



Structural Characteristics of the Cold-Water-Soluble Arabinoxylans from the White Flour of the Soft Wheat Variety Kadet

R. A. Hoffmann,^a M. Roza,^b J. Maat,^b J. P. Kamerling^a
& J. F. G. Vliegthart^{a,*}

^aBijvoet Center, Department of Bio-Organic Chemistry, Utrecht University, PO Box 80.075, NL-3508 TB Utrecht, The Netherlands

^bUnilever Research Laboratory, PO Box 114, NL-3130 AC Vlaardingen, The Netherlands

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ABSTRACT

Cold-water-soluble arabinoxylan polymers from the soft wheat variety Kadet were isolated by a stepwise $(\text{NH}_4)_2\text{SO}_4$ precipitation and freed from co-precipitating proteins by pronase digestion. The purified arabinoxylans, representing 0.5% of the dry weight of the white flour, were fractionated by graded precipitation with ethanol. Monosaccharide analysis revealed the major carbohydrate fractions to contain arabinoxylans only. The arabinoxylans are heterogeneous in molecular size. Determination of the carbohydrate composition in conjunction with methylation analysis and ^{13}C -NMR spectroscopy, indicated that they can be divided into specific groups, based on characteristic differences with respect to D-xylopyranose to L-arabinofuranose ratio and in 2,3,4-tri-:3,4-di-:4-mono-substituted D-xylopyranose ratio.

INTRODUCTION

White wheat flour, consisting principally of the ground endosperm of the wheat kernel, contains as the two main components starch and gluten-proteins, which are responsible for the functional properties of flour for making cereal products. The chemical nature and physical characteristics of flour performance are significantly influenced by the arabinoxylans, also known as pentosans or hemicelluloses, which represent only

*To whom correspondence should be addressed.

2.3–3.0% of the wheat flour (D'Appolonia, 1980; McCleary, 1986; Kühn & Grosch, 1988). The high water-binding capacity of arabinoxylans is responsible for the formation of clear, viscous solutions of water-soluble arabinoxylans. The viscosity of these solutions can be increased to a gel when peroxidase and H_2O_2 are added (Ciacco & D'Appolonia, 1982; Amadò & Neukom, 1985). It has been established that 4-hydroxy-3-methoxy-cinnamic acid (ferulic acid) which is ester-linked to the arabinoxylans, is involved in this oxidative gelation. Ferulic esters of neighbouring arabinoxylan chains can form a diferulic bridge (Geissmann & Neukom, 1973; Nishitani & Nevins, 1988), thus building an arabinoxylan network. In wheat flour also a tyrosine-ferulic ester crosslink is formed to give an arabinoxylan-protein network (Hoseney & Faubion, 1981; Fry, 1986). Enzymatic treatment of the arabinoxylan(-networks) with *endo*-xylanase causes a decrease in water-binding capacity (McCleary, 1986). It results in a very wet and sticky dough, and after baking in a decrease in loaf height and an increase in loaf volume (McCleary, 1986; Kühn & Grosch, 1988) replacing the normal silky crumb texture by a brittle crumpet-like structure.

Since the remarkable behaviour of the arabinoxylans must stem from their molecular structure, the investigation was aimed at gaining more insight into the structural diversity of the arabinoxylans present in the endosperm of wheat. As target of the investigation the flour of the European soft wheat variety Kadet was chosen.

MATERIALS AND METHODS

Isolation of water-soluble arabinoxylans

Pure-variety samples of the soft wheat variety Kadet (harvested 1982, southern Sweden) were used. Grains were thoroughly cleaned, brought to a moisture content of 16% and then gently blended in a MIAG Multomat blender, yielding 74% of white flour. Flour (600 g) was kneaded with 360 ml distilled water and the dough obtained was extracted six times with 0.1 M NaCl under constant kneading (Medcalf & Gilles, 1968). The remaining gluten complex was not used further. The pooled slurry was centrifuged at 10 000 g for 30 min, and the supernatant containing the water-soluble fraction (WSF), was decanted from the sedimented starch and tailings. The latter was used in additional studies (Hoffman *et al.*, 1991). The WSF was fractionated further, essentially as described by Fincher and Stone (1974). To the supernatant

$(\text{NH}_4)_2\text{SO}_4$ was added to a final concentration of 25% (w/w) and the precipitated starch and proteins (fraction WSSP) were removed by centrifugation at 10 000 g for 30 min. Then, the concentration of $(\text{NH}_4)_2\text{SO}_4$ in the supernatant was brought to 50% (w/w), and the precipitated water-soluble arabinoxylans (WSAX) were separated from the water-soluble arabinogalactan-peptide (WSAG) and starch fragments by centrifugation at 10 000 g for 30 min. To remove residual protein, the WSAX fraction dissolved in 200 ml 0.05 M NH_4Ac , pH 8.0, containing 0.015 M CaCl_2 , was heated for 5 min at 90°C, cooled to 37°C, and incubated for 48 h with four portions of 20 mg pronase (*Streptomyces griseus*, Boehringer) added at $t = 0, 6, 12$ and 24 h, respectively. The pH of the incubation was kept between 7.4 and 8.0. The digestion was stopped by heating the mixture for 5 min at 95°C and the coagulated pronase was removed by centrifugation (16 000 g, 20 min at 4°C). The supernatant was dialysed extensively against distilled water at 4°C, and the non-dialysable material, designated the water-soluble pronase treated arabinoxylan (WPAX), was lyophilized, yielding 3.12 g material (0.5% of total white flour).

