were 1.6, 1.7, and 2.6% (w/w), respectively, of the initial amount of barley grist before aqueous extraction. The fraction extracted with saturated barium hydroxide contained almost pure arabinoxylans (Table 1). Only minor amounts of glucose and galactose were present in this fraction. The xylose to arabinose (Xyl/Ara) ratio of 1.7 was higher than the ratio of 1.3 previously reported for alkali-extractable barley arabinoxylans (Viëtor, 1992) but

Table 1. Yield and monosaccharide content (mol %) of alkali-extractable polysaccharides from barley

Fraction/ subfraction	Yield (%)	[η] (dl/g)	Protein[c] (%)	Ara	Xyl	Glc	Gal	Man	Total Ara + Xyl	Xyl/Ara ratio
$Ba(OH)_2$	1.6[a]	4.5	2.2±0.2	35.9	60.9	2.3	0.9	—	96.8	1.7
$Ba(OH)_2/H_2O$	1.7[a]	5.0	1.0±0.3	26.7	36.6	34.8	1.9	—	63.3	1.4
$Ba(OH)_2/H_2O_{30}$	22.7[b]	4.8		2.1	2.4	89.3	—	6.3	4.5	1.2
$Ba(OH)_2/H_2O_{50}$	12.3[b]	2.9		7.3	18.1	73.6	—	1.1	25.4	2.5
$Ba(OH)_2/H_2O_{65}$	37.7[b]	5.2		22.1	43.7	34.0	0.2	—	65.8	2.0
$Ba(OH)_2/H_2O_{100}$	27.3[b]	3.2		42.8	50.8	4.7	1.7	—	93.6	1.2
NaOH	2.6[a]	2.7	2.5±0.3	14.2	44.0	40.9	0.9	—	58.2	3.1
$NaOH_{30}$	16.8[b]	3.5		3.4	18.7	77.9	—	—	22.1	5.5
$NaOH_{50}$	40.9[b]	2.6		8.0	36.4	55.6	—	—	44.4	4.5
$NaOH_{65}$	33.9[b]	3.5		15.8	50.9	32.9	0.4	—	66.7	3.2
$NaOH_{100}$	8.4[b]	2.0		36.3	59.0	1.6	3.0	—	95.3	1.6

[a] Yield of fractions based on the amount of barley grist before water extraction.
[b] Yield of subfractions based on the amount of material recovered after fractionation.
[c] Lowry method; $n = 3 \pm SD$.

within the range found in other cereal arabinoxylans (Izydorczyk & Biliaderis, 1995). The mechanism of the selective extraction of arabinoxylans using $Ba(OH)_2$ remains only partially understood, even though $Ba(OH)_2$ has been successfully used to extract these polymers from many cell wall materials, including wheat, barley, and sorghum (Bergmans *et al.*, 1996). It is believed that hydroxyl ions cause swelling of cellulose, disruption of hydrogen bonds between cellulose and hemicelluloses, and hydrolysis of ester bonds most likely connecting cell wall polysaccharides. The Ba^{2+} and borohydride ions, on the other hand, are thought to be responsible for the poor extractability of β-glucans (Bergmans *et al.*, 1996).

The fraction solubilized with water, after removal of $Ba(OH)_2$ extractable material, contained, in addition to arabinoxylans, 34.8% of β-glucans (based on the amount of glucose residues). Arabinoxylans in this fraction had a lower Xyl/Ara ratio (1.4) than in the $Ba(OH)_2$ fraction. Stepwise fractionation of the $Ba(OH)_2/H_2O$ fraction with ammonium sulphate was effective in separating the two populations of polysaccharides in this fraction; the yields of the resulting subfractions are given in Table 1. Subfraction $Ba(OH)_2/H_2O_{30}$, precipitated at 30% saturation of $(NH_4)_2SO_4$, contained almost pure β-glucans, whereas subfraction $Ba(OH)_2/H_2O_{100}$ was composed mainly of arabinoxylans. The two intermediate subfractions $Ba(OH)_2/H_2O_{50}$ and $Ba(OH)_2/H_2O_{65}$ contained the two polymers in different ratios. In general, the proportion of arabinoxylans in the fractions increased as the saturation of $(NH_4)_2SO_4$ increased. The Xyl/Ara ratio in arabinoxylan fractions changed from 2.5 to 1.2 with increasing saturation of the salt from 50 to 100%.

