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Sequential solvent extraction and structural characterization of polysaccharides from the endosperm cell walls of barley grown in different environments

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Abstract

The objective of this study was to examine the composition and molecular structure of the endosperm cell walls (CW) derived from barley grain grown in three environments in Canada, and differing in grain hardness, protein, and total β-glucan contents. The endosperm CW were isolated from barley, cv. Metcalfe, grown in Davidson, SK (Sample A), Hythe, AB (sample B), and Hamiota, MB (sample C). The CW were sequentially extracted with water at 65 °C, saturated $Ba(OH)_2$, again with water at 25 °C, and 1 M NaOH, resulting in fractions designated WE65, BaE, Ba/WE, and NaE, respectively. The monosaccharide analysis indicated the presence of β-glucans, arabinoxylans, and small amounts of arabinogalactans, glucomannans, and xyloglucans. Cellulose was detected in the CW remnants. The CW of sample A, exhibiting a lower grain hardness than sample B, contained the lowest amount of β-glucans, but the highest amount of arabinoxylans and the mannose-containing polysaccharides. The CW of sample C, characterized by very high protein content in the grain, contained the highest amount of β-glucans and the lowest amount of other polysaccharides. Polysaccharides in the CW of sample B, exhibiting the highest grain hardness, were characterized by the highest weight average molecular weights (M_w). β-Glucans in the CW of Sample B showed the highest ratio of DP3/DP4 and the longest cellulosic fragments in the polymeric chains. Of the three barley samples, arabinoxylans in the endosperm CW of sample A exhibited the lowest degree of branching, the highest amount of unsubstituted Xyl residues, and the highest ratio of singly to doubly substituted Xyl*p*. The highest water solubility of the CW of sample C was associated with the highest concentration of β-glucans, the lowest DP3/DP4 ratio, and the lowest M_w of the polymeric constituents. Arabinoxylans with the lowest amount of doubly substituted but the highest amount of unsubstituted xylose residues and long sequences of unsubstituted xylan regions were found in the NaE fractions. The NaE fractions showed a high ratio of →4)-Glc*p*-(1→ to →3)-Glc*p*-(1→ linkages and some →4)-Man*p*-(1→ linkages, indicating a high level of long cellulosic regions in β-glucan chains and the presence of glucomannans.
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Keywords: Barley; Endosperm cell walls; Hardness; Molecular structure; Polysaccharides

1. Introduction

Polysaccharides are the chief structural elements of the cell walls in various tissues making up the grain. In the starchy endosperm of barley, the predominant components of the walls are (1→3, 1→4)- β-D-glucans (~70%) and arabinoxylans (~20%), whereas levels of cellulose (~1–2%), glucomannans (~2%), and arabinogalactan–peptides are relatively low (Fincher 1975; Wilson et al., 2006; Woodward, Fincher, & Stone, 1983). The cell walls (CW) are dynamic structures whose composition and architecture are changing throughout the cell division, growth, and dif-

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ferentiation, and in addition undergoing further modification which can occur in response to environmental and pathogen-induced stresses (Wilson et al., 2006). As our understanding of the importance and contributions of CW and their constituents to human and animal nutrition and to agro-industrial processes such as paper and pulping, malting and brewing, milling, and bioethanol production grows, there is a need to clearly understand how the composition and properties of CW affect these processes and quality of grain-based products. For the malting and brewing industry the endosperm CW should be rapidly and uniformly degraded during germination as the residual CW are a barrier to the enzymes responsible for protein modification and starch hydrolysis during malting and mashing (Brennan et al., 1997; Palmer, 1992). In addition, high levels of CW polysaccharides in the wort cause increases in viscosity that may lead to filtration problems. Although high levels of β-glucans in the barley grain have been long regarded as deleterious to malting quality, the rate and extent of CW degradation cannot be reliably predicted based only on the content of these polymers or on the activity of β-glucanases, the enzymes responsible for their degradation (Bamforth, 1982,1994). The coexistence of several biopolymers in the cell walls, their spatial organization, and the nature of interactions among them contribute to the mechanical strength, permeability, and solubility, and therefore to enzymic susceptibility and digestibility of the cell walls. The extent of non-covalent self-associations among chains of β-glucans or arabinoxylans, as well as the extent of cross interactions between these polymers has been suggested to affect their extractability and solubility (Izydorczyk & MacGregor, 200). Such interactions (mostly through intermolecular hydrogen bonds) are governed by certain molecular characteristics of β-glucans and arabinoxylans (Izydorczyk & MacGregor, 2000; Izydorczyk, Macri, & MacGregor, 1998a; Izydorczyk, Macri, & MacGregor, 1998b) and hence, structural differences among these polysaccharides might contribute to the overall mechanical properties of the CW. Covalent cross-linking among arabinoxylans chains through esterified hydroxycinnamic acids also leads to changes in physicochemical, structural, and mechanical properties of the walls. Recently, Hrmova, Farkas, Lahnstein, and Fincher (2007) presented findings that raise the possibility of covalent linkages between xyloglucans and β-glucans and/or xyloglucans and cellulose in barley; these types of linkages could have important implications for wall rigidity, porosity, and digestibility.

The concentration of β-glucans in the whole barley grain is clearly influenced by genetic and environmental factors. Han et al. (1995) noted that the largest effect on the content of β-glucan in barley was associated with a locus on chromosome 2(2H). The total β-glucan content in the grain generally increases when barley is grown in hot and dry conditions (Morgan & Riggs, 1981; Swanston, Ellis, Perez-Vendrell, Voltas, & Molina-Cano, 1997) although, the content of β-glucan maybe reduced in response to short periods of very high temperature (Savin, Stone, Nicolas, & Wardlaw, 1997). MacNicol, Jacobsen, Keys, and Stuart (1993) suggested that the timing of the heat or drought stress is important and found that drought stress occurring late in the grain-filling period had no effect on β-glucan content. The content of arabinoxylans in barley also depends on genetic and environmental factors (Henry, 1986; HoltekjØlen, Uhlen, & Knudsen, 2007) but less is known about the effects of these factors of the properties of arabinoxylans. Recently, Toole et al. (2006) reported that the degree of branching in arabinoxylans, present in the endosperm CW of wheat, decreased during development, and that the rate of restructuring was faster when the plants were grown at a higher temperature and restricted water availability. The effect of genotypic and environmental factors on the properties of the barley endosperm CW has not been thoroughly studied. Ahluwalia and Ellis (1985) reported no significant differences in the amounts and composition of the CW polysaccharides and proteins among good and poor malting barley varieties, but observed significant differences in the solubility of β-glucans. Fincher (1975) observed only very small differences in the morphological appearance and composition of the endosperm CW isolated from two different barley genotypes. Barley genotypes expressing very low levels of β-glucans were shown to have thinner endosperm CW (Aastrup, Erdal, & Munck, 1985).

The objective of this study was to examine the differences in composition and molecular structure of polysaccharides in the endosperm CW derived from the Canadian malting barley cultivar Metcalfe, grown in three different environments in Canada and differing in grain hardness and contents of β-glucan and protein.

2. Experimental

2.1. Materials

Barley grain, variety Metcalfe, was grown in 2003 in three different eco-zones in Canada: moist mixed grassland region with dark brown soil (Davidson, Saskatchewan, SK); low boreal region with gray black soil (Hythe, Alberta, AB); and aspen parkland region with thick black soil (Hamiota, Manitoba, MB); the samples were designated A, B, and C, respectively. The mean temperatures for Davidson (location A) were 15.6, 18.8, 20.4, and 11.3 °C for June, July, August, and September, respectively. Similar temperatures were recorded for Hamiota (location C): 15.1, 18.0, 19.7, and 10.7 °C, respectively. Lower mean temperatures were recorded for Hythe (location B): 13.8, 16.3, 14.1, and 9.3 °C for the same months. Substantially higher amounts of precipitation occurred in Davidson (95, 44, 55,4, and 62.7mm in June, July, August, and September, respectively) than in Hythe (B) (44, 34.2, 18.8, and 24.8 mm) or in Hamiota (47, 32.6, 29, 28.2 mm).

The endosperm cell walls (CW) were isolated from the fibre-rich fractions (FRF) obtained by roller milling of

pearled barley (40% of the outer tissues removed; Satake, Type TM) according to the procedure described by Izydorczyk, Jacobs, and Dexter (2003). The FRF, enriched in the endosperm cell walls, were pin milled (Model 160Z, Alpine Corp., Augsburg, Germany) and dry-sieved on 250, 150, and 75 μm sieves to separate the starch granules from the cell wall material. The material with particle size above 75 μm was used for the isolation of the endosperm cell walls according to the methods of Mares and Stone (1973a), Ballance and Manners (1978), and Miller, Fulcher, Sen, and Arnason (1995). The pin-milled and sieved FRF were slurried in 70% aqueous ethanol and wet-sieved repeatedly on a 75 μm sieve. Repeated treatments of homogenization and sonication combined with wet-sieving using 1% sodium dodecyl sulfate (SDS) in 70% aqueous ethanol through the 75 μm screen were then carried out. The SDS was subsequently removed by washing with 70% ethanol and repeated wet sieving on a 75 μm sieve and filtration on a 53 μm pore size nylon cloth. The contaminating fragments of aleurone and husk tissues as well as the remaining cell contents were removed by repeated sedimentations; the crude cell wall material was suspended in 70% ethanol and allowed to settle for 5 min, and then the "fluffy" material remaining in suspension, that contained the endosperm cell walls, was collected by aspiration.

The isolated and purified endosperm cell walls (CW) were stored in 70% ethanol at room temperature rather than dried to avoid the dehydration that may cause changes in the physical state and solubility of the wall components.

2.2. Fractionation of endosperm cell wall material

The fractionation of the isolated CW is illustrated in Fig. 1. The isolated CW (~4 g wet weight) were filtrated through a 53 μm pore size nylon cloth to remove the alcohol and mixed with water (400 ml). The suspensions were extracted at 65 °C for 2 h and subsequently centrifuged (9000*g*, 20 min). The extraction was repeated twice. The extracts were combined and incubated with α-amylase (100 units porcine pancreas, Sigma, St. Louis, MO) overnight (pH 6.5, 10 mM $CaCl_2$ at 35 °C). The enzyme was inactivated by heat (95 °C, 20 min) and removed by centrifugation (9000*g*, 20 min). The water extract was dialyzed against distilled water (12,000–14,000 molecular weight cut off, 4 °C) for 3 days, freeze-dried and designated as the WE65 fraction. The water insoluble CW after the first extraction were suspended in a saturated $Ba(OH)_2$ solution (400 ml) containing 1% (w/v) $NaBH_4$ to prevent alkaline degradation. The suspension was stirred for 15 h at RT, and then centrifuged (9000*g*, 20 min). The residue was re-extracted with $Ba(OH)_2$ (300 ml) for 3 h at RT. The combined extracts were neutralized with acetic acid, dialyzed, and freeze-dried. This extract was designated as the BaE fraction. The remaining CW residue was suspended in distilled water (400 ml) and after stirring for 15 h at RT, the suspension was centrifuged (9000*g*, 20 min) and re-extracted with water (300 ml) for 3 h at RT. The combined extracts were neutralized, dialyzed and freeze-dried; this extract is referred to as the Ba/WE fraction. The insoluble CW residue was subsequently suspended in 1 N NaOH (400 ml) containing 1% (w/v) $NaBH_4$, stirred for 15 h at RT, centrifuged (9000*g*, 20 min) and re-extracted with NaOH (300 ml) for 3 h at RT. The combined extracts were neutralized, dialyzed and freeze-dried, as described above; this extract was designated as the NaE fraction. The CW remnants remaining after the sequential water and alkali extractions were neutralized, freeze-dried and designed as the Rem fraction.

2.3. Scanning electron microscopy

Intact CW and residues remaining after each extraction were examined by scanning electron microscopy (SEM). The samples were prepared for SEM by mounting them onto aluminium stubs covered with double-sided carbon adhesive discs and allowed to set for 24 h. The mounted samples were placed in a Hummer VII (Anatech, Ltd.) sputter coater and coated with a layer (50 nm) of gold and examined with a JEOL JSM-6400 scanning electron microscope at 10 KV.

2.4. Analytical methods

Protein content (%N × 5.7) in the grain and the CW-Rem fractions was determined by combustion nitrogen analysis (model FP-248 Leco Dumas CNA analyzer, St. Joseph, USA) calibrated with EDTA according to the AACC International (2000) Approved Method 46–30. Total β-glucan content was determined enzymatically using the Megazyme kit (Megazyme International, Bray, Ireland), according to the AACC International (2000) Approved Method 32–23. Grain hardness was measured by a single kernel characterization system (SKCS 4100, Perten Instruments Inc., IL). Protein content in the CW fractions was determined by the method of Lowry, Rosebrough, Farr, and Randall (1951) with bovine serum albumin used as standard (Sigma–Aldrich Canada, Ltd.).