Graded precipitation with ethanol

Precipitation of arabinoxylan from a stirred 2% WPAX (3.10 g) solution was effected by increasing slowly the concentration of ethanol, at room temperature. When a precipitate was formed, the mixture was stored for 2 h at 4°C and centrifuged (16 000 g, 30 min at 4°C). The pellet was redissolved in distilled water and lyophilized. Ethanol was added to the supernatant until the next precipitate was formed. In this way the fractions WPAX₄₃, WPAX₅₁, WPAX₅₅, WPAX₆₂, WPAX₆₅₋₇₁ and WPAX₇₅₋₈₅ were obtained, whereby the subscripts refer to the ethanol percentages at which precipitation occurred.

Gel permeation chromatography

Portions of 100 mg WPAX₄₃, WPAX₅₁ and WPAX₅₅, respectively, were fractionated on a Sephacryl S-500 column (159 × 2.2 cm, Pharmacia) eluted with distilled water (25 ml/h, 5-ml fractions). The eluate was monitored at 206 nm and carbohydrates were quantitatively determined by the phenol/sulphuric acid assay (Dubois *et al.*, 1956) with D-xylose as a standard. The Sephacryl S-500 column was calibrated with pullulan standards (Macherey-Nagel) from 48.0 kDa up to 853 kDa, giving a good linearity ($R^2 = 0.998$) between Log [Molecular mass] and exclusion volume.

Preparation of xylan and xylo-oligomers

Arabinoxylan from WPAX₄₃ (10 mg) was partially hydrolysed in 1.0 ml 3.5 mM HCl for 16 h at 85°C under nitrogen. The precipitated xylan was collected by centrifugation at 13 000 g, washed twice with distilled water, and lyophilized. To produce water-soluble xylo-oligomers, a partial hydrolysis of xylan (5 mg) was carried out in 1.0 ml 0.1 M HCl for 8 h at 85°C under nitrogen. After lyophilization, the residue was suspended in distilled water, centrifuged at 13 000 g, and the supernatant was fractionated on a Bio-Gel P-2 column (200–400 mesh, 50 × 1.0 cm, Bio-Rad) eluted with distilled water.

Monosaccharide analysis

Dry polysaccharide material (0.3 mg), dissolved in 1 ml methanolic 1 M HCl, was kept for 24 h at 85°C (Kamerling & Vliegthart, 1982, 1989). After neutralization with Ag₂CO₃ the mixture was treated with 25 μl acetic anhydride to prevent adhesion of methyl glycosides to the silver salts (Chambers & Clamp, 1971), stored in the dark for 4 h at room temperature, and centrifuged. The supernatant was concentrated to dryness under reduced pressure at 40°C, and the obtained mixture of methyl glycosides was dried over P₂O₅. To determine the absolute configuration, 0.3 mg methanolysed polysaccharide was treated with 1 ml (–) or (±)-2-butanolic 1 M HCl for 8 h at 85°C (Gerwig *et al.*, 1978) and concentrated to dryness, yielding butyl glycosides. The methyl and butyl glycosides were trimethylsilylated with 0.1 ml hexamethyldisilazane:chlorotrimethylsilane:pyridine (1:1:5, v/v/v) for 30 min at room temperature and analysed by GLC on a capillary SE-30 fused silica column (25 m × 0.34 mm, Pierce) using a Varian Aerograph 3700 gas chromatograph-Shimadzu C-R3A Integrator.

Methylation analysis

Dry polysaccharide (2 mg) was dissolved in 500 μl dry DMSO by sonication for at least 1 h, followed by stirring for 16 h at 40°C under nitrogen, and methylated according to Kvernheim (1987) using 100 μl butyllithium (15% in hexane) as base. Extraction, hydrolysis, reduction and acetylation were carried out as described by Harris *et al.* (1984). The partially methylated alditol acetates were analysed by GLC on a capillary CPSil 43 WCOT fused silica column (25 m × 0.34 mm,

Chrompack) and by GLC-MS (Carlo Erba GC/Kratos MS80/Kratos DS 55 combination) using the same GLC column.

