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Evidence of the presence of 2-*O*-β-D-xylopyranosyl-α-L-arabinofuranose side chains in barley husk arabinoxylan

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Abstract—(Glucurono)arabinoxylans were extracted from barley husks and degraded with *endo*-β-xylanase or subjected to periodate oxidation. The released oligosaccharide fragments were separated and isolated on Biogel-P2, and their structures were determined by NMR spectroscopy. The oligosaccharides identified consisted of β-D-(1→4)-linked xylopyranosyl residues, of which some were substituted at O-3 with α-L-arabinofuranosyl groups or at O-2 with 4-*O*-methylglucuronic acid. In addition to these substituents, a disaccharide side chain, 2-*O*-β-D-xylopyranosyl-α-L-arabinofuranose, attached at position O-3 of the main chain, was proved to exist in arabinoxylan from barley husks. The compound was fully characterized with NMR, and all ^{1}H and ^{13}C NMR signals were assigned. The arabinose to xylose ratio was low (~0.2) and no 2,3-disubstitution existed. No blocks of substituted xylose residues could be observed along the main chain.

Keywords: Barley husk; Arabinoxylan; Structure; NMR spectroscopy; Periodate oxidation; Enzymatic hydrolysis

1. Introduction

In recent years, efforts to develop a sustainable society have culminated in a greater use of renewable resources based on agricultural residues and wood biomass. A vast number of researchers are now focusing on the production of biofuels from renewable resources,[1] although there has also been a strong interest in the production of commercially interesting by-products from agricultural crops and wood toward new advanced materials.[2,3] Advantages are, in addition to being recovered from a renewable resource, that they are biodegradable, which facilitates composting and recycling, and that they give minimal environmental effects after incineration.[4]

Polysaccharides in cereals are produced in large quantities and of these, starch has been successfully converted into plastic materials by conventional thermoplastic processing.[5] The role of the hemicellulose, such as arabinoxylan and β-glucan in the cereal cell wall, is mainly as a matrix component, binding cellulosic microfibrils into composite structures. The use of hemicelluloses as a polymeric material is currently being investigated for a variety of applications.

The arabinoxylans in the starchy endosperm of cereal grains are polymers consisting of a backbone of (1→4)-linked β-D–xylopyranosyl (Xyl*p*) residues with side chains of terminal α-L-arabinofuranosyl (Ara*f*) residues preferentially linked to C-3, or to C-2 and C-3 and occasionally singly linked to C-2.[6–9] In the husks these structures can also be substituted with (1→2)-α-D-glucuronic acid or its 4-*O*-methyl derivative.[10] The structural heterogeneity of the arabinoxylan results in Ara/Xyl ratios

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from 0.3 to 1.0 in the aleurone layer and the husk fraction, respectively.[11]

In the previous work, we have focused on different methods for extraction of the hemicellulose fraction containing (glucurono)arabinoxylan from barley husks.[12] Our interest is in isolating arabinoxylans with high molecular weight, which can later be used to prepare oxygen barrier films or coatings. Since the structure–function relationships must be evaluated before these components can be used in materials, we have focused on the fine structure of the arabinoxylan, which is important for water mobility, and the mechanical properties of the films. This work focuses on the elucidation of the molecular architecture of the arabinoxylan in terms of substitution patterning and branching.

2. Results and discussion

2.1. Chemical composition

Barley husk arabinoxylan (BHAX) was isolated by alkali extraction. This resulted in complete removal of acetyl groups from the native arabinoxylan. The arabinoxylan consists of a heterogeneous set of structures with a varying degree of substituents that render them soluble or insoluble in aqueous solvents. The chemical composition of the water soluble and insoluble fractions, which were separated by centrifugation after neutralization, is shown in Table 1. The main difference lies in the content of arabinose and uronic acid. The Ara to Xyl ratio was higher for the soluble fraction. Low amounts of protein and lignin were also found.

Table 1. Chemical composition of barley husk arabinoxylan (BHAX)

Analysis	BHAX (total)	Water insoluble fraction	Water soluble fraction
Neutral sugars (% of total)			
Arabinose	15.1	11.3	18.8
Xylose	76.8	81.5	73.2
Glucose	7.1	5.9	7.4
Galactose	1.0	1.3	0.6
Ara/Xyl ratio	0.20	0.14	0.26
Uronic acid (% of dry weight)	3.76	3.21	4.12
Klason lignin (% of dry weight)	0.17	0.64	nd[a]
Protein (% of dry weight)	1.29	1.12	1.45

[a] Non-detectable.

