ELSEVIER

Carbohydrate Polymers **24** (1994) 61–71

0144-8617/94/$07.00

Studies on the structure of wheat-endosperm arabinoxylans

Marta S. Izydorczyk[1] & Costas G. Biliaderis[2]

Food Science Department, University of Manitoba Winnipeg, Manitoba, Canada R3T 2N2

(Received 4 February 1994)

Water-soluble arabinoxylans from a Canadian western red spring flour (cv. Laura) were fractionated by a graded ammonium-sulphate-precipitation technique into five fractions obtained at 55% (F55), 60% (F60), 70% (F70), 80% (F80), and 100% (F100) saturation with $(NH_4)_2SO_4$. The yields of the fractions (based on the total material recovered) were 37·5%, 22·3%, 23·6%, 11·9%, and 4·7%, respectively. The relative amount of Xyl*p* residues doubly substituted at C-2 and C-3 with Ara*f* increased from F55 to F100, whereas the amount of mono- and unsubstituted Xyl*p* residues decreased in the same order. The presence of short Ara*f* chains was more pronounced in F100 than in other samples. The content of ferulic acid was highest in F55. Fractions F55 and F100 were degraded with purified endo-β-xylanase from *Trichoderma viride*. The hydrolysate fragments were separated, quantified (Bio-Gel P-2, HP-SEC), and characterized (^{1}H-NMR and methylation analysis). F55 appeared to be built up from three structurally different regions. The first region, I_{55} (15%), was of relatively high DP and was high in terminal arabinose doubly linked to xylose residues at C-3 and C-2. The second region, II_{55} (40%), contained high amounts of terminal arabinoses linked to xylose residues at C-3. The third region, III_{55} (45%), contained high amounts of contiguously unsubstituted and C-3 monosubstituted xylose residues. Fraction F100 was built up mainly from the highly substituted region I_{100} (75%), enriched in C-2,3 di- and C-2 monosubstituted xylose residues as well as in short arabinose side chains. Region III_{100} (18%) contained a high proportion of unsubstituted xylose residues; however, the amount of disubstituted xylose residues in this region was still higher than that of monosubstituted residues.

INTRODUCTION

Among the minor constituents of wheat, which affect the physical properties, processing characteristics, and end-product quality of flours, is a family of cell-wall polysaccharides, arabinoxylans (Meuser & Suckow, 1986). These polymers have recently attracted the interest of many researchers because of some important physical properties that they exhibit. More specifically, arabinoxylans have been shown to affect the moisture distribution and rheological properties of dough (Jelaca & Hlynka, 1971; Patil *et al.*, 1975; Izydorczyk *et al.*, 1991; Michniewicz *et al.*, 1991), the loaf volume of bread (Delcour *et al.*, 1991), and retrogradation of starch (Gudmundsson *et al.*, 1991; Biliaderis & Izydorczyk, 1992).

It has long been established that wheat-endosperm arabinoxylans consist of a long backbone of (1 → 4)-linked β-D-xylopyranose residues to which α-L-arabinofuranose units are linked (Perlin, 1951). The attachment of these arabinose residues to the chain backbone, however, is still a matter of controversy. The two linkages of arabinofuranoses to C-3 and C-2,3 of xylose residues, as the only ones existing in arabinoxylans, have long been reported (Perlin, 1951; Goldschmid & Perlin, 1963; Westerlund *et al.*, 1990; Hoffmann *et al.*, 1991*a*, 1992*a*). However, proof of the presence of another linkage possibility, i.e. Ara*f* linked to C-2 of Xyl*p*, in arabinoxylans from beeswing bran of wheat

[1]To whom correspondence should be addressed.
[2]Present address: Department of Food Science and Technology, School of Agriculture, Aristotle University, Thessaloniki, Greece 54006.

kernel was provided by Brillouet and Joseleau (1987). Most recently, such a linkage has been reported in wheat-endosperm arabinoxylans (Gruppen, 1992; Izydorczyk & Biliaderis, 1993) as well as in arabinoxylan from rye bran (Ebringerova *et al.*, 1990), barley endosperm (Vietor *et al.*, 1992), and corn-cob heteroxylan (Ebringerova *et al.*, 1992).