Ferulic acid analysis

Polysaccharide fractions (3 mg) were de-esterified with 1 ml 0.5 M NaOH for 90 min at 60°C in a screw-cap tube under nitrogen according to Shibuya (1984). After acidification with 2 M HCl to pH 3.0 and extraction with ethyl acetate (4 × 2 ml), the combined organic phases were evaporated and the residue dissolved in 300 μl 0.05 M NaAc buffer, pH 4.0. Aliquots of 75 μl were analysed on a Kratos HPLC-system consisting of two Spectroflow 400 Solvent Delivery Systems, a Spectroflow 450 Solvent Programmer and a Rheodyne injection valve module, using a Chromospher C₁₈ reversed phase column (250 × 4.6 mm, Chrompack) and a gradient of 5–25% acetonitrile in 0.05 M NaAc buffer, pH 4.0, at a flow rate of 1.0 ml/min. The eluate was monitored at 290 nm by a Spectroflow 783 Programmable Absorbance Detector connected with a Spectra Physics SP4290 Integrator. Reference *trans* (82%)/*cis* (18%) ferulic acid was prepared from commercially available *trans*-ferulic acid (Fluka Chemie AG) by incubation with 0.5 M NaOH for 90 min at 60°C. For quantification *trans*-cinnamic acid was used as an internal standard. For the characterization by GLC-MS using a capillary BP1 WCOT fused silica column (25 m × 0.32 mm), HPLC fractions were lyophilized, dissolved in distilled water, acidified with 2 M HCl to pH 3.0, extracted with ethyl acetate (4 × 2 ml), and after evaporation derivatized with 20 μl *N*, *O*-bis(trimethylsilyl)acetamide:chloroform (1:3, v/v) in capped vials for 40 min at 30°C.

¹³C-NMR spectroscopy

Natural-abundance proton decoupled ¹³C-NMR spectroscopy was performed at 70°C on a Bruker MSL 300 FT spectrometer (Department of NMR Spectroscopy, Utrecht University) equipped with a 10-mm broad-band probe-head. Prior to ¹³C-NMR spectroscopy the samples were exchanged in ²H₂O (99.80% ²H, Merck), lyophilized and dissolved in ²H₂O (99.80% ²H) to obtain 3–5% (w/v) solutions. Chemical shifts (δ) are expressed in ppm downfield from external Me₄Si but were actually measured by reference to internal 1,4-dioxane (δ = 67.40 ppm) with an accuracy of 0.02 ppm. ¹³C-NMR spectra were recorded with a spectral width of 10 000 Hz and 16 k was used for the acquisition. An acquisition time of 0.819 s and a relaxation delay of 2 s was applied.

RESULTS

The monosaccharide analysis data of the water-soluble fraction WSF of the white flour of the variety Kadet are presented in Table 1, demonstrating relatively high amounts of Ara, Xyl, Gal and Glc. Graded $(\text{NH}_4)_2\text{SO}_4$ precipitation yielded at 25% (w/w) $(\text{NH}_4)_2\text{SO}_4$ fraction WSSP, containing besides proteins and starch also 10% of the total water-soluble arabinoxylans. At 50% (w/w) $(\text{NH}_4)_2\text{SO}_4$ the main (85%) water-soluble arabinoxylan fraction WSAX was obtained, contaminated with some protein material (Table 1). The supernatant WSAG contained in addition to arabinogalactan-peptide, protein and starch fragments, also 5% of the water-soluble arabinoxylans. To remove residual protein from WSAX, the material was treated with pronase, yielding WPAX with essentially the same carbohydrate composition as WSAX (Table 1). In the course of the pronase incubation the viscosity of the solution decreased slightly.

Graded precipitation with ethanol from an aqueous WPAX solution afforded seven carbohydrate-containing fractions. The monosaccharide analysis data and protein content are presented in Table 2. The relevant fractions WPAX₄₃, WPAX₅₁, WPAX₅₅ and WPAX₆₂, representing 83% of the total WPAX fraction, are composed predominantly of Xyl and Ara. Absolute configuration determination of Xyl and Ara, as carried out for the two major fractions WPAX₄₃ and WPAX₅₁, revealed exclusively D-Xyl and L-Ara. The relative amount of Xyl to Ara differed significantly between the four arabinoxylan fractions

TABLE 1

Monosaccharide and Protein Analysis Data of Fractions WSF, WSSP, WSAX, WSAG and WPAX

Fraction	Monosaccharides ^a							% Protein ^b
	Ara	Xyl	Man	Gal	Glc	Rha	GalA	
WSF	1.00	1.44	0.06	0.48	3.74	+	±	16
WSSP	1.00	2.34	0.06	1.08	5.22	-	±	n.d.
WSAX	1.00	2.02	+	0.07	0.03	±	±	10
WSAG	1.00	0.28	-	0.88	1.47	±	±	15
WPAX	1.00	2.02	-	0.07	+	-	-	0.6

^aExpressed as molar ratios relative to Ara.

^bDetermined by Coomassie Blue G-250 Pierce Protein assay according to Bradford (1976). n.d. = not determined.

Ara = arabinose; Xyl = xylose; Man = mannose; Gal = galactose; Glc = glucose; Rha = rhamnose; GalA = galacturonic acid.