2.2. Structure determination

The water soluble fraction of BHAX was analyzed by ^{1}H and ^{13}C NMR to determine the primary structure. The anomeric protons were used as starting points for the assignment of proton signals by COSY and TOCSY experiments. Carbon signals were assigned from HSQC and HMBC experiments. The inter-residue linkages were established from HMBC and ROESY spectra.

The ^{1}H NMR spectrum of this sample is shown in Figure 1. The anomeric signals are found in two spectral regions: δ 5.6–5.2 and δ 4.7–4.4. The ^{3}J values of coupling constants for these resonances are approximately 1.0–1.4 and 7.2–7.8 Hz, respectively, in accordance with the presence of α-L-Ara*f* and β-D-Xyl*p* residues. The α-L-Ara*f* anomeric proton region at 5.6–5.2 ppm shows two major peaks at 5.539 and 5.406 ppm in a 1:1 molar ratio.

Despite the 1:1 molar ratio, these chemical shifts do not correspond to those of anomeric protons of α-L-Ara*f* substituted at O-3 and O-2 of the same Xyl residues. Such substitution would give chemical shifts at 5.27 and 5.23 ppm, respectively.[13] Instead, the peak at 5.406 ppm resulted from the anomeric proton signal of α-L-Ara*f* (1→3)-linked to a β-D-Xyl*p* group, that is, monosubstituted Xyl (B) (Fig. 2). Starting from the anomeric signal at 5.539 ppm, the spin system of the second α-L-Ara*f* residue could be identified (Table 2). Consecutive assignment of the carbon resonances showed that the C-2 signal experienced a large deshielding to δ 90.02 ppm, indicating that the sugar is substituted at the 2-position. Evidence of the occurrence of a (1→2) glycosidic linkage between Ara and Xyl was also found in the HMBC experiment and ROESY experiments. The NOE between the H-1 of Xyl at 4.563 (E) and the H-2 of (A) at 4.287 together with the cross-peak between H-1 (E) and C-2 (A) at 90.02 ppm in the HMBC spectra confirmed the occurrence of a β-D-Xyl*p*-(1→2)-α-L-Ara*f*

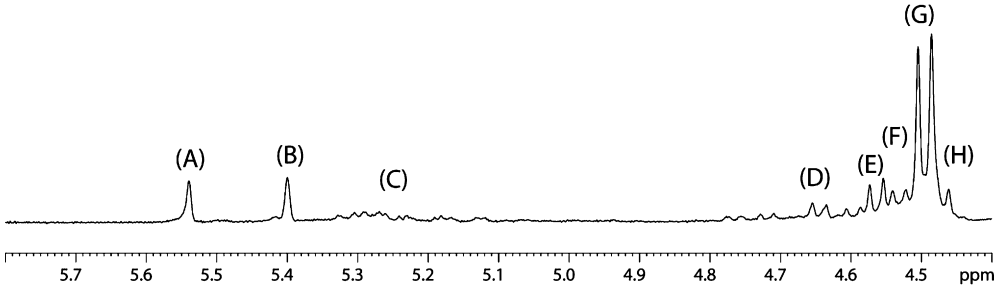

Figure 1. The anomeric region of a ^{1}H NMR spectrum of the water soluble fraction of BHAX (D_2O, 70 °C). A–H represent the various sugar residues in the schematic structure.

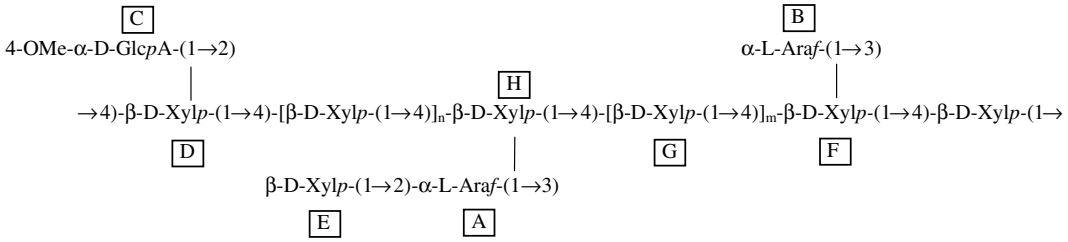

Figure 2. Structural features of the water soluble fraction of BHAX determined from ^{1}H and ^{13}C NMR analysis. A–H refer to NMR assignments.