The last fraction, extracted with 1 M NaOH, also contained the two polysaccharides: arabinoxylans (58.2%) and β-glucans (40.9%). In this fraction, however, β-glucans free from arabinoxylans could not be obtained by the ammonium sulphate fractionation technique. Even at a relatively low saturation level of the salt, arabinoxylans coprecipitated with β-glucans and constituted approximately 22% of subfraction $NaOH_{30}$. These results were rather unexpected, since so far all arabinoxylan populations required much higher salt concentration (at least 50%) to be forced out of solution. A structural feature which most notably distinguishes this arabinoxylan subfraction is the unusually low degree of substitution, as indicated by a very high Xyl/Ara ratio of 5.5. Subfractions obtained at a higher saturation level of $(NH_4)_2SO_4$ contained progressively more arabinoxylans and less β-glucans; $NaOH_{100}$ was composed of virtually pure arabinoxylans.

Linkage composition

Methylation analysis confirmed that the $Ba(OH)_2$ extracted polysaccharides comprised mainly arabinoxylans (Table 2). Most of the xyloses were present as unsubstituted residues, as revealed by the high content of 2,3-Me_2-Xyl. A considerable portion of xylose residues was also singly or doubly substituted. The ratio of unsubstituted to substituted xylose was 1.68, and the ratio of doubly to singly substituted xylose residues was 0.59. Compared with water-extractable arabinoxylans, the $Ba(OH)_2$ extracted polymers were substituted to a much lesser degree. Substituted arabinofuranosyl residues appeared to be present in small quantities, indicating the presence of short arabinan side chains. Substantially larger amounts of terminal xylose residues were found in alkali-extractable arabinoxylans compared with water-extractable ones (Izydorczyk *et al.*, 1997). Their presence might indicate more complicated structural features of side chains in the alkali-extractable polymers. It has been suggested that terminal xylose units might be linked through arabinose residues to the xylan chains (Vinkx *et al.*, 1995), but this possibility still awaits experimental assessment in barley arabinoxylans. Appreciable amounts of substituted arabinose and non-reducing end xylose residues were previously reported in rice bran hemicelluloses (Shibuya & Iwasaki, 1985), rye bran (Ebringerova *et al.*, 1990), and alkali-extractable rye arabinoxylans (Vinkx *et al.*, 1995).

Methylation analysis confirmed that fraction $Ba(OH)_2/H_2O$ comprised both arabinoxylans and β-glucans (Table 2). Fractionation with ammonium sulphate allowed for more detailed examination of glycosidic linkages in the individual subfractions. β-Glucans in fraction $Ba(OH)_2/H_2O$ contained glucose residues linked predominantly through β-(1→4) and β-(1→3). The ratio of β-(1→4) to β-(1→3) linkages in the subfractions ranged from 3.4 to 2.6, the highest being for $Ba(OH)_2/H_2O_{30}$ and the lowest for $Ba(OH)_2/H_2O_{65}$. Subfraction $Ba(OH)_2/H_2O_{30}$ contained also small amounts of 2-, 3-, and 6-substituted 1→4-linked glucose and 6-substituted 1→3-linked glucose residues. Only trace amounts of these residues were found in $Ba(OH)_2/H_2O_{65}$. Arabinoxylans in unfractionated $Ba(OH)_2/H_2O$ had a slightly higher degree of substitution, compared with those in the $Ba(OH)_2$ fraction, as shown by the lower ratio of unsubstituted to substituted xyloses (Table 2). However, the ratio of doubly to singly substituted xyloses was higher in $Ba(OH)_2$ than in $Ba(OH)_2/H_2O$. After fractionation with ammonium sulphate, arabinoxylans were found in subfractions $Ba(OH)_2/H_2O_{50-100}$. The amount of unsubstituted xyloses in the subfractions decreased from $Ba(OH)_2/H_2O_{50}$ to $Ba(OH)_2/H_2O_{100}$, but the level of monosubstituted xylose residues remained almost constant. Only traces of doubly substituted xyloses were found in $Ba(OH)_2/H_2O_{50}$. This is in stark contrast to water-extractable arabinoxylans, which were especially enriched with disubstituted xylose residues

Table 2. Linkage composition (mol %) of $Ba(OH)_2$ and $Ba(OH)_2/H_2O$ fractions and subfractions