TABLE 2

Monosaccharide and Protein Analysis Data of Fraction WPAX, Polysaccharide Fractions Precipitated with Ethanol from WPAX, and Final Supernatant

Fraction	Monosaccharides ^a						%		
	Ara	Xyl	Man	Gal	Glc	Rha	Yield	Carbohydrate ^b	Protein ^c
WPAX	1.00	2.02	—	0.07	+	—	100	83	0.6
WPAX ₄₃ ^d	1.00	2.25	—	—	+	—	50	87	0.3
WPAX ₅₁	1.00	1.75	—	—	+	—	21	84	0.4
WPAX ₅₅	1.00	1.89	—	—	+	—	8	80	0.4
WPAX ₆₂	1.00	2.44	±	+	+	±	4	80	0.7
WPAX ₆₅₋₇₁	1.00	0.88	±	0.54	+	±	8	55	0.7
WPAX ₇₅₋₈₅	1.00	1.55	+	0.45	0.19	+	2	55	0.8
Supernatant	1.00	2.34	0.11	0.23	1.14	+	7	29	2.2

^aExpressed as molar ratios relative to Ara.

^b± 5%, as determined by phenol/sulphuric acid assay.

^cDetermined by Coomassie Blue G-250 Pierce Protein assay according to Bradford (1976).

^dEthanol percentage at which precipitation occurred.

WPAX₄₃–WPAX₆₂. Methylation analysis using alditol acetate derivatives (Table 3) showed several structural differences between the arabinoxylan fractions. All arabinoxylans contain terminal Ara in the furanose form, as indicated by the presence of 2,3,5-Me₃-Ara. These residues are linked to the xylan-backbone through O-3 or O-2,3 as is evident from the occurrence of 2-Me-Xyl and Xyl, respectively. The small amounts of 2,5-, 3,5- and 2,3-Me₂-Ara indicate that 10–20% of the Ara_f residues can be extended through O-2, O-3, or O-5 by another Ara_f. Methylation analysis of the WPAX₄₃-derived xylan yielded 2,3-Me₂-Xyl (99%) and 2,3,4-Me₃-Xyl (1%) only, demonstrating that the xylan-backbone consists of either (1→4)-linked Xyl_p or (1→5)-linked Xyl_f residues. ¹H-NMR spectroscopy of xylo-oligomers, produced by mild acid hydrolysis of the WPAX₄₃-derived xylan, gave anomeric signals for internal (1→4)-linked β-Xyl_p residues at δ=4.48 ppm (δ_{acetone} = 2.225 ppm), proving the xylan-backbone to consist of a linear (1→4)-linked β-Xyl_p chain (full details will be published elsewhere). The distribution of the Ara side chains over the xylan-backbone is characterized by the relative amounts of unbranched (2,3-Me₂-Xyl) and branched (Xyl+2-Me-Xyl) Xyl residues in the backbone, and by the relative amounts of 2,3,4-trisubstituted (Xyl) and 3,4-disubstituted (2-Me-Xyl) Xyl residues. The methylation analysis data of the four major

TABLE 3

Partially Methylated Alditol Acetates from WPAX and the Major Cold-Water-Soluble Arabinoxylan Fractions Precipitated with Ethanol

Alditol acetate of	Relative mol. (%)					
	WPAX	WPAX ₄₃	WPAX ₅₁	WPAX ₅₅	WPAX ₆₂	WPAX ₆₅₋₇₁
2,3,5-Me ₃ -Ara ^a	27.5	29.1	31.3	33.2	22.2	26.5
2,5-Me ₂ -Ara	0.3	—	+	1.1	0.3	0.6
3,5-Me ₂ -Ara	1.0	0.7	1.7	+	0.8	0.5
2,3-Me ₂ -Ara	1.0	2.3	2.6	2.1	1.5	1.4
2,3,4-Me ₃ -Xyl	1.1	1.0	1.3	1.6	1.4	1.0
2,3-Me ₂ -Xyl	43.9	50.0	45.7	42.6	51.4	24.4
2-Me-Xyl ^b	12.1	10.7	8.4	10.3	14.4	7.9
Xyl	7.8	5.2	7.9	7.3	6.3	2.9
2,3,4,6-Me ₄ -Gal	—	—	—	+	0.3	+
2,4,6-Me ₃ -Gal	0.4	—	—	+	+	3.5
2,3,4-Me ₃ -Gal	—	—	—	—	—	1.3
2,4-Me ₂ -Gal	2.3	—	—	—	—	23.9
2,3,4,6-Me ₄ -Glc	+	—	—	+	+	+
2,4,6-Me ₃ -Glc	0.4	—	—	+	0.3	0.6
2,3,6-Me ₃ -Glc	0.9	—	+	0.5	1.0	1.2

^a2,3,5-Me₃-Ara = 2,3,5-tri-*O*-methyl-arabinose, etc.

^bNo 3-Me-Xyl has been identified.

Because of contamination with phthalic acid anhydride, the amount of terminal Ara is lower than indicated by its peak intensity.

arabinoxylan fractions (see Table 3) demonstrates that the ratio unbranched to branched Xyl residues decreases, from 3.1 to 2.4–2.5, with increase in ethanol percentage at which precipitation occurred. Furthermore, the ratio of 2,3,4-tri- to 3,4-disubstituted Xyl varies from 0.4 to 0.9.

