

Isolation and Characterization of Water-Extractable Arabinoxylan from Hull-less Barley Flours

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ABSTRACT

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A new procedure was developed for the isolation of highly purified water-extractable arabinoxylan (WE-AX) from hull-less barley flour. It included inactivation of endogenous enzymes, removal of proteins with silica gel, and removing β -glucans, arabinogalactan-peptides, and starch fragments by enzyme or solvent precipitation steps. WE-AX recovered by this isolation procedure represented, on average, 47% of all WE-AX present in hull-less barley flour. Purified WE-AX from flour of different hull-less European barley cultivars contained 84.9–91.8% AX and showed

small structural differences. The apparent peak molecular weight of the purified WE-AX was 730,000–250,000, and the arabinose-to-xylose ratio was 0.55–0.63. Proton nuclear magnetic resonance spectroscopy showed that the levels of un-, *O*-2 mono-, *O*-3 mono-, and *O*-2,*O*-3 disubstituted xylose residues were 59.1–64.7%, 8.2–10.0%, 5.7–10.6%, and 17.6–23.1%, respectively, and the ratio of di- to monosubstituted xylose was 0.90–1.54. Both *O*-3 mono- and disubstituted xylose residues occurred isolated or next to disubstituted xylose residues in the WE-AX chain.

Although the first hull-less barley cultivars were bred for use in the feed industry (swine and poultry) (Bhatty 1986a), their use in a variety of human foods (Bhatty 1986b, 1995a; Swanson and Penfield 1987; Newman et al 1990; Berglund et al 1992) increases because hull-less barley is easier to process than hulled barley and it is also a source of dietary fiber (Bhatty 1999). In contrast to regular hulled barley, hull-less or naked barley kernels, like wheat and rye, lose their hull during threshing. This feature offers clear advantages for processing and food use (Berglund et al 1992). Hull-less barley contains more protein, starch, and total and soluble β -glucan than hulled barley (Bhatty 1999). It is a good source of fiber in general and of soluble fiber, such as β -glucan and arabinoxylan (AX), in particular. The intake of dietary fiber from hull-less barley results in considerable health benefits (Newman and Newman 1991; Bhatty 1995a, 1999). Soluble fibers lower blood cholesterol levels and reduce the postprandial glycemic response, which is beneficial for diabetics (Klopfenstein 1988; Newman et al 1989; Bhatty 1999; Lu et al 2000). Better knowledge of the fiber components of hull-less barley might lead to an increased use in cereal-based products.

AX, the nonstarch polysaccharides that are major constituents of cereal cell walls, consist of a backbone of β -(1-4)-linked D-xylopyranosyl residues (xylose) to which α -L-arabinofuranose (arabinose) can be linked at the *O*-2 or *O*-3 positions or at both positions (*O*-2,*O*-3) (Fig. 1) (Perlin 1951; Viëtor et al 1992; Vinkx and Delcour 1996). Ferulic acid can be coupled to the *O*-5 position of arabinose through an ester linkage (Smith and Hartley 1983). AX are either water-extractable or water-unextractable. The WE-AX form highly viscous aqueous solutions, whereas the latter have strong water-binding capacity (Meuser and Suckow 1986; Rouau and Moreau 1993; Izydorczyk and Biliaderis 1995).

In contrast to hull-less barley AX, β -glucans were already intensively studied (Bhatty 1993, 1995b, 1999; Knuckles and Chiu 1999; Izydorczyk et al 2000). Limited information on WE-AX of hull-less barley flour is available in literature. Dervilly-Pinel et al (2001) partly purified and characterized WE-AX from one hull-less European barley flour that contained 44.7% AX with an arabinose-to-xylose (A/X) ratio of 0.58 and a molecular

weight (MW) of 255,000. Izydorczyk et al (2003) obtained WE-AX fractions of hull-less Canadian barley flours that contained mainly (>75%) β -glucan and <20% AX. While Oscarsson et al (1996) described the substitution levels of xylose residues of WE-AX from hull-less European and North American barley whole meal fractions which include significant levels of nonendosperm tissues, no information is available on the levels of un-, *O*-2 mono-, *O*-3 mono-, and *O*-2,*O*-3 disubstituted xylose residues in WE-AX from hull-less barley flour. Insight into the substitution profile of WE-AX is important for understanding enzyme degradability.

The hull-less barley character is controlled by a single recessive gene (Bhatty 1986a). It follows that WE-AX of hull-less and hulled barley flours can differ from each other, and lack in the literature of an isolation method to obtain highly purified WE-AX without contaminating substances from hull-less barley flour necessitated more research. The purpose of this study was, therefore, to develop a procedure for the isolation of highly purified WE-AX from hull-less barley flour, starting from existing procedures for WE-AX isolation from wheat flour (Faurot et al 1995; Izydorczyk and Biliaderis 1995; Loosveld et al 1997) but taking into account the presence of substantial levels of β -glucan in barley, and to gain insight into the structure of the purified WE-AX. The WE-AX were isolated from hull-less barley flours of different European hull-less barley cultivars (Andersson et al 2003) obtained within Solfibread, an EU-funded project aiming to produce health-promoting bread enriched in soluble fiber. Besides the analytical characterization of the hull-less barley flours, the AX content, apparent peak MW, and xylose substitution levels of the purified WE-AX were determined.

