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Heterogeneity in a water-extractable rye arabinoxylan with a low degree of disubstitution

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Abstract

From rye grain samples, water extractable arabinoxylan with a low degree of disubstitution (AX I) was isolated by fractionation on a DEAE-cellulose column. AX I of all rye samples had a similar relative composition of un- and monosubstituted xylose residues and with less than 5% disubstitution. It showed a shear thinning behaviour and the viscosity was related to molecular weight. AX I isolated from different rye varieties had different viscosities at the same concentration. One rye sample had a weight average molecular weight of 125 000 (polydispersity 2) according to calculations from both light scattering and viscosity data. Signals from un- and monosubstituted xylose residues in ^{13}C NMR spectra were assigned using ^{13}C-HSQC-DEPT and COSY techniques. AX I probably consisted of blocks or different molecules with sparsely and more densely substituted regions.

Keywords: Water-extractable rye arabinoxylan; Low degree of disubstitution; Viscosity; NMR

1. Introduction

Arabinoxylan is the predominant component of the dietary fibre complex in rye grain; hot water extractable arabinoxylan constitutes about 30% of the total arabinoxylan content (Nilsson et al., 1997; Pettersson & Åman, 1987), while room temperature water extractable arabinoxylan accounts for about 20% (Karlsson, 1988). Water extractable arabinoxylan from rye has previously been studied by Bengtsson and Åman (1990), who found that approximately 50% of that arabinoxylan was a fraction they called AX I, an arabinoxylan with approximately 50% O-3 monosubstituted (1,4)-linked xylose residues in the backbone and with a small proportion of disubstituted xylose residues. An arabinoxylan with a very high degree of disubstituted xylose residues (AX II) was isolated by enzyme degradation from a water extractable fraction and the authors hypothesized that water extractable arabinoxylan consists of a mixture of AX I and AX II type structures (Bengtsson, Anderson, Westerlund & Åman, 1992). Later Vinkx, Delcour, Verbruggen and Gruppen (1995); Vinkx, Reaynart, Grobet and Delcour (1993) applied ammonium sulphate precipitation on water extracts from rye grain and isolated several fractions of arabinoxylan with Ara:Xyl ratios from 0.50 to 1.42; among them an intact arabinoxylan with a high proportion of disubstitution (60%) and also with monosubstitution (14% at O-2 and 4% at O-3), hence confirming AX II as a polysaccharide present in water extracts of rye. Vinkx et al. (1993) on the basis of their fractionation and ^{1}H NMR studies, suggested that water extractable arabinoxylan consists of a range of structures.

Viscosity of arabinoxylan in solution, like that of other polysaccharides, is dependent on molecular weight. Arabinoxylan structure has also been shown to affect viscosity (Andrewartha, Phillips & Stone, 1979). Bengtsson et al. (1992) found a positive correlation between the proportion of disubstituted xylose residues and viscosity in water extracts of rye whole grain flour. Weight average molecular weight of rye water extractable arabinoxylan has been measured with gel permeation to 770 000 (Girhammar and Nair, 1992a).

Little is known about molecular weight and viscosity of highly purified water soluble arabinoxylan from rye. In this study, we have isolated, from three different rye whole grains, a water extractable arabinoxylan with a low degree of disubstitution and studied their structure, molecular weight distribution and viscosity. The distribution of substituents in a relatively large fragments obtained by incomplete xylanase degradation of the arabinoxylan has also

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Table 1
Chemical composition of the rye grain (% of dry matter), water extractability of arabinose and xylose residues (%), arabinose to xylose ratios of water extractable polysaccharides and relative distribution of water extractable xylose residues (%)

Component	Anna	Danko	Muskate	Amando[c]
Starch	64.3	66.6	64.4	63.4
Crude protein ($N \times 6.25$)	11.0	9.5	11.9	8.3
Crude fat	1.8	1.7	1.8	1.5
Ash	1.7	1.8	1.3	1.4
Mixed-linkage β-glucan	2.1	2.1	2.1	1.5
Dietary fibre[a]	13.2	14.2	13.1	16.1
Arabinose residues	2.7	3.3	2.8	3.5
Xylose residues	3.6	4.2	3.5	6.1
Mannose residues	0.7	0.6	0.7	0.8
Galactose residues	0.4	0.5	0.4	0.5
Glucose residues	4.2	4.1	4.3	3.9
Uronic acid residues	0.5	0.5	0.5	0.3
Klason lignin	1.0	1.0	0.9	1.1
Extractable glycosyl residues				
Arabinose	30.6	21.0	24.0	25.4
Xylose	42.3	33.5	36.0	27.7
Ara:Xyl ratio	0.53	0.49	0.53	0.53
mXyl[b]	27.5	31.9	26.2	35.5
dXyl[b]	11.8	7.8	11.9	8.9
uXyl[b]	60.8	60.3	61.9	55.6

[c] Previously published by Nilsson et al., 1997.
[a] Including traces of rhamnose and fucose.
[b] mXyl represents (1,3,4)-linked β-D-xylopyranosyl residues, uXyl represents (1,4)-linked β-D-xylopyranosyl residues and dXyl represents (1,2,3,4)-linked β-D-xylopyranosyl residues.

been studied. This information is of significance to understand the properties of the polysaccharide.

2. Experimental

2.1. Rye grain

Winter rye was obtained from Finland (cv. Anna), Sweden (cv. Danko and cv. Amando) and Canada (cv. Muskate). The samples (about 94% dry matter) were stored as whole kernels until they are analysed. Prior to chemical analysis the samples were ground in a Tecator cyclone sample mill to pass through a 0.5 mm screen.