The monosaccharide composition of intact CW and the CW fractions was determined (in duplicate) by high-performance liquid chromatography (HPLC). The intact CW was dried by a solvent exchange method before the monosaccharide analysis; the CW was initially dispersed in 100% ethanol, followed by dispersion in pure iso-propanol and then air-drying. The CW fractions were hydrolyzed in 1 M H_2SO_4 at 100 °C for 2 h. The intact CW and CW-Rem were initially hydrolyzed in 72% (w/w) H_2SO_4 at 4 °C for 1 h, and then in 1 M H_2SO_4 at 100 °C for 2 h. The hydrolyzates were neutralized with barium hydroxide and analyzed by HPLC equipped with a Dionex CarboPac PA1 column (4 × 250 nm), a PA1 guard column (4 × 50 mm) and a pulsed amperometric detector (PAD, Dionex Canada Ltd., Etobicoke ON). Hydrolysed samples

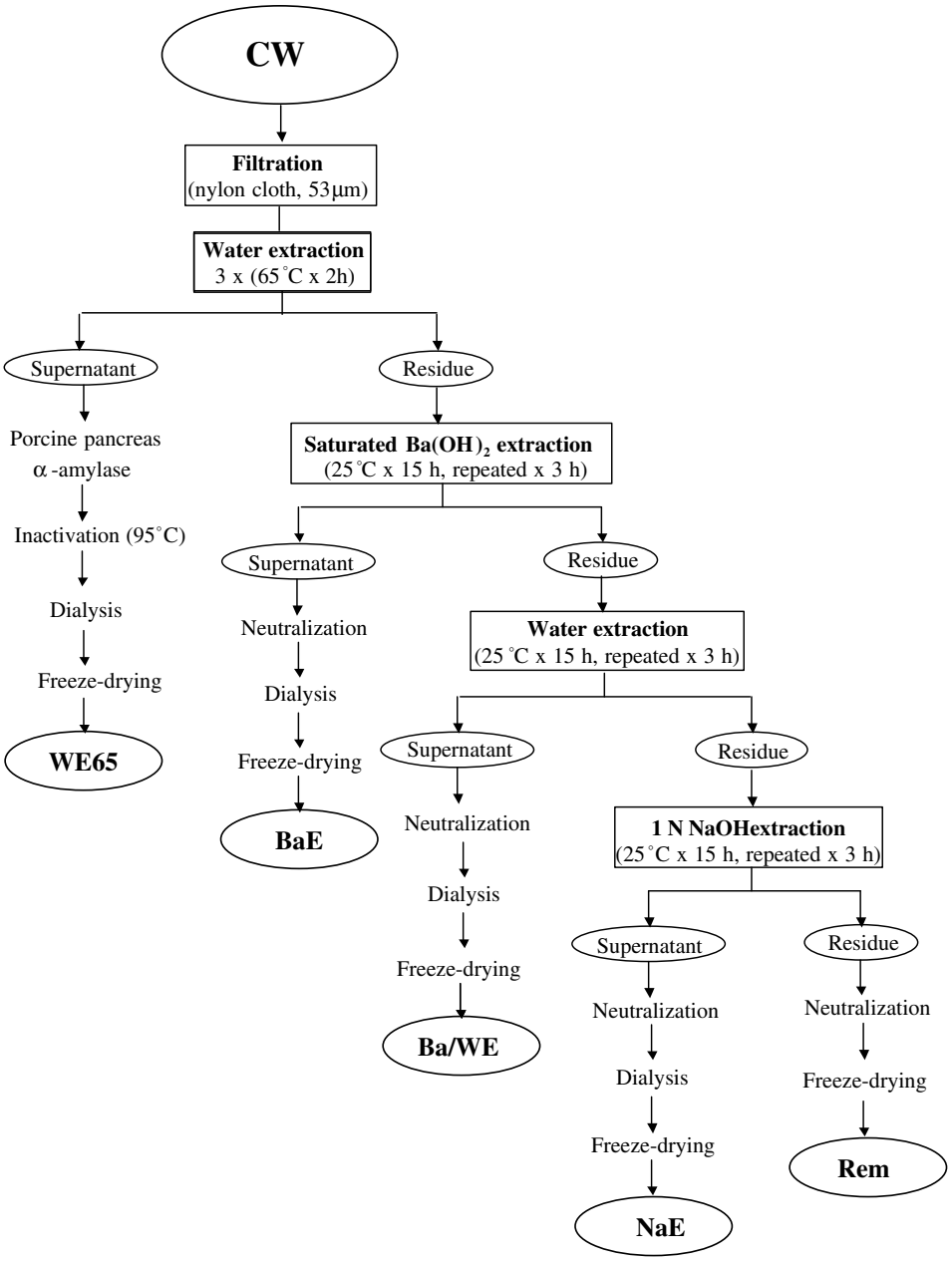

Fig. 1. Sequential fractionation of barley endosperm cell walls (CW) with water and alkali.

were eluted at 1.0 ml/min with 10 mM NaOH for 4 min, then with nanopure water for 26 min followed by a 2 min ramp to 100 mM NaOH, which was maintained for 6 min, then a 2 min return to the original starting conditions. Concentrated base (300 mM NaOH) was added to the post-column effluent at 0.5 ml/min (Dionex DQP-1 pump). Pulse potential, E (V), and duration, t (ms), were: $E_1 = 0.05$, $t_1 = 300$; $E_2 = 0.75$, $t_2 = 300$; $E_3 = -0.20$, $t_3 = 480$. The response time of the detector was 3 s, and the output range (sensitivity) was 10 K nA. Standard monosaccharides, arabinose (Ara), galactose (Gal), glucose (Glc), xylose (Xyl), and mannose (Man) were obtained from Sigma (St Louis, MO). The reported data represent means of duplicate analyses with a coefficient of variation (CV) not exceeding 5% in each case.

Linkage composition of the polysaccharides in the CW fractions was determined (in duplicates) by methylation analysis 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 gas-liquid chromatograph and mass spectroscopy (GLC-MS). Identification of the methylated alditol derivatives was possible by comparing the resultant spectra with those published (Carpita

& Shea, 1988). The effective carbon response factors, as given by Sweet, Shapiro, and Albersheim (1975), were used.

Digestion (in duplicates) of β-glucans in the CW fractions (2 mg/ml) with lichenase (*Bacillus subtilis*, EC 3.2.1.73, Megazyme International, Ireland, Ltd.; 2 U/mg, 10 mM phosphate buffer, pH 6.5, 40 °C, 20 h) and detection of the oligomers in soluble and insoluble digests was conducted as described in details by Izydorczyk et al. (1998a). Arabinoxylans in the CW fractions (2 mg/ml) were digested with endo-(1→4)-β-D-xylanase (*Trichoderma viride*, EC 3.2.1.8, Megazyme; 3 U/mg, 25 mM sodium acetate buffer, pH 4.7, 50 °C, 20 h). The enzyme was inactivated by heating at 95 °C (20 min). The solutions were filtered and the oligosaccharides were analyzed by the HPAEC system equipped with the CarboPac PA1 column, a PA1 guard column and the PAD detector. Eluent A and B were 150 mM NaOH and 500 mM sodium acetate in 150 mM NaOH, respectively. Digests were eluted at a flow rate of 1 ml/min at ambient temperature starting with 100% A, followed by a 10 min ramp to 70% A and 30% B, then a second gradient to 100% B from 10 to 45 min. Pulse potential, E (V), and duration, t (ms), 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 1 K nA. Xylose was obtained from Sigma (St. Louis, MO), whereas xylobiose, xylotriose, xylotetraose, xylopentaose, and xylohexaose were purchased from Megazyme (Megazyme International, Bray, Ireland). Intact masses and molecular structure of arabino-xylooligosaccharides in the digests were obtained from the mass spectrometry analysis. Enzymatic digests were desalted using the AG 50W-X8 resin, H+ form, (Bio-Rad), mixed with 10 mM NaCl and deposited onto the target. Matrix solution, saturated 2,5-dihydroxybenzoic acid (DHB) in 70%ACN/0.1%TFA, was added to the samples and allowed to air dry. MALDI-TOF (Matrix-Assisted Laser Desorption/Ionisation Time-Of-Flight) mass spectrometry was performed using a prototype QqTOF tandem mass spectrometer equipped with a nitrogen laser of 337 nm (Loboda, Krutchinsky, Bromirski, Ens, & Standing, 2000). After delayed extraction time, the ions were accelerated to 10 kV. The working mass range was 500–5000 amu at an orthogonal extraction frequency of 7500 Hz. A two-point calibration with singly-charged ions of dalargin, substance P and bee venom (monoisotopic masses 726.394, 1347.736 and 2845.762, respectively) was performed. Non-permethylated and permethylated (Ciukanu & Kerek, 1984) oligomers with m/z 569, 701, and 833 were analyzed on a MALDI QqTOF prototype to obtain high resolution spectra in the single-MS and tandem MS modes.

2.5. Molecular weight determination

Weight-average molecular weights (M_w) of polymers in the CW fractions were estimated by using high-performance size-exclusion chromatography with online multiangle laser light-scattering detection (HPSEC-MALLS). The HPSEC-MALLS system was comprised of a guard column (TSK PWH, TosoHaas GmbH, Stuttgart, Germany), two 3TSK-gel-packed columns (G5000 60 cm and G2500 30 cm, Toso Corp.), and refractive index (RI) and UV detectors (Waters 410 and Waters 490, respectively). Samples were dissolved in boiling water and filtered through 1.6 mm acrodisc glass fiber syringe filters (13 mm, Whatman Inc., Clifton, NJ). Solutions (0.2 ml, 1 mg/ml) were injected and eluted at 0.4 ml/min with 0.15 M sodium nitrate, containing 0.02% sodium azide, as a mobile phase at 25 °C. The calculations of M_w were performed using Astra 4.72 software (Wyatt Technology) with a $(dn/dc) = 0.145$. The analyses were conducted in duplicate.

3. Results and discussion

3.1. Composition of intact endosperm CW and the water- and alkali-extractable fractions

The endosperm cell walls (CW) were isolated from a Canadian malting barley variety, (cv. Metcalfe) grown in the same year in three environments differing in the soil type and weather conditions. As a result, the grain samples differed in grain hardness, protein and β-glucan contents. The total β-glucan content in whole grain was 4.2%, 4.6% and 4.8% (d.b.), whereas the protein content was 10.8%, 11.8% and 17.1% for samples A (Davidson, SK), B (Hythe, AB), and C (Hamiota, MB), respectively. Barley B had the highest hardness index (68.9), whereas A (59.3) and C (61.7) exhibited similar hardness values as determined by measurements using a single kernel characterization system (SKCS).