The degree of substitution and the distribution of arabinosyl substituents along the xylan backbone are of great importance, since they affect the capacity of arabinoxylans to interact with each other and/or with other polysaccharides and therefore alter certain physical and functional properties of these macromolecules. According to the model of Ewald and Perlin (1959) and Goldschmid and Perlin (1963), wheat-endosperm arabinoxylan consists mostly of highly branched regions, where singly (C-3) or doubly (C-2,3) substituted xyloses are separated by singly unsubstituted xylose residues. Relatively 'smooth' domains of at least between two and five (but possibly more) unsubstituted xylose units may also be present between the more-branched regions. On the basis of the structural evaluation of oligosaccharides generated by endo-1,4-D-xylanase digestion of arabinoxylans, Hoffmann *et al.* (1991*a*, 1992*a*) established that wheat arabinoxylans contained isolated and/or paired C-3 monosubstituted xylose residues, isolated disubstituted residues, and clusters of two disubstituted xylose units. Less frequently, elements containing monosubstituted xylose residues next to disubstituted residues were present. More recently, Gruppen (1992) proposed a model showing that the alkali-extractable arabinoxylans are composed of highly branched regions, mostly consisting of tetrameric repeating units of unsubstituted and doubly substituted (arabinofuranosylated) xylose residues, interlinked with less-branched regions, which include subregions of up to seven contiguous unsubstituted xylose residues. The highly branched regions are enriched in C-2,3 as well as C-2 substituted xylose. Rye arabinoxylans seem to differ markedly from those of wheat. On the basis of enzymic studies, Aman *et al.* (1990) proposed that rye arabinoxylans consist of at least two different polymers or two fractions of the polymer. The major polymer structure (arabinoxylan I) has a xylan chain substituted at C-3 (of xylose residues) with arabinosyl residues, whereas the minor polymer structure (arabinoxylan II) contains terminal arabinoses doubly linked to C-2 and C-3 of the same xylose residues. The successful separation of the minor fraction containing only non-substituted and disubstituted xylose residues (Vinkx *et al.*, 1993) supports the hypothesis that two separate polymers exist in rye arabinoxylans (even though the monosubstituted polymer has not yet been isolated).

The purpose of the present study was to elucidate further the structural characteristics of water-soluble wheat-endosperm arabinoxylans. The heterogeneous nature of arabinoxylans (Hoffmann *et al.*, 1992*a*; Izydorczyk & Biliaderis, 1992, 1993) necessitated, in our view, fractionation of arabinoxylans and structural characterization of each fraction separately. From these studies, models for two structurally distinct arabinoxylan fractions are proposed.

MATERIALS AND METHODS

Isolation, purification, and fractionation of arabinoxylans

Water-soluble arabinoxylans from Canada western red spring (CWRS) wheat class (cv. Laura) were isolated and purified according to the procedure of Izydorczyk *et al.* (1990). Flour was blended with three volumes of distilled water. After centrifugation (8000 × *g*, 5 min), the supernatant was immediately heated (90°C, 5 min). The denatured proteins were removed by adsorption on Vega clay (Pembina Mountain Clay, Winnipeg, MB, Canada). The extract was incubated with porcine pancreas α-amylase (EC 3.2.1.1, Type I-A, Sigma Chemical Co., St. Louis, MO, USA) for 24 h and then dialysed against distilled water. The enzyme was inactivated with heat (95°C, 5 min). The treatment with the enzyme was repeated. The purified arabinoxylan was fractionated by a graded ammonium-sulphate-fractionation technique. The material was dissolved (0·2% w/v) in phosphate buffer (0·1 M, pH 7), and $(NH_4)_2SO_4$ was added slowly up to 55% saturation; the solution was allowed to stand overnight at 25°C. The precipitated polysaccharide was collected by centrifugation (4000 × *g*, 15 min) and filtration on a glass-fibre paper, redissolved in distilled H_2O, and dialysed until free of $(NH_4)_2SO_4$; this fraction was designated F55. The saturation level of $(NH_4)_2SO_4$ in the remaining filtrate was subsequently adjusted to 60%, 70%, 80%, and 100%, stepwise. The corresponding precipitated polysaccharide fractions are designated F60, F70, F80, and F100.