Table 2. ^{1}H and ^{13}C NMR chemical shifts for BHAX in D_2O at 70 °C

		1	2	3	4	5eq	5ax	Me
α-L-Ara*f* (A)	^{1}H	5.539	4.287	4.092	4.26	3.815	3.736	
	^{13}C	107.5	90.02	77.2	85.6	62.6		
α-L-Ara*f* (B)	^{1}H	5.406	4.173	3.929	4.184	3.802	3.730	
	^{13}C	108.8	82.3	78.6	85.8	62.6		
α-D-Glc*p*A (C)	^{1}H	5.269	3.607	3.78	3.29			3.498
	^{13}C	99.13	[a]	[a]	[a]	[a]		60.9
β-D-Xyl*p* (D)	^{1}H	4.64	3.50	3.67	3.82	4.100	3.350	
	^{13}C	[a]	[a]	[a]	[a]	[a]		
β-D-Xyl*p* (E)	^{1}H	4.563	3.333	3.476	3.643	3.989	3.301	
	^{13}C	103.9	74.2	77.2	70.6	66.6		
β-D-Xyl*p* (F)	^{1}H	4.527	3.484	3.752	3.875	4.161	[a]	
	^{13}C	102.9	74.4	78.9	75.0	64.4		
β-D-Xyl*p* (G)	^{1}H	4.49	3.339	3.584	3.803	4.130	3.408	
	^{13}C	102.9	74.2	75.1	77.4	64.4		
β-D-Xyl*p* (H)	^{1}H	4.466	3.319	3.575	3.769	4.080	[a]	
	^{13}C	102.8	74.2	[a]	[a]	66.6		

[a] Not determined.

substitution. An NOE from H-1 of (A) to the H-3 at δ 3.575 ppm showed that the α-L-Ara*f* residue was (1→3)-linked to the β-D-Xyl*p* residue (H) of the xylan backbone, as depicted in Figure 2. The disaccharide side chain, 2-*O*-β-D-Xyl*p*-α-L-Ara*f* attached at O-3 of the main chain, has earlier been proved to exist in corn cob xylan.[14] NMR data for compounds with a similar substitution have been previously reported,[15–17] for heteroxylans from corn bran[18] and some arabinoxylans from rye[19] and wheat.[20]

A small amount of 4-*O*-methyl-α-D-glucuronic acid was also found to substitute the xylan backbone. The ^{1}H NMR chemical shifts for the 4-*O*-methyl glucuronic acid (C) and the Xyl (D) to which it is linked were very similar to the chemical shifts measured in (4-*O*-methyl-α-D-glucurono)-β-D-xylan.[21] The (1→2)-linkage between the two sugars was confirmed from the existence of an HMBC cross-peak between H-1 (C) and H-2 (D) (Figs. 1 and 2; Table 2).

Other signals of low intensity were present in the anomeric region between 5.2 and 5.4 ppm (Fig. 1) and probably originate from Glc, Gal or Man residues linked to the main chain or from polysaccharides non-covalently associated with the arabinoxylan fraction. However, reliable assignments could not be obtained because of the low sugar concentrations. For the Xyl residues, at least five different chemical environments were observed. The large signal at 4.49 ppm arises from the anomeric proton of the unsubstituted Xyl residues of the main chain. The remaining Xyl residues were monosubstituted and were assigned from the HMBC and NOESY spectra (Table 2; Fig. 2). The molar proportions of D-Xyl, α-L-Ara*f*-(1→3), β-D-Xyl*p*-(1→2)-α-L-Ara*f*-(1→3) and 4-*O*-methyl-α-D-Glc*p*A were determined by integration of the corresponding anomeric protons and were found to be 12:1:1:0.5. No information on whether the distribution of the side chains on the main xylan chain was random or in blocks could be obtained from the NMR study on this fraction of BHAX. To gain information on this the isolated arabinoxylan was fractionated and degraded by *endo*-β-xylanase or subjected to periodate treatment (see Section 3), and the released oligosaccharides were investigated by NMR spectroscopy.

2.3. Ethanol precipitation

To facilitate a structure investigation, the heterogeneous distribution was narrowed down by a sequential ethanol precipitation in steps of 10% (v/v). The fraction that precipitated at 60% (v/v) ethanol (BHAX-E) (Fig. 3b) was

almost free from protein (signals at 1–3 ppm) and lignin (7–8 ppm, see inset in Fig. 3) in comparison to BHAX (Fig. 3a) and was the fraction chosen for continued structure analysis. Figure 3 also showed that the ethanol precipitation led to an enrichment of the polymer containing β-D-Xyl*p*-(1→2)-α-L-Ara*f* substitution, simply by removing low molecular weight fragments, but did not affect the structure in any other way.