2.2. Chemical composition of rye grains

All analyses were carried out in duplicate and are reported on a dry matter basis. The dry matter content was determined by oven drying at 105°C for 16 h. Starch was determined enzymatically (Åman, Westerlund & Theander, 1994). Crude protein and ash were analysed according to standard methods (AOAC, 1984). Crude fat was extracted with petroleum ether in a Soxtec HT6 unit (Tecator AB, Sweden) after acid hydrolysis with 3 M HCl (Anon., 1971).

Dietary fibre was determined as the sum of neutral and acidic sugar residues and Klason lignin according to Theander, Åman, Westerlund, Andersson and Pettersson (1995). Mixed-linkage β-glucan was determined enzymatically (Åman & Graham, 1987). Soluble non-starch polysaccharides were extracted from the sample (400 mg) with a 8 ml sodium acetate buffer (0.1 M; pH 5.0) at 95 and 60°C during starch hydrolysis (Bengtsson et al., 1992). Polymers in the extract were isolated by ethanol precipitation, and the content of differently linked xylose and arabinose residues was determined by 400 MHz ^{1}H NMR and sugar analysis.

The sugar analysis was carried out as in Theander et al. (1995) with the following modifications: the starch removal step was omitted and 2–10 mg of fractions were weighed into 15 ml tubes, 500 μl 0.8 M sulphuric acid and 500 μl water containing 0.2 mg myo-inositol as internal standard was added to each tube, and thereafter the samples were autoclaved and analysed in the same manner as described in the method.

2.3. Isolation of water soluble arabinoxylan

Water-soluble arabinoxylan was essentially isolated as described by Bengtsson and Åman (1990), but precautions were taken not to reduce the molecular weight of the polymers. In this procedure, ground rye grains (300 g) were suspended in 90% aqueous ethanol (600 ml) and refluxed in a boiling water bath for 10 min. The insoluble residue was isolated by centrifugation ($3000 \times g$; 15 min) and washed with 600 ml of 90% aqueous ethanol and again isolated by centrifugation ($3000 \times g$; 15 min). The supernatants were discarded and the residue was partially dried in a vacuum-oven (40°C; 7 h), ground in a mortar and air-dried overnight. The dry residue was suspended in de-ionised water (500 ml) and treated in a shaking water-bath at 40°C for 30 min. More de-ionised water was added (400 ml) and the samples were further treated in the water bath for 60 min. Soluble components were isolated by centrifugation ($3000 \times g$; 15 min) and the residue washed with 140 ml de-ionised water and the supernatant again isolated by centrifugation ($3000 \times g$; 15 min). The combined supernatants were precipitated with ammonium sulphate (50 g/100 ml supernatant) during stirring and the mixture was left in a refrigerator for 48 h and the precipitate was isolated by centrifugation ($14\,600 \times g$; 15 min). The precipitate was suspended in hot de-ionised water (295 ml) and Termamyl (2.5 ml; 300L Novo Nordisk A/S, Copenhagen, Denmark) was added. The sample was treated in a boiling water bath for 30 min and cooled to room temperature. Crude arabinoxylan was precipitated by the addition of 95% aqueous ethanol (705 ml) during stirring. The mixture was left in a refrigerator overnight and the precipitate was then isolated by centrifugation ($3000 \times g$; 15 min) and washed with 80% aqueous ethanol (500 ml). To the pellet, 80% aqueous ethanol (2 ml) was added followed by a gentle addition of 50–100 ml de-ionised water (initially drop wise) during stirring and heated in a water bath (60°C) until a homogeneous gum was formed. The solution together with a 1–2 ml of chloroform was dialysed (Spectrapor, molecular cut off 12 000–14 000 Da)

Table 2
Yield (% of rye grain), content of sugar residues (% of dry fraction) and composition of differently linked residues (relative %) of arabinoxylan fractions (I–IV) eluted with water from DEAE-cellulose column

Fraction	Yield	Sugar residues			Ara:Xyl[a]	(Ara + Xyl):Glc[b]	Xylose residues[c]		
		Ara	Xyl	Glc			mxyl	dXyl	uXyl
Anna									
I	0.24	30.4	57.9	0.5	0.53	177	44.5	4.0	51.5
II	0.35	32.3	62.0	1.0	0.52	94	44.2	4.0	51.8
III	0.05	31.6	60.1	2.5	0.53	37	42.9	4.8	52.3
IV	0.07	23.5	45.2	2.1	0.52	33	41.7	5.1	53.2
Danko									
I	0.31	29.5	58.7	0.3	0.50	298	45.6	2.3	52.1
II	0.26	28.4	56.5	1.0	0.50	85	45.7	2.3	52.0
III	0.24	31.0	61.3	1.6	0.51	58	41.9	4.3	53.8
IV	0.09	27.5	53.8	3.5	0.51	23	44.3	3.4	52.3
Muskate									
I	0.10	29.9	60.6	Trace	0.49	–	42.5	3.4	54.1
II	0.24	32.2	61.5	Trace	0.52	–	41.0	5.7	53.3
III	0.13	32.4	58.4	2.3	0.55	39	38.2	8.6	53.2
IV	0.14	32.4	56.0	3.4	0.58	26	39.0	9.4	51.6

[a] Arabinose to xylose ratio.
[b] Sum of arabinose and xylose residues divided by glucose residues.
[c] Relative composition of xylose residues; mXyl represents (1,3,4)-linked β-D-xylopyranosyl residues, uXyl represents (1,4)-linked β-D-xylopyranosyl residues and dXyl represents (1,2,3,4)-linked β-D-xylopyranosyl residues.

against de-ionized water for at least 3 × 24 h. The suspension was then centrifuged (3000 × *g*; 15 min) and the supernatant was divided into two equal portions (about 500 ml each) which were stored at −18°C.