Chemical analysis

Protein content in arabinoxylan fractions was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as a standard. The monosaccharide composition of arabinoxylan fractions was determined by gas–liquid chromatography (GLC) Hewlett Packard Corporation, fused-silica column SP 2330, 30 × 0·75-mm i.d., 0·75-μm film thickness) of alditol acetates (Englyst *et al.*, 1982). Samples were hydrolysed with 1M H_2SO_4 for 2 h at 100°C. Allose was used as an internal standard. The content of feruloyl groups was determined spectrophotometrically by direct-absorbance measurements at 375 nm of freshly prepared solutions of arabinoxylans in 0·07M glycine–NaOH buffer (pH 10·0) by use of a molar-extinction coefficient of 31 600 (Fry, 1982).

Methylation of arabinoxylans was conducted according to the method of Ciucanu and Kerek (1984). The partially methylated alditol acetates were quantified by capillary GLC (fused-silica column SP 2330, 30 m × 0·75-mm i.d., 0·75-μm film thickness). Qualitative analysis of the partially methylated acetates was performed by coupled gas-liquid chromatography–mass spectrometry (GLC–MS) by use of a capillary column (SP 2330, 60 m × 0·25-mm i.d., 0·20-μm film thickness). Identification of the methylated alditol derivatives was possible by comparing the resultant spectra with those published (Carpita & Shea, 1988). The effective-carbon-response factors, as given by Sweet *et al.* (1975), were used for calculation of the molar quantities of permethylated products determined by GLC. Since 2-*O*-methyl-xylitol and 3-*O*-methyl-xylitol had very similar retention times, they were not resolved under the chromatographic conditions employed. However, their detection and quantification were achieved from the mass spectra by the integration of signals of fragment ions characteristic for these two derivatives, i.e. *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 129 were estimated to represent 20 and 25%, respectively, of the total ion current of the relevant derivatives. From these spectra, the ratio of C-3 to C-2 monosubstituted xylopyranose residues was calculated.

Periodate oxidation was performed by the repeated-oxidation/reduction procedure described by Aman and Bengtsson (1991). The oxidation and reduction procedures were repeated twice, and the final material was freeze-dried. The oxidized and reduced material was dissolved in 1 ml of 0·02M HCl and partially hydrolysed in a boiling-water bath for 20 min. The hydrolysates of the oxidized arabinoxylan were applied on a Bio-Gel P-2 (Bio-Rad Laboratories, Richmond, CA, USA) column (2·6 × 95 cm) and eluted with distilled, degassed H_2O (25 ml/h) at 25°C. Effluent fractions (2·5 ml) were collected and monitored for total carbohydrates by the phenol-sulphuric method (Dubois *et al.*, 1956).

Enzymic studies

Highly purified endo-β-D-xylanase (EC 3.2.1.8) from *Trichoderma viride* (specific activity 157 U/mg protein) devoid of α-L-arabinofuranosidase activity was purchased from MegaZyme (Aust.) Pty. Ltd (North Rocks, NSW, Australia). One unit of activity was defined as the amount of enzyme required to produce 1 μmole of product per minute under specified conditions (pH 4·5–5·0, temperature 40°C).

The degree of hydrolysis of arabinoxylan fractions by xylanase was calculated as the ratio of the amount of xylose-reducing sugar equivalent (Nelson–Somogyi method: Nelson, 1944; Somogyi, 1952) relative to the total carbohydrate content, and expressed as a percentage.