The isolated CW consisted predominantly of non-starch polysaccharides and contained 3.7–4.7% proteins (Table 1) and less than 1.1% of starch contaminants. In all three barley samples, the isolated CW consisted mainly of glucose, xylose and arabinose residues indicating the presence β-glucans, arabinoxylans, and possibly some xyloglucans. Small amounts of mannose (~2–5%) and galactose (<0.5%) might indicate the presence of arabinogalactans, glucomannans and/or (galacto)glucomannans. Because the ratio of Glc/Man in glucomannans or Ara/Gal in arabinogalactans is not known, their exact amount cannot be accurately determined from the monosaccharide analysis. Thus the amounts of Glc, Ara, and Xyl residues, obtained after acid hydrolysis (1M H_2SO_4) of the CW polymers, provide only estimates of the contents of β-glucans and arabinoxylans since some of these residues might constitute the other aforementioned polymers. The intact CW of sample B, exhibiting a higher grain hardness index than sample A, contained higher amounts of β-glucans but lower amounts of arabinoxylans and mannose- and galactose-containing polymers than the CW of sample A. The CW derived from sample C, characterized by very high grain protein content, had the highest amount of β-glucans and

Table 1
Composition of intact CW and CW fractions obtained upon sequential extraction with water and alkali

Sample/Fraction	Monosaccharide Composition (mg/100mg)					Ara/Xyl	Proteins (%)
	Ara	Gal	Glc	Xyl	Man		
Barley A							
Intact CW_A							
(1 M H_2SO_4)	9.4	0.3	43.8	16.2	3.6	0.58	4.7
(72% H_2SO_4)	11.2	0.5	50.9	18.7	4.8		
Fractions							
CW_A-WE65	3.1	0.4	83.1	4.9	0.2	0.63	0.9
CW_A-BaE	22.6	0.4	24.9	39.2	0	0.58	7.7
CW_A-Ba/WE	11.4	0.6	52.3	13.4	2	0.85	3.3
CW_A-NaE	9.9	0.7	27.4	22.8	19.3	0.43	7.8
CW_A-Rem							
(1 M H_2SO_4)	12.3	0.8	10	16.2	10.7	0.61	2.4
(72% H_2SO_4)	10.4	0.6	21.9	17.1	19.9		
Barley B							
Intact CW_B							
(1 M H_2SO_4)	7.6	0.3	46.8	11.1	3.1	0.67	3.7
(72% H_2SO_4)	8.2	0.3	49.8	12.3	3.2		
Fractions							
CW_B-WE65	2.3	0.2	72.3	3.4	1.1	0.68	1.2
CW_B-BaE	19.7	0.4	21.7	31.1	0	0.63	8
CW_B-Ba/WE	7.5	0.3	56	8.8	1.7	0.85	2.6
CW_B-NaE	9.9	0.7	33.7	18.3	12.3	0.54	6.7
CW_B-Rem							
(1 M H_2SO_4)	9	0.5	9	10.5	6		
(72% H_2SO_4)	11	0.5	22	14.3	16.5	0.77	2.9
Barley C							
Intact CW_C							
(1 M H_2SO_4)	7.1	0.3	53.3	10.4	1.9	0.72	4.5
(72% H_2SO_4)	7.8	0.3	62.3	10.8	2.2		
Fractions							
CW_C-WE65	2	0.2	72	3.3	0.8	0.61	1.2
CW_C-BaE	19.9	0.4	25.8	28.7	0	0.69	8.3
CW_C-Ba/WE	6	0.2	44.4	7.2	0.9	0.88	2.7
CW_C-NaE	10.1	0.5	39.5	16.2	8.7	0.62	4.8
CW_C-Rem							
(1 M H_2SO_4)	11.1	0.6	16.4	12.8	9.6	0.89	3.8
(72% H_2SO_4)	12.4	0.7	26.8	14.1	15.8		

[a]Results of duplicate analyses, CV < 5%.

the lowest amount of arabinoxylans and mannose- and galactose-containing polymers among the samples. The difference between the amounts of Glc residues determined after digestion of the CW material with 72% H_2SO_4 and 1 M H_2SO_4 theoretically indicates the content of cellulose in the preparations. The results shown in Table 1 imply a relatively low content of cellulose in the CW of sample B. However, the amounts of carbohydrates recovered after either type of hydrolysis for sample B (68.89% and 73.86%) was lower than for samples A and C, indicating difficulties in hydrolyzing the polysaccharide in the intact CW_A. These differences might indicate that the CW of sample B may contain a cellulosic skeleton that is stronger and more resistant to hydrolysis compare to that of A and C.

The relative monosaccharide composition (% mol) of fractions sequentially extracted from the intact CW with water and alkali is presented in Fig. 2. The WE65 fractions contained mainly β-glucans (89.10–90.7% of Glc residues), much smaller amounts of arabinoxylans (8.0–8.5% of Ara and Xyl), and only minute amounts of mannose- and galactose-containing polymers. The results are generally in agreement with previous reports, although relatively smaller amounts of β-glucans and more arabinoxylans were reported to be present in extracts obtained at lower temperatures (Ahluwalia & Ellis, 1985; Ballance & Manners, 1978; Fincher, 1975; Izydorczyk et al., 1998a; Storsley, Izydorczyk, You, Biliaderis, & Rossnagel, 2003). It appears that the extractability/solubility of β-glucans increases more steeply with the increasing extraction temperature than that of arabinoxylans, pointing to the differences in the degree and/or strength of binding of these polymers within the CW network. Some differences in the composition of the WE65 fractions were observed among the samples. The WE65 fraction of sample A contained the highest amount of arabinoxylans and galactose-containing polysaccharides, whereas the lowest amount of β-glucans and mannose containing polymers among the three samples. The WE65 fraction of the

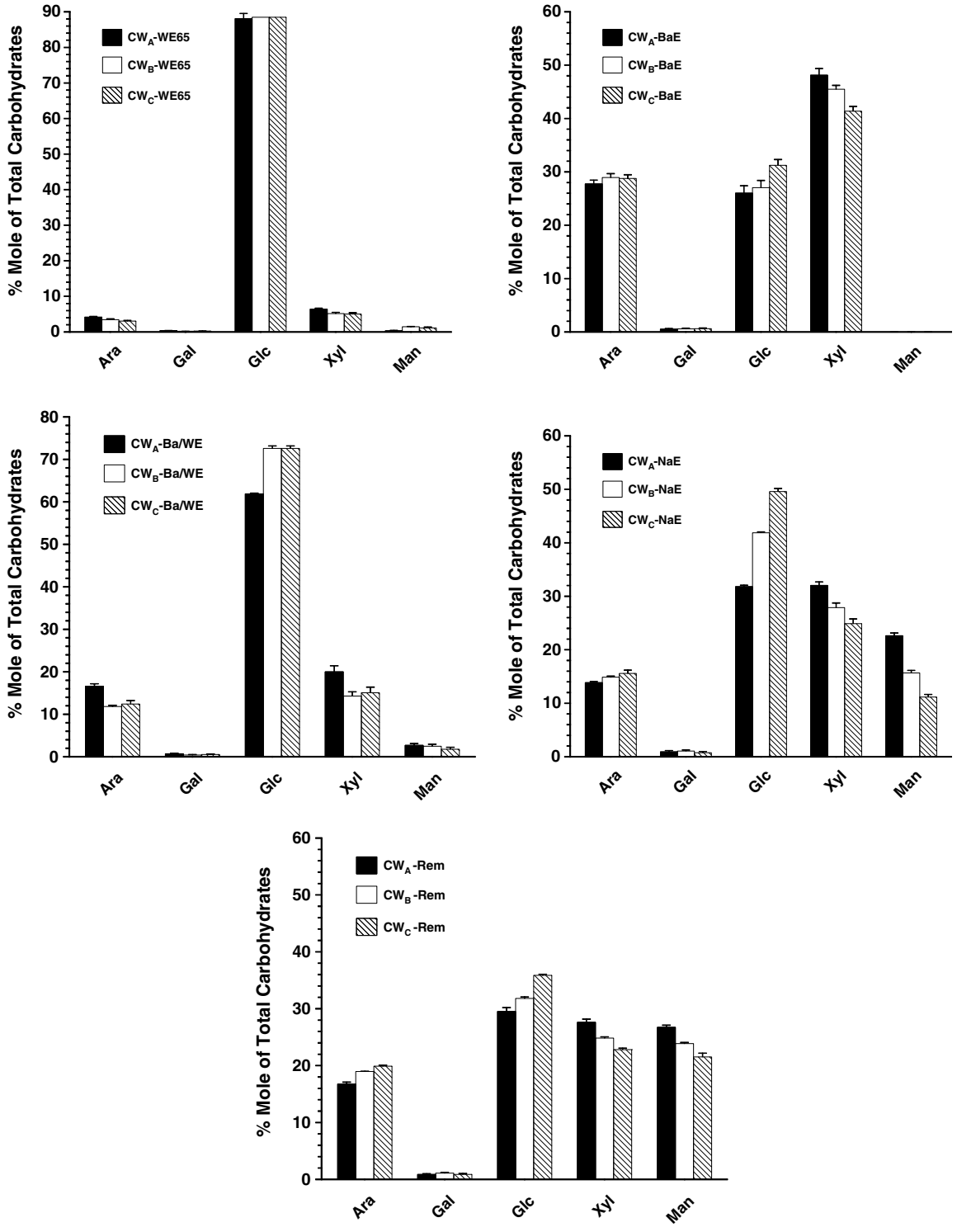

Fig. 2. Relative amounts of carbohydrates released sequentially from the CW with each solvent, expressed as% of total carbohydrates in each fraction.

CW of sample B contained slightly more Glc and Man residues than sample A.

The morphological features of the intact CW and the material remaining after each extraction stage were examined by SEM (Figs. 3 and 4). Following the extraction of the endosperm cell walls with water, the wall materials retained their structural integrity and thus, the wall–wall adhesion between adjacent cells was still evident (Fig. 3). However, the surface indentations due to starch granules and/or protein networks could no longer be seen. It appears that the water-soluble CW polymers, mostly composed of β-glucans, are encrusted onto the inner surface of

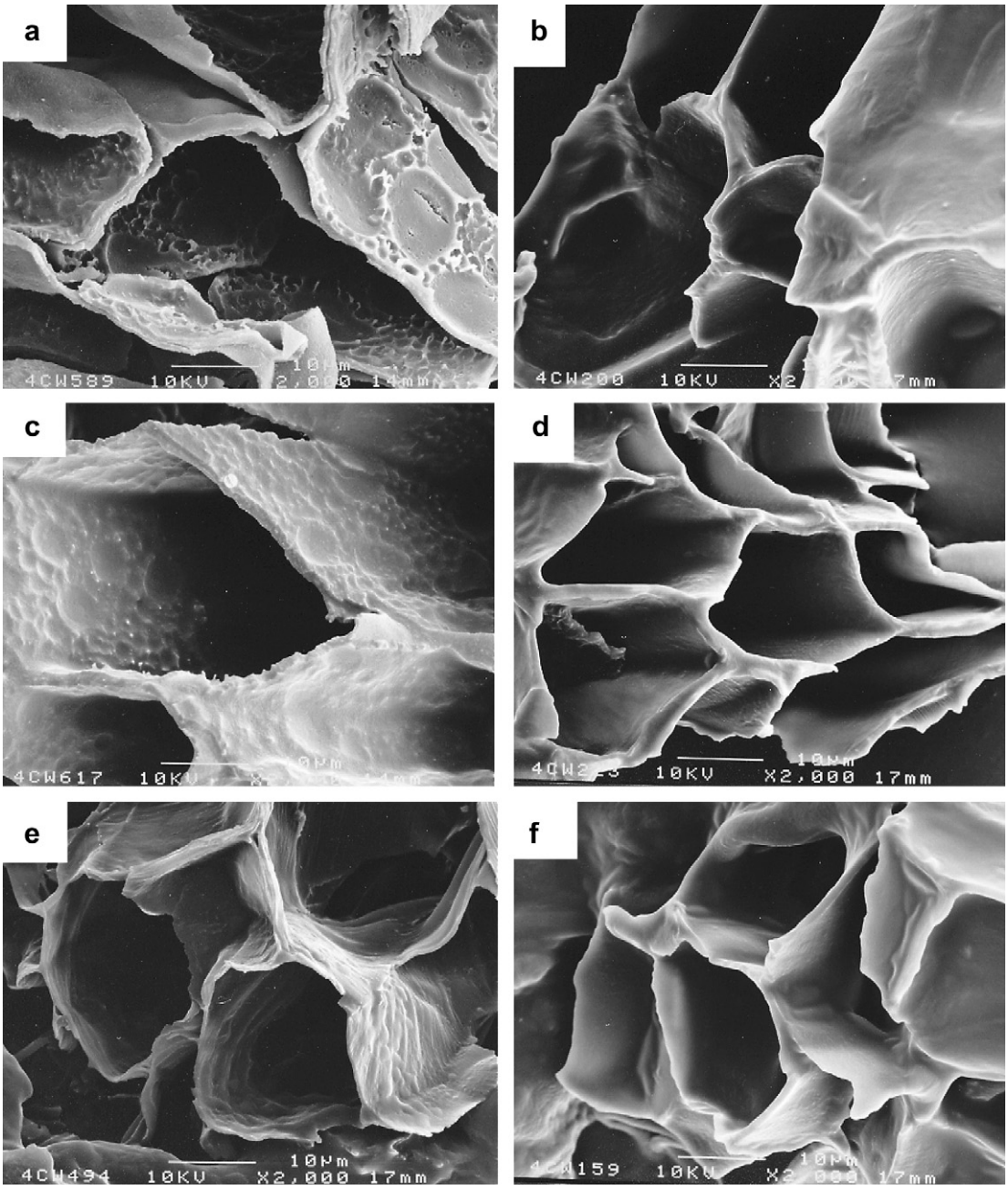

Fig. 3. Scanning electron micrographs of the intact and water-extracted CW: (a) intact CW of sample A, (b) CW of sample A after water extraction at 65 °C, (c) intact CW of sample B, (d) CW of sample B after water extraction at 65 °C, (e) intact CW of sample C, (f) CW of sample C after water extraction at 65 °C.

the walls rather than being distributed throughout the wall structure as previously suggested (Fincher, 1975; Mares & Stone, 1973a).