Gal and Glc occur as minor constituents in WPAX₅₅ and WPAX₆₂. The monosaccharide analysis data of WPAX₆₅₋₇₁, WPAX₇₅₋₈₅ and the final supernatant show relatively higher amounts of these constituents. The methylation analysis data of fraction WPAX₆₅₋₇₁ (Table 3) demonstrate the presence of Gal_p substituted at O-3 (2,4,6-Me₃-Gal), O-6 (2,3,4-Me₃-Gal) or at O-3,6 (2,4-Me₂-Gal). These results are consistent with those published for arabinogalactan(-peptides) from other wheat varieties (Neukom & Markwalder, 1975). The finding of 2,4,6-Me₃-Glc and 2,3,6-Me₃-Glc indicate that the presence of Glc is due to $\beta(1 \rightarrow 3)(1 \rightarrow 4)$ glucans (Åman & Graham, 1987; Bacic & Stone, 1980) and water-soluble starch.

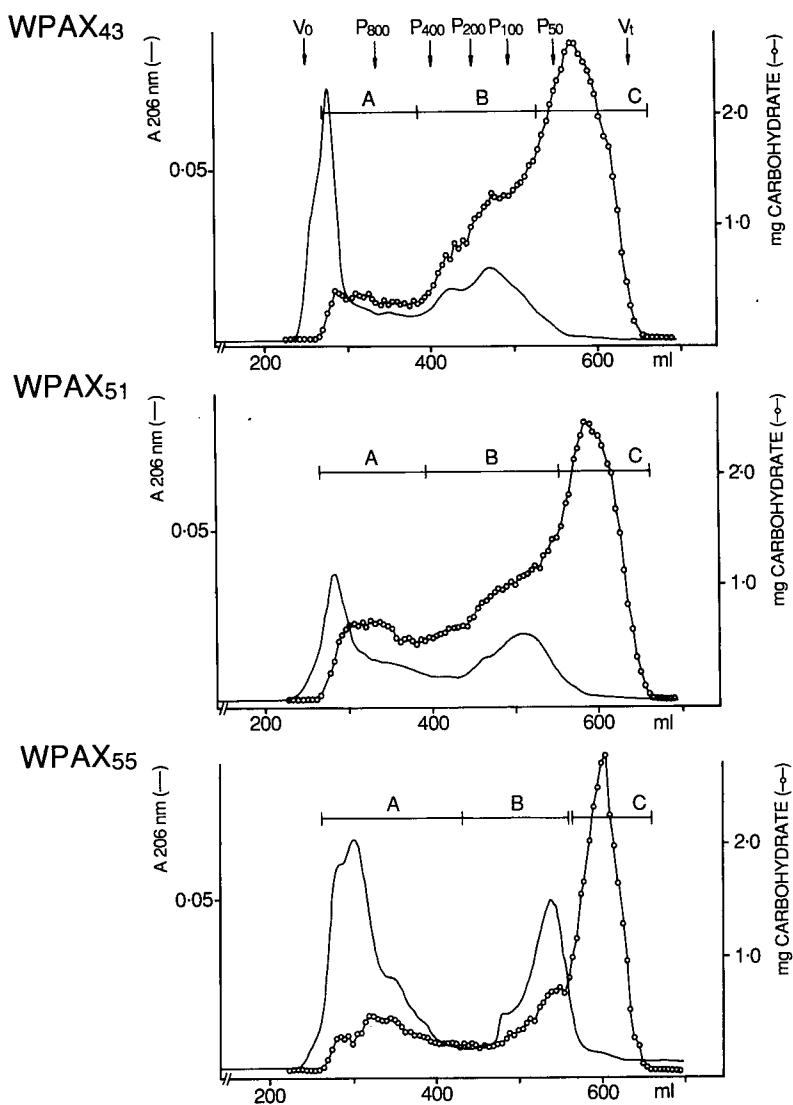


Fig. 1. Elution profiles of the three major WPAX fractions, WPAX₄₃, WPAX₅₁ and WPAX₅₅ on a Sephacryl S-500 gel permeation column (159 × 2.2 cm). P₈₀₀ = pullulan standard of 85.3×10^4 Da; P₄₀₀ = 38.0×10^4 Da; P₂₀₀ = 18.6×10^4 Da; P₁₀₀ = 10.0×10^4 Da; P₅₀ = 4.8×10^4 Da.

The elution profiles (Fig. 1) of WPAX₄₃, WPAX₅₁ and WPAX₅₅ on Sephacryl S-500 gave similar patterns, and in each case fractions were pooled as indicated. The high u.v.-peak in the void volume proved to be due to residual protein. Based on the monosaccharide composition of the Sephacryl S-500 fractions (Table 4) in conjunction with the methyl-

TABLE 4

Monosaccharide Composition and *trans*-Ferulic Acid Content of WPAX Fractions Obtained after Sephacryl S-500 Size Exclusion Chromatography

Fraction	Monosaccharides ^a						Ferulic acid ($\mu\text{g}/\text{mg}$ polysaccharide) ^b
	Ara	Xyl	Gal	Glc	Rha	%	
WPAX ₄₃	1.00	2.25	—	+	—	100	1.03
A	1.00	1.75	—	+	±	9	0.73
B	1.00	2.02	—	±	—	36	2.72
C	1.00	2.51	—	±	—	55	0.14
WPAX ₅₁	1.00	1.75	—	+	—	100	0.63
A	1.00	1.17	—	±	±	20	0.36
B	1.00	1.46	—	+	—	36	1.83
C	1.00	2.41	—	+	—	44	0.01
WPAX ₅₅	1.00	1.89	—	+	—	100	0.79
A	1.00	1.12	—	+	±	22	0.73
B	1.00	1.48	—	+	—	22	3.91
C	1.00	2.54	—	+	—	56	0.02

^aExpressed as molar ratios relative to Ara.