Component	Linkage type	$Ba(OH)_2$	$Ba(OH)_2/H_2O$	$Ba(OH)_2/H_2O_{30}$	$Ba(OH)_2/H_2O_{50}$	$Ba(OH)_2/H_2O_{65}$	$Ba(OH)_2/H_2O_{100}$
2,3,5-Me_3-Ara	(Ara*f*)1→	24.7	11.6 (21.0)[a]	tr	5.6 (18.9)	15.5 (22.0)	(23.0)
2,3,4-Me_3-Xyl	(Xyl*p*)1→	5.0	4.0 (7.2)	—	2.0 (6.7)	4.8 (7.0)	(9.2)
3,5-Me_2-Ara	→2(Ara*f*)1→	1.0	1.1 (2.0)	—	0.3 (1.0)	0.9 (1.4)	(2.2)
2,5-Me_2-Ara	→3(Ara*f*)1→	1.0	0.6 (1.1)	—	0.3 (1.0)	0.2 (0.3)	(1.3)
2,3-Me_2-Ara	→5(Ara*f*)1→	0.4	0.8 (1.4)	—	0.2 (0.7)	0.2 (0.3)	(2.4)
2,3-Me_2-Xyl	→4(Xyl*p*)1→	44.3	22.6 (40.9)	tr	15.2 (51.3)	29.0 (42.5)	(33.2)
2-Me-Xyl+	→3,4(Xyl*p*)1→	14.8	10.3 (18.6)	—	6.0 (20.3)	12.0 (17.6)	(20.3)
3-Me-Xyl	→2,4(Xyl*p*)1→						
Xyl	→2,3,4(Xyl*p*)1→	8.8	4.3 (7.7)	—	0.2 (0.7)	5.5 (8.1)	(8.5)
2,3,4,6-Me_4-Glc	(Glc*p*)1→	—	0.9	0.8	0.6	0.8	—
2,4,6,-Me_3-Glc	→3(Glc*p*)1→	—	9.8	20.8	17.9	8.3	—
2,3,6-Me_3-Glc	→4(Glc*p*)1→	—	32.2	70.1	47.7	21.7	—
2,6-Me_2-Glc	→3,4(Glc*p*)1→	—	0.1	2.2	1.5	0.5	—
3,6-Me_2-Glc	→2,4(Glc*p*)1→	—	0.1	1.3	0.4	tr	—
2,4-Me_2-Glc	→3,6(Glc*p*)1→	—	0.4	0.8	0.4	tr	—
2,3-Me_2-Glc	→4,6(Glc*p*)1→	—	0.6	3.9	1.0	0.5	—
(1→4)/(1→3) Glc Ratio		—	3.3	3.4	2.7	2.6	—
Unsub/Subs Xyl[b]		1.68	1.5	—	2.5	1.7	1.1
Doubl/Singl Xyl[c]		0.59	0.41	—	0.03	0.48	0.43

[a] Amounts in parentheses are based on arabinoxylan content only.
[b] 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→].
[c] Ratio of doubly [→2,3,4(Xyl)1→] to singly substituted xylose residues [→3,4(Xyl)1→ + →2,4(Xyl)1→].

(Izydorczyk *et al.*, 1997). The ratio of unsubstituted to substituted xylose residues declined from 2.5 to 1.1 as the Xyl/Ara ratio decreased, whereas the ratio of doubly to singly substituted residues increased from 0.03 to 0.48. It is not entirely clear why arabinoxylans in $Ba(OH)_2/H_2O$ fraction were not released by $Ba(OH)_2$, since the structural features of the two arabinoxylan populations were not substantially different. One possible explanation is that a portion of arabinoxylans is physically associated (entangled) with β-glucans and their release occurs only when β-glucans are solubilized (i.e., in the absence of $Ba(OH)_2$).

The results of methylation analysis of NaOH-extracted polysaccharides are given in Table 3. The data confirm that arabinoxylans and β-glucans were present in this fraction. In general, the arabinoxylans were enriched in unsubstituted xylose but had the lowest amount of doubly substituted residues. As a result, the ratio of unsubstituted to substituted xylose in this fraction was the highest (2.6) and that of doubly to singly substituted xylose the lowest (0.16) among the three arabinoxylan fractions. These parameters, therefore, seem to account for the differences in extractability of different populations of arabinoxylans. β-Glucans in the NaOH fraction exhibited a much higher ratio of (1→4) to (1→3)-linked glucose than their counterparts in $Ba(OH)_2/H_2O$ fraction (5.1 vs 3.3). This feature too might be responsible for the differences in the solubility of these polymers.

