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Structural Characteristics of the Warm-water-soluble Arabinoxylans from the Tailings of the Soft Wheat Variety Kadet

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ABSTRACT

Arabinoxylans were extracted from the tailings of the soft wheat variety Kadet with water at 70°C, and freed from protein by pronase digestion. The warm-water-soluble arabinoxylans, constituting 0·3% of the total flour, were fractionated by graded precipitation with ethanol and the four main fractions, covering 89% of the total, contained D*-xylose and* L*-arabinose in a ratio ranging from 2·21:1·00 to 1·26:1·00. Methylation analysis and* ^{13}*C-NMR spectroscopy revealed the arabinoxylans to consist of a (1→4)-linked β-*D*-xylopyranose-backbone substituted at O-3 or O-2,3 with terminal α-*L*-arabinofuranosyl residues. Differences in xylose to arabinose ratio were not only due to variations in the ratio unbranched to branched xylose, which ranged from 1·0 to 2·5, but also due to differences in the ratio 2,3,4-tri- to 3,4-di-substituted xylose, ranging from 0·4 to 2·1. Gel permeation chromatography revealed that the warm-water-soluble arabinoxylans consist solely of high-molecular-size material (> 40 kDa).*

INTRODUCTION

Wheat flour arabinoxylans (pentosans) can be divided into two types, namely, the water-soluble arabinoxylans and the arabinoxylans associ-

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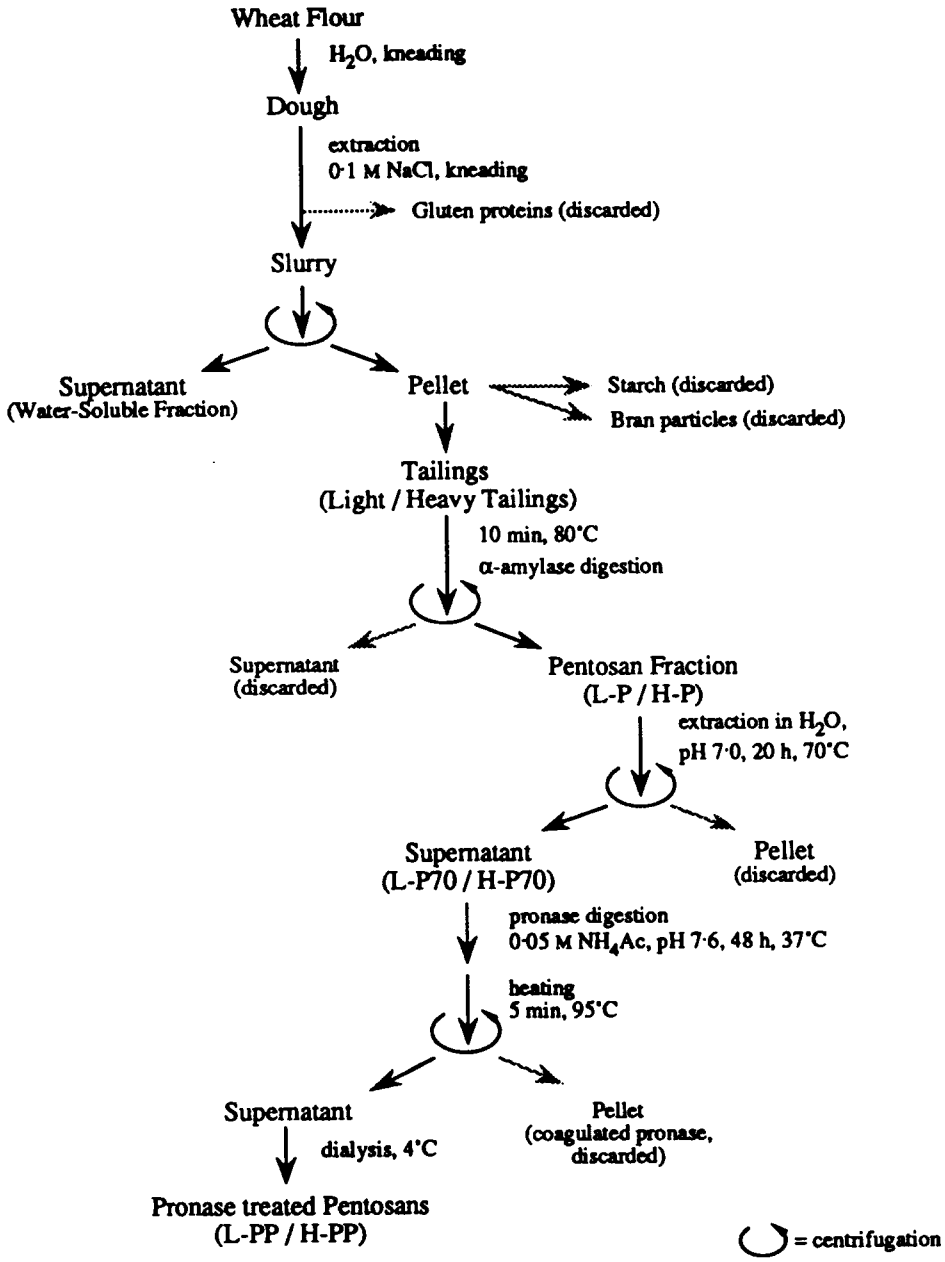
ated with the water-insoluble or 'tailing' fraction. These two fractions represent about 1·1–1·6% and 0·4–0·7% of the total wheat flour, respectively (Meuser *et al.*, 1981; Amadò & Neukom, 1985; Englyst & Cummings, 1985). Arabinoxylans are believed to have a functional role in dough development and an influence on baking performance of wheat and rye flour. Depolymerization of the arabinoxylans, using purified *endo*-xylanases, causes dramatical changes in dough properties and loaf characteristics. The dough becomes sticky and weak, and the subsequently baked loaf shows a decrease in crumb firmness and increase in the width/height ratio (Kühn & Grosch, 1988; McCleary, 1986). Fractionation-reconstitution experiments (Kühn & Grosch, 1989) demonstrate for rye flour that addition of the enzymatically treated insoluble arabinoxylan fraction increases the specific bread volume, whereas addition of the digested soluble fraction has the reverse effect. However, similar experiments performed on wheat flours gave conflicting results (McCleary *et al.*, 1986). This is probably caused by the use of different wheat-classes, varying in protein content. It has been suggested that differences in properties between the soluble and insoluble arabinoxylan flour fractions stem from differences in molecular structure (Meuser *et al.*, 1981; Ciacco & D'Appolonia, 1982).

