Structural Heterogeneity of Wheat Endosperm Arabinoxylans[1]

MARTA S. IZYDORCZYK and COSTAS G. BILIADERIS

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ABSTRACT

Water-soluble arabinoxylans from flours of 10 cultivars belonging to five classes of Canadian wheat were isolated and purified. Fractionation of arabinoxylans by a graded $(NH_4)_2SO_4$ technique gave three fractions: F65, F75, and F100. The numbers refer to the saturation level of the salt at which precipitation occurred. The yield of F65 was 53.3–85.9% of the total material recovered; F75 accounted for 10.2–35.7%; and F100 was only 3.9–11.0%. F65 showed the highest viscosity (3.15–5.24 dl/g). Viscosity decreased in F75 (2.23–3.60 dl/g) and F100 (0.88–1.21 dl/g). Ferulic acid was confined mostly to F65. The xylose-to-arabinose ratio was the highest for F65 (2.23–1.53) and much lower for F75 and F100 (1.22–1.05 and 1.18–1.03, respectively). F65 was a high molecular mass arabinoxylan fraction with the highest amount of xyloses carrying only one arabinose side chain residue (at C-2 or C-3), but it had the lowest content of xylose residues substituted with arabinoses at both C-2 and C-3. F75 and F100, with decreasing molecular mass of arabinoxylan fractions, had a decreased amount of singly substituted xyloses but an increased amount of doubly branched xyloses. The occurrence of unsubstituted Xyl*p* residues was much higher in the high molecular mass arabinoxylan fractions. The short Ara*f* side chains were more commonly encountered in low molecular mass arabinoxylan fractions.

Polysaccharides, as secondary gene products, usually exhibit a high degree of microheterogeneity and, therefore, belong to a class of polydisperse polymers (Aspinall 1982). Polydispersity may be reflected in the degree of polymerization as well as in the abundance, type, distribution, or degree of polymerization of side-chain substituents on a regular or irregular backbone. Arabinoxylans are typical heterogeneous polysaccharides. They consist of a long backbone of (1→4)-linked β-D-xylopyranose residues to which α-L-arabinofuranose units are linked (Perlin 1951, Amado and Neukom 1985). The attachment of these arabinose residues to the chain backbone has long been a matter of controversy. All three possible linkage combinations (arabinose linked to C-2, C-3, or C-2,3 of a xylose residue) have been reported; however, the occurrence and the relative amount of these linkages vary greatly from one report to another (Amado and Neukom 1985, Bengtsson and Aman 1990, Ebringerova et al 1990, Hoffmann et al 1991). Another structural feature of arabinoxylans is ferulic acid covalently linked through an ester linkage to arabinose residues (Smith and Hartley 1983).

Wheat arabinoxylans have generated much interest among cereal chemists and technologists since they have been proven to have significant influence on water balance of dough (Jelaca and Hlynka 1971, Patil et al 1975), rheological properties of dough (Meuser and Suckow 1986, Michniewicz et al 1991), retrogradation of starch (Gudmundsson et al 1991, Biliaderis and Izydorczyk 1992), and bread quality (McCleary 1986, Delcour et al 1991). Because the fine molecular structure of arabinoxylans dictates their functional behavior and physicochemical properties, this investigation was aimed at gaining more insight into the structural characteristics and heterogeneity of arabinoxylans, as well as the structural diversity of these polymers, depending on the wheat cultivar and growth location.

[1]Contribution 223 of the Food Science Department, University of Manitoba Food Science Department, University of Manitoba, Winnipeg, Canada.

MATERIAL AND METHODS

Water-soluble arabinoxylans from five distinct Canadian wheat classes were isolated and purified according to the procedure of Izydorczyk et al (1990): Canada western red spring (CWRS) (cv. Selkirk, Lancer, Katepwa) from three locations (Melfort, Indian Head, Brandon); Canada western soft white spring (cv. Fielder); Canada prairie spring (cv. HY 355, Oslo); Canada western red winter (cv. Norstar); and Canada utility (cv. Glenlea). The purified arabinoxylans were fractionated by a graded ammonium sulphate fractionation technique. The polymer (0.2%, w/v) was dissolved in phosphate buffer (0.1*M*, pH 7.0), and ammonium sulphate was added slowly to obtain 65% saturation level; the solution was left overnight at 20°C, then the precipitated polysaccharides were collected by centrifugation and filtration on a glass-fiber paper, redissolved in H_2O, dialyzed until free of $(NH_4)_2SO_4$, and lyophilized. This fraction was designated F65. Two other fractions, F75 and F100, were also obtained. The numbers refer to the saturation level of $(NH_4)_2SO_4$ at which precipitation occurred.

Gel-filtration chromatography was performed on a Sepharose CL-4B column (2.4 × 85 cm). Elution was achieved with degassed 0.3% NaCl containing 0.05% NaN_3 at a flow rate of 25 ml/hr at 25°C. Total (V_t) and void (V_0) volumes were determined with xylose and blue dextran, respectively. Effluent fractions (5 ml) were analyzed for total carbohydrates by the phenol-sulfuric method (Dubois et al 1956).