2.4. Fractionation on DEAE-cellulose

DEAE-cellulose (40 g, DE-23, Whatman) was washed with 0.5 M HCl and 0.5 M NaOH and activated with saturated $Na_2B_4O_7$ as described by the supplier. The crude arabinoxylan extract (500 or 250 ml for the highly viscous extract from Muskate) was applied on the column (5 × 12 cm^2) and eluted with de-ionized water (Neukom, Deuel, Heri & Kündig, 1960). Fractions (10 ml) were collected and their carbohydrate content determined using the phenol–sulphuric acid method (Dubois, Gilles, Hamilton, Rebers & Smith, 1956). The carbohydrate containing fractions were pooled into a total of four fractions (I–IV) which were dialysed against de-ionized water (at least 3 × 24 h using Spectrapor, molecular cut of 12 000–14 000), and freeze dried.

2.5. Size exclusion chromatography

Combined fractions I and II from Anna, Danko and Muskate were separated on sequentially connected size exclusion columns (μHydrogel, 2000, 500 and 250) at

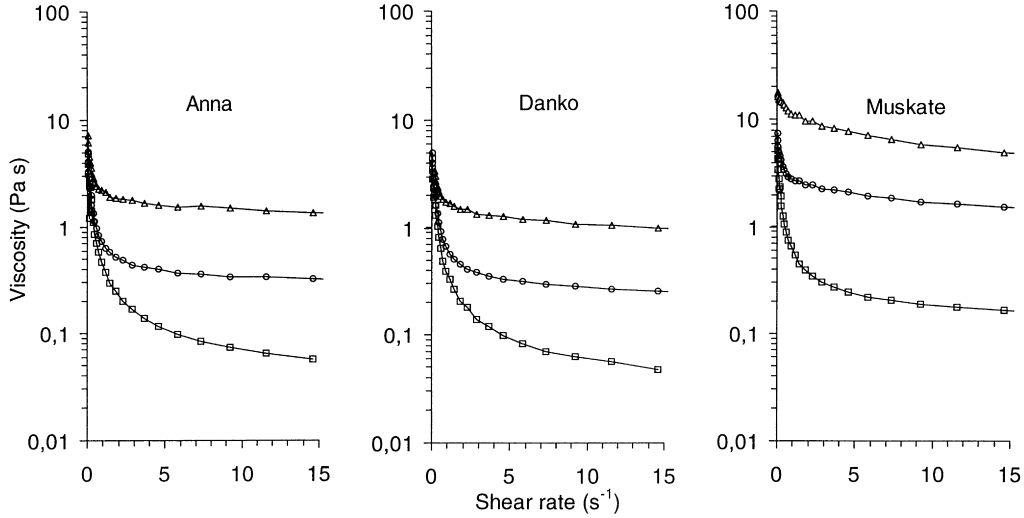

Fig. 1. Viscosity of AX I solutions of three concentrations 0.5 (□), 1.0 (○) and 1.5% (△) from Anna, Danko and Muskate, at different shear rates.

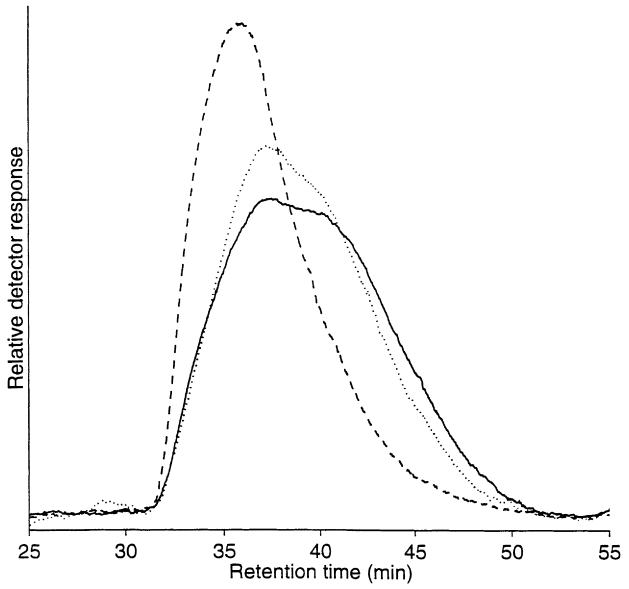

Fig. 2. Size exclusion chromatography of AX I from Muskate (- - -), Anna (· · ·) and Danko (—). Areas of the peaks are normalized.

70°C using a M-590 HPLC pump, M-715 automatic injector and a M-411 refractive index detector (Millipore/Waters division, Milford, MA, USA). Eluent was 50 mM sodium hydroxide and a flow rate 0.5 ml/min. Samples were prepared at 100–200 mg/ml and 100 μl injected. Molecular weights were estimated by comparison with the retention volumes of pullulan standards with molecular weights of 853 000, 186 000, 48 000 and 12 200.