2.4. NMR analysis of enzymatically degraded arabinoxylan

Fraction A11 contained free Ara and Xyl and fraction A10 contained xylobiose (spectra not shown). Fraction A9 contained predominantly xylotriose (Fig. 4). Fraction A8 contained two main components, two tetrasaccharides, each containing Ara and Xyl at a molar ratio of 1:3. The two α-L-Ara*f* anomeric signals at δ 5.406 and δ 5.317 ppm were characteristic for α-L-Ara*f* groups (1→3)-linked to an internal and terminal non-reducing β-D-Xyl*p* group, respectively. Fraction A7 contained predominantly one component, a pentasaccharide with Ara and Xyl at a ratio of 1:4. The chemical shift of the α-L-Ara*f* anomeric signal at δ 5.406 ppm was diagnostic of an internal (1→3)-linked residue and the compound was identified by NMR as β-D-Xyl*p*-(1→4)-[α-L-Ara*f*-(1→3)]-β-D-Xyl*p*-(1→4)-β-D-Xyl*p*-(1→4)-β-D-Xyl*p* with the ^{1}H and ^{13}C data reported in Table 3. These NMR data are in very good agreement with the data previously reported for the same structure.[13] The ^{1}H NMR spectrum showed that fraction A6 contained mainly one component (>95%), a hexasaccharide with Ara and Xyl at a ratio of 1:5. The signal at 5.539 ppm was identified as the anomeric proton of a α-L-Ara*f* residue (1→3)-linked to a β-D-Xyl*p* group, but also linked by its C-2 to another β-D-Xyl*p* residue. The C-2 signals of α-L-Ara*f* exhibited a characteristic glycosylation downfield shift to δ 89.9 ppm (Table 4), in agreement with a (1→2) linkage, whose occurrence was confirmed from the HMBC- and ROESY experiments (Table 5). To our knowledge, such a structure and the

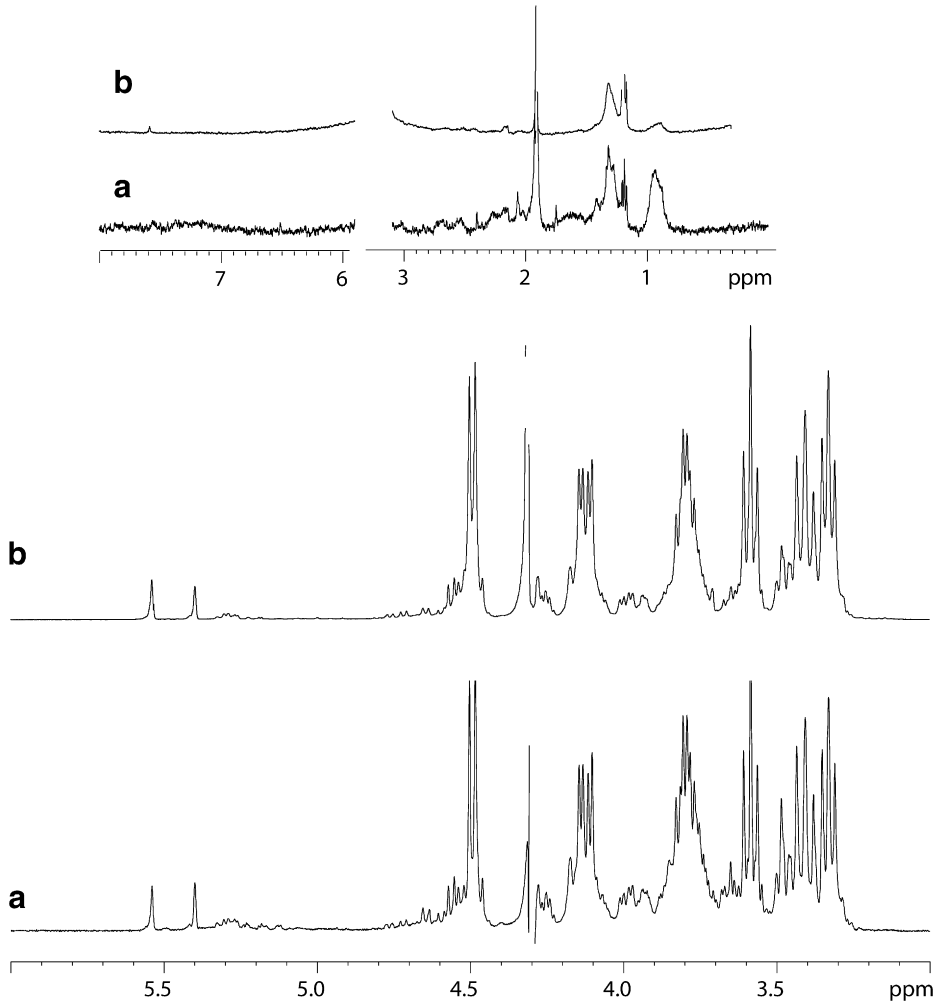

Figure 3. ^{1}H NMR spectra of (a) BHAX and (b) BHAX-E. The inset shows specific protein and lignin regions.