^b ± 5%.

tion analysis data (Table 5), the following variations in structural characteristics are observed:

- (i) The low molecular mass carbohydrate polymers of fractions WPAX_{43C}, WPAX_{51C} and WPAX_{55C}, covering 51% of the WPAX arabinoxylans, have a Xyl to Ara ratio of 2.4–2.5 and a 2,3,4-tri- to 3,4-disubstituted Xyl ratio of 0.3–0.4.
- (ii) The arabinoxylans of WPAX_{43B} have a Xyl to Ara ratio of 2.0 and a 2,3,4-tri- to 3,4-disubstituted Xyl ratio of 0.7.
- (iii) The arabinoxylans of WPAX_{43A} have a Xyl to Ara ratio of 1.7 and a 2,3,4-tri- to 3,4-disubstituted Xyl ratio of 1.0.
- (iv) The arabinoxylans of WPAX_{51B} and WPAX_{55B} have a Xyl to Ara ratio of 1.5, and a 2,3,4-tri- to 3,4-disubstituted Xyl ratio of 1.3–1.5.
- (v) The arabinoxylans of WPAX_{51A} and WPAX_{55A} have a Xyl to Ara ratio of 1.1–1.2, and a 2,3,4-tri- to 3,4-disubstituted Xyl ratio of 1.8–2.1.
- (vi) The unbranched regions (2,3-Me₂-Xyl) in the arabinoxylans are less abundant with increase in molecular mass of the polymer.

TABLE 5

Partially Methylated Alditol Acetates from Cold-Water-Soluble Arabinoxylan Fractions after Sephadryl S-500 Separation. Also Given are the Ratios Unbranched (2,3-Me₂-Xyl) to Branched (2-Me-Xyl + Xyl) Xylose and Trisubstituted (Xyl) to Disubstituted (2-Me-Xyl) Xylose

Alditol acetate of	Relative mol. (%)								
	W _{43A}	W _{43B}	W _{43C}	W _{51A}	W _{51B}	W _{51C}	W _{55A}	W _{55B}	W _{55C}
2,3,5-Me ₃ -Ara ^a	34.8	30.9	27.6	36.7	35.3	26.1	44.9	37.7	25.5
2,5-Me ₂ -Ara	0.3	+	—	0.7	+	+	2.7	1.8	1.4
3,5-Me ₂ -Ara	1.4	0.9	0.5	3.5	2.2	0.6	0.5	0.3	+
2,3-Me ₂ -Ara	2.9	2.3	3.1	4.1	2.7	1.8	3.0	2.0	1.6
2,3,4-Me ₃ -Xyl	1.4	1.0	1.0	1.7	1.3	1.1	1.2	1.2	1.4
2,3-Me ₂ -Xyl	40.8	46.5	53.7	34.2	39.9	51.7	26.6	36.3	50.9
2-Me-Xyl ^b	8.6	10.1	9.4	6.0	6.7	12.2	6.4	8.7	13.3
Xyl	8.5	7.1	3.1	10.9	10.4	5.0	13.4	11.7	4.9
2,3,4,6-Me ₄ -Gal	—	—	—	—	—	—	—	—	+
2,4,6-Me ₃ -Gal	—	—	—	—	—	—	—	—	0.3
2,3,4,6-Me ₄ -Glc	—	—	—	—	—	—	—	—	+
2,4,6-Me ₃ -Glc	—	—	—	—	—	—	—	—	0.2
2,3,6-Me ₃ -Glc	—	—	—	+	—	+	+	—	0.6
<u>2,3-Me₂-Xyl</u>	2.4	2.7	4.3	2.0	2.3	3.0	1.3	1.8	2.8
<u>2-Me-Xyl + Xyl</u>									
<u>Xyl</u>	1.0	0.7	0.3	1.8	1.5	0.4	2.1	1.3	0.4
<u>2-Me-Xyl</u>									

^a2,3,5-Me₃-Ara = 2,3,5-tri-*O*-methyl-arabinose, etc.

^bNo 3-Me-Xyl has been identified.

- (vii) The unbranched regions (2,3-Me₂-Xyl) in arabinoxylans having the same branching pattern (Xyl to 2-Me-Xyl ratio) are less abundant with increase in ethanol percentage at which precipitation occurred.