2.6. Viscosity measurements

Arabinoxylan water solutions of 0.5, 1.0 and 1.5% (pooled fractions I and II from Anna, Danko and Muskate) were prepared and the viscosity was measured with a Bohlin VOR rheometer using a concentric cylinder system (C14) in the shear rate range from 0.0581 to 23.2/s at 25°C.

2.7. Molecular size distribution and xylanase treatment

The arabinoxylan prepared from Amando (pooled fractions I and II) was used for molecular size distribution and enzyme treatment experiments. Arabinoxylan (2 ml; 0.5 mg/ml) was fractionated on a S-500 column (0.5 ml/min) with 0.1 M NaCl and fractions of 2.5 ml were collected, evaporated using a SpeedVac Concentrator SVC 100H (SAVANT) and analysed for sugar composition.

Arabinoxylan from Amando (10 ml; 0.5 mg/ml) was treated with a 200 μl (800 GPU) pure endo-xylanase (EC 3.2.1.8; Danisco Ingredients, Denmark) for 30 min at 40°C. This enzyme has previously been shown that it does not contain any arabinofuranosidic activity (Eriksson, 1997). The enzyme was inactivated by treatment in a boiling water bath for 30 min. After enzyme treatment, the arabinoxylan solution (3 ml) was separated on a Biogel P-6 column at 0.5 ml/min with a 0.1 M NaCl as eluent. Fractions of 2 ml were collected, evaporated using the SpeedVac Concentrator and analysed for sugar composition. To collect enough material for ^{1}H NMR analysis the separation was repeated six times and the corresponding fractions pooled in four approximately equally sized fractions and lyophilized.

2.8. NMR analyses

Dried samples (2–10 mg) were dissolved in D_2O and evaporated to dryness. This procedure was repeated 4–5 times, and samples were finally dissolved in 1 ml D_2O. Proton nuclear magnetic resonance (^{1}H NMR) spectra (400 MHz) were recorded at 85°C on a Bruker DRX 400 instrument or a Varian VXR 400 instrument. Pulse repetition time was 4.2 s and radio frequency pulse angle 30°. The relative distribution of (1,4)-linked (uXyl), (1,3,4)-linked (mXyl) and (1,2,3,4)-linked (dXyl) xylose residues was calculated from the sugar analysis and integrals of the ^{1}H NMR spectra as described by Westerlund, Andersson, Åman and Theander (1990). ^{13}C NMR spectra (101 MHz) were recorded at 80°C on a Bruker DRX 400 instrument. Pulse repetition time was 5.5 s, radio frequency pulse angle 90° and 40 000 scans were recorded. Heteronuclear singular quantum coherence-distortionless enhancement by the polarization transfer (^{13}C-HSQC-DEPT) spectrum was recorded at 80°C on a Bruker DRX 400 instrument with 20 transients over 512 increments (zerofilled to 1K) and 2K data points with spectral widths of 1600 Hz in F_2 and 10 064 Hz in F_1. Pulse repetition time was 1.64 s. The delays were adjusted according to a coupling constant ^{1}J(CH) of 145 Hz. Gaussian apodization was applied in both dimensions. Correlation spectroscopy 90° (COSY) using gradients spectrum was recorded at 80°C on a Bruker DRX 400 instrument with eight scans over 256 increments (zerofilled to 512) and 2K data points with spectral widths of 1600 Hz in both F_1 and F_2. Pulse repetition time was 2.13 s. Sine bell apodization was applied in both dimensions.

2.9. Molecular weight determination

Molecular weight determination was performed on a high performance size exclusion chromatography (HPSEC) system consisting of an inline degasser (Shimadzu DGU-4A), a pump (Shimadzu LC-10AD) and a column (PL aquagel-OH Mixed 8 μm 300×7.5 mm^2, Polymer Laboratories). The eluent was 0.1 M Na_2HPO_4 containing 0.02% NaN_3 at 1.0 ml/min. Detectors were refractive index (RI) and viscometer (Dual detector model 250, Viscotek Corp.) and multiple-angle laser light scattering (MALLS; Dawn DSP equipped with a He–Ne laser 632.8 nm, Wyatt Technology Corp., Santa Barbara, CA, USA). Columns and RI detector were controlled at 30°C. Arabinoxylan was dissolved in eluent at a concentration of 3.1 mg/ml and 20 μl was injected. Data for the molecular weight determination by light scattering detection was analysed using ASTRA software (version 4.5, Wyatt Technology Corp., Santa Barbara, CA, USA) and a d*n*/d*c* value of 0.147 was