The apparent viscosities of aqueous solutions of arabinoxylan fractions were measured with Ubbelohde capillary viscometers (International Research Glassware, Kenilworth, NJ) at 25°C. The limiting viscosities [η] were calculated from the Huggins equation (Huggins 1942).

The content of feruloyl groups was estimated spectrophotometrically by direct absorbance measurements at 375 nm of freshly prepared arabinoxylan fractions in 0.07*M* glycine-NaOH buffer (pH 10) assuming a molar extinction coefficient of 31,600 (Fry 1982).

Monosaccharide composition of arabinoxylan fractions was

determined by gas-liquid chromatography (Hewlett Packard fused silica column SP2330; 30 × 0.75 mm, i.d.; 0.75 μm film thickness) of alditol acetates (Englyst et al 1982). Samples were hydrolyzed with 1*M* H_2SO_4 for 2 hr at 100°C. Allose was used as an internal standard.

Natural-abundance proton decoupled ^{13}C-nuclear magnetic resonance spectroscopy (75.9 MHz) was performed at 85°C using a Bruker AM 300FT spectrometer. The fractions (2.0%, w/v) were dissolved in H_2O containing 10% D_2O. Approximately 30,000 pulses were recorded. Chemical shifts (δ) are expressed in parts per million downfield from external Me_4Si, but they were actually measured by reference to internal 1,4-dioxane (δ = 67.4 ppm).

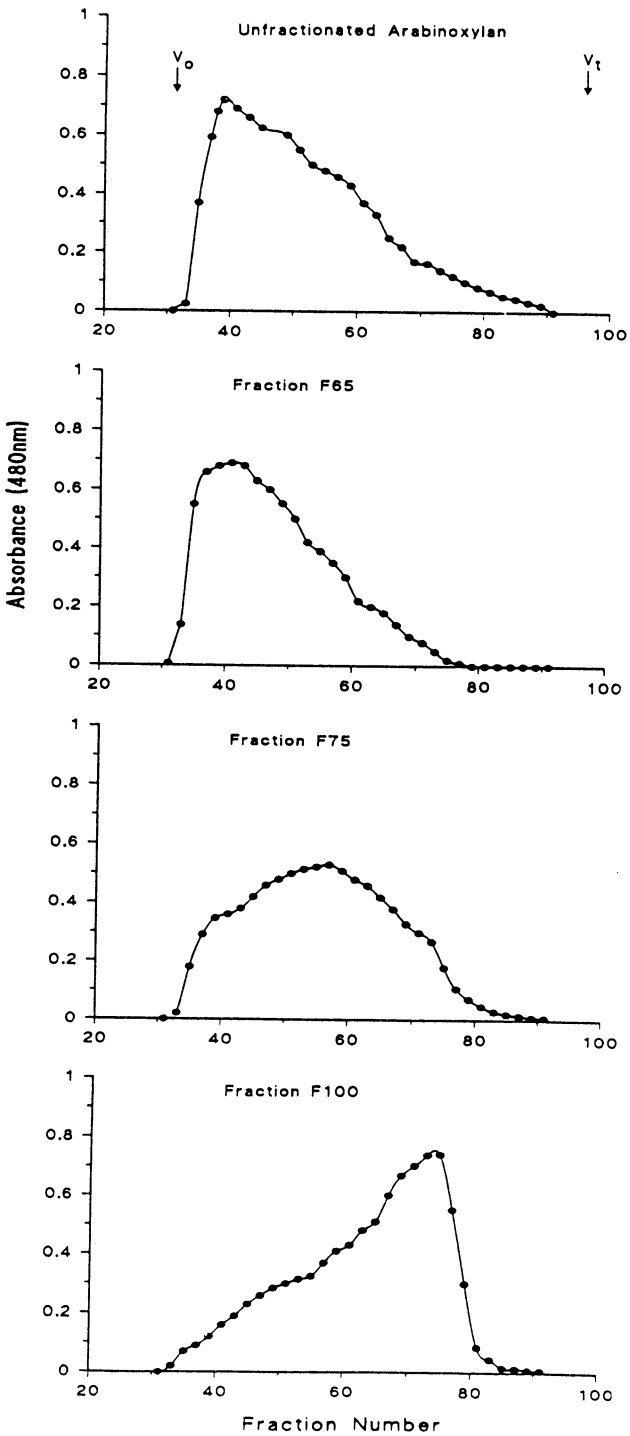

Fig. 1. Gel-filtration chromatography on a Sepharose CL-4B column (2.5 × 85 cm, 0.3% NaCl and 0.05% NaN_3, flow rate 25 ml/hr, 25°C) of the unfractionated arabinoxylan (isolated from cv. Katepwa, location Brandon). Fractions obtained by fractional precipitation with $(NH_4)_2SO_4$. V_t and V_0 = total and void volumes, respectively.