In this investigation, structural characteristics of the arabinoxylans extracted with warm water from the tailings of the soft wheat variety Kadet are described and the structural differences are discussed between these arabinoxylans and the cold-water-soluble arabinoxylans from the same variety (Hoffmann *et al.*, 1991).

MATERIALS AND METHODS

Isolation of arabinoxylans from tailings (Scheme 1)

The slurry obtained after kneading of 2·0 kg flour of the soft wheat variety Kadet (harvested 1982, southern Sweden) was centrifuged at 10000 ***g*** for 30 min, yielding a water-soluble fraction (Hoffmann *et al.*, 1991) and a pellet containing three layers: a spongy gray top-layer of tailings, a huge white bottom layer of starch, and a brown border consisting of small bran fragments. The tailings were scraped off, suspended in 1 liter 0·1 M NaCl and centrifuged at 10000 ***g*** for 30 min to minimize starch content. The tailings were again carefully scraped off and separated, according to their firmness and location in the layer, into two fractions called the light and heavy tailings, representing 2·5% (49 g) and 5·2% (104 g) of the wheat flour, respectively. The light and heavy tailings


Wheat Flour
H_2O, kneading
Dough
extraction
0·1 M NaCl, kneading
Gluten proteins (discarded)
Slurry
Supernatant
(Water-Soluble Fraction)
Pellet
Starch (discarded)
Bran particles (discarded)
Tailings
(Light / Heavy Tailings)
10 min, 80°C
α-amylase digestion
Supernatant
(discarded)
Pentosan Fraction
(L-P / H-P)
extraction in H_2O,
pH 7·0, 20 h, 70°C
Supernatant
(L-P70 / H-P70)
Pellet
(discarded)
pronase digestion
0·05 M NH_4Ac, pH 7·6, 48 h, 37°C
heating
5 min, 95°C
Supernatant
dialysis, 4°C
Pellet
(coagulated pronase,
discarded)
Pronase treated Pentosans
(L-PP / H-PP)
= centrifugation

Scheme 1

were each suspended in distilled water and heated for 10 min at 80°C, to eliminate possible enzyme activity. Then, both fractions were exhaustively incubated with α-amylase (type IIa from *Bacillus*, Sigma) with intermediate centrifugation (10000 ***g*** for 15 min) at room temperature until the supernatant was freed of starch (KJ-staining). The pellets were extensively washed with distilled water at room temperature until clear supernatants were gained, yielding 14·6 g (0·7% of the flour) light pentosan (L-P) and 8·5 g (0·4% of the flour) heavy pentosan (H-P).