A more detailed analysis of the least soluble subfraction $NaOH_{30}$ revealed that its constituents had rather unusual molecular characteristics. The arabinoxylans, which constituted approximately 30% of this fraction, had a very high ratio (5.3) of unsubstituted to substituted xyloses. The presence of sequences of contiguously unsubstituted xylose residues is, therefore, highly possible in these arabinoxylans. Another unusual feature of this fraction that merits comment was the complete absence of doubly substituted xylose residues. β-Glucans, which represented approximately 78% of $NaOH_{30}$ subfraction (Table 1), exhibited a very high ratio of (1→4) to (1→3) linkages (6.6). Such a high value indicates the presence of longer blocks of adjacent 1→4 linkages in this fraction. These results support the hypothesis that the presence of contiguously unsubstituted xylan segments in arabinoxylans and of cellulose-like regions in β-glucans render these polymers less soluble. The results indicate also that both polymers exhibit structural features that would permit some intermolecular alignment between polymer chains and/or non-covalent interactions of arabinoxylans with β-glucans. The problems associated with separation of β-glucans from arabinoxylans in this subfraction might, therefore, be due to some interactions between these polymers. Non-covalent interactions of β-glucans with arabinoxylans have already been considered as responsible for water unextractability of these polymers; however, until now no structural prerequisites for such interactions have been revealed in either β-glucans or arabinoxylans, and no actual interactions have been demonstrated experimentally.

Hydrolysis of β-glucans with lichenase

Fine structural features of water-unextractable β-glucans were further investigated by enzymic hydrolysis with lichenase. This enzyme hydrolyses only the β-(1→4) linkages between glucose residues which are linked at C-O-3 position, and information about the degree of polymerization of β-(1→4) linkages may be deduced from chromatographic analysis of the released oligosaccharides. The proportions of the soluble products released by lichenase from $Ba(OH)_2/H_2O$ and NaOH fractions and subfractions are given in Table 4. The tri- and tetrasaccharide components from β-glucans in $Ba(OH)_2/H_2O$ accounted for 88.8% of the total analysed. There was a gradual decline of oligosaccharides with DP 5–8 from 3.8 to 0.8%, followed by an increase in peak size at DP 9 to 1.5% of the total. Oligosaccharides with DP 10–13 accounted for about 2.5%. The general profiles of the oligosaccharides released from the three subfractions of $Ba(OH)_2/H_2O$ containing β-glucans were similar; however, the proportion of individual fragments differed slightly. There was a progressive decrease in the amount of tri- and tetrasaccharides with concomitant increase in the amount of higher oligosaccharides with DP 10–13 from $Ba(OH)_2/H_2O_{30}$ to $Ba(OH)_2/H_2O_{65}$. The tri-/tetrasaccharide ratio was relatively constant for all subfractions.

β-Glucans in the NaOH fraction exhibited a slightly different structure. The amount of tri- and tetrasaccharides was lower than in the $Ba(OH)_2/H_2O$ fraction and accounted for 85.6% of the total oligosaccharides. On the other hand, the ratio of DP 3/DP 4 oligosaccharides in NaOH (2.41) was higher than in $Ba(OH)_2/H_2O$ (2.12) or in the water-extractable β-glucans [1.76 and 2.13 for 40°C (WE40) and 65°C (WE65) extracts, respectively] (Izydorczyk *et al.*, 1997). Also the amount of oligomers with DP 10–13 in the NaOH fraction (5.5%) was substantially higher than in $Ba(OH)_2/H_2O$ (2.5%) and in water-extractable β-glucans (1.1 and 1.7% in WE40 and WE65, respectively) (Izydorczyk *et al.*, 1997).