Methylation of arabinoxylans was conducted according to the method of Ciucanu and Kerek (1984) as modified by Aspinall (*personal communication*). Added to the arabinoxylan solutions (1–2 mg per 500μl of DMSO) were finely powdered NaOH (20 mg) and methyl iodide (100 μl). The mixture was stirred for 2 hr in a closed vial at 25°C. Water (4 ml) and methylene chloride (3 ml) were then added, and the methylene chloride layer was washed four times with 4 ml of water and dried (Na_2SO_4). The dried CH_2Cl_2 layer containing the partially methylated polysaccharide was then evaporated to dryness under N_2. The methylated polysaccharides were hydrolyzed with 4*M* trifluoroacetic acid (6 hr, 100°C) and, after evaporation, water (0.3 ml), 1% ammonium aqueous solution (1 drop), and sodium borodeuteride ($NaBD_4$) (10 mg) were added to the hydrolyzed samples. The reaction vials were closed and kept stirring at room temperature overnight. The methylated products were converted to alditol acetates. One drop of acetic acid was added to destroy any excess of $NaBD_4$. The samples were then evaporated to dryness, and 0.5 ml of 5% acetic acid in methanol was added to the vials and evaporated to dryness; this was repeated three times. After addition of acetic anhydride (0.5 ml), the reaction mixtures were heated at 100°C for 2 hr. The excess of acetic anhydride was destroyed by addition of a few drops of ethanol and evaporated to dryness. Methylene chloride was then added to dissolve the partially methylated acetated alditols. The organic layer was dried by passing through a small anhydrous Na_2SO_4 column and evaporating to dryness. The partially methylated alditol acetates were analyzed by capillary gas-liquid chromatography (fused silica column, SP2330; 30 × 0.75 mm, i.d.; 75 μm film thickness) and by coupled gas-liquid chromatography-mass spectrometry (capillary column, SP2330; 60 m × 0.25 mm, i.d.; 0.20 μm film thickness). The effective carbon response factors, as given by Sweet et al (1975), were used for calculation of molar quantities of the permethylated products determined by gas-liquid chromatography. Because 1,3,4,5-tetra-o-acetyl-(1-deuterio)-2-o-methyl-xylitol (C-3 singly substituted xyloses) and 1,2,4,5-tetra-o-acetyl-(1-deuterio)-3-o-methyl-xylitol (C-2 singly substituted xyloses) had very similar retention times, they could not be resolved under the chromatographic conditions employed. However, their detection and quantitation was achieved from the mass spectra by integration of signals of fragment ions characteristic for these two derivatives: m/e 118 (specific for 2-o-methyl-xylitol) and m/e 129 (specific for 3-o-methyl-xylitol). The intensities of signals at m/e 118 and m/e 129 were estimated to represent 20 and 25%, respectively, of the total ion current of the respective derivatives. From these spectra, the ratio of C-3 to C-2 monosubstituted xylopyranose residues was calculated.

RESULTS AND DISCUSSION

Purified arabinoxylan from wheat flour exhibits a broad distribution of molecular mass upon gel-filtration chromatography with Sepharose CL-4B (Fig. 1). Fractionation of arabinoxylan by graded $(NH_4)_2SO_4$ technique yielded three polymer fractions that differed significantly in their elution profiles (Fig. 1). The fraction obtained at the 65% saturation level of $(NH_4)_2SO_4$ (F65) eluted mostly in the vicinity of the V_0. In contrast, the elution profile of the fraction obtained at 100% saturation level (100%) shifted towards the lower molecular mass end. F75 eluted between the two extreme peaks of F65 and F100. Previous studies with arabinoxylans from a CWRS flour had shown that fractional precipitation with $(NH_4)_2SO_4$ affords fractions differing not only in molecular mass but also in fine molecular structure (Izydorczyk and Biliaderis 1992a). Consequently, in this investigation, purified arabinoxylans from 10 different wheat cultivars were subjected to graded precipitation with this salt. The quantities of each fraction, based on the amount of material (dried weight) recovered after fractionation, are presented in Table I. In all cases, most of the polysaccharide precipitated at 65% saturation with $(NH_4)_2SO_4$. However, there were differences in the yield of F65 among the cultivars, as indicated in Table I. The highest yield

value was obtained for Katepwa (location Brandon) and the lowest for Oslo. F75 accounted for 10.2–35.7% of the total material recovered after fractionation; F100 accounted for only 3.9–11.0%. The large variation in the yields of the three fractions among the cultivars implies substantial differences in the composition of the arabinoxylan preparations in terms of structure and molecular mass of constituent polymeric species.