To extract arabinoxylans, 13·6 g L-P and 7·5 g H-P were suspended in 1 liter and 0·5 liters water, respectively, and after adjusting the pH to 7·0 with 0·05 M ammonia, heated for 20 h at 70°C under nitrogen. After centrifugation at 20000 ***g*** for 20 min the supernatants were decanted, and the pellets were extracted twice. The pH did not change during the extraction. The obtained supernatants were lyophilized, yielding 4·95 g (36%) L-P70 and 1·77 g (24%) H-P70, respectively. To remove residual protein, 4·55 g L-P70 and 1·50 g H-P70 were each dissolved in 200 ml 0·05 M NH_4Ac, pH 7·6, containing 0·015 M $CaCl_2$ and incubated for 48 h at 37°C with four portions of 20 mg pronase (Boehringer) added at $t = 0$, 6, 12 and 24 h, respectively. The pH of the incubations was kept between 7·2 and 7·8. The digestions were stopped by heating the mixtures for 5 min at 95°C, and the coagulated pronase was removed by centrifugation (16000 ***g***, 20 min at 4°C). The supernatants were dialysed extensively against distilled water at 4°C, and the non-dialysable material was lyophilized, yielding 3·71 g (82%) L-PP and 0·90 g (60%) H-PP, representing an overall yield of 0·22% and 0·06%, respectively, of the total white flour of Kadet.

Graded precipitation with ethanol

Precipitation of arabinoxylans from a stirred, clear 2% L-PP (3·71 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 (16000 ***g***, 30 min at 4°C). The pellet was redissolved in water and lyophilized. To the supernatant, ethanol was added until the next precipitate was formed. In this way the fractions L-PP_{35}, L-PP_{44}, L-PP_{53}, L-PP_{60}, L-PP_{66}, L-PP_{74} and L-PP_{80} were obtained, whereby the subscripts refer to the ethanol percentages at which precipitation occurred.

Size-exclusion chromatography

Portions of 75 mg L-PP_{35}, L-PP_{44}, L-PP_{53} and L-PP_{60}, respectively, were fractionated on a Sephacryl S-500 gel permeation column (150 × 3·0 cm,

Pharmacia) eluted with water (37 ml/h, 9-ml fractions). The eluate was monitored at 206 nm and carbohydrates were quantitatively determined by the resorcinol/sulfuric acid microassay (Monsigny *et al.*, 1988) 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 ($R^2 = 0{\cdot}98$) between log [molecular mass] and exclusion volume.

Monosaccharide analysis

Monosaccharide analysis, including absolute configuration determination, was carried out by GLC on a capillary SE-30 fused silica column (25 m × 0·32 mm, Pierce) using a Varian Aerograph 3700 gas chromatograph. The methyl glycosides were prepared by methanolysis (1·0 M HCl/methanol, 85°C, 24 h) and an aliquot converted into (−)-/(±)-2-butyl glycosides by subsequent butanolysis (1·0 M HCl/(−)-/(±)-2-butanol, 85°C, 8 h), respectively. The obtained glycosides were trimethylsilylated (Gerwig *et al.*, 1978; Kamerling & Vliegenthart, 1982, 1989).

Methylation analysis

Polysaccharides (1 mg), dissolved in DMSO, were methylated twice with methyliodide using butyllithium (15% in hexane) as base (Kvernheim, 1987). 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 × 0·32 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). The extracted ferulic acid (Hoffmann *et al.*, 1991) was 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 ChromSpher 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. For quantification *trans*-cinnamic acid was used as an internal standard.

^{13}C-NMR spectroscopy

Natural-abundance proton decoupled ^{13}C-NMR spectroscopy was performed at 70°C on a Bruker WP-200 FT spectrometer (Bijvoet Center, Department of NMR Spectroscopy, Utrecht University) equipped with a 5-mm broad-band probe-head. Prior to ^{13}C-NMR spectroscopy the samples were exchanged once in $^{2}H_2O$ (99·80% ^{2}H, Merck), lyophilized and dissolved in $^{2}H_2O$ (99·80% ^{2}H) to obtain 3–5% (w/v) solutions. Chemical shifts (δ) are expressed in ppm downfield from external Me_4Si but were actually measured by reference to internal 1,4-dioxane ($\delta = 67{\cdot}4$).