In both fractions, $Ba(OH)_2/H_2O$ and NaOH, large amounts of water-insoluble precipitate, 8.4 and 10.5% (w/w), respectively, were released by β-glucans on digestion with lichenase (Table 5). These amounts were considerably higher than those precipitated from water-extractable β-glucans (Izydorczyk *et al.*, 1997). The least soluble subfractions $Ba(OH)_2/H_2O_{30}$ and $NaOH_{30}$ were especially rich in this insoluble material, but the

Table 3. Linkage composition (mol %) of NaOH fraction and subfractions

Component	Linkage type	NaOH	$NaOH_{30}$	$NaOH_{50}$	$NaOH_{65}$	$NaOH_{100}$
2,3,5-Me_3-Ara	(Ara*f*)1→	8.1 (13.7)[a]	2.5 (8.6)	6.1 (11.7)	7.6 (11.1)	(15.1)
2,3,4-Me_3-Xyl	(Xyl*p*)1→	5.2 (8.8)	2.8 (9.7)	3.6 (6.9)	4.5 (6.6)	(5.5)
3,5-Me_2-Ara	→2(Ara*f*)1→	1.3 (2.2)	0.7 (2.4)	1.7 (3.2)	2.4 (3.5)	(3.3)
2,5-Me_2-Ara	→3(Ara*f*)1→	1.0 (1.7)	0.4 (1.3)	0.2 (0.4)	0.2 (0.3)	(3.3)
2,3-Me_2-Ara	→5(Ara*f*)1→	1.3 (2.2)	0.5 (1.7)	1.1 (2.1)	1.8 (2.6)	(3.0)
2,3-Me_2-Xyl	→4(Xyl*p*)→	30.4 (51.6)	18.4 (66.7)	30.4 (52.8)	38.4 (56.2)	(44.2)
2-Me-Xyl +	→3,4(Xyl*p*)→	10.0 (16.9)	3.6 (12.4)	7.9 (15.1)	11.3 (16.5)	(21.1)
3-Me-Xyl	→2,4(Xyl*p*)→					
Xyl	→2,3,4(Xyl*p*)1→	1.6 (2.7)	—	1.2 (2.3)	2.1 (3.1)	(4.4)
2,3,4,6-Me_4-Glc	(Glc*p*)1→	1.4	0.5	0.5	0.9	—
2,4,6,-Me_3-Glc	→3(Glc*p*)1→	6.1	8.8	11.4	7.9	tr
2,3,6-Me_3-Glc	→4(Glc*p*)1→	31.2	58.9	34.8	22.0	tr
2,6-Me_2-Glc	→3,4(Glc*p*)1→	0.1	1.0	0.6	0.4	—
3,6-Me_2-Glc	→2,4(Glc*p*)1→	tr	—	0.1	0.3	—
2,4-Me_2-Glc	→3,6(Glc*p*)1→	tr	—	0.3	0.1	—
2,3-Me_2-Glc	→4,6(Glc*p*)1→	0.2	1.1	tr	tr	—
(1→4)/(1→3) Glc Ratio		5.1	6.6	3.0	2.8	—
Unsub/Subs Xyl[b]		2.6	5.3	3.0	2.8	1.7
Doubl/Singl Xyl[c]		0.16	—	0.15	0.19	0.21

[a] Amounts in parentheses are based on arabinoxylan content only.
[b] 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→].
[c] Ratio of doubly [→2,3,4(Xyl)1→] to singly substituted xylose residues [→3,4(Xyl)1→ + →2,4(Xyl)1→].

Table 4. Composition of water-soluble oligosaccharides (mol %) released by lichenase from β-glucan fractions[a]

DP	$Ba(OH)_2/H_2O$	$Ba(OH)_2/H_2O_{30}$	$Ba(OH)_2/H_2O_{50}$	$Ba(OH)_2/H_2O_{65}$
3	60.1	61.3	60.5	59.8
4	28.8	29.2	28.5	28.9
5	4.0	4.1	4.1	4.2
6	2.0	2.1	1.9	2.0
7	0.7	0.7	0.7	0.7
8	0.8	0.7	0.9	0.9
9	1.6	1.4	1.6	1.6
10–13	2.0	0.5	1.6	2.0
3+4 total	88.9	90.5	89.0	88.7
3:4 ratio	2.09	2.10	2.12	2.07
DP	NaOH	$NaOH_{30}$	$NaOH_{50}$	$NaOH_{65}$
3	61.6	63.4	61.7	61.2
4	26.1	26.7	25.3	25.7
5	4.0	4.1	4.0	4.1
6	1.8	1.9	1.9	1.8
7	0.7	0.6	0.7	0.7
8	0.8	0.7	0.8	0.9
9	1.4	1.3	1.5	1.5
10–13	3.5	1.3	3.9	4.0
3+4 total	87.7	90.1	87.0	86.9
3:4 ratio	2.36	2.37	2.43	2.38

[a] All fractions, except $Ba(OH)_2/H_2O_{50}$ and $NaOH_{50}$, were analysed in duplicate.