The differences in the molecular weight distribution among F65, F75, and F100 were confirmed by $[\eta]$ measurements (Table II). In all cases, F65 showed the highest $[\eta]$. It decreased in F75 and F100. In the F65 fractions, arabinoxylans from CWRS had considerably higher viscosity than arabinoxylans from other classes. However, differences in $[\eta]$ of F65 were observed even within a single wheat cultivar. The $[\eta]$ was higher for the Katepwa sample grown in the Brandon area than it was for Katepwa grown at other locations. Similar trends were observed for F75 fractions. No major differences in $[\eta]$ were observed for F100, regardless of their origin. The content of ferulic acid also varied widely among the fractions (Table II). Although ferulic acid was found in all fractions, most of it was confined to those of high molecular weight. In general, F65 contained more than twice the amount of ferulic acid of the remaining samples (with the exception of F65 from HY 355). Again, differences in the ferulic acid content of F65 were found within the same wheat class.

Monosaccharide composition and the molar ratio of arabinose-xylose-glucose are presented in Table III. Arabinose and xylose were the main constituents of all fractions. Galactose residues were not found in the arabinoxylan preparations. The degree of substitution of the xylan backbone with the arabinose residues plays an important role in determining the shape of molecule, and, hence, its solution properties (Andrewartha et al 1979). As indicated in Table III, the xylose-to-arabinose ratio was the highest for F65. For the other fractions, this ratio was generally much lower, suggesting a much higher degree of substitution in F75 and F100 than in the F65 fractions. The distribution of glucose was very uneven among the fractions. With the exception of Fielder and Glenlea, this monosaccharide was found mainly in F65 fractions.

The results of the graded $(NH_4)_2SO_4$ fractionation show that arabinoxylans from various wheat cultivars are very heterogeneous and are composed of polysaccharide populations differing in xylose-to-arabinose ratio, in ferulic acid content, and in molecular mass. Interestingly, a definite relationship appears to exist among these three structural parameters. As shown in Figure 2, the population of arabinoxylan polymers with the least degree of substitution (high xylose-to-arabinose ratio) and the highest ferulic acid was also characterized by high molecular mass, as indicated by the high $[\eta]$ values. In contrast, low xylose-to-arabinose ratios and a low ferulic acid content were concomitant with low $[\eta]$ values. Multiple regression analysis also indicated a relatively strong linear relationship between $[\eta]$ and the two structural parameters, xylose-to-arabinose ratio and ferulic acid content (F in mg/ml):

$$[\eta] = -1.25 + 2.63 \text{ xyl/ara} + 0.42F$$
$$r = 0.79$$

TABLE I
Yields (%)[a] of Arabinoxylan Fractions Obtained by Fractional Precipitation with Ammonium Sulphate

Cultivar and Location	Fractions		
	F65	F75	F100
Katepwa			
Melfort	83.1	10.5	6.4
Indian Head	78.3	14.4	7.3
Brandon	85.9	10.2	3.9
Selkirk	72.4	19.6	8.0
Lancer	84.0	11.0	5.0
Fielder	80.1	11.7	8.2
HY 355	81.5	11.7	6.8
Oslo	53.3	35.7	11.0
Norstar	67.0	25.5	7.5
Glenlea	55.3	34.4	10.3

[a]Based on total amount (dried weight) of material recovered. Data represent means of triplicate fractionation experiments; the coefficient of variation was less than 5.0% of the mean values in all cases.

TABLE II
Limiting Viscosity and Ferulic Acid Content of Arabinoxylan Fractions

Cultivar and Location	Limiting Viscosity, dl/g[a]			Ferulic Acid, mg/g[b]		
	F65	F75	F100	F65	F75	F100
Katepwa						
Melfort	4.69	3.36	1.21	1.71 ± 0.05	0.57 ± 0.03	0.50 ± 0.03
Indian Head	4.53	3.42	1.21	1.69 ± 0.06	0.48 ± 0.01	0.34 ± 0.04
Brandon	5.24	3.60	1.15	1.51 ± 0.04	0.54 ± 0.03	0.52 ± 0.04
Selkirk	4.44	2.73	0.95	1.29 ± 0.03	0.39 ± 0.05	0.47 ± 0.03
Lancer	4.51	2.65	0.90	1.10 ± 0.05	0.45 ± 0.03	0.28 ± 0.04
Fielder	3.87	2.33	1.10	1.75 ± 0.06	0.71 ± 0.04	0.79 ± 0.05
HY 355	3.84	2.55	1.20	1.37 ± 0.05	0.80 ± 0.05	0.74 ± 0.04
Oslo	3.75	2.23	1.14	1.20 ± 0.04	0.51 ± 0.05	0.36 ± 0.04
Norstar	3.15	2.28	0.88	1.69 ± 0.04	0.59 ± 0.03	0.48 ± 0.03
Glenlea	3.63	2.59	1.17	1.45 ± 0.03	0.54 ± 0.04	0.66 ± 0.05

[a]$n = 3$; the coefficient of variation was less than 3.0% of the mean values in all cases.
[b]$n = 3 \pm$ SD.