RESULTS

The monosaccharide compositions of the tailings, and tailings-subfractions of the white flour of the variety Kadet are presented in Table 1. For the tailings relatively high amounts of Ara, Xyl, Man, Gal and Glc were found. Most of the bound Glc was removed by α-amylase treatment (→ L-P/H-P), suggesting that the major part of Glc originates from starch. The content of Man and Gal decreased also during this step. Gal could stem from water-soluble arabinogalactan which was enclosed in the tailings and Man probably could be derived from β-glucomannans

TABLE 1
Monosaccharide Analysis Data of the Tailings and Tailings Fractions

Fraction	*Monosaccharides*[a]						
	Ara	*Xyl*	*Man*	*Gal*	*Glc*	*Rha*	*GalA*
Tailings	1·00	2·00	0·19	0·30	23·54	±	+
L-P	1·00	1·93	0·10	0·06	0·32	+	+
H-P	1·00	1·93	0·08	0·12	0·44	±	±
L-P70	1·00	1·76	0·04	0·05	0·43	−	±
H-P70	1·00	1·89	0·04	0·12	0·44	−	−
L-PP	1·00	1·93	0·02	0·02	0·05	−	±
H-PP	1·00	1·99	0·03	0·03	0·16	±	±

[a]Expressed as molar ratios relative to Ara. Ara = arabinose; Xyl = xylose; Man = mannose; Gal = galactose; Glc = glucose; Rha = rhamnose; GalA = galacturonic acid.

(Mares & Stone, 1973; Neukom & Markwalder, 1975). During the whole working-up procedure of the light and heavy tailings minor changes in the Xyl to Ara ratio occurred. These changes are caused by the release of trace amounts of Ara during the extraction at 70°C. By dialysis, after pronase digestion, free Ara is removed. In principle it cannot be excluded that the arabinoxylans, soluble at 70°C, have a slightly different Xyl to Ara ratio. Extraction at higher temperatures yielded more soluble material but also mono- and oligo-saccharides, as demonstrated by TLC (results not shown). The difference in monosaccharide composition between each set of light and heavy pentosan fractions are only marginal (Table 1). Differences in texture between the light and heavy tailings are attributed to differences in starch content, sustained by the larger weight loss during α-amylase treatment of the heavy tailings relative to the light tailings. Further investigations were carried out on the light pentosan fraction L-PP only.

Graded precipitation with ethanol from an aqueous L-PP solution afforded eight carbohydrate-containing fractions. The monosaccharide analysis data and protein contents are given in Table 2. Going from L-PP_{35} to L-PP_{80}, a decrease in the Xyl to Ara ratio is observed. The most relevant arabinoxylan fractions L-PP_{35} to L-PP_{60}, representing 89% of

TABLE 2
Monosaccharide and Protein Analysis Data of Fraction L-P, L-PP, Polysaccharide Fractions Precipitated with Ethanol from L-PP, and Final Supernatant

Fraction	*Monosaccharides*[a]							*Yield (%)*	*Carbohydrate*[b] *(%)*	*Protein*[c] *(%)*
	Ara	*Xyl*	*Man*	*Gal*	*Glc*	*Rha*	*GalA*			
L-P	1·00	1·93	0·10	0·06	0·16	±	±	–	69	n.d.
L-PP	1·00	1·93	0·02	0·02	0·05	–	±	100	91	1·0
L-PP_{35}[d]	1·00	2·21	+	+	0·03	–	+	55	85	0·5
L-PP_{44}	1·00	1·59	+	+	+	–	–	22	86	0·3
L-PP_{53}	1·00	1·27	0·03	+	+	+	+	8	87	0·3
L-PP_{60}	1·00	1·26	0·05	0·02	0·02	–	–	4	95	1·5
L-PP_{66}	1·00	1·23	0·09	0·05	0·07	+	+	2	79	9·3
L-PP_{74}	1·00	1·25	0·16	0·21	0·26	0·04	0·04	1	58	4·3
L-PP_{80}	1·00	0·53	0·11	0·15	0·35	0·03	0·04	1	60	1·9
Supernatant	1·00	0·47	0·22	0·18	1·28	0·03	+	3	27	7·7

[a]Expressed as molar ratios relative to Ara.
[b]± 5%, as determined by resorcinol/sulfuric acid microassay.
[c]Determined by Coomassie Blue G-250 Pierce Protein assay according to Bradford (1976). n.d. = not determined.
[d]Ethanol percentage at which precipitation occurred.

the total L-PP fraction, are composed of D-Xyl and L-Ara. Between 53 and 74% ethanol no significant changes in the Xyl to Ara ratio were detected in the precipitates, but the content of other monosaccharides was increasing. Even at higher ethanol-percentages the content of Glc and non-carbohydrate components is significant.