Table 5. Composition of oligosaccharides (mol %) in water-insoluble precipitate released during lichenase digestion of some fractions[a]

DP	$Ba(OH)_2/H_2O$ (ppt)	$Ba(OH)_2/H_2O_{30}$ (ppt)	NaOH (ppt)	$NaOH_{30}$ (ppt)
3	16.4	19.6	25.9	24.0
4	9.0	9.8	11.5	10.6
5	1.7	1.7	2.1	2.0
6	1.2	1.2	1.0	1.4
7	1.0	1.2	0.6	1.1
8	3.3	3.9	2.1	3.7
9	16.5	19.8	14.4	14.7
10	6.1	7.1	5.5	5.6
11	12.3	11.8	12.1	10.5
12	10.8	8.8	9.4	8.9
13	7.4	5.4	6.1	5.9
14	5.0	3.4	3.8	3.8
15	3.4	2.2	2.4	2.6
16	2.1	1.4	1.3	1.7
17	1.6	1.0	0.7	1.2
18	0.9	0.6	0.4	0.9
19	0.7	0.6	0.3	0.8
20	0.5	0.5	0.2	0.8

[a] The yield of the precipitate based on the amount of β-glucans present in the samples was 8.4%, 15.0%, 10.5%, and 16.5% for $Ba(OH)_2/H_2O$, $Ba(OH)_2/H_2O_{30}$, NaOH, and $NaOH_{30}$, respectively. All precipitates, except those of $Ba(OH)_2/H_2O_{30}$, were analysed singly.

more soluble subfractions $Ba(OH)_2/H_2O_{65}$ and $NaOH_{65}$ contained only traces of it. A mixture of oligomers with DP ranging from 3 to 20 was found in the precipitate and methylation analysis confirmed the presence of cellulose-like fragments with β-(1→4) linkages. In the material released by the action of lichenase on $Ba(OH)_2/H_2O$, the major components were oligosaccharides with DP 3, 9, 11, and 12, although identifiable peaks were analysed up to DP 20. In the material generated by lichenase from NaOH fraction, in addition to large amounts of DP 9, 11, and 12 a high content of DP 3 and 4 was also detected. These results were rather unexpected since the precipitate was extensively washed to avoid contamination from the soluble portion of the digested fractions. It is possible, however, that some of the smaller fragments were physically entrapped in the high mass of the precipitating material. It is prudent to assume that the cellulose-like segments must be of certain optimal length to form stable lateral junctions (most likely

stabilized by hydrogen bonds) and subsequently insoluble precipitates. If, however, the mass of the aggregating segments is large, smaller fragments, due to cooperative forces, might also be drawn into the aggregates. This would explain the presence of oligomers with DP 5–13 in the soluble portion of the digests (although in rather small amounts), as well as in the precipitates.

Molecular size distribution

Figure 1 shows the gel permeation pattern of the $Ba(OH)_2$ fraction. The extracted polymers were clearly polydisperse with apparent molecular weights in a very broad range. The bulk of the polysaccharide material eluted in the very high molecular weight region.

The elution pattern of $Ba(OH)_2/H_2O$ shows that this fraction comprised two populations of polysaccharides that differed considerably in hydrodynamic volumes (Fig. 2). HPSEC of the subfractions revealed that β-glucans were responsible for a peak eluted in a lower molecular weight region, as both subfractions, $Ba(OH)_2/H_2O_{30}$ and $Ba(OH)_2/H_2O_{50}$ containing the majority of β-glucans, eluted in this region. A small peak in the elution profile of $Ba(OH)_2/H_2O_{50}$ at higher molecular weights very likely indicates the presence of small amounts of arabinoxylans in this fraction. Arabinoxylan-enriched subfractions $Ba(OH)_2/H_2O_{65}$ and $Ba(OH)_2/H_2O_{100}$ eluted at low retention times and had very high apparent molecular weights, or at least very high hydrodynamic volumes.