The thickness of the intact endosperm cell walls, estimated from the SEM micrographs, ranged from 0.5 to 1.6, 0.5 to1.7 and 0.8 to 2.3 μm for samples A, B, and C, respectively. The thickness of the cell walls remaining after the water extraction ranged from 0.3 to 1.4 μm for all samples. These results imply that the thickness of the CW was reduced by approximately 20% for samples A and B, and by almost 45% for sample C. The greater water solubility of the CW of sample C was also confirmed from a greater yield of the CW_C-WE65 fraction (45.9%) compared to the yields of CW_A-WE65 (31.8%) and CW_B-WE65 (29.3%).

The subsequent extraction of the CW material with saturated barium hydroxide solubilized more arabinoxylans than any other polymers. While the hydroxyl ions are believed to disrupt the hydrogen and covalent bonds, loosen up the cell wall matrix and consequently release various CW polysaccharides, the Ba^{2+} and borohydride ions are thought to specifically interact with pentose sugars of arabinoxylans and preferentially ease their extractions

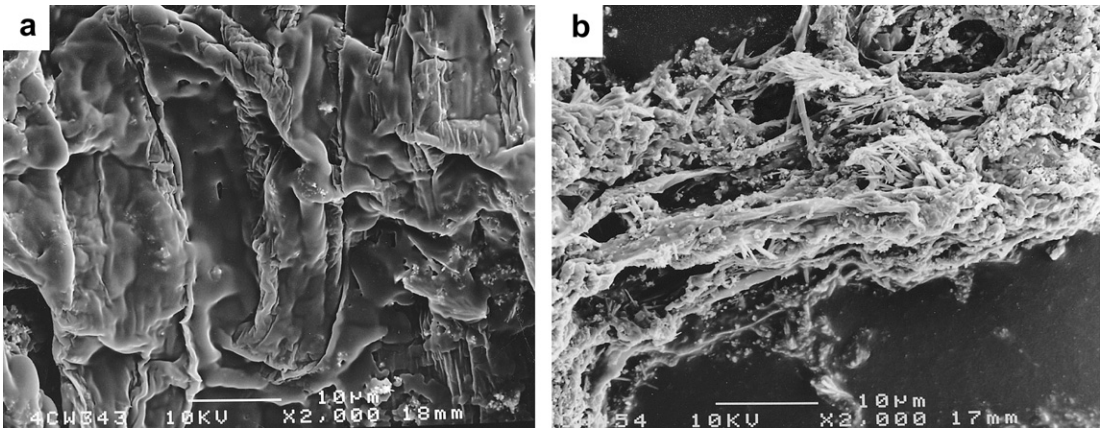

Fig. 4. Scanning electron micrographs of CW residue of sample A remaining after water and $Ba(OH)_2$ extractions (a) and after subsequent extraction with NaOH (b).

(Bergmans, Beldman, Gruppen, & Voragen, 1996; Gruppen, Hamer, & Voragen, 1991; Li, Cui, & Kakuda, 2006; Verbruggen, Beldman, & Voragen, 1995). The BaE fractions contained 68.8–74.3% of Ara and Xyl residues, 25–30.5% of Glc residues, and only very small amounts of galactose containing polymers (Fig. 2). No mannose-containing polysaccharides were extracted with $Ba(OH)_2$ (Table 1 and Fig. 2). The portion of β-glucans extracted with $Ba(OH)_2$ possibly represents a population of these polymers associated with arabinoxylans. The CW_A-BaE fraction contained relatively more arabinoxylans than CW_B-BaE and CW_C-BaE fractions. Following the extraction of the CW with saturated $Ba(OH)_2$, the general morphological features of endosperm cells disappeared, indicating the importance of covalent linkages in the overall architecture of the CW (Fig. 4a).

The subsequent water extraction of the CW material, remaining after the first two extractions, removed more hexose- than pentose-containing polysaccharides (Fig. 2). The Ba/WE extracts contained approximately 60–70% of β-glucans, 25–35% arabinoxylans, 1.5–2.5% of mannose-containing polysaccharides, and 0.36–0.65% of galactose-containing polymers. The preceding extraction of arabinoxylans with $Ba(OH)_2$ seemed to loosen up the CW structure and eased the extraction of additional portion of β-glucans with water. It has been suggested that arabinoxylans may restrict the extraction of β-glucans in barley cell walls either through non-covalent topological associations between these polymers (Izydorczyk & MacGregor, 2000) or through physical entrapment of β-glucans in the covalently-linked network of arabinoxylans chains. It has been shown that arabinoxylan-hydrolyzing enzymes, including xylanases, arabinofuranases, xyloacetylesterase and feruloyl esterase, can release β-glucans from the CW materials (Bamforth & Kanauchi, 2001; Kanauchi & Bamforth, 2001, 2002; Moore, Bamforth, Kroon, Bartolome, & Williamson, 1996).

The least soluble fractions obtained with sodium hydroxide, the NaE fractions, contained a considerable amount of mannose (~10–20%) in addition to glucose (~30–50%) and Ara + Xyl (~40–45%) residues (Table 1, Fig. 2). The relative proportion of arabinoxylans and β-glucans varied greatly among the samples: the CW_A-NaE contained more Ara + Xyl (45.3%) than Glc residues (31.6%), the CW_B-NaE contained almost equal amounts of these polymers (42.0 and 41.7%, respectively), whereas the CW_C-NaE contained less Ara + Xyl (39.4%) than Glc residues (49.2%). The CW-NaE fraction of sample A contained the highest relative amount of mannose-containing polysaccharides (22.3% of Man residues), whereas the CW-NaE of sample C the smallest (10.8%).

The SEM micrographs of the NaOH insoluble residue of the endosperm CW, the CW-Rem fraction, (Fig. 4b) revealed further corrosion of the matrix and collapse of the structure compared to the CW material remaining after the $Ba(OH)_2$ extraction (Fig. 4a). The CW-Rem fractions exhibited fibrous and porous appearance. Small differences in the relative molar composition of the CW remnants were found among the samples. The CW-Rem consisted mostly of Ara + Xyl (42.2–43.7%), followed by Glc (29.0–35.85%) and Man residues (21.0–26.5%) (Table 1, Fig. 2). As shown in Table 1, the CW-Rem contained a considerable amount of Glc and Man residues determined after the hydrolysis of the CW remnants with 72% H_2SO_4, indicative of the presence of cellulose and glucomannans in the insoluble skeleton the CW. Previous studies (Ballance & Manners, 1978; Fincher, 1975; Mares & Stone, 1973a) revealed the presence of a microfibrillar phase in the residues remaining after exhaustive extraction of barley and wheat CW with water, alkali and other extractants. The major constituent of the microfibrillar phase was identified as cellulose on the basis of its monosaccharide composition (Ballance & Manners, 1978; Fincher, 1975; Mares & Stone, 1973a) and the disappearance of the microfibrils after digestion of the residue by cellulosin (Mares & Stone, 1973b). It has also been proposed that glucomannans with few or no substitutes on the linear backbone many crystallize into insoluble aggregates and form non-covalent associations

with the crystalline cellulose in vitro (Chanzy, Grosrenaud, Joseleau, Dube, & Marchessault, 1982). Similar associations might be expected in the cell wall matrix, resulting in a tightly bound network of all wall polysaccharides. The protein content of each fraction showed that the major portion of the proteinaceous material in the wall preparations was alkali-extractable. Thus, the protein levels in the water-soluble fractions were low, ranging from 0.9 to 1.2%, but higher in the alkali-extractable fractions (Table 1).

3.2. Molecular features of the CW polysaccharides

3.2.1. Hydrolysis of β-glucans with lichenase

The molecular structure of β-glucans in water and alkali extracts was investigated by examining the oligosaccharides obtained after hydrolysis of β-glucans with lichenase. Lichenase, a (1→3)(1→4)-β-D-glucan-4-glucanohydrolase, specifically cleaves the (1→4)-glycosidic bond of the 3-substituted glucose residues in β-glucans yielding oligomers with different degree of polymerization (DP). As previously established, analysis of lichenase digests with high-performance anion-exchange chromatography (HPAEC) revealed that the major structural elements of β-glucans were 3-O-β-cellobiosyl-D-glucose (DP3) and 3-O-β-cellotriosyl-D-glucose (DP4) (Izydorczyk et al., 1998a;b; Lazaridou, Biliaderis, Micha-Screttas, & Steele, 2004; Wood, Weisz, & Blackwell, 1994). Cellodextrin-like oligosaccharides (DP ⩾ 5) were also released during lichenase digestion, however, they are more difficult to quantify because of their lower abundance and their tendency to aggregate and precipitate out of solution once released from the β-glucan chains.

The distribution of oligosaccharides in the aqueous digests of the CW fractions is presented in Table 2. The DP3 and DP4 fragments predominated, representing 88.94–91.81%, 89.16–92.12%, and 87.92–91.55% of soluble oligosaccharides in the digests of the CW fractions of sample A, B, and C, respectively. Among the water- and alkali-extractable CW fractions, the highest DP3/DP4 ratio was found for β-glucans in the Ba/WE fraction, whereas the lowest for those present in the NaE fractions. In general, β-glucans present in every CW fraction of sample B exhibited a higher ratio of DP3/DP4 than their counterparts of sample A. The lowest ratio of DP3/DP4 was found for β-glucans present in the CW of sample C.

The aqueous digests of the CW fractions also contained considerable amounts of cellulose-like fragments with DP ⩾ 5 (Table 2). Among the water- and alkali-extractable CW fractions, the highest amount of the higher oligomers was found in β-glucans contained in the NaE fractions. β-Glucans present in every CW fraction of sample C exhibited a slightly higher amount of the cellulose-like fragments than their counterparts of samples A and B. The analysis of the precipitates formed upon the digestion of the CW fractions with lichenase confirmed the presence of very long cellulose-like fragments in the β-glucan chains. Among the different samples, the longest fragments with DP up to 28 were found for β-glucans contained in the WE65 fraction of sample B (Fig. 5, Table 3).

Compared to the populations of β-glucans present in the water- and-alkali-soluble fractions, β-glucans remaining in the insoluble CW remnants exhibited relatively low DP3/DP4 ratio, except for those in the CW_B-Remnants. The level of the cellulose-like fragments in the aqueous digest of the CW remnants was relatively low and only fragments with DP up to 10 were detected. β-Glucans remaining in the insoluble CW remnants are probably covalently bound with xylans, glucomannans or cellulose (most likely along the cellulose-like segments) and, therefore, they are resistant to enzymic hydrolysis and solubilization.

The ratio of DP3/DP4 and the presence of cellulose-like fragments in the β-glucan chains have been shown to be important determinants of their solubility, extractability and aggregation properties. Two possible mechanisms for intermolecular associations between β-glucan chains have been proposed in the literature. One involves the side-by-side associations of cellulose-like segments of more than three contiguous β-(1→4)-linked glucosyl units, leading to formation of aggregates (Fincher & Stone, 1986), and the other refers to the interactions of chain segments with consecutive cellotriosyl units linked by β-(1→3) bonds that might form extended junction zones (Bohm & Kulicke, 1999). The latter has been supported by findings that a helix made up of at least three consecutive cellotriosyl residues could constitute a stable ordered motif in β-glucan chains, as shown by X-ray analysis (Tvaroska, Ogawa, Deslandes, & Marchessault, 1983), and that the β-(1→3) linkages could be directly involved in the ordered conformation of barley β-glucans, as revealed by ^{13}C CP/MAS NMR spectroscopy (Morgan, Roberts, Tendler, Davies, & Williams, 1999). Bohm and Kulicke (1999) and Lazaridou et al. (2004) showed that an increasing DP3/DP4 ratio increases the tendency of cereal β-glucans to form aggregates and enhances their gelation potential. Moreover, high ratios of DP3/DP4 and large amounts of long cellulose-like fragments (DP ⩾ 5) in β-glucan chains have been associated with decreased solubility or extractability of these polysaccharides from cereal grains (Izydorczyk et al., 1998a,b).