TABLE III
Monosaccharide Composition of Arabinoxylan Fractions[a]

Cultivar and Location	Fraction		
	F65	F75	F100
Katepwa			
Melfort	1.00:1.83:0.07	1.00:1.20:0.0	1.00:1.13:0.0
Indian Head	1.00:2.32:0.20	1.00:1.22:0.0	1.00:1.16:0.0
Brandon	1.00:1.72:0.06	1.00:1.18:0.0	1.00:1.05:0.0
Selkirk	1.00:1.60:0.05	1.00:1.07:0.0	1.00:1.07:0.0
Lancer	1.00:1.70:0.06	1.00:1.05:0.0	1.00:1.03:0.0
Fielder	1.00:1.84:0.0	1.00:1.22:0.08	1.00:1.18:0.0
HY 355	1.00:1.90:0.40	1.00:1.19:0.0	1.00:1.15:0.0
Oslo	1.00:1.72:0.10	1.00:1.21:0.0	1.00:1.16:0.0
Norstar	1.00:1.90:0.40	1.00:1.19:0.0	1.00:1.15:0.0
Glenlea	1.00:1.53:0.12	1.00:1.15:0.01	1.00:1.15:0.02

[a]Molar ratios of Ara-Xyl-Glu.

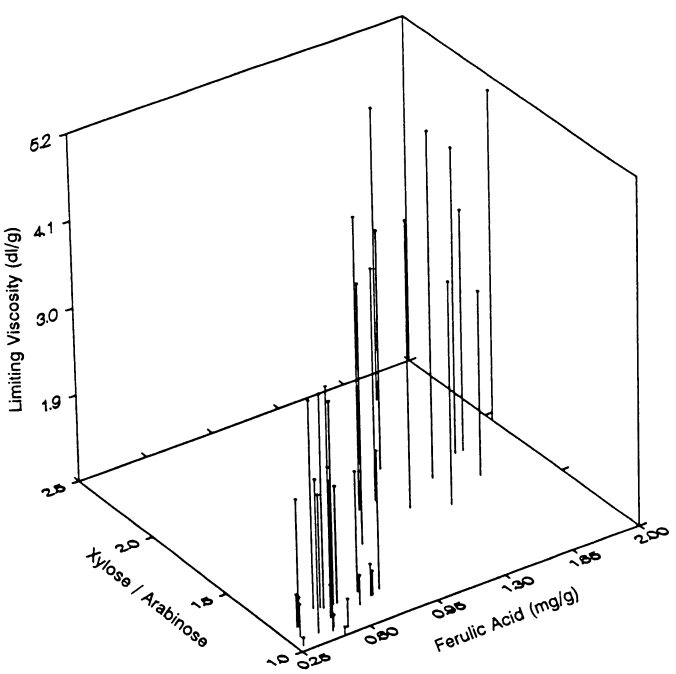

Fig. 2. Relationships of three structural parameters of wheat arabinoxylan fractions.

To identify and compare in more detail the molecular features of arabinoxylan fractions, all polymers were analyzed by ^{13}C-nuclear magnetic resonance spectroscopy. According to the data published by Bock et al (1984), Ebringerova et al (1990), and Hoffmann et al (1991), the resonances in the region of 108–110 ppm and 100.8–102.5 ppm are attributed to anomeric carbons of α-L-Ara*f* residues and β-D-Xyl*p*, respectively (Fig. 3). The peaks at 109.4 and 108.7 ppm correspond to C-1 of α-L-Ara*f* residues linked to a single xylose at C-2 and C-3, respectively. The peak at 108.4 ppm corresponds to C-1 of α-L-Ara*f* linked to a xylose residue at C-3 only. An upfield shoulder of the 109.4 resonance observed in the spectra might originate from small amounts of α-L-Ara*f* linked to β-D-Xyl*p* at C-2 only. The resonance at 100.7 ppm is attributed to C-1 of β-D-Xyl*p* doubly substituted (at C-2 and C-3) with arabinoses. The 101.9-ppm resonance has been assigned to C-1 of unsubstituted xylose residues adjacent to mono- and disubstituted xyloses at their nonreducing end (Hoffmann et al 1991). The remaining unsubstituted, as well as the mono-substituted, xyloses contribute to the signal at 102.4 ppm (Hoffmann et al 1991). Other regions of resonances in the carbon spectra are those for C-2, C-4, and C-5 of Ara*f* residues. Arabinoses linked simultaneously to the same xylose (at C-2 and C-3) give rise to the following resonances: α-L-Ara*f*-(1→2): $\delta_{C\text{-}2}$ 82.30, $\delta_{C\text{-}4}$ 85.08, $\delta_{C\text{-}5}$ 62.18; and α-L-Ara*f*-(1→3): $\delta_{C\text{-}2}$ 81.97, $\delta_{C\text{-}4}$ 85.31, $\delta_{C\text{-}5}$ 62.18. The arabinose residues linked to the xylose at C-3 only are responsible for the $\delta_{C\text{-}2}$ 81.69, $\delta_{C\text{-}4}$ 85.58, and $\delta_{C\text{-}5}$ 62.31 peaks. The relative signal intensities in the anomeric region of Xyl*p* and Ara*f* were consistent with those of the C-2, C-3, and C-5 resonances of Ara*f* residues. From these resonances the relative amounts of di-, mono-, and unsubstituted Xyl*p* residues and arabinoses linked to a single xylose at C-2 and C-3 simultaneously, as opposed to arabinoses linked to xylose residues at C-3 only, can be deduced. Therefore, ^{13}C-nuclear magnetic resonance spectroscopy is a suitable molecular probe for detecting differences in branching patterns of arabinoxylans. To reveal further diversities among arabinoxylan polymers from various cultivars, two structural parameters were derived from the integration of resonances in the anomeric regions of the carbon spectra. One of them quantifies the doubly branched xyloses, and the other gives the amounts of arabinoses linked to a xylose residue at C-3 only. Both parameters are presented in Table IV. In general, F65 contained less doubly substituted xyloses than the other fractions. Within the F65 group, arabinoxylans of Katepwa (at all three locations) had the lowest percentage of xylose residues that carried substituents at both C-2 and C-3. Similar observations were also made for F75 and F100. When the amount of doubly substituted xyloses was plotted against the [η] (Fig. 4) for all fractions, certain trends became apparent. F100 concentrated within the low [η] end with relatively high amounts of doubly substituted xyloses (≈40%). The next group, with [η] of 2.2–2.7 dl/g and still with a relatively high content of doubly substituted xylose residues (≈35–42%), contained F75, with the exception of Katepwa. F65 were clearly divided into two groups: arabinoxylans of CWRS (all Katepwa samples, Lancer, and Selkirk) with distinctively high [η] (>4.4 dl/g) and low content of doubly branched xyloses. The remaining F65