Arabinoxylan digests were obtained by incubation of arabinoxylan fractions (0·1% w/v in 0·1M acetate buffer, pH 4·5) with xylanase (10 U) for various time periods (from 5 min to 48 h) at 40°C. The enzymic reaction was stopped by rapid heating of the digests at 95°C for 10 min. The fragmentation products of arabinoxylan were separated (on an analytical scale) by high-performance-size-exclusion chromatography (HP-SEC) by use of a series of gel-permeation columns (KS 802–Ultrahydrogel 500-Ultrahydrogel 250-Ultrahydrogel 120; Waters, Milford, MA, USA) and a model 410 refractive-index detector (Waters, Milford, MA, USA). Retention times and peak areas were calculated with a Maxima 820 Chromatography Workstation software package (Waters, Milford, MA, USA). All samples were run isocratically at 70°C and at a flow rate of 0·8 ml/min with degassed sodium acetate buffer (0·1M, pH 4·5) as the eluent. All xylo-oligosaccharide (X_1–X_5) standards were purchased from MegaZyme (Aust.) Pty. Ltd (North Rocks, NSW, Australia). Maltoheptaose and maltohexaose were obtained from Boehringer Mannheim Canada Ltd (Laval, QU, Canada).

To obtain oligosaccharide fragments on a preparatory scale, arabinoxylan digests (obtained by incubation of 40 mg polysaccharide with 20 U of xylanase for 24 h) were applied to a Bio-Gel P-2 column (2·6 × 95 cm, Bio-Rad Labs, Richmond, CA, USA) and eluted with water (25 ml/h) at 25°C. Fractions (2·6 ml) were assayed for total carbohydrate content by the phenol-sulphuric method (Dubois *et al.*, 1956). From the digests of F55 and F100, appropriate fractions were pooled, and each pooled sample was freeze-dried. The procedure was repeated a number of times to obtain appreciable amounts of oligosaccharides. The Bio-Gel P-2 column was calibrated with a mixture of xylo-oligosaccharides. The oligosaccharide fractions were subjected to methylation analyses (conditions described above) and ^{1}H-NMR spectroscopy by using a Bruker AM 300 FT spectrometer operated at 27°C. The oligosaccharide fractions (2–5 mg) were dissolved in D_2O (1 ml). Chemical shifts (δ) were expressed in ppm downfield from internal sodium 4,4-dimethyl-4-silapentane-1-sulphonate (DSS) (Aldrich Chemical Co., Inc., Milwaukee, WI, USA) but were actually measured by reference to internal acetone (δ 2·225).

RESULTS AND DISCUSSION

Fractionation of arabinoxylans

Stepwise addition of ammonium sulphate to the solution of purified arabinoxylan resulted in five polysaccharide fractions. The yield and composition of each fraction are presented in Table 1. Differences in the molecular-size distribution among the fractions were revealed by limiting-viscosity, [η], measurements. Frac-

Table 1. Yield and composition of arabinoxylan fractions

Fraction	Yield[a] (%)	[η] (dl/g)	Proteins[b] (%)	Ferulic acid[c] (mg/g)
F55	37·5	5·77	0·71 ± 0·04	2·16 ± 0·05
F60	22·3	4·09	0·54 ± 0·05	0·95 ± 0·03
F70	23·6	3·50	0·52 ± 0·06	0·52 ± 0·02
F80	11·9	2·4	0·31 ± 0·06	0·35 ± 0·03
F100	4·7	1·1	0·25 ± 0·05	0·31 ± 0·03

[a]Based on total amount of material recovered. Data represent means of at least triplicate fractionation experiments; the coefficient of variation was less than 5% of the mean values in all cases.
[b]Lowry method; $n = 3 \pm SD$.
[c]Spectrophotometric measurements (375 nm) of solutions after adjustment of pH to 10·0 with glycine-NaOH; extinction coefficient 31 000 (Fry, 1982).