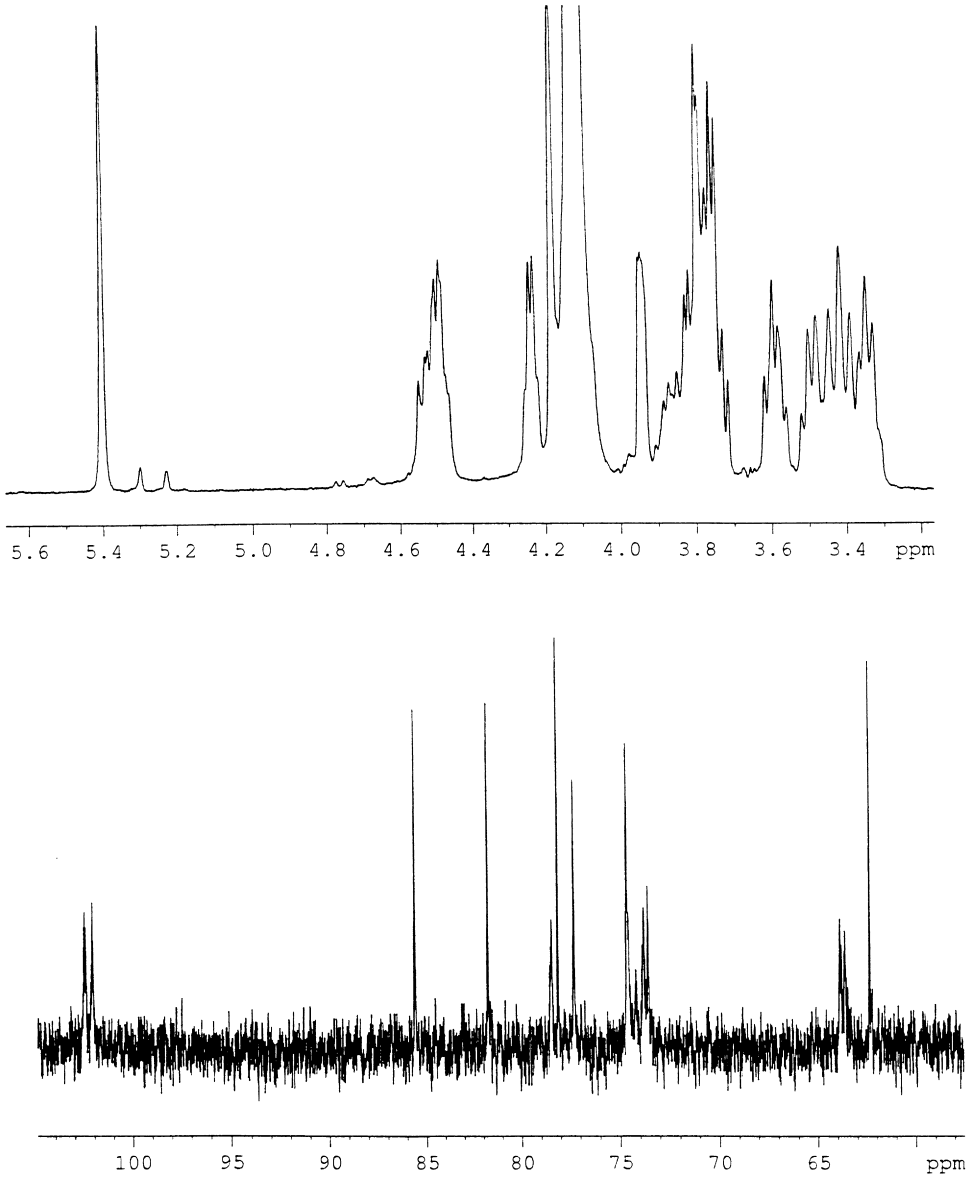

Fig. 3. ^{1}H and ^{13}C NMR spectra of AX I from Amando.

used. Calculations based on viscometry data were carried out using TriSEC software (version 3.0, Viscotek Corp., Houston, TX, USA)

3. Results and discussion

3.1. Rye grain composition

The chemical composition differed between the rye samples (Table 1). Amando had the lowest protein and highest dietary fibre content with a high proportion of arabinoxylan and a low proportion of β-glucan. The extractability of the arabinoxylan from Anna was higher than for the other samples. The chemical composition of the rye samples was generally in agreement with previous results (Nilsson et al., 1997). However, the samples in this study had a lower total dietary fibre content, a higher extractability of xylose residues and a higher proportion of uXyl in the water extractable arabinoxylan fraction. Arabinose to xylose ratio (Ara:Xyl) was also low compared to the previously published results (0.53–0.71; Bengtsson et al., 1992; Nilsson et al., 1997). Differences in dietary fibre content and water extractability may partly be explained by environmental conditions (Plaami, Saastamoinen & Kumpulainen, 1989).

3.2. Isolation of AX I

Rye samples were extracted with water and the extract separated on a DEAE-cellulose column into four fractions (Table 2). Yield varied between 0.07 and 0.35% of rye grain