MATERIALS AND METHODS

Specialty chemicals were thermostable α -amylase, Termamyl 120L (Novo Nordisk, Copenhagen, Denmark), amyloglucosidase from *Aspergillus niger* (Megazyme, Bray, Ireland), lichenase from *Bacillus subtilis* (Megazyme), and silica gel, Stabifix super (Stabifix Brauerei-Technik KG, Graefelfing/Munich, Germany). The enzymes were free from AX-degrading enzyme side activities. Azurine-cross-linked wheat arabinoxylan tablets (Xylazyme AX) were obtained from Megazyme. Standard P-82 pullulans were purchased from Showa Denko K.K. (Tokyo, Japan). Deuterium oxide (D_2O) was obtained from Acros Organics (Geel, Belgium). All reagents were of at least analytical grade.

Three different Swedish hull-less barley (*Hordeum vulgare* L.) cultivars, SW 1290, SW 1291, and SW 8775, were obtained from Svalöf Weibulls AB (Svalöf, Sweden). SW 1290 was grown at two different locations in Sweden, Landskrona (SW 1290 L) and

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Haga (SW 1290 H). The hull-less barleys were tempered to 14.3% moisture content and milled on a laboratory mill (Bühler MLU-202, Uzwil, Switzerland). Milling yields and the detailed analytical data of the milling fractions were described by Andersson et al (2003).

Standard Analyses

Moisture and ash contents of the different fractions were estimated according to Approved Methods 44-15A and 08-01 (AACC 2000), respectively. The protein content ($N \times 6.25$) was determined using the Dumas method, an adaptation of the AOAC Official Method (1995) to an automated Dumas protein analysis system (EAS varioMax N/CN, Elt, Gouda, The Netherlands).

Determination of Endoxylanase Activity

Endoxylanase activity was estimated by a colorimetric method using azurine-cross-linked wheat arabinoxylan tablets (Xylazyme AX) as substrate. Samples were suspended in sodium acetate buffer (25 mM, pH 5.0) (1:5 w/v). After shaking (30 min, 4°C) and centrifugation (12,000 × g, 20 min, 4°C), the supernatant was filtered. The filtrate (1.0 mL) was used for estimation of the endoxylanase activity. After preincubation (10 min, 40°C), a substrate tablet was added. The suspension was incubated for 14 hr at 40°C. The reaction was stopped by the addition of 1.0% (w/v) Tris(hydroxymethyl)aminomethane solution (10.0 mL) and vigorous vortex mixing. After 10 min at room temperature, the tubes were shaken and the contents were filtered. The extinction values (590 nm) were measured with an UV/visible spectrophotometer (Ultraspec III, Pharmacia Biotech, Uppsala, Sweden) and corrected for the loss of color of the native substrate tablet and for color originating from the filtrate. The endoxylanase units (U) are defined as extinction values per hour and per gram of fraction under the conditions of the assay.

Isolation and Purification of WE-AX from Hull-less Barley Flour

The hull-less barley flour was boiled under reflux in 80% ethanol (1:5 w/v, 120 min) to inactivate endogenous enzymes. The resi-

due was filtered, washed with ethanol (80%), and air-dried at room temperature (RT). The hull-less barley flour (100 g) was further extracted with deionized water (1:5 w/v, 60 min, 4°C) under continuous stirring. After centrifugation (10,000 × g, 30 min, 4°C), ethanol (95%) was added stepwise to the supernatant under continuous stirring at RT to a concentration of 20% to remove β-glucans (Wang et al 1998). The mixture was stirred for 30 min at RT, kept at 4°C during 4 hr, and centrifuged (10,000 × g, 30 min, 4°C). The supernatant was brought to a final ethanol concentration of 65%. The mixture was stirred (30 min, RT), kept at 4°C overnight, and centrifuged (10,000 × g, 30 min, 4°C). Under these conditions, WE-AX precipitated while arabinogalactan-peptide (AGP) remained in the supernatant (Loosveld et al 1997). The precipitate was dissolved in deionized water and heated to 75°C. Residual starch was hydrolyzed by addition of Termamyl (3.5 mL), and the suspension was incubated (60 min, 90°C) under continuous stirring. After cooling to RT, the suspension was centrifuged (10,000 × g, 20 min, 18°C) and the supernatant was adjusted to pH 4.5 with 0.1M HCl. The supernatant was incubated (15 hr, 60°C) with amyloglucosidase (450 μL) under continuous stirring, then boiled (20 min) to inactivate the enzymes and centrifuged (10,000 × g, 20 min, 18°C) after cooling to RT. Proteins were removed from the supernatant with silica gel (protein-to-silica gel ratio 1:7 w/w) (Maes and Delcour 2002). The suspension of silica gel and deionized water (1:5 w/v) was added to the supernatant. The mixture was adjusted to pH 4.8 with 1.0M HCl and it was stirred for 30 min at RT. After centrifugation (10,000 × g, 30 min, 18°C), the supernatant was dialyzed against deionized water (28 hr, 4°C) and ethanol was added stepwise to a final concentration of 65%. The mixture was stirred (30 min, RT), kept at 4°C overnight and the precipitate, which contained the WE-AX, was recovered by centrifugation (10,000 × g, 30 min, 4°C) and lyophilization. Because this fraction contained significant levels of galactose and glucose besides arabinose and xylose, further purification was needed. The lyophilized material was solubilized in deionized water and ethanol was added to a concentration of 20%. The mixture was stirred (30 min, RT) and kept at 4°C