Table 2. Monosaccharide composition of arabinoxylan fractions

Fraction	Molar composition %			
	Ara	Xyl	Glc	Xyl/Ara
F55	32·2	63·9	3·9	1·98
F60	40·1	59·9	—	1·49
F70	44·2	55·8	—	1·25
F80	47·0	53·0	—	1·13
F100	47·6	52·4	—	1·10

tion F55 showed the highest limiting viscosity (5·77 dl/g) and was followed by F60, F70, and F80. An unusually low [η] was found for fraction F100. The content of proteins in arabinoxylan fractions was highest for F55 and then progressively decreased towards F100. Ferulic acid was found in all fractions, but most of it was confined to F55; this fraction contained more than twice the amount of ferulic acid found in the remaining samples. Monosaccharide composition and the molar ratio of arabinose:xylose:glucose are presented in Table 2. Arabinose and xylose were the main monosaccharide constituents of all fractions. Fraction F55 has the least-substituted xylan backbone, as indicated by the highest xylose-to-arabinose ratio. For the other fractions, this ratio progressively decreased from F55 to F100. In addition to xylose and arabinose, fraction F55 also contained small amounts of glucose.

Glycosidic-linkage analysis

Methylation analyses (Table 3) revealed several structural differences among the arabinoxylan fractions. All arabinoxylans contained terminal arabinose in the furanose form, as indicated by the presence of 2,3,5-Me_3-Ara. The relative amounts of this residue changed depending on the fraction; generally, there was a progressive increase in terminal Ara from F55 to F100.

The presence of small quantities of 3,5-Me_2-Ara, 2,5-Me_2-Ara, and 2,3-Me_2-Ara indicated that short arabinose side chains, extended through C-2, C-3, or C-5, might also be present in arabinoxylans. Their occurrence was especially prominent in fraction F100, where approximately 20% of all arabinoses appeared in short side chains.

Most of the xyloses in arabinoxylan were present as unsubstituted residues, as evidenced by the high content of 2,3-Me_2-Xyl. The amount of this derivative was highest in F55 and F60 fractions and then decreased stepwise towards F100. The amount of monosubstituted xylose residues was twice as high in F55 as in the remaining samples. In addition to C-3 monosubstituted xylose residues, the mass spectra of the alditol derivatives also indicated the presence of C-2 monosubstituted residues (3-Me-Xyl). The presence of the latter in arabinoxylans has been rarely reported in the literature, which is probably due to the fact that alditol acetates of 2-Me-Xyl and 3-Me-Xyl have identical retention times under most chromatographic conditions, and these two derivatives cannot, therefore, be resolved. Their detection and quantification were only possible from the

Table 3. Monosaccharide residue and linkage distribution of arabinoxylan fractions

Alditol acetate	Mode of linkage	RT[a]	Molar composition (%)				
			F55	F60	F70	F80	F100
2,3,5 Me_3Ara	Ara*f*-(1→	0·66	28·5	35·4	40·0	44·0	38·3
3,5 Me_2Ara	→2)-Ara*f*-(1→	1·05	0·7	1·1	1·3	1·7	5·0
2,5 Me_2Ara	→3)-Ara*f*-(1→	1·17	—	0·4	0·6	0·6	0·7
2,3 Me_2Ara	→5)-Ara*f*-(1→	1·34	2·2	1·4	1·8	1·8	4·4
2,3,4 Me_3Xyl	Xyl*p*-(1→	0·85	1·1	1·2	1·8	2·4	4·8
2,3 Me_2Xyl	→4)-Xyl*p*-(1→	1·44	36·9	37·3	30·2	25·0	20·0
2 MeXyl+ 3MeXyl	→3,4)-Xyl*p*-(1→ + →2,4)-Xyl*p*-(1→	2·08	20·8 (23·4)[b]	10·4 (9·9)	11·4 (8·5)	10·5 (6·0)	10·3 (4·1)
Xyl	→2,3,4)-Xyl*p*-(1→	2·76	9·8	12·8	12·9	14·0	16·5

[a]Retention times of the corresponding alditol acetates on SP2330 relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol.
[b]Numbers in parentheses are ratios of C-3 to C-2 monosubstituted Xyl*p*.

mass spectra by the integration of signals of fragment ions characteristic for these two derivatives. As can be gathered from Table 3, the ratio of C-3 to C-2 mono-substituted xylose residues (given in parentheses) decreased consistently from F55 to F100, which revealed that the low-molecular-weight fractions had a much higher content of xylose residues substituted exclusively at C-2. The presence of xylose residues carrying two substituents, at C-2 and C-3 simultaneously, is evident from the occurrence of alditol acetate of Xyl. An increase in the amount of doubly substituted xyloses was observed with a decrease in the molecular size of the fractions (from F55 to F100). Another trend observed was in the amount of terminal non-reducing end xyloses, which predictably increased from the high- to the low-molecular-weight arabinoxylan fractions.