Methylation analysis data (Table 3) demonstrate the arabinoxylans to be composed of a (1→4)-linked Xyl_p-backbone branched at O-2,3 or O-3 by terminal Ara_f (Hoffmann *et al.*, 1991). These data also show that the arabinoxylans present in L-PP_{53} and L-PP_{60} are similar in structure. The monosaccharide analysis data (Table 2) in conjunction with the methylation analysis data (Table 3) revealed that the decrease in the Xyl to Ara ratio from 2·21 to 1·26 in the four main arabinoxylan fractions L-PP_{35} to L-PP_{60} is caused by the combination of two factors:

(i) a decrease in the ratio unbranched (2,3-Me_2-Xyl) to branched (2-Me-Xyl + Xyl) residues in the xylan-backbone from 2·5 to 1·4.

TABLE 3

Partially Methylated Alditol Acetates from L-PP and the Major L-PP Arabinoxylan Fractions Precipitated with Ethanol. Also given are the Ratios Unbranched (2,3-Me_2-Xyl) to Branched (2-Me-Xyl + Xyl) Xylose and Trisubstituted (Xyl) to Disubstituted (2-Me-Xyl) Xylose

Alditol acetate of	*Relative mol (%)*				
	L-PP	*L-PP_{35}*	*L-PP_{44}*	*L-PP_{53}*	*L-PP_{60}*
2,3,5-Me_3-Ara[a]	21·9	23·3	27·7	32·1	34·7
3,5-Me_2-Ara	1·2	0·3	1·7	3·5	3·7
2,3-Me_2-Ara	1·3	1·0	0·5	1·5	1·6
2,3,4-Me_3-Xyl	0·7	0·6	0·9	2·2	2·2
2,3-Me_2-Xyl	47·2	52·5	42·1	33·1	30·8
2-Me-Xyl[b]	14·1	14·6	11·4	8·5	8·5
Xyl	10·3	6·1	14·4	15·5	14·3
2,3,4,6-Me_4-Glc	0·3	+	±	+	+
2,4,6-Me_3-Glc	+	–	–	–	–
2,3,6-Me_3-Glc	2·1	1·3	0·4	0·9	1·2
2,3,4-Me_3-Gal	±	–	–	–	–
2,4-Me_2-Gal	±	–	–	–	±
2,3,4,6-Me_4-Man	±	–	–	–	+
2,3,6-Me_3-Man	0·7	–	0·8	2·1	3·0
2,3-Me_2-Xyl/ 2-Me-Xyl + Xyl	1·9	2·5	1·6	1·4	1·4
Xyl/2-Me-Xyl	0·7	0·4	1·3	1·8	1·7

[a] 2,3,5-Me_3-Ara = 2,3,5-tri-*O*-methyl-arabinose, etc.

[b] No 3-Me-Xyl has been identified.