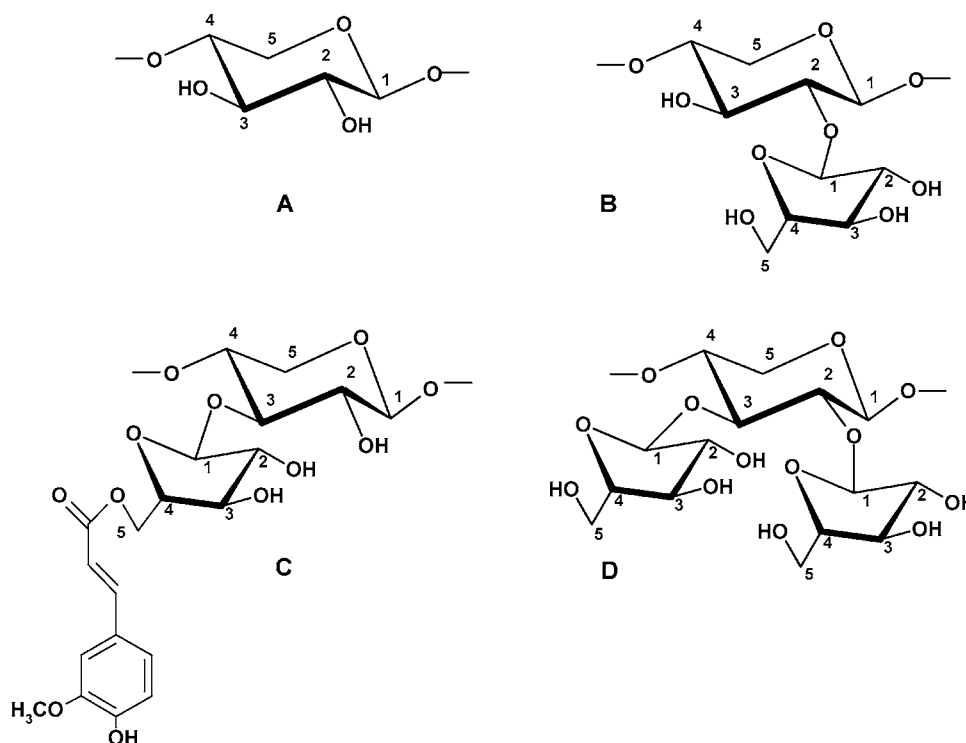


Fig. 1. Structural elements of arabinoxylans: **A**, unsubstituted D-xylopyranosyl (xylose) residue; **B**, xylose residue substituted at the *O*-2 position with L-arabinofuranose (arabinose); **C**, xylose residue substituted at the *O*-3 position with arabinose; and **D**, xylose residue substituted at the *O*-2 and *O*-3 position with arabinose. Structure **C** shows the link of ferulic acid to the *O*-5 position of an arabinose residue.

overnight. After centrifugation (10,000 × *g*, 30 min, 4°C), WE-AX were precipitated from the supernatant with ethanol (final concentration 65%). The mixture was stirred (30 min, RT), kept at 4°C overnight, and centrifuged (10,000 × *g*, 30 min, 4°C). The precipitate was dissolved in deionized water and incubated (2 hr, 40°C) with lichenase (100 μL) to remove residual β-glucan fragments (Dervilly-Pinel et al 2001). The suspension was boiled (20 min) to inactivate the enzyme and centrifuged (10,000 × *g*, 15 min, 18°C). Finally, the supernatant was dialyzed (68 hr, 4°C) against deionized water and lyophilized to obtain highly purified WE-AX. The coefficient of variation calculated for the yield of WE-AX recovered with this isolation procedure was <3.2%.

Carbohydrate Composition

Carbohydrate compositions of the flour fractions, aqueous extracts thereof, and isolated WE-AX samples were estimated by gas-liquid chromatography of alditol acetates as described by Englyst and Cummings (1984). Aqueous extracts were obtained by suspension of the flour samples (1.0 g) in deionized water (1:10 w/v) followed by shaking (30 min, 4°C), centrifugation (3,000 × *g*, 10 min, 4°C), and filtration. The flour (0.1 g) and purified WE-AX fractions (0.01 g) were hydrolyzed in 2.0M trifluoroacetic acid (TFA) (5.0 mL) at 110°C for 120 min and 60 min, respectively, and the aqueous extracts (2.5 mL) in 4.0M TFA (2.5 mL) at 110°C for 60 min. Reduction was with sodium borohydride and acetylation with acetic anhydride. The formed alditol acetates (1.0 μL) were separated on a polar column (30 m × 0.32 mm i.d.; 0.2 μm film thickness) (SP-2380, Supelco, Bellefonte, PA) in a chromatograph (Agilent 6890 series, Wilmington, DE) equipped with autosampler, splitter injection port (split ratio 1:20), and flame ionization detector. The carrier gas was helium. Separation was at 225°C, injection and detection at

270°C. The coefficient of variation of the analysis was <5.0%. AX content was defined as 0.88 × the sum of xylose and arabinose, after correction of the arabinose content for the presence of AGP based on an arabinose-to-galactose ratio of 0.7 for barley flour and with the assumption that all the arabinose of AGP is present in the aqueous extract (Loosveld et al 1997).