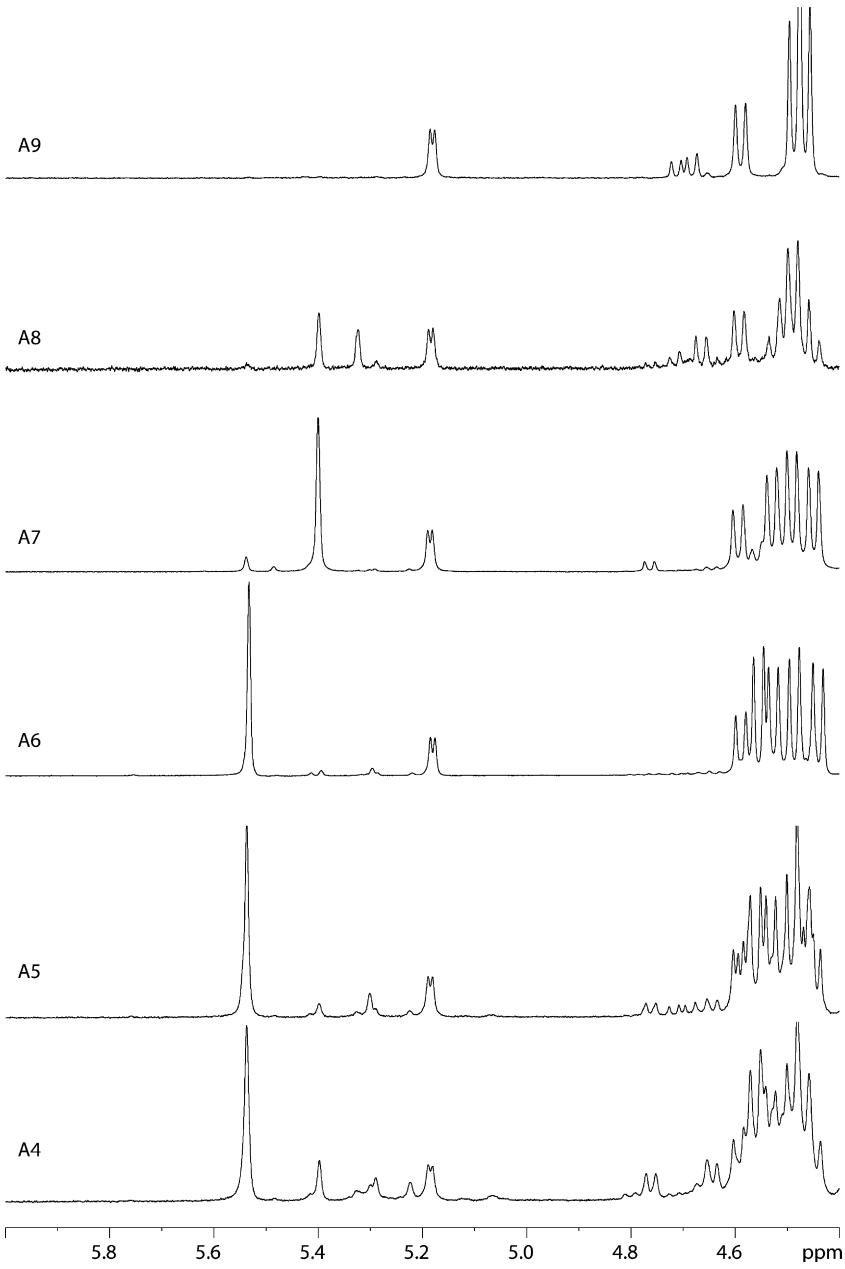

Figure 4. The anomeric region of a ^{1}H NMR spectrum of fractions A9–A4 obtained after enzymatic hydrolysis of BHAX-E.

corresponding NMR data have not previously been described. The compound was therefore fully characterized as (β-D-Xyl*p*-(1→4)-[β-D-Xyl*p*-(1→2)-α-L-Ara*f*-(1→3)]-β-D-Xyl*p*-(1→4)-β-D-Xyl*p*-(1→4)-β-D-Xyl*p*) and all ^{1}H and ^{13}C NMR signals were assigned (Table 4).