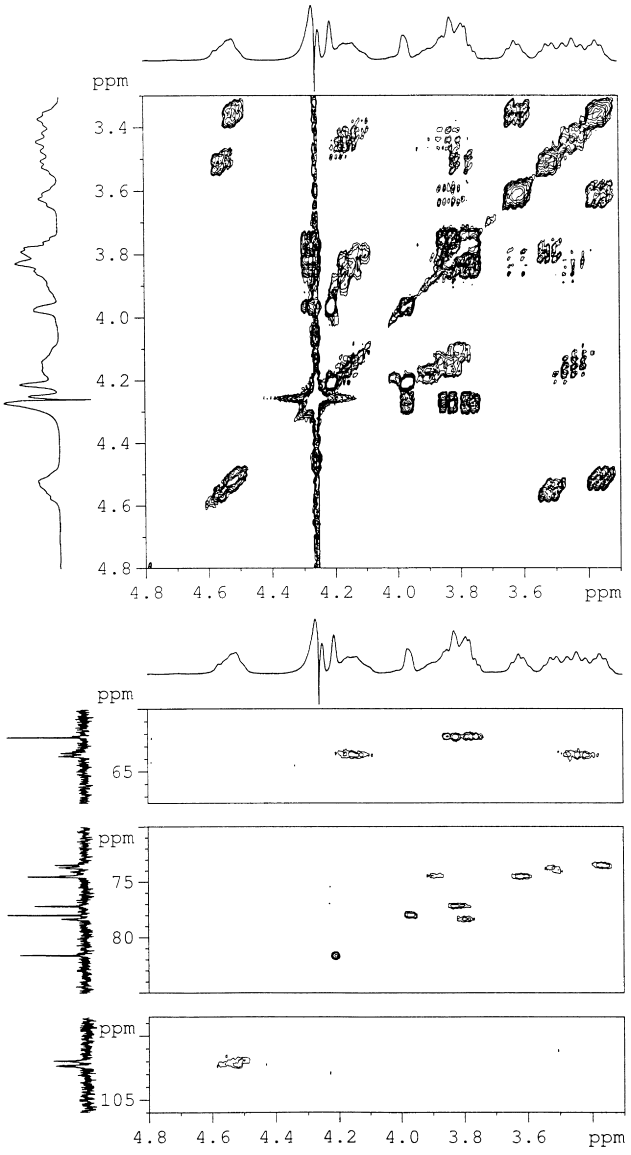

Fig. 4. COSY and ^{13}C-HSQC-DEPT spectra of AX I from Amando.

generally with the highest yields in the first two fractions. Muskate produced a highly viscous arabinoxylan solution and this might explain the lower yield of the sum of the first two fractions. All fractions had A:X-ratios ranging from 0.49 to 0.58 and arabinoxylan content (calculated as the sum of arabinose and xylose residues) ranging from 65.0 to 94.3%. Fractions I and II contained less than 1% of glucose residues and all isolated fractions had contents below 3.5%. Fractions I and II of each rye sample, respectively, had similar composition of m-, d- and uXyl, especially for Anna and Danko where they were virtually identical. A higher content of dXyl was found in fraction II compared to I for Muskate. Fractions III and IV had higher contents of dXyl compared to fractions I and II of each rye sample, respectively. Muskate had highest content of dXyl in fractions III and IV. Generally, the differences observed between samples were small since the structure of the fractions isolated are highly dependent on the fractionation method used. As the structural features of fractions I and II were similar for each sample, they were combined and are hereafter referred to as AX I.

3.3. Characterization of AX I

Viscosity measurements at different shear rates and size exclusion chromatography (SEC) were performed on water solutions of AX I. All solutions showed a shear thinning behaviour with a logarithmically high viscosity at increasing concentration (Fig. 1). AX I from Muskate had higher viscosity than AX I from Anna and Danko. SEC also revealed that AX I from Muskate had a substantially higher molecular weight than the other two samples (Fig. 2), which is probably the reason for the high viscosity. Anna had somewhat higher molecular weight than Danko, and also a slightly higher viscosity. Bengtsson et al. (1992) suggested that the AX II type of structure (rich in dXyl) gives a higher contribution to viscosity of water extracts than the AX I type of structure. In this study of purified AX I, this could not fully explain the large differences in viscosity between the rye samples.

A large amount of AX I from Amando was prepared, and the ^{1}H and ^{13}C NMR spectra showed an arabinoxylan with a low degree of disubstituted xylose units, much like the spectra of AX I previously reported by Bengtsson and Åman (1990) (Fig. 3). Previous reports on the assignment of the different carbon signals of xylose residues in the ^{13}C NMR spectrum have been based on literature references and on the chemistry of arabinoxylan (Bengtsson & Åman, 1990) and by 2D (two-dimensional) NMR techniques (Hoffman, Kamerling & Vliegenhart, 1992). Our ^{13}C signal assignments for C-3 and C-5 agree with those reported by Hoffman et al. (1992) and the assignments for C-3, C-4 and C-5 agree with that of Bengtsson et al. (1992). The chemical

Table 3
Chemical shifts (^{13}C NMR) of unsubstituted and monosubstituted xylose residues from rye grain arabinoxylan compared to data on rye and wheat arabinoxylan from other authors

	Chemical shifts (ppm)[a]				
	C-1	C-2	C-3	C-4	C-5
Rye					
uXyl[b]	102.1	73.6	74.6	77.3	63.9
mXyl[b]	102.5	73.8 and 74.2	78.4	74.6	63.6
Rye[c]					
uXyl[b]	102.5	73.9	74.6	77.3	63.9
mXyl[b]	102.0	73.6	78.5	74.6	63.7
Wheat[d]					
uXyl[b]	102.09 and 102.48	73.56 and 73.84	74.64	77.29	63.85
mXyl[b]	102.43	74.37	78.38	78.13	63.59

[a] Relative to internal standard dioxan.
[b] uXyl represents (1,4)-linked β-D-xylopyranosyl residues and mXyl represents (1,3,4)-linked β-D-xylopyranosyl residues.
[c] From Bengtsson and Åman (1990).
[d] From Hoffman, Roza, Maat, Kamerling and Vliegenhart (1991a).