The elution profile of the NaOH fraction reveals the presence of several polysaccharide populations (Fig. 3). The NaOH-extracted arabinoxylans most likely

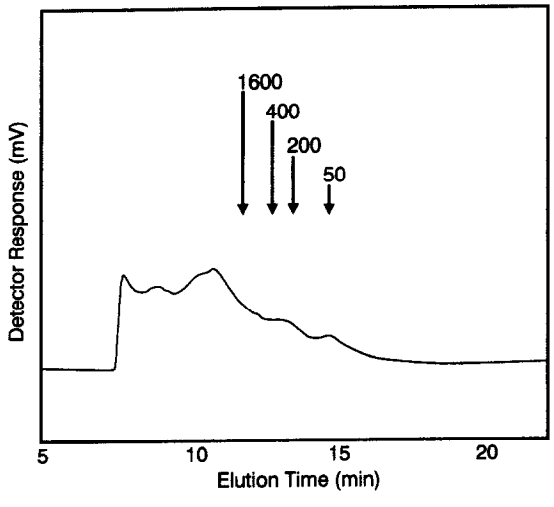

Fig. 1. High performance size exclusion chromatography of the $Ba(OH)_2$ fraction with refractive index detection. Arrows (↓) indicate elution time of pullulan standards with various molecular weights (MW): 1600, MW 1660×10^3; 400, MW 380×10^3; 200, MW 186×10^3; and 50, MW 48×10^3.

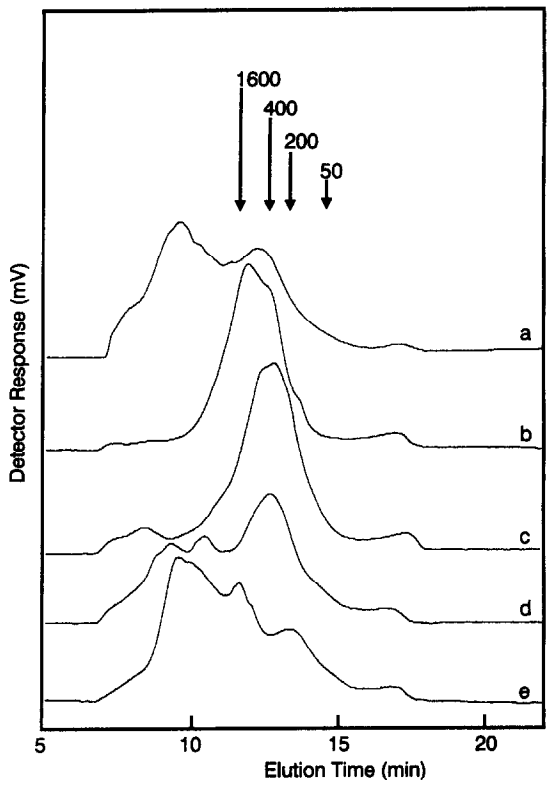

Fig. 2. High performance size exclusion chromatography of the $Ba(OH)_2/H_2O$ fraction and subfractions with refractive index detection: (a) $Ba(OH)_2/H_2O$ unfractionated; (b) $Ba(OH)_2/H_2O_{30}$; (c) $Ba(OH)_2/H_2O_{50}$; (d) $Ba(OH)_2/H_2O_{65}$; (e) $Ba(OH)_2/H_2O_{100}$. Arrows (↓) indicate elution time of pullulan standards, see caption in Fig. 1.

exhibited higher molecular weights than NaOH-extracted β-glucans as subfractions enriched in arabinoxylans appeared in a very high molecular weight region. The broad elution profiles and the presence of many shoulders might indicate either a heterogeneous nature of arabinoxylans or possibly some intermolecular interactions. Arabinoxylans are known to assume a fully extended rod-like conformation in solution, and their molecular weights are often overestimated by SEC (Andrewartha *et al.*, 1979). The present study has revealed that the alkali-extractable arabinoxylans possess structural characteristics that would allow for intermolecular alignment over short sequences of continuously unsubstituted xylose residues, which would lead to the formation of H-bond-stabilized macrostructures. All these factors, therefore, would probably lead to overestimation of molecular weights of arabinoxylans by SEC.