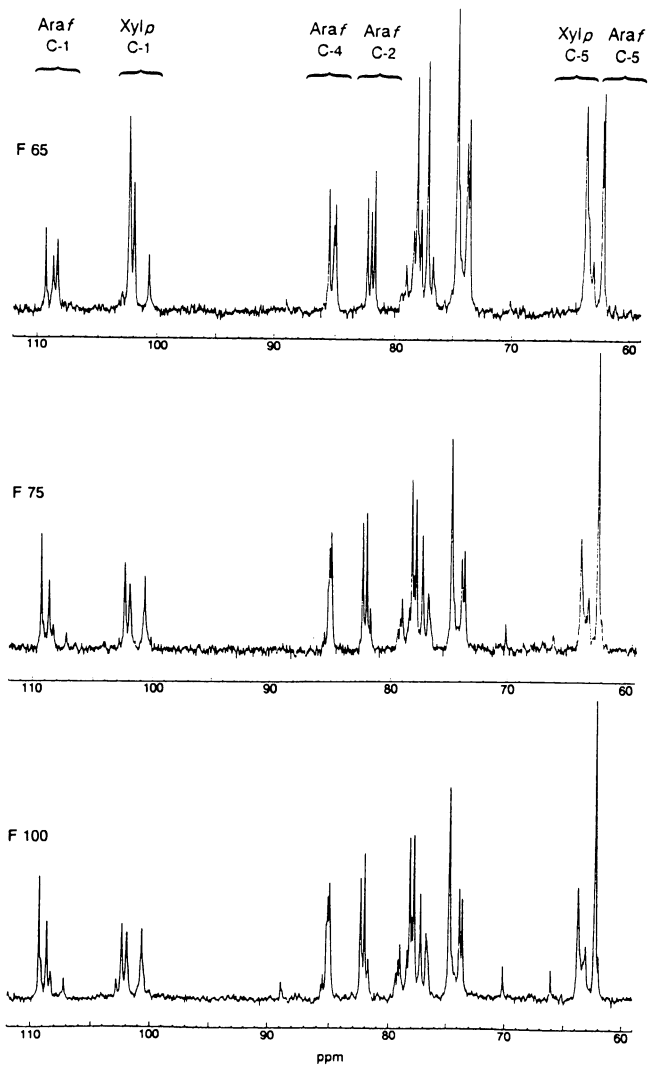

Fig. 3. Typical ^{13}C-nuclear magnetic resonance spectra of arabinoxylan fractions from a representative arabinoxylan preparation (cv. Katepwa, location Brandon). The chemical shifts were assigned relative to 1,4-dioxane.