Trans-ferulic acid, which can be linked to O-5 of terminal L-Ara₄ (Smith & Hartley, 1983; Kato & Nevins, 1985; Mueller-Harvey *et al.*, 1986), proved to be the only cinnamic acid derivative linked to the water-soluble arabinoxylans. In the fractions WPAX_{43B}, WPAX_{51B} and WPAX_{55B} relatively high amounts of *trans*-ferulic acid were present, whereas in the fractions WPAX_{43C}, WPAX_{51C} and WPAX_{55C} the

amounts were almost negligible (Table 4). This is in good agreement with the u.v.-absorption pattern in Fig. 1.

The WPAX_{43C}, WPAX_{43A} and WPAX_{51B} Sephacryl S-500 fractions, having characteristic differences in distribution of Ara_f along the xylan-backbone, were further investigated by ¹³C-NMR spectroscopy, to relate intensities of relevant signals in the ¹³C-NMR spectra (Fig. 2) with quantitative methylation analysis data (Table 5). The ¹³C-NMR data, together with those of a reference compound, are compiled in Table 6. The assignments were confirmed by ¹H-¹³C COSY measurements on WPAX_{51B} (will be published elsewhere). In the ¹³C-NMR spectra of the three arabinoxylans (Fig. 2) clearly distinguishable clusters of signals are present. The chemical shift values of the anomeric carbon signals of

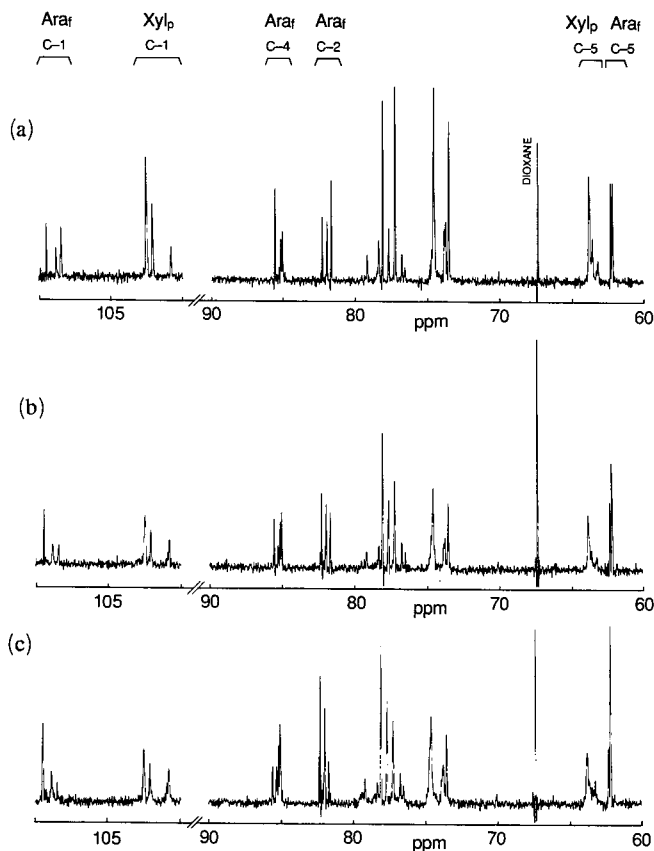


Fig. 2. ¹³C-NMR spectra (75.47 MHz) at 70°C of WPAX_{43C} (a), WPAX_{43A} (b) and WPAX_{51B} (c), respectively, relative to internal 1,4-dioxane ($\delta = 67.40$ ppm).

TABLE 6
 ^{13}C -NMR Chemical Shift Data of the Cold-Water-Soluble Arabinoxylans from Kadet (WPAX Fractions), together with some Reference Data

Residue ^b	Chemical shifts (ppm) ^a				
	C-1	C-2	C-3	C-4	C-5
Rye grain arabinoxylan ^c					
β -D-Xyl _p	102.5	73.9	74.6	77.3	63.9
Element B					
β -D-Xyl _p	102.0	73.6	78.5	74.6	63.7
α -L-Ara _f (1 → 3)	108.5	81.8	78.1	85.6	62.4
Wheat endosperm arabinoxylan ^d					
β -D-Xyl _p	102.48	73.56	74.60	77.28	63.85
β -D-Xyl _p (adj)	102.09				63.85
Element A					
β -D-Xyl _p	100.79				63.21
α -L-Ara _f (1 → 2)	109.46	82.30 82.41 ^e		85.08	62.18
α -L-Ara _f (1 → 3)	108.84	81.97		85.17 85.31 ^e	62.18
Element B					
β -D-Xyl _p	102.48	73.77	78.11	74.60	63.59
α -L-Ara _f (1 → 3)	108.44	81.69	78.11	85.58	62.31

^aRelative to internal 1,4-dioxane ($\delta = 67.40$ ppm).

^bElement A = →4) [α -L-Ara_f(1 → 2)] [α -L-Ara_f(1 → 3)]- β -D-Xyl_p(1 →

Element B = →4) [α -L-Ara_f(1 → 3)]- β -D-Xyl_p(1 →

β -D-Xyl_p = →4)- β -D-Xyl_p(1 →

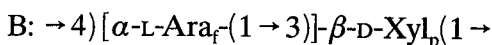
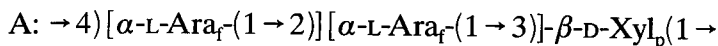
β -D-Xyl_p(adj) = →4)- β -D-Xyl_p(1 → adjoining element A and element B at the non-reducing end.