The intrinsic viscosity values (Table 1) have to be interpreted with caution since most alkali-extracted fractions and subfractions contained both polymers, which contribute to the overall values of $[\eta]$. Subfractions $Ba(OH)_2/H_2O_{30}$ and $Ba(OH)_2/H_2O_{50}$,

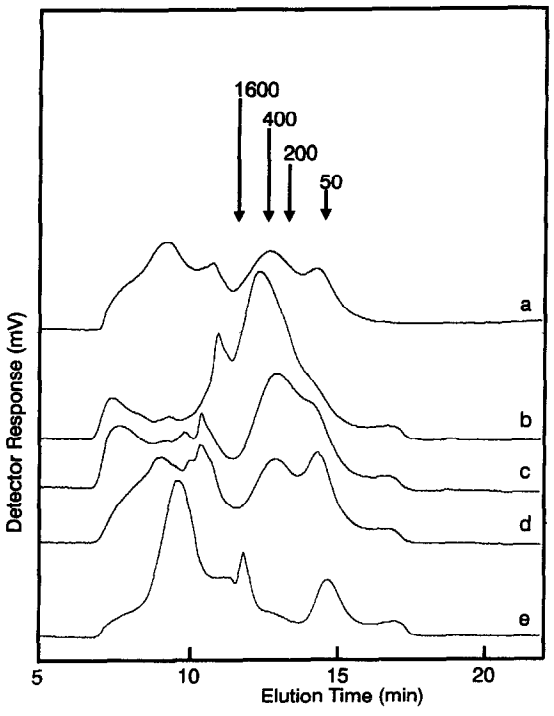

Fig. 3. High performance size exclusion chromatography of the NaOH fraction and subfractions with refractive index detection: (a) NaOH unfractionated; (b) $NaOH_{30}$; (c) $NaOH_{50}$; (d) $NaOH_{65}$; (e) $NaOH_{100}$. Arrows (↓) indicate elution time of pullulan standards, see caption in Fig. 1.

containing largely β-glucans, eluted as broad but relatively homogeneous peaks with peak maxima of approximately 900,000 and 400,000, respectively. The limiting viscosity values of 4.8 and 2.9 dl/g, respectively, reflected differences in molecular weight of these β-glucan fractions. However, subfractions containing relatively pure arabinoxylans ($Ba(OH)_2$/H_2O_{100} and $NaOH_{100}$) eluted in extremely high molecular weight regions but exhibited relatively low values of $[\eta]$. These contradictory results might be related to overestimation of molecular weights by SEC as explained above.

CONCLUSIONS

The present study revealed that several structural parameters distinguish alkali-extractable β-glucans and arabinoxylans from their water-extractable counterparts. The alkali-extractable β-glucans, in general, were characterized by higher ratios of β-(1→4)/(1→3) linkages, greater amounts of long, continuously linked β-(1→4) segments, and higher ratios of cellotriosyl/cellotetraosyl units than the water-extractable β-glucans (Izydorczyk *et al.*, 1997). The sum total of all these structural features of alkali-extractable β-glucans are most likely related to their non-extractability with water.

The presence of cellulose-like fragments in β-glucans may have very significant practical implications. Upon degradation of β-glucans with lichenase, these fragments are released and are capable of forming macroaggregates. The solubility of these cello-oligosaccharides is probably a sensitive function of their concentration, DP, temperature, and/or solvent conditions. Also, depending on their amount, they are capable of physically entrapping smaller degradation products, otherwise water-soluble, and, therefore, increasing the overall mass of the precipitating material. Their presence in mash or beer could, therefore, cause a risk that, under certain environment conditions determined by time/temperature/solvent, they might form aggregates that would subsequently result in filtering problems.

The alkali-extractable arabinoxylans constituted a large portion of the non-starch polysaccharides found in the malting barley. Several structural features, most notably very high Xyl/Ara ratio and a very low degree of substitution, distinguish the alkali-extractable arabinoxylans from their water-extractable counterparts (Izydorczyk *et al.*, 1997). Certain populations of arabinoxylans displayed structural characteristics that would enable them to self-associate or to interact with β-glucans. Theoretically, less substituted arabinoxylans should be more prone to enzymic hydrolysis by xylanases. However, because of possible intermolecular associations with other arabinoxylans and/or β-glucans, these polymers might in fact be protected from enzymic attack due to reduced accessibility of xylanases to their substrates. In conclusion, if alkali-extractable β-glucans and arabinoxylans dissolve and are inadequately degraded during malting and mashing, they have the potential to cause problems in filtration and haze formation to an even greater extent than their water-extractable counterparts.

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