High-Performance Size-Exclusion Chromatography

The apparent MW distribution of the purified WE-AX samples was studied by high-performance size-exclusion chromatography (HPSEC) on a Shodex SB-806 HQ column (300 mm × 8 mm i.d.) with a Shodex SB-G guard column (50 mm × 6 mm i.d.) from Showa Denko K.K. (Tokyo, Japan). The separation range of the SB-806 HQ column is between 1,000 and 20,000,000. Purified WE-AX (6.0 mg) were solubilized in 0.3% NaCl (2.0 mL). Elution of the samples (20 μL) was with 0.3% NaCl (0.5 mL/min at 30°C) on a Kontron 325 pump system (Kontron, Milan, Italy) with autoinjection. The separation was monitored with a refractive index (RI) detector (VDS Optilab, Berlin, Germany). MW markers were Shodex standard P-82 pullulans (1.5 mg/mL) with MW of 78.8 × 10⁴, 40.4 × 10⁴, 21.2 × 10⁴, 11.2 × 10⁴, 4.73 × 10⁴, 2.28 × 10⁴, 1.18 × 10⁴, and 0.59 × 10⁴ and glucose.

Proton Nuclear Magnetic Resonance Spectroscopy

WE-AX samples (4.0 mg) were dissolved in D₂O (1.0 mL), stirred (8 hr) and lyophilized. This step was repeated three times. The lyophilized samples were then dissolved in D₂O (0.5 mL) and analyzed by proton nuclear magnetic resonance (¹H-NMR) spectroscopy. The ¹H-NMR spectra were recorded with a NMR-spectrometer (Bruker AMX 500 MHz, Karlsruhe, Germany) at 85°C. Pulse repetition time was 2.0 sec, and the number of scans was 128. The proportions of un-, O-2 or O-3 mono-, and O-2,O-3

TABLE I
Ash and Protein Contents (% dm), Endoxylanase Activities (U), Total and Water-Extractable (WE) Monosaccharide Contents After Hydrolysis (% dm), and Estimated Arabinoxylan (AX) Contents (% dm) from Hull-less Barley Flour Fractions

	SW 1290 L	SW 1290 H	SW 1291	SW 8775
Ash	0.76	0.92	0.89	1.04
Protein	10.30	11.50	11.40	9.70
Endoxylanase activity	0.19	0.05	0.03	0.14
Total monosaccharide after hydrolysis ^a				
Ara	0.80	0.83	0.66	0.71
Xyl	0.94	1.01	0.77	0.85
Man	0.25	0.25	0.18	0.23
Gal	0.23	0.26	0.26	0.28
Glc	90.50	88.90	87.10	91.70
Total AX	1.45	1.53	1.16	1.27
WE monosaccharide after hydrolysis				
Ara	0.20	0.18	0.13	0.16
Xyl	0.24	0.24	0.14	0.19
Man	0.11	0.12	0.15	0.15
Gal	0.12	0.14	0.14	0.16
Glc	3.78	2.46	2.54	2.84
WE-AX	0.31	0.28	0.15	0.21

^a Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose. AX = [(Ara - 0.7 × Gal_{WE}) + Xyl] × 0.88.

TABLE II
Yield (%), Monosaccharide Contents After Hydrolysis (% dm), Arabinoxylan (AX) Contents (% dm), and Arabinose-to-Xylose (A/X) Ratio of the Purified WE-AX from Hull-less Barley Flour Fractions

Purified WE-AX	Yield	Monosaccharides ^a					AX ^b	A/X ^c
		Ara	Xyl	Man	Gal	Glc		
SW 1290 L	49.2	40.55	64.11	1.91	0.45	0.63	91.82	0.63
SW 1290 H	47.9	39.80	63.78	1.81	0.44	0.76	90.88	0.62
SW 1291	41.9	35.48	61.56	1.54	0.89	1.23	84.85	0.57
SW 8775	47.0	35.00	63.36	2.22	0.44	0.88	86.29	0.55

^a Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose.

^b [(Ara - 0.7 × Gal_{WE}) + Xyl] × 0.88.

^c (Ara - 0.7 × Gal_{WE})/Xyl.

disubstituted xylose were calculated by combining the gas-liquid chromatography results with the $^1\text{H-NMR}$ spectral data according to the method of Roels et al (1999), which relies on the approach by Westerlund et al (1990). The level of *O*-2 monosubstituted xylose was estimated as the difference between the integrals for the two peaks of arabinose residues linked to disubstituted xylose (Oscarsson et al 1996).

RESULTS AND DISCUSSION

Chemical Composition of Hull-less Barley Flours

The chemical compositions of the different hull-less barley flours are listed in Table I. The ash content was 0.76–1.04%, whereas the protein content was 9.7–11.5%. The endoxylanase activity was the highest in the flour of SW 1290 L (0.19 U) and the lowest for SW 1291 (0.03 U). The total AX and WE-AX contents were highest in the flour of SW 1290 H (1.53%) and SW 1290 L (0.31%), respectively, while the contents were lowest in SW 1291 (1.16% total AX and 0.15% WE-AX). The water-extractability of AX from hull-less barley flour was low compared with that from wheat flour and varied between 13% for SW 1291 and 21% for SW 1290 L. Typical total AX content ranges of wheat flour found in literature are 1.4–2.5%, and 25–40% of the AX are water-extractable (Meuser and Suckow 1986; Cleemput et al 1993).