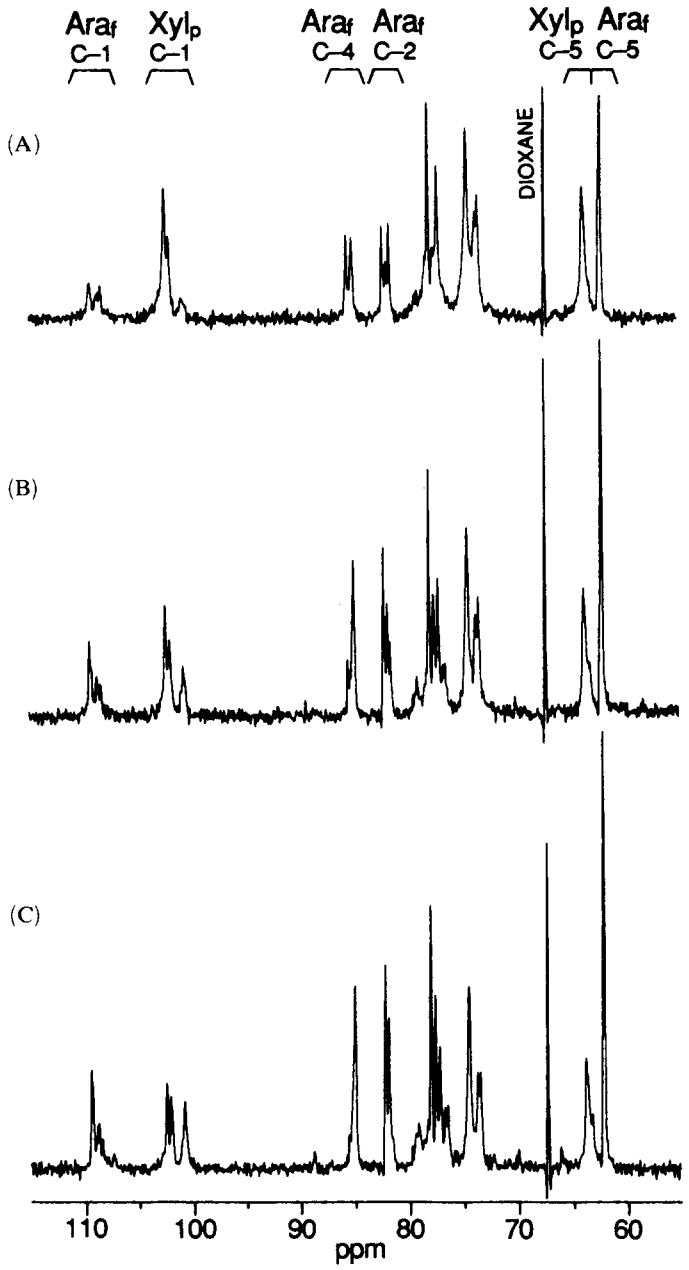


Fig. 1. ^{13}C-NMR spectra (50 MHz) at 70°C of L-PP$_{35}$ (A), L-PP$_{44}$ (B) and L-PP$_{53}$ (C), respectively, relative to internal 1,4-dioxane ($\delta = 67{\cdot}4$ ppm).

(ii) an increase in the ratio 2,3,4-trisubstituted (Xyl) to 3,4-disubstituted (2-Me-Xyl) Xyl_p in the xylan-backbone from 0·4 to 1·7–1·8.

As reported (Hoffmann *et al.*, 1991), ^{13}C-NMR spectroscopy is a suitable method to identify differences in branching pattern of arabinoxylans. In Fig. 1 the ^{13}C-NMR spectra of L-PP_{35}, L-PP_{44} and L-PP_{53} are presented. They were interpreted on guidance of the earlier reported data for the cold-water-soluble arabinoxylans. The ^{13}C-NMR data are compiled in Table 4. Changes in relative signal intensities in the anomeric regions and especially in the Ara_f C-2 and C-4 regions are in accordance with the differences in Ara_f distribution (ratio 2,3,4-trisubstituted to 3,4-disubstituted Xyl_p) over the xylan-backbone as found by methylation analysis. This is evident by comparing the signals derived from α-L-Ara_f-(1→2) ($\delta_{C\text{-}2}$ = 82·3, $\delta_{C\text{-}4}$ = 85·1) and α-L-Ara_f-(1→3) ($\delta_{C\text{-}2}$ = 82·0, $\delta_{C\text{-}4}$ = 85·1) of element A {→4)[α-L-Ara_f-(1→2)][α-L-Ara_f-(1→3)]-β-D-Xyl_p(1→} with the signals derived from α-L-Ara_f-(1→3) ($\delta_{C\text{-}2}$ = 81·7, $\delta_{C\text{-}4}$ = 85·6) of element B {→4)[α-L-Ara_f-(1→3)]-β-D-Xyl_p(1→}, going from L-PP_{35} to L-PP_{53}.

TABLE 4

^{13}C-NMR Chemical Shift Data of the L-PP Arabinoxylans from the Tailings of the Soft Wheat Variety Kadet[a]

Residue[b]	*Chemical shifts (ppm)*[c]				
	C-1	*C-2*	*C-3*	*C-4*	*C-5*
β-D-Xyl_p	102·5	73·6	74·6	77·3	63·8
β-D-Xyl_p-(adj)	102·1				63·8
Element A					
β-D-Xyl_p	100·8				63·2
α-L-Ara_f-(1→2)	109·5	82·3		85·1	62·2
α-L-Ara_f-(1→3)	108·8	82·0		85·1	62·2
Element B					
β-D-Xyl_p	102·5	73·8	78·1	74·6	63·6
α-L-Ara_f-(1→3)	108·5	81·7	78·1	85·6	62·3

[a]Assignments were carried out by comparison with data published by Bengtsson & Åman (1990) and Hoffmann *et al.* (1991).

[b]Element 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 or element B at the non-reducing end.

[c]Relative to internal 1,4-dioxane (δ = 67·4 ppm).