3.2.2. Linkage composition

The glycosidic linkage composition in the water- and alkali-extractable CW fractions confirmed their complex composition and structural heterogeneity of the polysaccharide constituents (Table 4). In agreement with the monosaccharide analysis, the WE65 fractions consisted mostly of β-glucans, the BaE fractions of arabinoxylans, whereas the composition of Ba/WE and NaE fractions was complicated by the presence of the mannose-containing polymers. The ratio of β-(1→4)/β-(1→3) linked Glc in the WE65 and BaE fractions indicate the relative amount of these linkages in the β-glucan chains, as the amount of Man residues in these fractions was relatively

Table 2
Distribution of oligosaccharides (peak area,%) [a] released during lichenase digestion of the CW fractions

DP	Sample A					Sample B					Sample C				
	WE65	BaE	Ba/WE	NaE	CW-Rem	WE65	BaE	Ba/WE	NaE	CW-Rem	WE65	BaE	Ba/WE	NaE	CW-Rem
											61.87	61.77	63.06	58.51	57.56
3	62.05	63.4	64.66	60.92	60.29	63.54	63.79	64.91	61.44	63.88					
4	29.46	28.45	26.95	28.02	30.44	27.96	27.82	27.22	27.72	28.53	29.47	29.4	28.49	29.41	32.46
5	3.93	3.85	3.62	3.65	5.47	3.7	3.58	3.81	3.71	4.5	3.73	3.66	3.53	3.73	4.17
6	1.93	1.79	1.78	1.74	1.55	1.95	1.75	1.76	1.76	1.78	2.02	1.87	1.82	1.98	2.01
7	0.35	0.4	0.49	0.26	0.41	0.44	0.39	0.4	0.3	–	0.42	0.37	0.49	0.52	0.4
8	0.48	0.45	0.43	0.5	0.68	0.46	0.44	0.38	0.47	–	0.55	0.48	0.46	0.57	0.75
9	1.17	0.98	1.22	1.77	0.82	1.25	1.29	0.98	1.72	1.3	1.25	1.44	1.35	1.84	1.05
10	0.17	0.14	0.19	0.39	0.34	0.18	0.19	0.11	0.39		0.18	0.22	0.22	0.36	0.18
11	0.22	0.18	0.26	0.7		0.23	0.29	0.17	0.63		0.23	0.32	0.28	0.67	–
12	0.12	0.09	0.17	0.53		0.13	0.18	0.1	0.5		0.13	0.2	0.15	0.51	–
13	0.06	0.1	0.09	0.4		0.06	0.12	0.07	0.41		0.07	0.12	0.08	0.52	–
14	0.04	0.11	0.06	0.64		0.04	0.12	0.09	0.5		0.05	0.11	0.06	0.84	1.42
15	0.02	0.02	0.01	0.21		0.02	0.02	0.01	0.17		0.02	0.03	0.01	0.21	
16	0.01	0.01	0.04	0.11		0.01	0.01		0.1		0.01		0.01	0.11	
17	0.01	0.01	0.04	0.06		0.01	0.01		0.06					0.07	
18	0	0		0.04		0			0.03					0.05	
19		0		0.02		0.01			0.03					0.03	
20		0.03		0.05					0.03					0.02	
21		0							0					0.01	
22		0.05							0.03					0.02	
DP3+DP4	91.5	91.81	91.61	88.94	90.73	91.5	91.61	92.12	59.16	92.44	91.34	91.17	91.55	87.92	90.2
DP3/DP4	2.11	2.23	2.4	2.17	1.98	2.27	2.29	2.38	2.22	2.24	2.1	2.1	2.21	1.99	1.77
DP$\geqslant$5	8.5	8.19	8.39	11.06	9.27	8.5	8.39	7.88	10.84	7.56	8.66	8.83	8.45	12.08	9.98

[a] Results of duplicate analyses, CV < 3.5%.

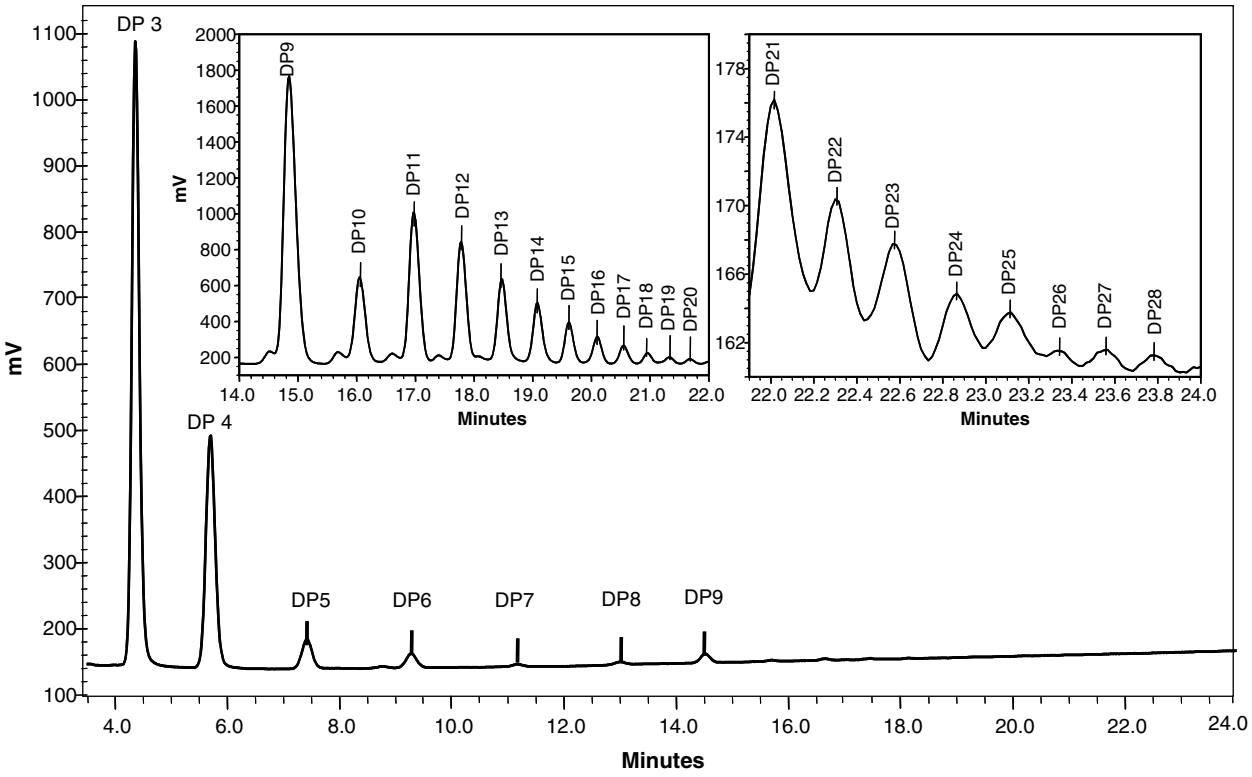

Fig. 5. High-performance anion exchange chromatography profile of oligosaccharides in the water-soluble material released during lichenase digestion of the WE65 fraction sample B; insets show the profile of oligosaccharides from the water-insoluble material released from lichenase treatment of this sample.

Table 3
Distribution of oligosaccharides (peak area, %)[a] in the water-insoluble material (precipitates) released during digestion of the fractions with lichenase

DP	Sample A		Sample B		Sample C	
	WE65	BaE	WE65	BaE	WE65	BaE
9	38.47	34.59	36.28	27.19	38.23	34.48
10	10.86	9.30	9.67	8.59	10.63	13.89
11	16.48	21.30	16.20	22.13	16.30	21.58
12	12.41	16.21	13.11	16.89	12.37	14.95
13	8.31	8.80	8.66	9.97	8.19	7.05
14	5.20	6.59	6.05	10.70	5.51	5.30
15	3.22	1.85	3.74	2.90	3.24	2.03
16	2.10	0.76	2.44	1.15	2.13	0.59
17	1.33	0.18	1.57	0.47	1.38	0.15
18	0.69	0.18	0.85		0.75	
19	0.46	0.23	0.57		0.53	
20	0.26		0.37		0.33	
21	0.11		0.20		0.18	
22	0.03		0.10		0.09	
23	0.04		0.09		0.07	
24	0.02		0.05		0.03	
25			0.03		0.04	
26			0.00			
27			0.01			
28			0.01			

[a] Results of duplicate analyses, CV < 3.5%.

Table 4
Linkage composition (mol%)[a] determined by methylation analysis of glucose and mannose-containing polysaccharides in CW fractions

Linkage type	Fractions			
	WE65	BaE	Ba/WE	NaE
Sample A				
→3 (Glc*p*) 1→	23.8	6.6	17.9	7.6
→4 (Glc*p*) 1→	62.8	19.4	48.8	24.5
→4 (Man*p*) 1→	1.1	0.5	2.4	32.0
Ratio				
→4 (Glc*p*) 1→/				
→3 (Glc*p*) 1→	2.64	2.93	2.73	3.20
Sample B				
→3 (Glc*p*) 1→	24.3	7.1	19.9	10.0
→4 (Glc*p*) 1→	64.0	20.0	54.1	33.7
→4 (Man*p*) 1→	0.8	0.7	2.6	20.1
Ratio				
→4 (Glc*p*) 1→/				
→3 (Glc*p*) 1→	2.63	2.82	2.72	3.37
Sample C				
→3 (Glc*p*) 1→	24.3	7.6	19.2	11.2
→4 (Glc*p*) 1→	68.3	22.5	54.9	43.2
→4 (Man*p*) 1→	-	0.5	1.2	12.5
Ratio				
→4 (Glc*p*) 1→/				
→3 (Glc*p*) 1→	2.81	2.95	2.86	3.86

[a] Results reported as % mol of total glycosidic linkages in the fractions. Results of duplicate analyses, CV < 3.5%.

low. The ratio of β-(1→4)/β-(1→3) linked Glc in the WE65 fraction of sample C was considerably higher than that in the WE65 of sample A and B. Combined with the results obtained with lichenase digestion, it appears that water-soluble β-glucans in the endosperm of sample C were charac-

terized by the lowest DP3/DP4 ratio and the highest amount of the cellulose-like fragments among the samples, whereas β-glucans in sample B by the highest DP3/DP4 ratio and the lowest amount of cellulose-like fragments. Similar trends were observed for β-glucans confined in the $Ba(OH)_2$ and Ba/WE fractions (Tables 2 and 4).

The relatively high proportion of [→4 (Man*p*) 1→] linkages in the NaE fractions implied the presence of glucomannans in the water insoluble skeleton of the barley endosperm CW. In agreement with the monosaccharide analysis, the highest amount of the β-(1→4)-linked Man residues was found in sample A and the lowest in Sample C.

The degree of branching and mode of substitution in arabinoxylans were investigated by monosaccharide and glycosidic linkage analyses. The general structure of arabinoxylans in the endosperm CW was similar to that reported in previous studies (Ballance, Hall, & Manners, 1986; Gruppen, Hamer, & Voragen, 1992; Izydorczyk et al., 1998a,b; Vietor, Angelino, & Voragen, 1992). Arabinoxylans consisted of a linear backbone of β-(1→4)-linked xylopyranosyl residues partly substituted with single α-arabinofuranosyl residues at either C(O)-2, or C(O)-3 and at both C(O)-2 and C(O)-3 positions of the xylose residues, as evidenced by the occurrence of [→4 (Xyl*p*) 1→], [→2,4 (Xyl*p*) 1→], [→3,4 (Xyl*p*) 1→] and [→2,3,4 (Xyl*p*) 1→] linkages, respectively (Table 5). Most of the Ara residues were linked to Xyl residues as single units, although small quantities of [→2 (Ara*f*) 1→] and [→3 (Ara*f*) 1→] indicated the presence of short arabinan chains especially in the alkali-extractable arabinoxylans.

In general, arabinoxylans present in the CW of sample A exhibited the lowest degree of branching, whereas those in sample C the highest. This was evident from the Ara/Xyl ratio in the intact CW as well as in the water- and alkali-extractable fractions (Tables 1 and 5). Among different fractions, arabinoxylans in the NaE fractions exhibited the lowest Ara/Xyl ratio (0.37–0.52), whereas those in Ba/WE the highest (0.80–0.88). The ratio of Ara/Xyl in arabinoxylans contained in the WE65 (0.61–0.68) was similar to that of arabinoxylans in the BaE fractions (0.59–0.70), but the substitution pattern was different. The water-extractable arabinoxylans confined in the WE65 fractions contained much higher amount of doubly substituted Xyl residues than those in the BaE fractions (Table 5). Arabinoxylans in the BaE fractions, on the other hand, contained substantially more singly that doubly substituted Xyl*p* than the WE65 fractions, but generally had similar amounts of unsubstituted Xyl*p* and, therefore, similar ratios of Ara/Xyl. Overall, arabinoxylans in WE65 exhibited the highest amount of doubly substituted Xyl*p* among the fractions. Of the three barley samples, arabinoxylans in the WE65 of sample C exhibited the highest amount of doubly substituted Xyl residues but the lowest amount of unsubstituted Xyl*p*; consequently they had the highest ratio of doubly to singly substituted Xyl residues but the lowest ratio of unsubstituted to substituted Xyl*p*. Similar trends were observed for arabinoxylans in the BaE fractions of different samples.