Isolation and Purification of WE-AX from Hull-less Barley Flour

Although laborious, the isolation method developed in this study is effective. All steps are deemed essential to obtain highly purified WE-AX. Before extraction with water, the flours were boiled under reflux in 80% ethanol to inactivate endogenous enzymes. The inactivation step had no effect on the AX molecular weight or content in the water extract of the boiled flours compared with the original flours. This step was thus needed because preliminary tests showed that without prior inactivation of the flour, AX of low apparent molecular weight were obtained due to enzymic degradation during the purification process (*results not shown*). Silica gel removed proteins, and several ethanol precipitation steps and enzymic treatments were used to remove β -glucans, AGP, and starch fragments from the water extract. WE-AX yields from different hull-less barley flours obtained with the developed procedure were 42–49% (Table II). On average, 47% of the total WE-AX of hull-less barley flour was recovered.

Table II also shows the composition of purified WE-AX of the flour fractions from the different hull-less barley cultivars. Apart from the high levels of arabinose and xylose, only small levels of

mannose, galactose, and glucose were present. The AX contents of the purified WE-AX were 84.9–91.8%, which was much higher than the value obtained by Dervilly-Pinel et al (2001) (44.7%) or Izydorczyk et al (2003) (<20%). The A/X ratios of the purified WE-AX, which are a measure for the degree of substitution of the AX backbone, were 0.55–0.63 (Table II) and were comparable with figures reported by Dervilly-Pinel et al (2001) and Dervilly et al (2002) for WE-AX of a hull-less European barley flour (0.58) and hulled European barley flour (0.61 and 0.64, respectively). For WE-AX fractions obtained after stepwise precipitation with ammonium sulphate of WE-AX isolated from hulled Canadian barley grist, Izydorczyk et al (1998) found A/X ratios of 0.61–0.82. For WE-AX fractions fractionated by size-exclusion chromatography from WE-AX of hulled European barley flour, Dervilly et al (2002) reported values of 0.40–0.92. A/X ratios (0.55–0.63) reported here are in the range of the values for wheat flour found in literature of 0.53–0.71 (Izydorczyk et al 1991) and 0.50–0.61 (Cleemput et al 1993).

Molecular Weight of Purified WE-AX

Figure 2 shows the HPSEC apparent MW profiles of the purified WE-AX. The apparent peak MW of the WE-AX varied between 730,000 (for SW 1290 H) and 250,000 (for SW 1291). The lower MW of WE-AX from SW 1291 is most probably cultivar-dependent and not due to the action of endoxylanases after harvesting because this flour had the lowest endoxylanase activity (Table I). The small shoulder at low MW ($\approx 4.7 \times 10^4$) can probably be ascribed to a small remaining contamination by AGP (Loosveld et al 1998). Dervilly-Pinel et al (2001) found MW 255,000 and 237,000 for WE-AX of hull-less and hulled European barley flour, respectively, and Dervilly et al (2002) reported MW 215,000–370,000 for different WE-AX fractions of hulled European barley flour. These values,

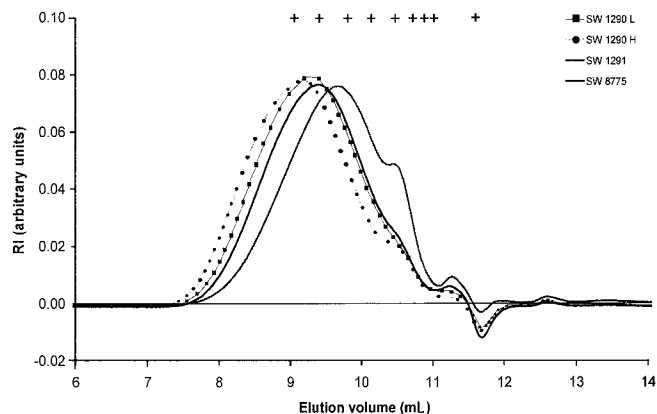


Fig. 2. HPSEC apparent molecular weight profiles of WE-AX purified from flour fractions of hull-less barleys SW 1290 L, SW 1290 H, SW 1291, and SW 8775. Molecular weight markers (+) from left to right are 78.8×10^4 , 40.4×10^4 , 21.2×10^4 , 11.2×10^4 , 4.73×10^4 , 2.28×10^4 , 1.18×10^4 , 0.59×10^4 and glucose.

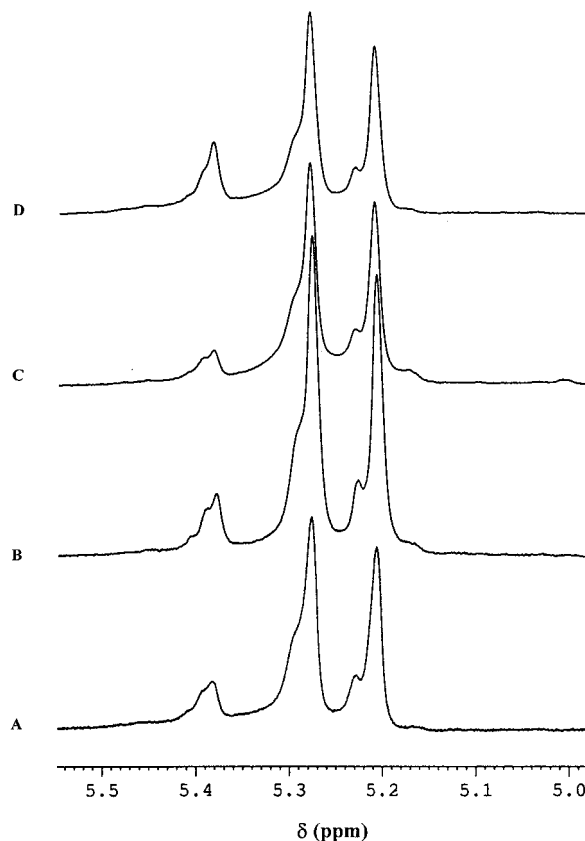


Fig. 3. Anomeric regions of arabinose protons in $^1\text{H-NMR}$ (D_2O , 85°C , 500 MHz) of WE-AX purified from hull-less barley flours SW 1290 L (A), SW 1290 H (B), SW 1291 (C), and SW 8775 (D).