Size exclusion chromatography of the four major arabinoxylan fractions L-PP_{35} to L-PP_{60} on Sephacryl S-500 gave similar elution patterns. The elution profiles of L-PP_{35}, L-PP_{44} and L-PP_{53}/L-PP_{60} (Fig. 2) demonstrate that the arabinoxylans are composed of a range of high-

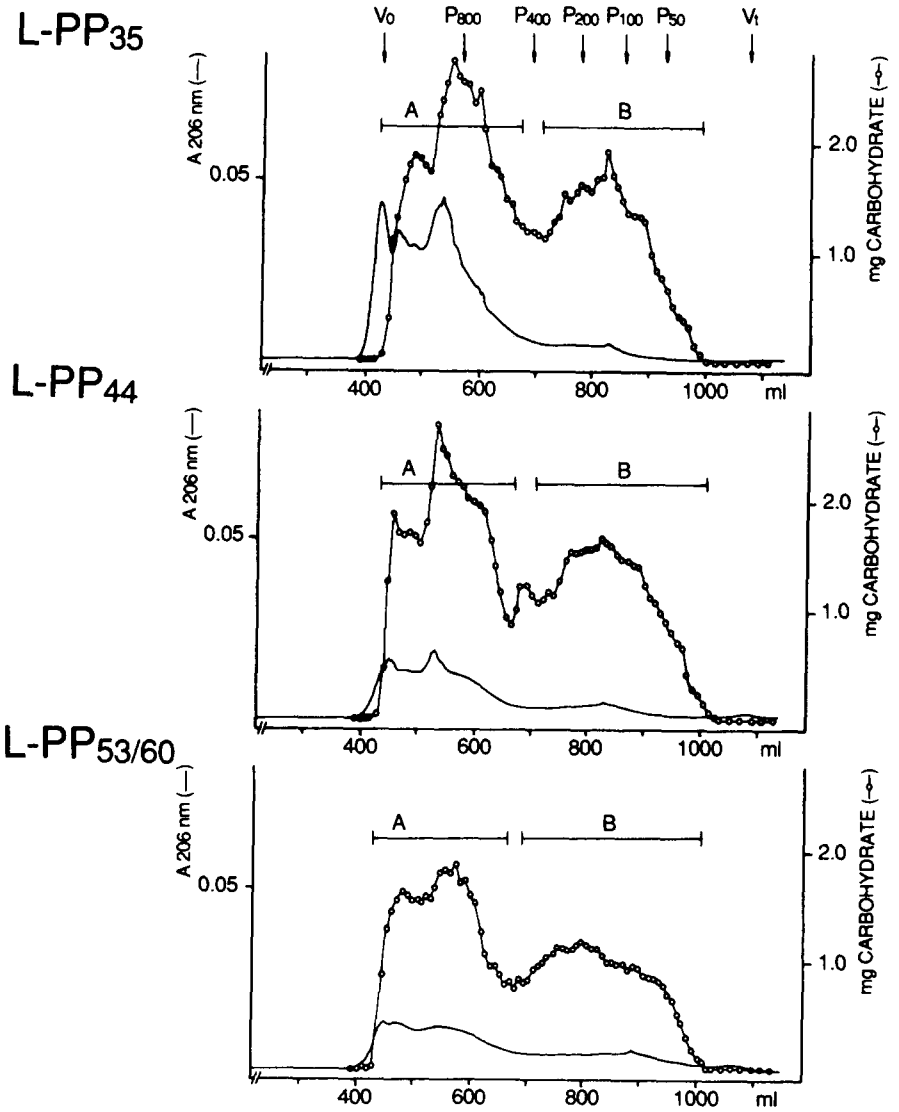


Fig. 2. Elution profiles of the three major L-PP fractions, L-PP_{35}, L-PP_{44} and L-$PP_{53/60}$ on a Sephacryl S-500 gel permeation column (150 × 3·0 cm). The UV absorption of the void volume fractions is caused by the presence of some residual protein. P_{800} = pullulan standard of 85·3 × 10^4 Da; P_{400} = 38·0 × 10^4 Da; P_{200} = 18·6 × 10^4 Da; P_{100} = 10·0 × 10^4 Da; P_{50} = 4·8 × 10^4 Da.

molecular-size polymers (> 40 kDa). In each case fractions were pooled as indicated. In Table 5 the relative amounts and monosaccharide compositions of the Sephacryl S-500 fractions are given. There are only minor differences in the Xyl to Ara ratio between the subfractions A and B of the same L-PP fraction; invariably subfraction A has the lowest ratio. Methylation analysis (Table 6) showed that the polymers in subfraction A had a slightly higher ratio 2,3,4-tri- to 3,4-disubstituted Xyl_p than the polymers of subfraction B of the same L-PP fraction. It can also be seen