Table 4
Average molecular size and polydispersity of AX I from Amando as detected by multi-angle laser light scattering (MALLS) and viscometry combined with 90° light scattering angle

	MALLS	Viscometry
Number average molecular weight ($\bar{M}_n$)	61 700	67 300
Weight average molecular weight ($\bar{M}_w$)	124 000	125 000
Z average molecular weight ($\bar{M}_z$)	226 000	226 000
$\bar{M}_w/\bar{M}_n$	2.0	1.9
$\bar{M}_z/\bar{M}_n$	3.7	3.4

shifts of the anomeric protons have previously been assigned to 4.48 and 4.51 for uXyl and mXyl, respectively (Hoffman, Leeflang, de Barse, Kamerling & Vliegenhart, 1991b). This information was used in combination with COSY and ^{13}C-HSQC-DEPT to assign the anomeric ^{13}C signals of AX I from Amando (Fig. 4). The overlapped and asymmetric cross-peak cluster at 102.0–102.5 ppm from m- and uXyl indicated that the down field ^{13}C NMR signal corresponded to mXyl (Table 3). C-2 ^{13}C signals at 73.6, 73.8 and 74.2 ppm gave cross peaks at 3.35, 3.5 and 3.5 ppm, respectively, indicating that the down field ^{13}C signal refers to mXyl. The anomeric signal of uXyl at 102.48 reported by Hoffman et al. (1992) was detected as a split belonging to the mXyl by ^{13}C-HSQC-DEPT in this investigation and our C-2 assignment for peaks at 73.8 and 74.2 was assigned to mXyl. The split ^{13}C signal at 74.6 was assigned to C-4 in mXyl and C-3 in uXyl which was not in agreement with that of Hoffman et al. (1992).

The molecular size of AX I from Amando was calculated with light scattering and viscosity data (Table 4). Molecular weight measurements were in good agreement between the two methods; a slightly higher number average molecular weight from the viscometry calculations being the only noticeable difference. Polydispersity of the arabinoxylan was around two, showing that there was a large span of molecular weights distributed between 15 000–650 000. From the viscometric detector, weight average intrinsic viscosity was calculated to be 274 ml/g with a range of 110–691 ml/g. Molecular weight measurements have previously been performed on water extractable rye arabinoxylan with a 71.7% content of polysaccharide residues with an Ara:Xyl ratio of 0.74 (Girhammar & Nair, 1992a). Average molecular weight was found to be 770 000 (and polydispersity of 8.5) as determined by GPC while ultra centrifugation gave an average molecular weight of 275 000 and an intrinsic viscosity of 560 ml/g (Girhammar & Nair, 1992b).

3.4. Distribution of arabinosyl substituents

Arabinoxylan homogeneity can be studied with the help of structure analysis of fractions obtained by SEC. Similar Ara:Xyl ratios over such a molecular size interval is, however, not proof of a homogeneous distribution of arabinosyl substituents along the xylan chain. There may still be blocks of more sparsely or densely substituted regions within or between molecules. Xylanases are known to generally have a preference for sites on the arabinoxylan chain with several consecutive uXyl and therefore to be less active on highly substituted xylans (Kormelink, Gruppen, Viëtor & Voragen, 1993). Thus, it should be possible to reveal differently substituted regions in arabinoxylan chains by incomplete xylanase treatment followed by fractionation of the fragments released. This hypothesis was tested on AX I from Amando.

AX I from Amando was distributed over a Sephacryl S-500 column with a peak maximum at about Kd 0.45 and a complete recovery of arabinoxylan (101%) (Fig. 5).

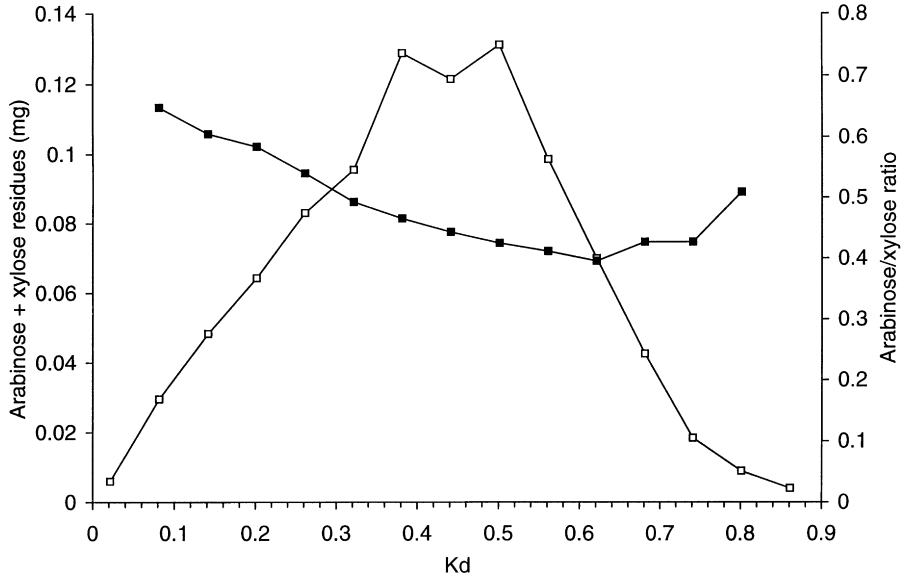

Fig. 5. Fractionation of AX I from Amando on a Sephacryl S-500 column. The amount of arabinoxylan in each fraction (□), calculated as the sum of arabinose and xylose residues, is presented, as well as the arabinose to xylose ratio (■).