The molecular weight of the major components in fractions A6 and A7 was confirmed from MALDI-TOF mass spectra. Fraction A7 showed a mass spectrum with a major $(M+Na)^{+}$ ion with *m*/*z* of 701, indicating a molecular weight of 678 and corresponding to a molecule consisting of five pentoses. Two minor components with *m*/*z* of 569 $(M+Na)^{+}$ and 833 $(M+Na)^{+}$ indicated that fraction A7 also contained molecules with four and six pentoses, respectively. The MALDI-TOF mass spectrum showed that the major component in fraction A6 had a *m*/*z* of 833 $(M+Na)^{+}$, which is consistent with a molecule consisting of six pentoses.

The NMR spectra showed that fractions A5 and A4 mainly consisted of arabinoxylans with the same building block, β-D-Xyl*p*-(1→2)-α-L-Ara*f*-(1→3), as in A6 but with longer Xyl chains. Other minor components containing either internal or terminal α-L-Ara*f*-(1→3)-linked to the xylan chain were also present (anomeric signals at δ 5.406 and δ 5.315 ppm). In fraction A4, the 5.2–5.4 ppm region showed the presence of additional anomeric signals that were not assigned.

Table 3. ^{1}H and ^{13}C NMR chemical shifts for glycosyl residues of fraction A7 (X_4–X_3(A)–X_2–X_1) in D_2O at 70 °C

		1	2	3	4	5eq	5ax
α-L-Ara*f* (A)	^{1}H	5.406	4.183	3.937	4.260	3.812	3.751
	^{13}C	108.9	82.13	78.6	86.1	62.8	
β-D-Xyl*p* (X_4)	^{1}H	4.454	3.274	3.449	3.618	3.952	3.292
	^{13}C	102.7	74.4	77.1	70.7	66.5	
β-D-Xyl*p* (X_3)	^{1}H	4.535	3.485	3.772	3.863	4.153	3.433
	^{13}C	102.9	74.4	78.8	75.1	64.1	
β-D-Xyl*p* (X_2)	^{1}H	4.497	3.328	3.595	3.803	4.133	3.414
	^{13}C	103.0	74.1	75.2	74.4	64.2	
β-D-Xyl*p* (X_1)	^{1}H	4.600	3.280	3.580	3.795	4.075	3.320
	^{13}C	97.9	75.5	75.2	77.8	66.5	
α-D-Xyl*p* (X_1)	^{1}H	5.191	3.568	3.791	[a]	3.840	3.779
	^{13}C	93.3	72.9	72.4	[a]	60.46	

[a] Not determined.

Table 4. ^{1}H and ^{13}C NMR chemical shifts for glycosyl residues of fraction A6 (X_5–X_4(A–X_3)–X_2–X_1) in D_2O at 70 °C

		1	2	3	4	5eq	5ax
α-L-Ara*f* (A)	^{1}H	5.539	4.288	4.092	4.268	3.816	3.754
	^{13}C	107.6	89.9	77.15	85.4	62.5	
β-D-Xyl*p* (X_5)	^{1}H	4.447	3.282	3.445	3.612	3.949	3.324
	^{13}C	102.7	74.9	[a]	70.6	66.6	
β-D-Xyl*p* (X_4)	^{1}H	4.533	3.493	3.579	3.864	4.158	3.432
	^{13}C	102.8	74.3	78.9	75.0	64.1	
β-D-Xyl*p* (X_3)	^{1}H	4.560	3.303	3.484	3.645	3.993	3.365
	^{13}C	103.9	74.4	77.1	70.6	66.8	
β-D-Xyl*p* (X_2)	^{1}H	4.492	3.348	3.594	3.802	4.133	3.416
	^{13}C	103.0	74.1	75.3	77.8	64.1	
β-D-Xyl*p* (X_1)	^{1}H	4.594	3.284	3.584	3.792	4.073	3.407
	^{13}C	97.9	75.5	75.3	77.9	64.9	
α-D-Xyl*p* (X_1)	^{1}H	5.186	3.566	3.785	[a]	3.839	3.775
	^{13}C	93.4	72.9	72.5	[a]	60.4	

[a] Not determined.

Fractions A3–A1 (spectra not shown) contained a mixture of higher oligosaccharides and were not analyzed further.

These results indicated that the enzymatic degradation of unbranched Xyl*p* residues and of α-L-Ara*f*-(1→3)-branched Xyl*p* residues was faster than the degradation of β-D-Xyl*p*-(1→2)-α-L-Ara*f*-(1→3)-branched Xyl residues. It also indicates that the two types of Ara substituents are not located on the neighboring Xyl residues.