As mentioned above, arabinoxylans extracted with water after the $Ba(OH)_2$ extractions exhibited the highest Ara/Xyl ratio and contained almost equal amounts of unsubstituted and substituted Xyl*p* as well as almost equal amounts of doubly and singly substituted Xyl*p*. Relatively small differences in the molecular features of arabinoxylans in the Ba/WE fractions were observed among the three barley samples. The arabinoxylans most resistant to solubilization, confined in the NaE fractions, exhibited the lowest degree of branching, the highest amount of unsubstituted Xyl*p* and singly substituted Xyl*p*, and the lowest amount of doubly substituted Xyl*p* among the fractions. Of the

Table 5
Linkage composition (mol%)[a] determined by methylation analysis of arabinoxylans in CW fractions

Linkage type	Fractions			
	WE65	BaE	Ba/WE	NaE
Sample A				
(Ara*f*) 1→	37.9	33.5	39.6	23.6
→2 (Ara*f*) 1→	-	0.7	2.9	2.1
→3 (Ara*f*) 1→	-	0.7	1.8	1.2
→4 (Xyl*p*) 1→	36.8	38.7	28.7	52.4
→2,4 (Xyl*p*) 1→	3.2	3.3	5.1	2.4
→3,4 (Xyl*p*) 1→	6.3	9.2	8.0	11.7
→2,3,4 (Xyl*p*) 1→	15.8	8.0	13.8	6.6
Usub/Sub Xyl[b]	1.9	1.89	1.07	2.54
Doub/Sing Xyl[c]	1.7	0.65	1.06	0.47
Ara/Xyl	0.6	0.59	0.80	0.37
Sample B				
(Ara*f*) 1→	37.8	39.2	42.0	26.2
→2 (Ara*f*) 1→	-	0.9	2.0	2.4
→3 (Ara*f*) 1→	-	0.7	1.5	1.5
→4 (Xyl*p*) 1→	36.6	36.2	26.5	45.8
→2,4 (Xyl*p*) 1→	2.4	4.0	4.5	3.3
→3,4 (Xyl*p*) 1→	6.1	9.0	8.5	11.8
→2,3,4 (Xyl*p*) 1→	15.8	10.2	14.5	9.0
Usub/Sub Xyl[b]	1.46	1.56	0.94	1.89
Doub/Sing Xyl[c]	1.68	0.78	1.10	0.61
Ara/Xyl	0.62	0.69	0.84	0.43
Sample C				
(Ara*f*) 1→	40.6	39.9	42.2	30.2
→2 (Ara*f*) 1→	–	0.7	2.8	2.4
→3 (Ara*f*) 1→	–	0.7	1.8	1.7
→4 (Xyl*p*) 1→	33.3	34.0	26.9	38.4
→2,4 (Xyl*p*) 1→	6.0	4.6	4.6	3.8
→3,4 (Xyl*p*) 1→	2.0	8.3	7.4	12.4
→2,3,4 (Xyl*p*) 1→	18.0	11.6	14.4	11.3
Usub/Sub Xyl[b]	1.28	1.39	1.00	1.40
Doub/Sing Xyl[c]	2.25	0.90	1.20	0.70
Ara/Xyl	0.68	0.70	0.88	0.52

[a] Results expressed as % mol of total linkages of Ara*f* and Xyl*p* residues in each fraction. Results of duplicate analyses, CV < 3.6%.

[b] Ratio of unsubstituted xylose residues [→4 (Xyl*p*) 1→] to the sum of singly and doubly substituted xylose residues [→2,4 (Xyl*p*) 1→ + →3,4 (Xyl*p*) 1→ + →2,3,4 (Xyl*p*) 1→].

[c] Ratio of doubly [→2,3,4 (Xyl*p*) 1→] to singly substituted xylose residues [→2,4 (Xyl*p*) 1→+→3,4 (Xyl*p*) 1→].

three barley samples, arabinoxylans in the NaE of sample A exhibited the highest ratio of unsubstituted to substituted Xyl*p*, and the lowest ratio of doubly to singly substituted Xyl*p*.

Additional information about the substitution patterns in arabinoxylans was derived from digestion of the CW fractions with endo-(1→4)-β-D-xylanase from *T. viride*, the enzyme that requires at least three contiguously unsubstituted xylose residues in the chain for its hydrolytic action (Biely, Vrsanska, Tenkanen, & Kluepfel, 1997; Wong, Tan, & Saddler, 1988). Analysis of the enzymic digests of the WE65 fraction by MALDI-TOFMS produced spectra of signals with the mass values that were in agreement with the expected masses for sodiated $[M+Na]^+$ pentose oligomers with DP 4–21 (Fig. 6a, inset). The maximum signal intensity was at m/z 833, which is equivalent to DP = 6. The enzymic digests of the BaE fractions produced similar spectra, although the signal intensities for several oligomers were somewhat different (Fig. 6b, inset). The xylanase digests were also fractionated by HPAEC and assignment of the eluted xylo- and arabinoxylo-oligosaccharides was based on the elution time of standards and mass spectrometry (Fig. 6). In agreement with previous studies (Fernandez, Obel, Scheller, & Roepstorff, 2003; Ordaz-Ortiz et al., 2004), arabinoxylo-oligomers with DP 1–6 were clearly resolved and identified; they constituted between 50% and 80% of the eluting species and, therefore were used as rough indicators of the substitution patterns in arabinoxylans chains (Fig. 6 and Table 6).

Considering the specific mode of action of the xylanase and the fact that substituents on the xylan backbone restrict access of the enzyme to its substrate (Vietor, Kor-

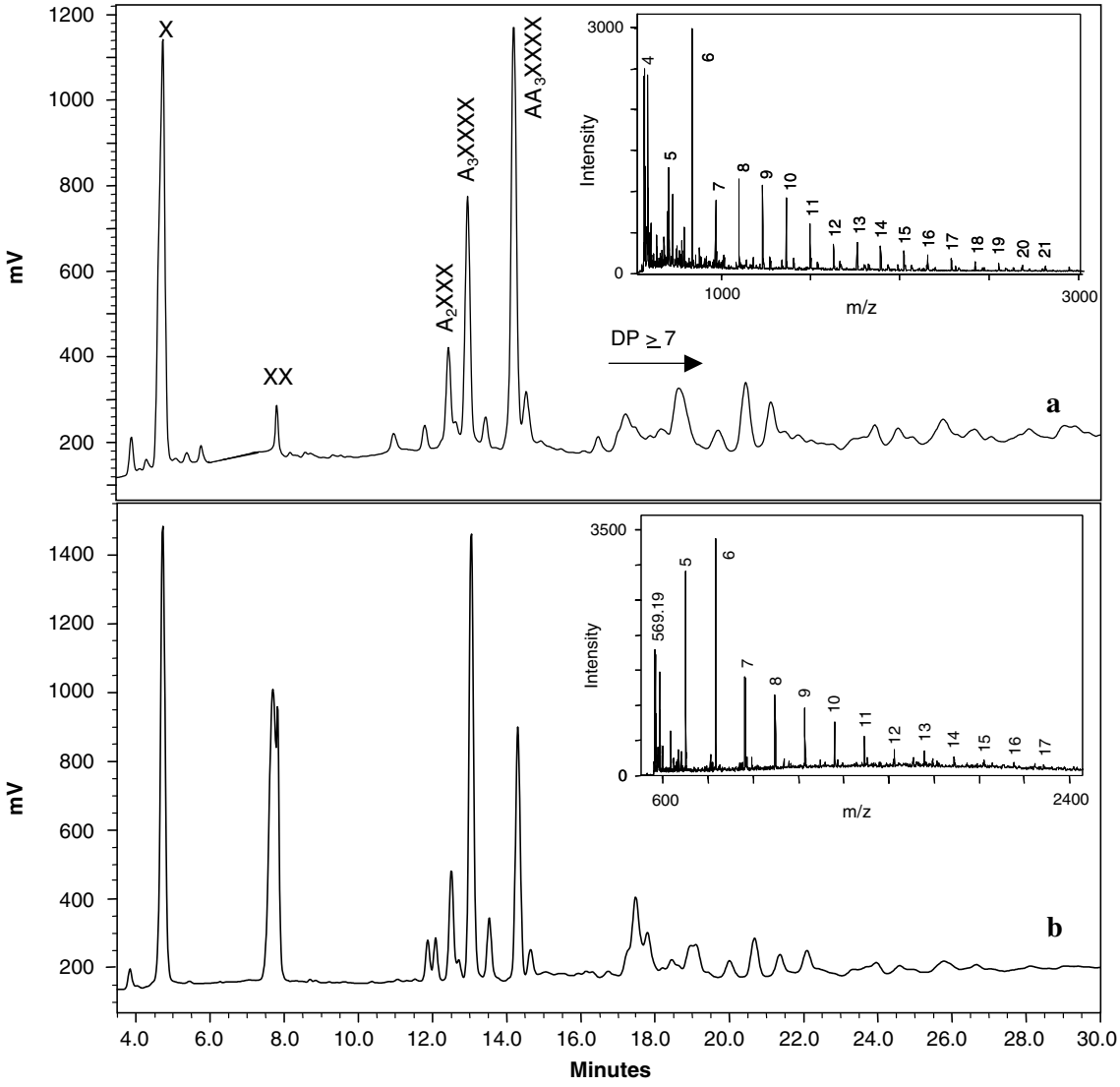

Fig. 6. High-performance anion exchange chromatography profile of xylo- and arabino-xylooligosaccharides released during endo-xylanase digestion of the WE65 fractions (a) and BaE fraction (b) of sample B. Insets shows the corresponding MALDI-TOF-MS spectra of the oligosaccharides; number above the signals indicate the degree of polymerization. X, xylose; XX, xylobiose; A_2XXX, arabino-xylotriose with arabinose residue attached to the 2nd xylose residue; A_3XXXX, arabino-xylotetraose with arabinose residue attached to the 3rd xylose residue, $(AA)_3XXXX$, diarabino-xylotetraose with two arabinose residues attached to the third xylose residue.

Table 6
Distribution of oligosaccharides (peak area,%)[a] released during digestion of the CW fractions with endo-xylanase

Digestion products	Fractions		
	WE65	BaE	NaE
Sample A			
X + XX[b]	12.7	33.9	46.7
DP4 A_2XXX[c]	4.9	6.1	7.5
DP5 A_3XXXX[d]	9.9	18.6	21.2
DP6 $(AA)_3XXXX$[e]	20.4	11.0	4.3
⩾DP7	52.2	30.4	20.3
Sing/Doub Sub Xyl[f]	0.72	2.24	6.7
Sample B			
X + XX[b]	12.8	28.5	47.8
DP4 A_2XXX[c]	5.0	5.9	7.7
DP5 A_3XXXX[d]	10.1	15.1	22.8
DP6 $(AA)_3XXXX$[e]	21.8	13.5	5.1
⩾DP7	50.3	36.9	16.7
Sing/Doub Sub Xyl[f]	0.69	1.55	6.0
Sample C			
X + XX[b]	11.9	27.7	39.9
DP4 A_2XXX[c]	4.5	6.2	7.2
DP5 A_3XXXX[d]	8.4	14.2	21.3
DP6 $(AA)_3XXXX$[e]	23.4	16.0	7.2
⩾DP7	51.7	35.8	24.4
Sing/Doub Sub Xyl[f]	0.55	1.3	3.9

[a] Results of duplicate analyses, CV < 3.8%.
[b] X, xylose; XX, xylobiose.
[c] A_2XXX, arabino-xylotriose with arabinose residue attached to the 2nd xylose residue.
[d] A_3XXXX, arabino-xylotetraose with arabinose residue attached to the 3rd xylose residue.
[e] $(AA)_3XXXX$, diarabino-xylotetraose with two arabinose residues attached to the third xylose residue.
[f] Ratio of $A_2XXX + A_3XXXX/AA_3XXXX$.

melink, Angelino, & Voragen, 1994), the presence of xylose and xylobiose (X and XX peaks) in the digests could roughly indicate the unsubstituted xylan regions in the arabinoxylans chains. The amount of these species increased in the CW fractions in the order WE65 < BaE < NaE (Table 6), confirming the impact of the contiguously unsubstituted Xyl*p* on the extractability of arabinoxylans from the CW. Of the three barley samples, arabinoxylans from the CW fractions of samples A and B seemed to have higher amounts of the unsubstituted xylan regions than those of sample C (Table 6); this was especially evident for arabinoxylans in the NaE fractions.