^cFrom Bengtsson and Åman (1990).

^dThe assignment of β -D-Xyl_p, and element B was done by comparison with data published by Kovac *et al.* (1980), Bock *et al.* (1984), Bradbury and Jenkins (1984), Kato and Nevins (1985), Brillouet and Joseleau (1987), Saavedra *et al.* (1988), Bengtsson and Åman (1990).

^eTentative assignment based on the assumption that small chemical shift differences occur when two elements A are linked together.

Ara_f ($\delta = 108.4$ – 109.5 ppm) and Xyl_p ($\delta = 100.8$ – 102.5 ppm) indicate that Ara_f has α and Xyl_p has β configuration (Joseleau *et al.*, 1977; Bock & Pedersen, 1983). The signal intensities of the well-resolved Ara_f C-2, C-4 and C-5 resonances give detailed information about the relative amounts of the two branching elements A and B.



A relationship between the signal intensities of $\alpha\text{-L-Ara}_f\text{(1}\rightarrow 2)$ ($\delta_{\text{C-2}} = 82.30/82.41$ ppm, $\delta_{\text{C-4}} = 85.08$ ppm, $\delta_{\text{C-5}} = 62.18$ ppm) and $\alpha\text{-L-Ara}_f\text{(1}\rightarrow 3)$ ($\delta_{\text{C-2}} = 81.97$ ppm, $\delta_{\text{C-4}} = 85.17/85.31$ ppm, $\delta_{\text{C-5}} = 62.18$ ppm) of element A on one hand and $\alpha\text{-L-Ara}_f\text{(1}\rightarrow 3)$ ($\delta_{\text{C-2}} = 81.69$ ppm, $\delta_{\text{C-4}} = 85.58$ ppm, $\delta_{\text{C-5}} = 62.31$ ppm) of element B on the other hand in conjunction with the relative abundance of the two elements, as given by the ratio 2,3,4-tri- to 3,4-disubstituted Xyl (methylation analysis), is obvious in these structural-reporter-group regions. The increase in the relative Ara_f signal intensities, in the above mentioned structural-reporter-group regions, of element A relative to element B is observed with increase in the ratio 2,3,4-tri- to 3,4-disubstituted Xyl residues in the xylan-backbone going from $\text{WPAX}_{43\text{C}}$ via $\text{WPAX}_{43\text{A}}$ to $\text{WPAX}_{51\text{B}}$ (Table 5).

DISCUSSION

The cold-water-soluble arabinoxylans represent 0.5% of the total white flour from the soft wheat variety Kadet. As demonstrated in this paper, they constitute a heterogeneous group of polysaccharides with respect to molecular mass, D-Xyl_p to L-Ara_f ratio, and Ara side chain distribution along the xylan-backbone. The molecular size distribution of these arabinoxylans is similar to those published for the other soft wheat varieties Diplomat and M Huntsman (Meuser *et al.*, 1981), and show that the arabinoxylans have a molecular mass ranging from 25 to more than 1000 kDa. The largest group of arabinoxylans, about 50% of the total, have a molecular mass of 25–40 kDa. Characteristic for this group is the high Xyl to Ara ratio of 2.5 and the low 2,3,4-tri- to 3,4-disubstituted Xyl ratio of 0.3. The arabinoxylan polymers having a molecular mass above 40 kDa have a lower Xyl to Ara ratio (2.0 to 1.1) and a higher 2,3,4-tri- to 3,4-disubstituted Xyl ratio (0.7–2.1). Differences in precipitation behaviour of arabinoxylans with the same branching pattern are attributed to differences in the ratio unbranched to branched Xyl residues in the xylan-backbone. The presence of small amounts of extended Ara_f side chains found in the Kadet arabinoxylans are commonly encountered in most arabinoxylans (Medcalf & Gilles, 1968; Carpita *et al.*, 1985; Shibuya *et al.*, 1985; Nishitani & Nevins, 1988).

In the ¹³C-NMR spectra the relative-intensity changes of the signals in the anomeric region as well as in the C-2, C-4 and C-5 Ara_f regions

confirm the methylation analysis results presented in Table 5. This emphasizes the suitability of ^{13}C -NMR spectroscopy in the structural elucidation of arabinoxylan polymers.

The presence of *trans*-ferulic acid as the sole cinnamic acid derivative bound to the endospermic arabinoxylans has already been established (Neukom, 1976; Fry, 1979; Yeh *et al.*, 1980). This investigation demonstrates that the amounts of *trans*-ferulic acid differ significantly between the various fractions. The ferulic acid content of the major endospermic arabinoxylan group is even negligible.

The present results show that there is a wide diversity of arabinoxylan structures, even within one single wheat variety. Each characteristic group of arabinoxylans will presumably have a different effect on flour performance, and thus on the final cereal product. To establish these effects, more research on well defined arabinoxylan fractions has to be done.

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