Table 5. Intra-residual NMR connectivities observed for anomeric protons in NOESY and HMBC spectra used for determination of the linkages positions in fraction A6

	NOE	HMBC
5.539 (H-1 A)	3.759 (H-3 X4)	78.9 (C-3 X4)
4.560 (H-1 X3)	4.281 (H-2 A)	89.9 (C-2 A)
4.533 (H-1 X4)	3.801 (H-4 X2)	77.8 (C-4 X2)
4.494 (H-1 X2)	3.792 (H-4 X1)	77.9 (C-4 X1)
4.447 (H-1 X5)	3.864 (H-4 X4)	75.0 (C-4 X4)

2.5. NMR analysis of periodate treated arabinoxylan

Separation of periodate treated arabinoxylan on Biogel-P2 resulted in ten fractions (B1–B10). B10 and B9 were identified as mixtures of low molecular weight components and were not further studied. The ^{1}H NMR spectrum of B8–B6 revealed doublets at 4.45–4.57 ppm (Fig. 5), corresponding to the anomeric protons of the Xyl*p* in glycerol xylosides containing 1–3 Xyl residues.[6] Fractions B7-B6 also contained an Ara residue attached to O-3. Since this Ara*f* residue was resistant to $NaIO_4$ it must have been substituted at O-2 and/or O-3, which confirms the presence of terminal Xyl residues on some branches. No oligosaccharides larger than five sugar units were obtained, indicating that the distribution of substituents along the xylan chain was random and not in blocks.

Periodate oxidation confirmed the presence of β-D-Xyl*p*-(1→2)-α-L-Ara*f*-(1→3) substituents in arabinoxylan from barley husks, as also shown by enzymatic degradation. The influence of these substituents on water holding capacity and material properties is currently under investigation.

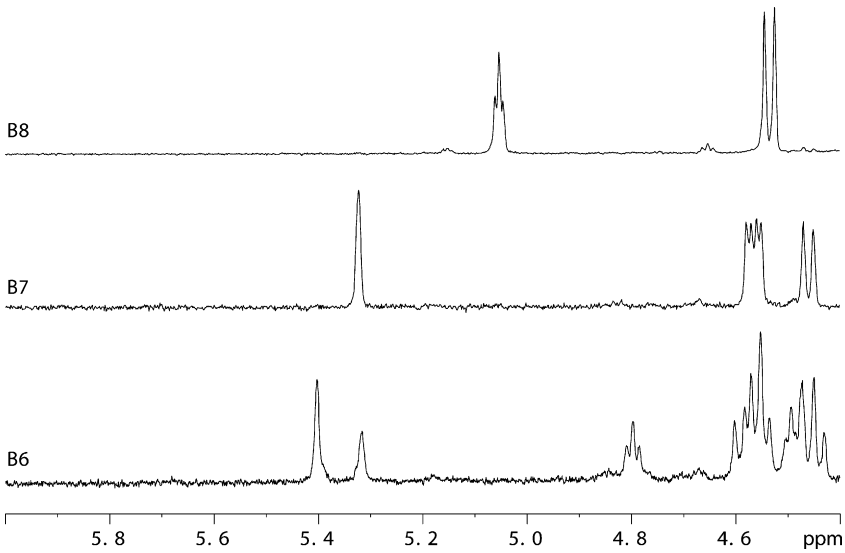

Figure 5. The anomeric region of a ^{1}H NMR spectrum of fractions B8–B6 obtained after periodate oxidation of BHAX-E.

3. Experimental

3.1. Material

Barley husks (*Hordeum vulgare*, cultivar Cindy) were obtained from Lyckeby Stärkelsen, Kristianstad, Sweden. Cindy is a high-amylopectin (waxy) barley where the starch consists of 96% amylopectin. The raw material was first ground in a mill to a particle size of less than 1 mm. Enzymes used were thermostable α-amylase (Termamyl Ultra 300L, Novozymes, Copenhagen, Denmark), amyloglucosidase (special quality for starch determination) from *Aspergillus niger* (Roche Diagnostics, Mannheim, Germany), protease from *Bacillus licheniformis* (Subtilisin A, Megazyme, Bray, Ireland) and *endo*-β-xylanase M3 from *Trichoderma longibrachiatum* (Megazyme).