Arabino-xylooligomers with DP 4 $[(A)_2XXX]$ and DP 5 $[(A)_3XXXX]$ are indicative of the chain fragments singly substituted with Ara*f*, whereas with DP 6 $[(A)_3XXXX]$ of fragments carrying Xyl*p* residues doubly substituted with Ara*f*. Digestion of the CW fractions with xylanase revealed that more singly than doubly substituted arabino-xylooligomers were released from the alkali-extractable fractions, while the opposite was observed for the water-extractable fractions (Table 6). The highest amount of doubly substituted arabino-xylooligomers was found in WE65, followed by BaE and NaE. Among the barley samples, arabinoxylans in all fractions of sample C exhibited the highest amount of doubly substituted arabino-xylooligomers. The ratio of singly to doubly substituted arabino-xylooligomers was highest in the CW fractions of sample A, followed by B and C. The trends in the amounts of singly and doubly substituted arabino-xylooligomers corresponded with the amounts of singly and doubly substituted *Xylp* determined by methylation analysis (Tables 5 and 6).

The results presented in this study clearly indicate that the degree and pattern of substitution in the arabinoxylans chains can influence certain properties of these polymers such as the solubility, ability to self-associate or to interact with other polymers. However, in accordance with previous studies it was concluded that the differences in the degree of branching, as determined from the ratio of Ara/Xyl, may not necessarily govern their solubility or extractability (Ballance & Manners, 1978; Fincher, 1975; Mares & Stone, 1973a; Medcalf, D'Appolonia & Gilles, 1968). As shown in this study, arabinoxylans extracted from the endosperm CW with water were not significantly more branched that those extracted with $Ba(OH)_2$, as indicated by the Ara/Xyl ratio, but the former exhibited a different pattern of substitution signified by a higher amount of doubly substituted Xyl*p*. The least soluble arabinoxylans contained in the NaE fractions had the lowest amounts of disubstituted Xyl*p* residues, the highest amount of unsubstituted Xyl*p* domains, as well as the lowest Ara/Xyl ratio. In addition to the high amounts of unsubstituted β-(1→4)-linked Xyl*p* in arabinoxylans, the NaE fractions contained relatively high amounts of β-(1→4)-linked Glc residues, originating likely from the long cellulosic regions in β-glucan and/or glucomannan chains. The presence of arabinoxylans, β-glucans, and glucomannans in the NaE fractions in almost equivalent amounts and the specific molecular features of these polymers provide further evidence for intermolecular interactions among them in the endosperm CW of barley. This corroborates the earlier hypothesis for interactions between lowly substituted arabinoxylans and β-glucans put forward by Izydorczyk and MacGregor (2000). Taking this view further, such intermolecular associations can affect the resilience, strength and porosity of the wall matrix, and, therefore, reduce the solubility and digestibility of the cell wall constituents.

In general, substantial differences in the amount and structural features of arabinoxylans from the endosperm CW of barley grain grown in different environments were found in this study. Previous studies indicated possible relations between structural characteristics of arabinoxylans and hardness and/or other grain parameters. Kavitha and Chandrashekar (1992, 1993) suggested that the occurrence and a high amount of unbranched regions in arabinoxylans and cellulose-like segments in β-glucans contributed to the rigidity and strength of sorghum grain. Vietor, Voragen, and Angelino (1993) reported that arabinoxylans in feed barley exhibited a lower Ara/Xyl ratio than those in good malting varieties. On the other hand, no differences in the degree of branching in arabinoxylans isolated from the endosperm cell walls of barley differing in

malting grade or wheat varying in milling quality were found (Fincher, 1975; Mares & Stone, 1973a). It appears that the distribution of arabinosyl substituents along the xylan backbone plays an important role in the properties of these polysaccharides and consequently affects the properties of the CW. Arabinoxylans with less regular substitution pattern and long sequences of unsubstituted xylose residues may be prone to self-associations or interactions with other cell wall polysaccharides. Moreover, the extent of cross-linking between the adjacent chains of arabinoxylans through the diferulate bridges needs to be further explored in relation to the CW rigidity and grain hardness.

3.2.3. Molecular weight distribution

The HPSEC elution profiles of the species confined in the water- and alkali-extractable fractions are presented in Fig. 7. The broad and asymmetrical peaks indicated the polydisperse nature of the constituents as well as heterogeneous composition of the fractions. The response from the UV detector (data not shown) indicated the presence of UV-absorbing material especially in the high and low molecular weight regions of the alkali-extracts that contained a considerable amount of proteins. The weight average molecular weight (M_w) and the molecular weight at the peak (M_p) for the Ba/WE and NaE fractions cannot be assigned with any certainty to any particular polymeric constituents. Instead the values indicate the average M_w and M_p of all polymers present in these fractions. The WE65 and BaE fractions were less heterogeneous in composition and contained mostly β-glucans (~80%mol) and arabinoxylans (~70%), respectively; therefore, the M_w and M_p values for the WE65 and BaE fractions reflect mostly the molecular weights of β-glucans and arabinoxylans, although the contribution from other polymers cannot be excluded. In general, the water-extractable polymers exhibited higher molecular weights than the alkali-extractable ones (Table 7). The highest M_w and M_p values were obtained for β-glucans in the WE65 fraction of sample B, whereas the lowest for those of sample C. The BaE fractions exhibited a much broader elution profile than WE65, comprising species of very high and relatively low molecular weights. The M_p values of polymers in BaE were substantially lower than those in the WE65 fractions, however the M_w were still very high. The M_w and M_p values of fractions Ba/WE and NaE were similar. The M_w and M_p values of polymers in the alkali-extractable fractions were the highest in sample B and the lowest in sample C (Table 7). Medcalf, D'Appolonia, and Gilles (1968) found the molecular weight of the water-soluble pentosans higher in a hard wheat variety than in a soft. Moreover, it has been previously shown that β-glucans (Lazaridou, Biliaderis, & Izydorczyk, 2003) and arabinoxylans (Izydorczyk & Biliaderis, 1995) having high molecular sizes can form strong gel networks with well-organized junction zones. Thus, the possibility for contribution of the high molecular weight wall polymers to the rigidity of endosperm cell walls and probably to the hardness of the grain could not be excluded.

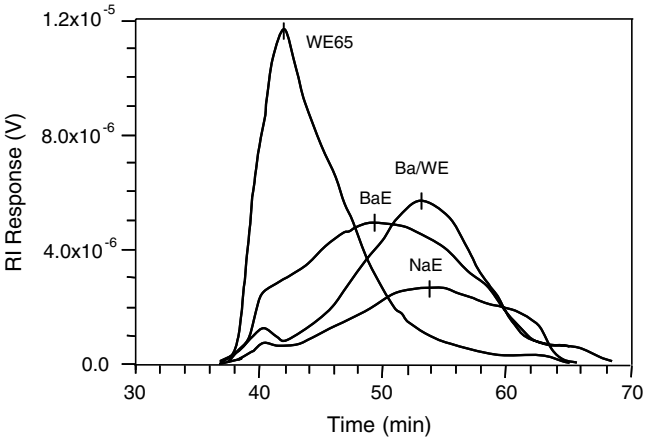

Fig. 7. High performance size exclusion chromatography profiles of the water and alkali extracted CW fractions of barley sample B.

4. Conclusions

Sequential extraction of the isolated endosperm CW with water and alkali solvents allowed for a detailed analysis of the major CW constituents. Substantial differences

Table 7
Weight average molecular weight of the CW fractions

Sample/M_w	Fractions			
	WE65	BaE	Ba/WE	NaE
Sample A				
Average M_w ($\times 10^{-3}$)	1,680 ± 13[a]	1,280 ± 7	520 ± 7	590 ± 9
Mol. wt. at Peak M_p ($\times 10^{-3}$)	1,220 ± 19	360 ± 7	100 ± 2	140 ± 3
Sample B				
M_w ($\times 10^{-3}$)	1,800 ± 25	1,380 ± 22	950 ± 10	1,090 ± 13
Mol. wt. at Peak M_p ($\times 10^{-3}$)	1,350 ± 27	360 ± 8	130 ± 2	120 ± 2
Sample C				
M_w ($\times 10^{-3}$)	1,300 ± 23	970 ± 17	530 ± 9	480 ± 7
Mol. wt. at Peak M_p ($\times 10^{-3}$)	920 ± 19	210 ± 4	130 ± 3	150 ± 2

[a] Mean values ± standard deviation ($n = 6$); HPSEC recovery of each fraction was >92%.

in the composition and molecular structure of the major CW polysaccharides were found for the endosperm CW isolated from barley cultivar Metcalfe, grown in three different environments in Canada. The CW of sample A, grown in the region with a relatively high mean temperature and precipitation and exhibiting a lower grain hardness than sample B, contained the lowest amount of β-glucans, but the highest amount of arabinoxylans and the mannose-containing polysaccharides. The CW of sample C, with very high protein content in the grain, contained the highest amount of β-glucans and the lowest amount of other polysaccharides. Polysaccharides present in the CW of sample B, grown in the region with a lower mean temperature and precipitation and exhibiting the highest grain hardness, were characterized by the highest weight average molecular weights. Moreover, β-glucans in the CW of Sample B showed the highest ratio of cellotriose to cellotetraose units (DP3/DP4) and the longest cellulosic fragments in the polymeric chains; these molecular characteristics have been shown to increase the propensity of β-glucan chains for self-association. Of the three barley samples, arabinoxylans in the endosperm CW of sample A exhibited the lowest degree of branching, the highest amount of unsubstituted Xyl residues, and the highest ratio of singly to doubly substituted Xyl*p*.

The solubility of the CW was shown to be related to their composition and molecular characteristics of the CW constituents. The highest water solubility of the CW preparations was associated with the highest concentration of β-glucans in the CW, the lowest DP3/DP4 ratio, and generally the lowest molecular weight of the polymeric constituents. The degree of branching and substitution pattern in arabinoxylans also had a role in the solubility of the CW preparations. The least soluble fractions of the endosperm CW, contained arabinoxylans with the lowest amount of doubly substituted but the highest amount of unsubstituted xylose residues and long sequences of unsubstituted xylan regions. In addition to the presence of lowly substituted arabinoxylans in the least soluble CW fractions, the monosaccharide and glycosidic linkage analyses revealed a high ratio of →4)-Glc*p*-(1→ to →3)-Glc*p*-(1→ linkages as well as the presence of →4)-Man*p*-(1→ linkages, indicating a high level of long cellulosic regions in β-glucan chains and the presence of glucomannans. The monosaccharide analysis of the CW remnants remaining after the combined water- and alkali-extractions indicated the presence of a small amount of cellulose. The findings of this study further support the theory about self-associations of polymeric chains along structurally regular segments of arabinoxylans and β-glucans, as well as the presence of heterotypic interactions among glucomannans, arabinoxylans, β-glucans, and cellulose in the endosperm CW of barley grain. This study has also demonstrated that large differences in composition of the endosperm CW and molecular structure of the major CW polysaccharides can be expected for barley grain grown in different environments.

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References

AACC International (2000). *Approved methods of the AACC* (10th ed.). St. Paul, MN, USA: The Association.

Ahluwalia, B., & Ellis, E. (1985). Studies of β-glucan in barley, malt and endosperm cell walls. In R. D. Hill & L. Munck (Eds.), *New approaches to research on cereal carbohydrates* (pp. 285–290). Amsterdam, The Netherlands: Elsevier Science B.V..

Aastrup, S., Erdal, K., & Munck, L. (1985). Low β-glucan barley mutants and their malting behaviour. In *Proceedings of the 20th congress of the European brewery convention* (pp. 387–393). Oxford: Oxford University Press.

Ballance, G. M., Hall, R. S., & Manners, D. J. (1986). Studies of some arabinoxylans from barley endosperm walls. *Carbohydrate Research, 150*, 290–294.

Ballance, G. M., & Manners, D. J. (1978). Structural analysis and enzymic solubilization of barley endosperm cell-walls. *Carbohydrate Research, 61*, 107–118.

Bamforth, C. W. (1982). Barley β-glucans. Their role in malting and brewing. *Brewers Digest, 57*, 22–27, p.35.

Bamforth, C. W. (1994). β-Glucan and β-glucanases in malting and brewing: Practical aspects. *Brewers Digest, 69*, 12–16, 21.

Bamforth, C. W., & Kanauchi, M. (2001). A simple model for the cell wall for the starchy endosperm in barley. *Journal of the Institute of Brewing, 107*, 235–240.

Bergmans, M. E. F., Beldman, G., Gruppen, H., & Voragen, A. G. J. (1996). Optimisation of the selective extraction of (glucorono)arabinoxylans from wheat bran: Use of barium hydroxide and calcium hydroxide solution at elevated temperatures. *Journal of Cereal Science, 23*, 235–245.