TABLE 5

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

Fraction	*Monosaccharides*[a]							*Ferulic acid*
	Ara	*Xyl*	*Man*	*Gal*	*Glc*	*GalA*	%	*μg/mg polysaccharide*[b]
L-PP_{35}	1·00	2·21	+	+	0·03	+	100	
A	1·00	2·17	−	+	0·03	+	60	1·7
B	1·00	2·27	+	−	+	−	35	0·8
L-PP_{44}	1·00	1·59	+	+	+	−	100	
A	1·00	1·48	−	+	+	−	53	0·7
B	1·00	1·62	0·03	+	+	−	42	0·4
L-PP_{53}	1·00	1·27	0·03	+	+	+	100	
A	1·00	1·23	+	+	+	+	52	0·3
B	1·00	1·37	0·05	+	+	−	43	0·3
L-PP_{60}	1·00	1·26	0·05	0·02	0·02	−	100	
A	1·00	1·14	+	+	+	+	46	0·4
B	1·00	1·28	0·09	0·02	0·03	−	48	0·3

[a]Expressed as molar ratios relative to Ara.
[b]± 5%.

TABLE 6

Partially Methylated Alditol Acetates from Major L-PP Arabinoxylan Fractions after Sephacryl S-500 Separation Giving the Ratios Unbranched[a] (2,3-Me_2-Xyl) to Branched[b] (2-Me-Xyl + Xyl) Xylose and Trisubstituted (Xyl) to Disubstituted (2-Me-Xyl) Xylose

Alditol acetate of	*Relative mol (%)*							
	PP_{35A}	PP_{35B}	PP_{44A}	PP_{44B}	PP_{53A}	PP_{53B}	PP_{60A}	PP_{60B}
2,3-Me_2-Xyl/ 2-Me-Xyl + Xyl	2·5	2·3	1·7	1·6	1·1	1·4	1·0	1·2
Xyl/2-Me-Xyl	0·5	0·4	1·5	1·1	2·1	1·8	2·0	1·7

[a]2,3-Me_2-Xyl = 2,3-di-*O*-methyl-xylose, etc.
[b]No 3-Me-Xyl has been identified.

that for L-PP_{35} and L-PP_{44} the ratio unbranched to branched Xyl_p of subfraction A was slightly higher than of subfraction B. For L-PP_{53} and L-PP_{60} the reverse holds. All Sephacryl S-500 fractions contain significant amounts of *trans*-ferulic acid (Table 5). However, most ferulic acid was connected to the arabinoxylans of L-PP_{35}.

DISCUSSION

The warm-water-soluble arabinoxylans, extracted at 70°C from the tailings of the soft wheat variety Kadet, represent 0·3% of the total flour. They form a heterogeneous group of polysaccharides with respect to molecular mass, D-Xyl_p to L-Ara_f ratio, and distribution of Ara side chains along the xylan-backbone. The warm-water-soluble arabinoxylans contain, in contrast to the main portion of the cold-water-soluble arabinoxylans, high-molecular-size polymers only (> 40 kDa). The ratio 2,3,4-tri- to 3,4-di-substituted Xyl_p (branching pattern) of the arabinoxylans extracted from the tailings is the same as found for the cold-water-soluble arabinoxylans, ranging from 0·4 to 2·1. However, in the case when both have the same branching pattern the warm-water-soluble arabinoxylans have a relatively lower amount of unbranched Xyl_p residues. This is clearly demonstrated by the two major groups of water-soluble arabinoxylans:

Ratio	*Relative mol (%)*	
	WPAX$_{43C,51C,55C}$	*L-PP*$_{35A,35B}$
Xyl/Ara	2·4–2·5	2·2–2·3
2,3,4-Trisubstituted Xyl/ 3,4-disubstituted Xyl	0·3–0·4	0·4–0·5
Unbranched Xyl/branched Xyl	2·8–4·3	2·3–2·5

All arabinoxylans extracted from the tailings contain a significant amount of *trans*-ferulic acid. In the major cold-water-soluble arabinoxylan fractions ($WPAX_{43C,51C,55C}$) *trans*-ferulic acid is nearly absent. This observation supports the hypothesis that ferulic acid plays a role in the formation of water-insoluble polymer clusters.

The differences between the arabinoxylans from the tailings and the cold-water-soluble arabinoxylans, in particular the differences in size-distribution and ferulic acid content, are most likely responsible for the variation in baking properties found for flours having different ratios of soluble to insoluble arabinoxylans (Kühn & Grosch, 1989).

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