TABLE III
Proportions (%) of Un-, Mono-, and Disubstituted Xylose^a and Ratios of Di- to Monosubstituted Xylose Residues (Di/Mono) in Purified WE-AX from Hull-less Barley Flours

Purified WE-AX	u-Xyl	2-Xyl	3-Xyl	2,3-Xyl	Di/Mono ^b
SW 1290 L	59.1	10.0	9.0	21.9	1.15
SW 1290 H	61.1	8.3	7.5	23.1	1.46
SW 1291	64.7	8.2	5.7	21.4	1.54
SW 8775	62.8	9.0	10.6	17.6	0.90

^a Percentages of total xylose occurring as un-, *O*-2 mono-, *O*-3 mono-, and *O*-2,*O*-3 disubstituted xyloses.

^b (2,3-Xyl)/[(2-Xyl) + (3-Xyl)].

together with those mentioned in literature for purified WE-AX of wheat flours (MW >200,000) (Izydorczyk et al 1991; Cleemput et al 1993), are in the same MW range obtained in this study. Alkaline treatment with 2.0*N* NaOH (Dervilly et al 2000), did not change the MW of the purified WE-AX (*results not shown*).

Proton Nuclear Magnetic Resonance Spectroscopy of Purified WE-AX

Figure 3 shows the anomeric regions of arabinose protons in the ¹H-NMR spectra of the purified WE-AX. The peak at δ 5.38 ppm represents the anomeric protons of arabinose linked to the *O*-3 position of xylose residues, while the two peaks at δ 5.28 and δ 5.20 ppm are from anomeric protons of arabinose residues linked to *O*-2 and *O*-3 of the same xylose residue. The unresolved signals downfield from the peaks at δ 5.28 and δ 5.20 ppm result from two neighboring disubstituted xylose residues in the AX chain (Hoffmann et al 1992; Vinkx et al 1993), which indicates that the purified WE-AX contained both isolated and paired disubstituted xylose residues (Cleemput et al 1995). The presence of a shoulder downfield from the peak at δ 5.38 ppm represents the presence of *O*-3 monosubstituted next to disubstituted xylose (Hoffmann et al 1992). The *O*-2 monosubstitution of xylose cannot be detected directly by ¹H-NMR spectroscopy because its signal (δ 5.28 ppm) overlaps with that of disubstituted xylose (Vinkx et al 1995). Therefore, the content of *O*-2 monosubstituted xylose was estimated as the difference between the integrals of the two peaks of arabinose residues linked to disubstituted xylose (Oscarsson et al 1996).

The proportions of un-, mono-, and disubstituted xylose in the purified WE-AX of the hull-less barley flours are listed in Table III. On average, the levels of un-, *O*-2 mono-, *O*-3 mono-, and *O*-2,*O*-3 disubstituted xylose in the purified WE-AX from the hull-less barley flours were 62.0, 8.9, 8.2, and 21.0%, respectively. More specifically, the levels of unsubstituted xylose varied at 59.1–64.7% and were in line with earlier results for WE-AX from barley whole meal fractions and hulled barley flour. Oscarsson et al (1996) mentioned unsubstituted xylose contents of 47–62% for WE-AX of different hull-less and hulled European barley whole meal fractions, and Dervilly et al (2002) noted a value of 64% for purified WE-AX from a hulled European barley flour. The contents of *O*-2 and *O*-3 monosubstituted xylose were 8.2–10.0% and 5.7–10.6%, respectively (Table III). The latter levels were low compared with results for *O*-3 monosubstituted xylose obtained by Oscarsson et al (1996) (12–20%), while the *O*-2 monosubstituted xylose levels (8.2–10.0%) were in the same range (6–16%). Dervilly et al (2002) reported values of 5.0 and 9.0%, respectively, for *O*-2 and *O*-3 monosubstituted xylose of WE-AX from a hulled European barley flour. About 17.6–23.1% of the xylose residues were disubstituted (Table III), which was in line with data by Oscarsson et al (1996) (16–24%) and Dervilly et al (2002) (22%) for European barley whole meal fractions and a hulled European barley flour, respectively. In general, Oscarsson et al (1996) found no consistent differences between substitution levels of xylose from hull-less and hulled barley whole meal. We concluded that the average level of *O*-3 monosubstituted xylose (8.2%) in hull-less European barley flour WE-AX is lower than that in hull-less and hulled European

barley whole meal fractions (15%) (Oscarsson et al 1996) and that wheat flour WE-AX of has a higher *O*-3 monosubstituted xylose content (14–20%) and a much lower *O*-2 monosubstituted xylose content (0.3–1.9%) (Cleemput et al 1995) than WE-AX of hull-less European barley flour.

The ratios of di- to monosubstituted xylose residues (di/mono) of the purified WE-AX were 0.90–1.54 (Table III) and were in accordance with values in literature (0.87–1.81) for WE-AX of different wheat flours (Cleemput et al 1993), indicating a variation in WE-AX structure between the hull-less barley cultivars.