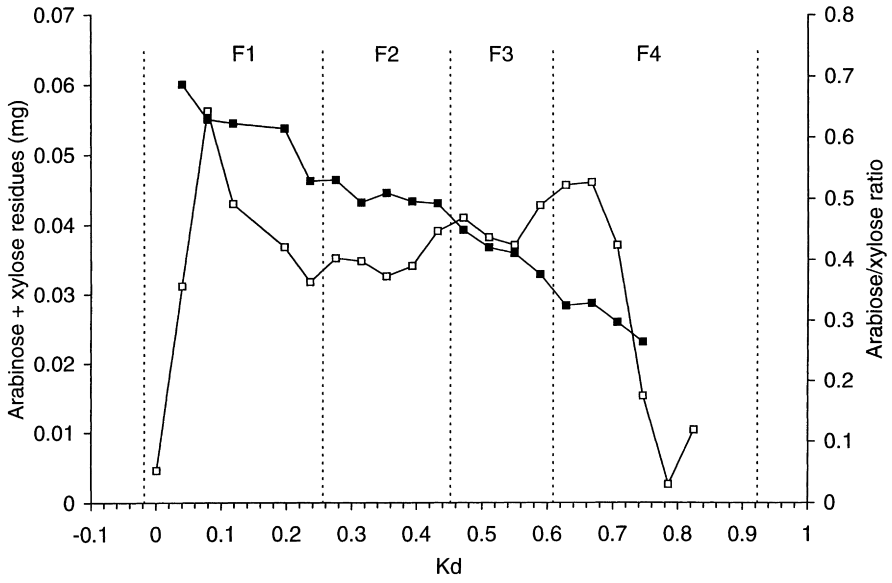

Fig. 6. Fractionation of xylanase treated AX I from Amando on a Biogel P-6 column. The amount of arabinoxylan (□) in each fraction, calculated as the sum of arabinose and xylose residues, is presented, as well as the arabinose to xylose ratio (■). Pooled fractions collected (F1–F4) are shown with a dashed line.

Arabinose to xylose ratio decreased from 0.65 close to the void volume to 0.40 at Kd 0.65. A small increase in the ratio could be noticed close to the included volume. The change of Ara:Xyl ratio over the column could be due to the heterogeneous distribution of mXyl in the arabinoxylan. It may also be explained by a contaminating arabinoxylan with a higher content of dXyl, since a mixture of these arabinoxylans would give a decreasing Ara:Xyl ratio if they had different molecular size distributions.

In order to obtain relatively large AX I fragments which might reveal an uneven distribution of substituents according to the hypothesis, AX I from Amando (Ara:Xyl ratio 0.46) was treated with an endo-xylanase devoid of arabinofuranosidic activity. The conditions for the incomplete degradation were optimized to give fragments with a molecular range covering that of the Biogel P-6 column (Fig. 6). Recovery of arabinoxylan was complete (102%) and the polysaccharide was distributed over the column in a polymodal fashion. No accumulation of monomers or very small fragments was detected, confirming the endo-xylanase activity of the enzyme used. The Ara:Xyl ratio decreased linearly from 0.69 close to the void volume to 0.26 close to the included volume. The eluted material was pooled into four fractions (F1–F4) as indicated in Fig. 6 and the fractions analysed with ^{1}H NMR and sugar analysis (Table 5). The relative content of dXyl decreased from 4.0% in F1 to 0.6% in F4, but this could not fully explain the decrease in Ara:Xyl ratio over the column. It was also evident that the relative content of mXyl decreased and uXyl increased from F1 to F4. Thus, the xylanase treatment produced an arabinoxylan with a degree of mono- as well as disubstitution that decreased with the molecular weight. The decrease of Ara:Xyl ratio was more pronounced compared to that observed for untreated AX I. The average chain length of the xylan backbone fragments in the isolated fractions was estimated from the relative amount of anomeric ^{1}H NMR signals of reducing end xylose residues and decreased from 52 in F1 to 4 in F4. The fragments with Kd above 0.56 were less substituted than the least substituted part of the observed before xylanase treatment. Thus, these fragments must have been regions of larger polymers. These larger polymers could contain more dense regions or separate polymers of different structures could exist. The presence of separate polymers of different structures seem less likely, however, due to the apparent homogeneity of the first two fractions from the DEAE-cellulose column.

Table 5
Recovery of arabinose and xylose residues, arabinose to xylose ratio, relative distribution and degree of polymerization of the xylan backbone of m- and uXyl of xylanase treated AX I from Amando and fractions (F1–F4) after separation on a Biogel P-6 column

	Recovery(%)		Ara/Xyl	Xylose residues[a]			
	Ara	Xyl		mXyl	dXyl	uXyl	DP^{b}_{n}
AX I	–	–	0.46	40.3	2.7	57.1	–
F1	24.3	17.8	0.62	54.2.	4.0	41.8	52
F2	25.3	23.6	0.49	45.2	1.8	53.0	20
F3	24.7	24.8	0.46	42.9	1.6	55.5	10
F4	28.5	37.8	0.34	33.3	0.6	66.2	4

[a] mXyl represents (1,3,4)-linked β-D-xylopyranosyl residues, uXyl represents (1,4)-linked β-D-xylopyranosyl residues and dXyl represents (1,2,3,4)-linked β-D-xylopyranosyl residues.

[b] Degree of polymerisation of the xylan backbone. Calculated as the sum of the α and β ^{1}H NMR signals from reducing end xylose residues divided with the ^{1}H NMR signals from non-reducing xylose.

4. Conclusion

Water extractable arabinoxylan from rye grain with a low degree of branching (AX I) can be isolated by fractionation on a DEAE-cellulose column. Similar relative proportions of m-, d- and uXyl were found for all rye samples. AX I showed a shear thinning behaviour and the viscosity was dependent on molecular weight. AX I from one rye sample had an average molecular weight of 125 000 and a polydispersity of two according to light scattering as well as viscometric calculations. AX I from rye grain is probably heterogeneous in its distribution of mXyl, consisting of blocks with sparsely or more densely substituted regions. The strategy to use incomplete degradation proved useful for the study of relatively large regions in the polysaccharides as compared to previous investigations on small fragments.

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