3.2. Isolation and purification of the arabinoxylan

The isolation method, as described earlier,[12] involved a mild acid hydrolysis followed by delignification using sodium chlorite. The fiber was then extracted with 1 M sodium hydroxide for 16 h at room temperature and the product was collected by dialysis (MW cut-off 12–14,000) and freeze-drying. The arabinoxylan had a purity of 83%, and the remainder consisted mainly of starch, proteins and lignin. In order to elucidate the structural characteristics of the arabinoxylan, further purification was required. Lignin was removed by dissolution of 25 g of the isolated arabinoxylan in 1 L water, and the pH was adjusted to 4.0 with glacial acetic acid. Ten grams of sodium chlorite (80% purity) was added and the temperature was raised to 75 °C. After 2 h of extraction, the mixture was allowed to cool to room temperature and the arabinoxylan was recovered by dialysis and freeze-drying. Starch was removed by enzymatic hydrolysis. Lignin-free arabinoxylan (20 g) was dissolved in 1 L phosphate buffer at pH 6.0. Thermostable α-amylase (200 μL) was added and the bottle was placed in an 90 °C water bath. The bottle was shaken every 15 min. After 3 h the temperature was slowly reduced to 60 °C and 200 μL amyloglucosidase was added. To remove proteins, 200 μL protease was added simultaneously. After 16 h the temperature was raised to 100 °C for 15 min in order to inactivate the enzymes. Barley husk arabinoxylan (BHAX) was recovered by dialysis and freeze-drying.

3.3. Composition analysis

The lignin content and neutral sugar composition of the samples were analyzed by the Uppsala method for the determination of total dietary fiber.[22] The protein content was calculated from the nitrogen content ($N * 6.25$), determined by the Kjeldahl method.[23]

3.4. Precipitation with ethanol

A sequential precipitation with ethanol in steps of 10% (v/v) was performed on BHAX. After mixing, cooling on ice for 1 h and centrifugation (10 min, 1300 g), the pellets were collected and subjected to nuclear magnetic resonance (NMR) analysis. The material that precipitated at 60% (v/v) ethanol (BHAX-E) was chosen for further analysis.

3.5. Enzymatic hydrolysis

BHAX-E was hydrolyzed for 24 h at 50 °C. The assay mixture consisted of 1% arabinoxylan solution (0.05 M Na-phosphate, pH 6.0) and 5 U/mL *endo*-β-xylanase.

After 24 h of hydrolysis, the enzyme was inactivated by a 15-min treatment in a boiling water bath.

3.6. Periodate oxidation and Smith degradation

BHAX-E (100 mg) was dissolved in 100 mL of 0.02 M sodium metaperiodate, adjusted to pH 4.5 with glacial acetic acid.[6,24] The reaction flask was covered with aluminum foil and kept in darkness for 24 h at 4 °C. The excess of periodate was destroyed by an addition of 1 mL ethylene glycol, and the solution was dialyzed against distilled water for 48 h and thereafter concentrated to about 20 mL. The product was then reduced with sodium borohydride (20 mg) and the mixture was left for 24 h at room temperature. The sample was then dialyzed against distilled water for 24 h and freeze-dried. The oxidation and reduction procedures were repeated twice. The final product (40 mg) was dissolved in 0.02 M HC1 to a final concentration of 1%, and partially hydrolyzed in a water bath at 60 °C for 2 h.

3.7. Fractionation on Biogel-P2

The hydrolysates were applied on a Biogel P-2 column (94 × 1.6 cm, Bio-Rad Lab., Hercules, CA, USA) and eluted with water (0.5 mL/min). The refractive index and UV absorption were registered continuously and fractions of 5 mL were collected. The void fractions of each sample consisted of approximately 50% of the total amount of injected material and were not further studied. Fractions were pooled according to peak occurrence, and the samples were concentrated and freeze-dried. Eleven fractions were obtained from the enzymatically hydrolyzed arabinoxylan (A1–A11) and ten fractions from periodate oxidized arabinoxylan with Smith hydrolysis (B1–B10).

3.8. NMR spectroscopy

The ^{1}H and ^{13}C NMR spectra were acquired on BRUKER 400 or 600 MHz DRX spectrometers (Bruker Spectrospin Canada, Milton, Ont., Canada). The two-dimensional NMR experiments ^{1}H–^{1}H COSY, TOCSY, NOESY, ROESY, ^{1}H–^{13}C HSQC, and HMBC were run using standard pulse sequences from the BRUKER library. The samples were deuterium exchanged and dissolved in D_2O (0.5 mL), and the NMR experiments were run at 70 °C. The chemical shifts were referenced to internal acetone setting the ^{1}H signal to δ_H 2.20 ppm and the ^{13}C signal to δ_C 30.89 ppm.

3.9. Mass spectrometry

MALDI-TOF mass spectrometry was carried out on a Bruker Reflex III mass spectrometer (Bruker Daltonics, Leipzig, Germany) using 2,5-dihydroxybenzoic acid as the matrix.

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