CONCLUSIONS

The total AX and WE-AX contents of the hull-less European barley flours were 1.16–1.53% and 0.15–0.31%, respectively. The water extractability of AX from hull-less barley flour was 13–21%. A procedure was developed for the isolation of highly purified WE-AX from hull-less barley flour. In part, it was based on earlier procedures developed for purification of wheat flour AX. The WE-AX recovered by this isolation procedure represented, on average, 47% of all WE-AX present in hull-less barley flour. The A/X and di/mono ratios, and apparent peak MW of the purified WE-AX indicated small differences in the WE-AX structure between the hull-less barley cultivars. The ¹H-NMR spectral data indicated that both *O*-3 mono- and disubstituted xylose residues occurred isolated or next to disubstituted xylose residues in the WE-AX chain. The levels of un-, *O*-2 mono-, *O*-3 mono-, and disubstituted xylose of the purified WE-AX showed little variation and were comparable with those from a hulled barley flour described by Dervilly et al (2002). Only the level of *O*-3 monosubstituted xylose from hull-less barley flour WE-AX was lower compared with the level reported for WE-AX from hull-less and hulled barley whole meal (Oscarsson et al 1996).

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LITERATURE CITED

- American Association of Cereal Chemists. 2000. Approved Methods of the AACC, 10th Ed. Methods 08-01, 44-15A. The Association: St. Paul, MN.
- Andersson, A. A. M., Courtin, C. M., Delcour, J. A., Fredriksson, H., Schofield, J. D., Trogh, I., Tsiami, A. A., and Åman, P. 2003. Milling performance of North European hull-less barleys and characterization of resultant millstreams. *Cereal Chem.* 80:667-673.
- Association of Official Analytical Chemists. 1995. Official Methods of Analysis, 16th Ed. Method 990.03, AOAC: Washington, DC.