TABLE IV
Amounts (%) of Doubly Substituted Xylose Residues and Arabinose Residues Linked to Xyloses Exclusively at C-3[a]

Cultivar and Location	Doubly Substituted Xylose[b]			1→3-Linked Arabinose[c]		
	F65	F75	F100	F65	F75	F100
Katepwa						
Melfort	13.0	30.2	33.3	37.0	20.4	12.8
Indian Head	11.7	31.2	34.9	42.1	10.2	15.4
Brandon	13.5	30.4	32.9	45.7	10.6	12.0
Selkirk	17.3	36.2	34.3	34.2	16.8	13.0
Lancer	17.2	42.4	43.2	34.4	11.0	26.0
Fielder	19.0	36.9	38.2	34.0	7.8	10.0
HY 355	15.0	35.3	38.5	34.0	16.6	14.2
Oslo	22.1	38.8	40.0	28.0	10.0	8.0
Norstar	20.0	35.5	40.0	34.2	12.9	12.0
Glenlea	26.1	40.2	40.5	19.9	8.6	8.6

[a] Results calculated from integration of resonances in the respective anomeric region of the ^{13}C-nuclear magnetic resonance spectra.
[b] →2,3,4)-Xyl*p*-(1→; based on total amount of xylose residues.
[c] Based on total amount of arabinose residues.

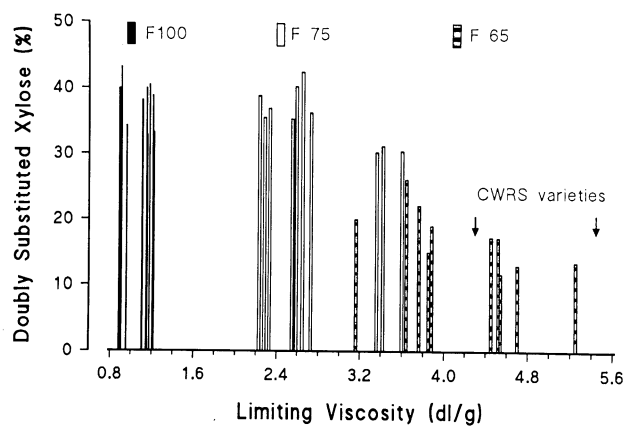

Fig. 4. Relationship of two structural parameters for three arabinoxylan fractions from Canada western red spring (CWRS) wheat.

fractions had [η] of 3.15–3.87 dl/g and an intermediate amount of xyloses carrying two substituents. Moreover, a relatively strong linear association ($r = 0.83$) between these two structural variables ([η] and the doubly substituted xyloses) was found. The other structural parameter derived from the ^{13}C-nuclear magnetic resonance spectra also points to some distinct differences in branching patterns among the fractions. This is evident by comparing the amounts of arabinoses linked to xylose residues exclusively at C-3 in F65 fractions with those in F75 and F100. The ^{13}C-nuclear magnetic resonance analyses provided clear evidence that the high molecular weight population of arabinoxylan fractions is characterized by a relatively small percentage of doubly substituted xyloses and by a high content of singly substituted xylose residues (as indicated by the higher content of arabinoses linked at C-3 only); the opposite holds for the low molecular weight population of arabinoxylan polymers.

The results of methylation analyses of the three arabinoxylans extracted from a single cultivar of wheat (Katepwa) grown in three different locations are compiled in Table V. These data confirmed that the main residues in the arabinoxylan fractions were terminal arabinofuranoses and unsubstituted xylopyranosyl units. In all three cases, the relative amounts of these two residues changed depending on the fraction. Generally, there was a progressive increase in terminal Ara*f*, with a concurrent decrease in unsubstituted Xyl*p* residues from F65 to F100. Small quantities of substituted arabinoses (at C-2, C-3, and C-5) indicated that short oligomeric side chains might also be present in arabinoxylans. Their occurrence was especially prominent in F100, where approximately 10% of the arabinose residues appeared in short side chains. The mass spectra of the alditol derivatives also indicated the presence of both C-3 and C-2 monosubstituted xyloses of the chain backbone (Table V). Interestingly, the ratio of C-3 to C-2 monosubstituted xylopyranoses decreased consistently from F65 to F100 for all three Katepwa arabinoxylan samples. Comparisons of the amounts of mono-, di-, and unsubstituted xyloses in all fractions are also presented in Table V. The composition of F65 differed slightly among the samples: in F65 of Melfort, 10.6% of the xylose residues carried a substituent at both C-2 and C-3; 24.5% carried a substituent at C-3 or C-2 only; and 62.3% were unsubstituted. In F65 of Indian Head, these values were 9.3, 24.6, and 63.9%. In F65 of Brandon, these values were 11.5, 26.6, and 57.7%. Small differences among the samples also became evident when the ratio of unbranched-to-branched xylose residues was calculated; the ratio was the highest for Indian Head (1.88), followed by Melfort (1.77) and Brandon (1.55). Slight structural differences were also found among fractions F75 and F100.

In addition to Xyl*p* and Ara*f*, glucopyranosyl residues linked at C-3 and C-4 were detected upon methylation (Table V), suggesting that glucose in arabinoxylan preparations originates from β-(1→3),(1→4)-glucans. This also explains why, upon stepwise fractionation with $(NH_4)_2SO_4$, glucose amassed mainly in fractions obtained at the lowest saturation level of the salt (in F65). Low concentration of this salt has, in fact, been used to selectively precipitate β-glucans from water extracts of barley in which arabinoxylans are also present (Aspinall 1982).