Biely, P., Vrsanska, M., Tenkanen, M., & Kluepfel, D. (1997). Endo-β-1,4-xylanase families: Differences in catalytic properties. *Journal of Biotechnology, 57*, 151–166.

Bohm, N., & Kulicke, W. M. (1999). Rheological studies of barley (1(3)(1(4)-β-D-glucan in concentrated solution: Mechanistic and kinetic investigation of the gel formation. *Carbohydrate Research, 315*, 302–311.

Brennan, C. S., Amor, M. A., Harris, N., Smith, D., Cantrell, I., Griggs, D., & Shewryll, P. R. (1997). Cultivar differences in modification patterns of protein and carbohydrate reserves during malting of barley. *Journal of Cereal Science, 26*, 83–93.

Carpita, N. C., & Shea, E. M. (1988). Linkage structure of carbohydrates by gas chromatography-mass spectroscopy (GC-MS) of partially methylated alditols. In C. J. Biermann & G. D. MacGinnis (Eds.), *Analysis of carbohydrates by GLC and MS* (pp. 157–216). Boca Raton, FL: CRL Press.

Chanzy, H. D., Grosrenaud, A., Joseleau, J. P., Dube, M., & Marchessault, RH. (1982). Crystallization behaviour of glucomannan. *Biopolymers, 21*, 301–319.

Ciucanu, I., & Kerek, F. (1984). A simple and rapid method for the permethylation of carbohydrates. *Carbohydrate Research, 131*, 209–217.

Fernandez, L. E. M., Obel, N., Scheller, H. V., & Roepstorff, P. (2003). Characterization of plant oligosaccharides by matric-assisted laser desorption/ionization and electron spray mass spectrometry. *Journal of Mass Spectrometry, 38*, 427–437.

Fincher, G. B. (1975). Morphology and chemical composition of barley endosperm cell walls. *Journal of the Institute of Brewing, 81*, 116–122.

Fincher, G. B., & Stone, B. A. (1986). Cell walls and their components in cereal grain technology. In Y. Pomeranz (Ed.), *Advances in cereal science and technology* (pp. 207–295). American Association of Cereal Chemists.

Gruppen, H., Hamer, R. J., & Voragen, A. G. J. (1991). Barium hydroxide as a tool to extract pure arabinoxylans from water-insoluble cell wall material of wheat flour. *Journal of Cereal Science, 13*, 275–290.

Gruppen, H., Hamer, R. J., & Voragen, A. G. J. (1992). Water-unextractable cell wall material from wheat flour 1. Extraction of polymers with alkali. *Journal of Cereal Science, 16*, 41–51.

Han, F., Ullrich, S. E., Chirat, S., Menteur, S., Jestin, L., Sarrafi, A., Hayes, P. M., Jones, B. L., Blake, T. K., Wesenberg, D. M., et al. (1995). Mapping of β-glucan content and β-glucanase activity loci in barley grain and malt. *Theoretical and Applied Genetics, 91*, 921–927.

Henry, R. J. (1986). Genetic and environmental variation in the pentosan and β-glucan contents of barley and their relation to malting quality. *Journal of Cereal Science, 4*, 269–277.

Holtekjølen, A. K., Uhlen, A. K. & Knudsen, S. H. (2007). Barley carbohydrate composition varies with genetic and abiotic factors. *Acta Agriculturae Scandinavica, Section B, Soil and Science*, 1–8.

Hrmova, M., Farkas, V., Lahnstein, J., & Fincher, G. B. (2007). A barley xyloglucan xyloglucosyl transferase covalentlylinks xyloglucan, cellulosic substrates, and (1,3;1,4)-β-D-glucans. *Journal of Biological Chemistry, 282*, 12951–12962.

Izydorczyk, M. S., & Biliaderis, C. G. (1995). Cereal arabinoxylans: Advances in structure and physicochemical properties. *Carbohydrate Polymers, 28*, 33–48.

Izydorczyk, M. S., Jacobs, M., & Dexter, J. E. (2003). Distribution and structural variation of nonstarch polysaccharides in milling fractions of hull-less barley with variable amylose content. *Cereal Chemistry, 80*, 645–653.

Izydorczyk, M. S., Macri, L. J., & MacGregor, A. W. (1998a). Structure and physicochemical properties of barley non-starch polysaccharides – I. Water-extractable β-glucans and arabinoxylans. *Carbohydrate Polymers, 35*, 249–258.

Izydorczyk, M. S., Macri, L. J., & MacGregor, A. W. (1998b). Structure and physicochemical properties of barley non-starch polysaccharides – II. Alkali-extractable β-glucans and arabinoxylans. *Carbohydrate Polymers, 35*, 259–269.

Izydorczyk, M. S., & MacGregor, A. W. (2000). Evidence of intermolecular interactions of β-glucans and arabinoxylans. *Carbohydrate Polymers, 41*, 417–420.

Kanauchi, M., & Bamforth, C. W. (2001). Release of β-glucan from cell walls of starchy endosperm of barley. *Cereal Chemistry, 78*, 121–124.

Kanauchi, M., & Bamforth, C. W. (2002). Enzymic digestion of walls purified from the starchy endosperm of barley. *Journal of the Institute of Brewing, 108*, 73–77.

Kavitha, R., & Chandrashekar, A. (1992). Content and composition of nonstarch polysaccharides in endosperms of sorghums varying in hardness. *Cereal Chemistry, 69*, 440–443.

Kavitha, R., & Chandrashekar, A. (1993). Characterization of cell wall components from the endosperm of sorghum varieties varying in hardness. *Carbohydrate Polymers, 22*, 107–115.

Lazaridou, A., Biliaderis, C. G., & Izydorczyk, M. S. (2003). Molecular size effects on rheological properties of oat β-glucans in solutions and gels. *Food Hydrocolloids, 17*, 693–712.

Lazaridou, A., Biliaderis, C. G., Micha-Screttas, M., & Steele, B. R. (2004). A comparative study on structure–function relations of mixed linkage (1→3), (1→4) linear β-D-glucans. *Food Hydrocolloids, 18*, 837–855.

Li, W., Cui, S. W., & Kakuda, Y. (2006). Extraction, fractionation and physical characterization of wheat β-glucans. *Carbohydrate Polymers, 63*, 408–416.

Loboda, A. V., Krutchinsky, A. N., Bromirski, M., Ens, W., & Standing, K. G. (2000). A tandem quadrupole/time-of-flight mass spectrometer with a matrix-assisted laser desorption/ionization source: Design and performance. *Rapid Communication in Mass Spectrometry, 14*, 1047–1057.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry, 193*, 265–275.

MacNicol, P. K., Jacobsen, J. V., Keys, M. M., & Stuart, I. (1993). Effects of heat and water stress on malt quality and grain parameters of Schooner barley grown in cabinets. *Journal of Cereal Science, 18*, 61–68.

Mares, D. J., & Stone, B. A. (1973a). Studies on wheat endosperm. I. Chemical composition and ultrastructure of the cell walls. *Australian Journal of Biological Sciences, 26*, 793–812.

Mares, D. J., & Stone, B. A. (1973b). Studies on wheat endosperm. II. Properties of the wall components and studies on their organization in the wall. *Australian Journal of Biological Sciences, 26*, 813–830.

Medcalf, D. G., D'Appolonia, B. L., & Gilles, K. A. (1968). Comparison of chemical composition and properties between hard red spring and durum wheat endosperm pentosans. *Cereal Chemistry, 45*, 539–549.

Miller, S. S., Fulcher, R. G., Sen, A., & Arnason, J. T. (1995). Oat endosperm cell walls: I. Isolation, composition, and comparison with other tissues. *Cereal Chemistry, 72*, 421–427.

Moore, J., Bamforth, C. W., Kroon, P. A., Bartolome, B., & Williamson, G. (1996). Ferulic acid esterase catalyses the solubilization of β-glucans and pentosans from the starchy endosperm cell walls of barley. *Biotechnology Letters, 18*, 1423–1426.

Morgan, G., & Riggs, T. J. (1981). Effects of drought on yield and on grain and malt characters in spring barley. *Journal of the Science of Food and Agriculture, 32*, 339–346.

Morgan, K. R., Roberts, C. J., Tendler, S. J. B., Davies, M. C., & Williams, P. M. (1999). A ^{13}C CP/MAS NMR spectroscopy and AFM study of the structure of Glucagel™, a gelling β-glucan from barley. *Carbohydrate Research, 315*, 169–179.

Ordaz-Ortiz, J. J., Guillon, F., Tranquet, O., Dervilly-Pinel, G., Tran, V., & Saulnier, L. (2004). Specificity of monoclonal antibodies generated against arabinoxylans of cereal grains. *Carbohydrate Polymers, 57*, 425–433.

Palmer, G. H. (1992). Cereal science and malting technology – The future. *Journal of the American Society of Brewing Chemists, 50*, 121–130.

Savin, R., Stone, P. J., Nicolas, M. E., & Wardlaw, I. F. (1997). Effects of heat stress and moderately high temperature on grain growth and malting quality of barley. *Australian Journal of Agricultural Research, 48*, 615–624.

Storsley, J. M., Izydorczyk, M. S., You, S., Biliaderis, C. G., & Rossnagel, B. (2003). Structure and physicochemical properties of β-glucans and arabinoxylans isolated from hull-less barley. *Food Hydrocolloids, 17*, 831–844.

Swanston, J. S., Ellis, R. P., Perez-Vendrell, A., Voltas, J., & Molina-Cano, J. L. (1997). Patterns of barley grain development in Spain and Scotland and their implication for malting quality. *Cereal Chemistry, 74*, 456–461.

Sweet, D. P., Shapiro, R. H., & Albersheim, P. (1975). Quantitative analysis by various G.L.C. response factor theories for partially methylated and partially ethylated alditol acetates. *Carbohydrate Polymers, 40*, 217–225.

Toole, G. A., Wilson, R. H., Parker, M. L., Wellner, N. K., Wheeler, T. R., Shewry, P. R., & Mills, E. N. C. (2006). The effect of environment on endosperm cell-wall development in *Triticum aestivum* grain filling: An infrared spectroscopic imaging study. *Planta, 225*, 1393–1403.

Tvaroska, I., Ogawa, K., Deslandes, Y., & Marchessault, R. H. (1983). Crystalline conformation and structure of lichenan and barley β-glucan. *Canadian Journal of Chemistry, 61*, 1608–1616.

Verbruggen, M. A., Beldman, G., & Voragen, A. G. J. (1995). The selective extraction of glucoronoarabinoxylans from sorghum endosperm cell walls using barium and potassium hydroxide solutions. *Journal of Cereal Science, 21*, 271–282.

Vietor, R. J., Angelino, S. A. G. F., & Voragen, A. G. J. (1992). Structural features of arabinoxylans from barley and malt cell wall material. *Journal of Cereal Science, 23*, 235–245.

Vietor, R. J., Kormelink, F. J. M., Angelino, S. A. G. F., & Voragen, A. G. J. (1994). Substitution patterns of water-unextractable arabinoxylans from barley and malt. *Carbohydrate Polymers, 24*, 113–118.

Vietor, R. J., Voragen, A. G. J., & Angelino, S. A. G. F. (1993). Composition of non-starch polysaccharides in wort and spent grain from brewing trials with malt from a good quality barley and a feed barley. *Journal of the Institute of Brewing, 99*, 243–248.

Wilson, S. M., Burton, R. A., Doblin, M. S., Stone, B. A., Newbigin, E. J., Fincher, G. J., & Bacic, A. (2006). Temporal and spatial appearance of wall polysaccharides during cellularization of barley (*Hordeum vulgare*) endosperm. *Planta, 224*, 655–667.

Wood, P. J., Weisz, J., & Blackwell, B. A. (1994). Structural studies of (1→3)(1→4)-β-D-glucans by^{13} C-nuclear magnetic resonance spectroscopy and by rapid analysis of cellulose-like regions using high-performance anion-exchange chromatography of oligosaccharides released by lichenase. *Cereal Chemistry, 71*, 301–307.

Woodward, J. R., Fincher, G. B., & Stone, B. A. (1983). Water-soluble (1→3),(1→4)-β-D-glucans from barley (*Hordeum vulgare*) endosperm. II. Fine structure. *Carbohydrate Polymers, 3*, 207–225.

Wong, K. K. Y., Tan, L. U. L., & Saddler, J. H. (1988). Multiplicity of β-14-xylanase in microorganisms: Functions and applications. *Microbiological Reviews, 52*, 305–317.