- Berglund, P. T., Fastnaught, C. E., and Holm, E. T. 1992. Food uses of waxy hull-less barley. *Cereal Foods World* 37:707-714.
- Bhatty, R. S. 1986a. The potential of hull-less barley—A review. *Cereal Chem.* 63:97-103.
- Bhatty, R. S. 1986b. Physicochemical and functional (breadmaking) properties of hull-less barley fractions. *Cereal Chem.* 63:31-35.
- Bhatty, R. S. 1993. Extraction and enrichment of (1→3),(1→4)-β-D-glucan from barley and oat brans. *Cereal Chem.* 70:73-77.
- Bhatty, R. S. 1995a. Hull-less barley bran: A potential new product from an old grain. *Cereal Foods World* 40:819-823.
- Bhatty, R. S. 1995b. Laboratory and pilot plant extraction and purification of β-glucans from hull-less barley and oat brans. *J. Cereal Sci.* 22:163-170.
- Bhatty, R. S. 1999. Review—The potential of hull-less barley. *Cereal Chem.* 76:589-599.
- Cleemput, G., Roels, S. P., Van Oort, M., Grobet, P. J., and Delcour, J. A. 1993. Heterogeneity in the structure of water-soluble arabinoxylans in European wheat flours of variable bread-making quality. *Cereal Chem.* 70:324-329.
- Cleemput, G., van Oort, M., Hessing, M., Bergmans, M. E. F., Gruppen, H., Grobet, P. J., and Delcour, J. A. 1995. Variation in the degree of D-xylose substitution in arabinoxylans extracted from a European wheat flour. *J. Cereal Sci.* 22:73-84.
- Dervilly, G., Saulnier, L., Roger, P., and Thibault, J.-F. 2000. Isolation of homogeneous fractions from wheat water-soluble arabinoxylans. Influence of the structure on their macromolecular characteristics. *J. Agric. Food Chem.* 48:270-278.
- Dervilly, G., Leclercq, C., Zimmermann, D., Roue, C., Thibault, J.-F., and Saulnier, L. 2002. Isolation and characterization of high molar mass water-soluble arabinoxylans from barley and barley malt. *Carbohydr. Polym.* 47:143-149.
- Dervilly-Pinel, G., Rimsten, L., Saulnier, L., Andersson, R., and Åman, P. 2001. Water-extractable arabinoxylan from pearled flours of wheat, barley, rye and triticale. Evidence for the presence of ferulic acid dimers and their involvement in gel formation. *J. Cereal Sci.* 34:207-214.
- Englyst, H. N., and Cummings, J. H. 1984. Simplified method for the measurement of total non-starch polysaccharides by gas-liquid chromatography of constituent sugars as alditol acetates. *Analyst* 109:937-942.
- Faurot, A.-L., Saulnier, L., Bérot, S., Popineau, Y., Petit, M.-D., Rouau, X., and Thibault, J.-F. 1995. Large scale isolation of water-soluble and water-insoluble pentosans from wheat flour. *Lebensm. Wiss. Technol.* 28:436-441.
- Hoffmann, R. A., Kamerling, J. P., and Vliegenthart, J. F. G. 1992. Structural features of a water-soluble arabinoxylan from the endosperm of wheat. *Carbohydr. Res.* 226:303-311.
- Izydorczyk, M. S., and Biliaderis, C. G. 1995. Cereal arabinoxylans: Advances in structure and physicochemical properties. *Carbohydr. Polym.* 28:33-48.
- Izydorczyk, M., Biliaderis, C. G., and Bushuk, W. 1991. Comparison of the structure and composition of water-soluble pentosans from different wheat cultivars. *Cereal Chem.* 68:139-144.
- Izydorczyk, M. S., Macri, L. J., and MacGregor, A. W. 1998. Structure and physicochemical properties of barley non-starch polysaccharides. I. Water-extractable β-glucans and arabinoxylans. *Carbohydr. Polym.* 35:249-258.
- Izydorczyk, M. S., Storsley, J., Labossiere, D., MacGregor, A. W., and Rossnagel, B. G. 2000. Variation in total and soluble β-glucan content in hull-less barley: Effects of thermal, physical, and enzymic treatments. *J. Agric. Food Chem.* 48:982-989.
- Izydorczyk, M. S., Jacobs, M., and Dexter, J. E. 2003. Distribution and structural variation of nonstarch polysaccharides in milling fractions of hull-less barley with variable amylose content. *Cereal Chem.* 80:645-653.
- Klopfenstein, C. F. 1988. The role of cereal beta-glucans in nutrition and health. *Cereal Foods World* 33:865-869.
- Knuckles, B. E., and Chiu, M.-C. M. 1999. β-Glucanase activity and molecular weight of β-glucans in barley after various treatments. *Cereal Chem.* 76:92-95.
- Loosveld, A.-M. A., Grobet, P. J., and Delcour, J. A. 1997. Contents and structural features of water-extractable arabinogalactan in wheat flour fractions. *J. Agric. Food Chem.* 45:1998-2002.
- Loosveld, A., Maes, C., van Casteren, W. H. M., Schols, H. A., Grobet, P. J., and Delcour, J. A. 1998. Structural variation and levels of water-extractable arabinogalactan-peptide in European wheat flours. *Cereal Chem.* 75:815-819.
- Lu, Z. X., Walker, K. Z., Muir, J. G., Mascara, T., and O'Dea, K. 2000. Arabinoxylan fiber, a byproduct of wheat flour processing, reduces the postprandial glucose response in normoglycemic subjects. *Am. J. Clin. Nutr.* 71:1123-1128.
- Maes, C., and Delcour, J. A. 2002. Structural characterisation of water-extractable and water-unextractable arabinoxylans in wheat bran. *J. Cereal Sci.* 35:315-326.
- Meuser, F., and Suckow, P. 1986. Non-starch polysaccharides. Pages 42-61 in: *Chemistry and Physics of Baking*. J. M. V., Blanshard, P. J., Frazier, and T. Galliard, eds. R. Soc. Chem.: London.
- Newman, R. K., and Newman, C. W. 1991. Review—Barley as a food grain. *Cereal Foods World* 36:800-805.
- Newman, R. K., Newman, C. W., and Graham, H. 1989. The hypocholesterolemic function of barley β-glucans. *Cereal Foods World* 34:883-886.
- Newman, R. K., McGuire, C. F., and Newman, C. W. 1990. Composition and muffin-baking characteristics of flours from four barley cultivars. *Cereal Foods World* 35:563-566.
- Oscarsson, M., Andersson, R., Salomonsson, A.-C., and Åman, P. 1996. Chemical composition of barley samples focusing on dietary fibre components. *J. Cereal Sci.* 24:161-170.
- Perlin, A. S. 1951. Structure of the soluble pentosans of wheat flours. *Cereal Chem.* 28:382-393.
- Roels, S. P., Collado, M., Loosveld, A.-M., Grobet, P. J., and Delcour, J. A. 1999. Variation in the degree of D-xylose substitution in water-extractable European durum wheat (*Triticum durum* Desf.) semolina arabinoxylans. *J. Agric. Food Chem.* 47:1813-1816.
- Rouau, X., and Moreau, D. 1993. Modification of some physicochemical properties of wheat flour pentosans by an enzyme complex recommended for baking. *Cereal Chem.* 70:626-632.
- Smith, M. M., and Hartley, R. D. 1983. Occurrence and nature of ferulic acid substitution of cell-wall polysaccharides in graminaceous plants. *Carbohydr. Res.* 118:65-80.
- Swanson, R., and Penfield, M. P. 1987. Whole-grain yeast bread production and consumer acceptability using hull-less barley grown in Alaska. *Agroborealis* 19:16-19.
- Viëtor, R. J., Angelino, S. A. G. F., and Voragen, A. G. J. 1992. Structural features of arabinoxylans from barley and malt cell wall material. *J. Cereal Sci.* 15:213-222.
- Vinx, C. J. A., Reynaert, H. R., Grobet, P. J., and Delcour, J. A. 1993. Physicochemical and functional properties of rye nonstarch polysaccharides. V. Variability in the structure of water-soluble arabinoxylans. *Cereal Chem.* 70:311-317.
- Vinx, C. J. A., Delcour, J. A., Verbruggen, M. A., and Gruppen, H. 1995. Rye water-soluble arabinoxylans also vary in their 2-monosubstituted xylose content. *Cereal Chem.* 72:227-228.
- Vinx, C. J. A., and Delcour, J. A. 1996. Rye (*Secale cereale* L.) arabinoxylans: A critical review. *J. Cereal Sci.* 24:1-14.
- Wang, L., Miller, R. A., and Hoseney, R. C. 1998. Effects of (1→3)(1→4)-β-D-glucans of wheat flour on breadmaking. *Cereal Chem.* 75:629-633.
- Westerlund, E., Andersson, R., Åman, P., and Theander, O. 1990. Effects of baking on water-soluble polysaccharides in white bread fractions. *J. Cereal Sci.* 12:33-42.

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