The findings of the present study have demonstrated that wheat endosperm arabinoxylans constitute a heterogeneous group of polysaccharides with respect to molecular mass, D-Xyl*p* to L-Ara*f* ratio, ferulic acid content, and Ara*f* side-chain distribution along the xylan backbone. However, the evidence presented here may also indicate that not all molecular characteristics are diffused by microheterogeneity. First, because the main biological function of these polymers is that of a structural material in the cell wall (Fincher and Stone 1986), the configuration of the glycosidic bonds of the xylan backbone (β-1→4) is the most conservative feature in all arabinoxylans. Second, even among the most variable molecular characteristics of arabinoxylans, some relationships and tendencies seem to exist. Thus, the population of arabinoxylans with high molecular mass shows the highest xyl/ara ratio, the highest ferulic acid content, and the highest amount of xyloses carrying only one arabinose residue (at C-3 or C-2), but it has the lowest content of xylose residues substituted with arabinoses at both C-2 and C-3. With decreasing molecular mass of arabinoxylans the xyl/ara ratio, ferulic acid, and singly substituted xyloses decrease, whereas the amount of xyloses doubly branched increases. Furthermore, the relative amounts of singly substituted Xyl*p* at C-2 versus C-3, increase with decreasing molecular mass of the arabinoxylan fractions. The occurrence of unsubstituted Xyl*p* residues has been much higher in the high molecular mass population of arabinoxylans. The short Ara*f* side chains have been more commonly encountered in low molecular mass arabinoxylans. The relative amounts of the high, medium, and low molecular mass populations of arabinoxylans seem to vary greatly with the source of these polysaccharides (i.e., wheat class, cultivar, and growth location). Each characteristic group of arabinoxylans exhibits different physicochemical properties, as demonstrated previously (Izydorczyk and Biliaderis 1992a,b); therefore, the overall effect of arabinoxylans on the technological performance of the respective flours may also vary greatly.

TABLE V
Molar Composition (%) for Monosaccharide Residue and Linkage Distribution of Selected Arabinoxylan (cv. Katepwa from three locations) Fractions

		Melfort			Indian Head			Brandon		
Alditol Acetate[a]	Mode of Linkage	F65	F75	F100	F65	F75	F100	F65	F75	F100
$2,3,5Me_3Ara$	Ara*f*-(1→	37.3	47.4	49.5	37.3	45.8	48.9	36.5	49.3	45.7
$3,5Me_2Ara$	→2)-Ara*f*-(1→	0.3	2.0	3.3	0.7	2.4	3.4	1.1	2.0	3.7
$2,5Me_2Ara$	→3)-Ara*f*-(1→	0.2	0.2	0.2	0.2	0.5	0.3	0.5	0.2	0.3
$2,3Me_2Ara$	→5)-Ara*f*-(1→	1.1	0.8	1.4	0.6	1.3	1.4	1.6	0.8	1.6
$2,3,4Me_3Xyl$	Xyl*p*-(1→	1.6	2.4	3.7	1.3	2.2	3.2	1.6	2.6	4.2
$2,3Me_2Xyl$	→4)-Xyl*p*-(1→	37.7	29.4	25.5	37.8	29.6	25.4	34.7	27.4	24.7
2MeXyl + 3MeXyl	→2,4)-Xyl*p*-(1→ + →2,4)-Xyl*p*-(1→	14.8 (26.7)[b]	6.4 (6.7)	5.5 (4.8)	14.5 (32.0)	6.8 (7.8)	6.2 (4.9)	15.6 (31.9)	5.9 (6.9)	6.9 (4.9)
Xyl	→2,3,4)-Xyl*p*-(1→	6.4	11.4	10.8	5.5	11.3	11.2	6.7	11.6	12.8
$2,3,6Me_3Glu$	→4)-Glu*p*-(1→	0.1	...	...	0.6	...	...	0.6	...	...
$2,4,6Me_3Glu$	→3)-Glu*p*-(1→	0.5	...	...	1.5	...	...	1.2	...	...
Doubly Xyl		10.6	23.0	23.7	9.3	22.5	24.2	11.5	24.4	26.3
Singly Xyl		24.5	12.9	12.1	24.6	13.6	13.5	26.6	12.5	14.3
Unsubs Xyl		62.3	59.3	56.0	63.9	59.1	55.2	59.2	57.7	50.8
Unsubs/Subs Xyl		1.77	1.61	1.56	1.88	1.64	1.46	1.55	1.56	1.26

[a] Doubly substituted xylose residues: →2,3,4)-Xyl*p*-(1→; based on total amount of xylose residues. Singly substituted xylose residues: →3,4)-Xyl*p*-(1→ or →2,4)-Xyl*p*-(1→. Unsubstituted xylose residues: →4)-Xyl*p*-(1→.
[b] Numbers in parentheses refer to ratio of C-3 to C-2 monosubstituted Xyl*p*.

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