Periodate oxidation

The arrangement of branching in arabinoxylan fractions was investigated by the periodate-oxidation technique. Table 4 shows the relative amounts of glycerolxylosides obtained after periodate oxidation and subsequent mild hydrolysis of some arabinoxylan fractions. In the high-molecular-weight arabinoxylan fractions, most of the branched material was present as single isolated residues (>50%) or as blocks of two contiguous xylose residues. Much less frequently, arabinoses were attached to three contiguous xylopyranosyl residues. In the low-molecular-weight arabinoxylan fractions, the occurrence of blocks containing three contiguously substituted xyloses increased substantially; in addition, the presence of the higher homologous was also observed.

Degree of hydrolysis

The progress of enzymic hydrolysis of arabinoxylan fractions with time was followed by measuring the amount of reducing sugars released (Fig. 1). The rate (indicated by the sharp increase in reducing sugars during the initial period of hydrolysis, i.e. <15 min) and extent (indicated by the final amount of reducing sugars released after 48 h) of hydrolysis were highest for fraction F55 and progressively decreased from F55 to F100.

Table 4. Relative distribution[a] (%) of glycerolxylosides of some arabinoxylan fractions after periodate oxidation and mild acid hydrolysis

Fraction	Tetramer	Trimer	Dimer	Monomer
F55	—	7·3	36·5	56·2
F70	0·5	10·5	35·4	53·6
F80	5·7	14·5	36·0	43·8
F100	7·0	15·3	36·5	41·5

[a]Expressed as percentage of total carbohydrates.

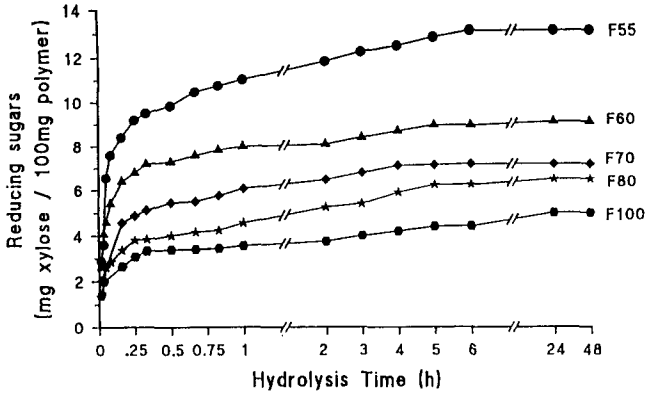


Fig. 1. Progress of enzymic hydrolysis of arabinoxylan fractions with time. Arabinoxylan fractions (0·05% w/v in acetate buffer, 0·1M, pH 4·5) were treated with 10 U xylanase at 40°C. The reaction was monitored by determination of reducing sugars.

The degree of hydrolysis was 11·5, 8·3, 7·0, 6·7, and 5·4% for F55, F60, F70, F80, and F100, respectively.

Distribution of oligosaccharides

The mixtures of oligosaccharides, obtained upon xylanase digestion of the arabinoxylan fractions for various time periods, were fractionated by HP-SEC. Assignments of eluted oligosaccharides as monomers, dimers, and higher oligosaccharides were based on the elution times of standard xylo-oligosaccharides. These assignments do not imply that the fractionated oligomers were pure xylo-oligosaccharides. Instead, a mixture of arabinosylxylo-oligomers was found, and the individual fractions were assigned according to their elution times in relation to the respective linear xylo-oligosaccharide standards. The patterns of fragments with the degree of polymerization (DP) $\leqslant 6$ found in the digests of individual fractions suggest that the high-molecular-weight fractions (F55 and F60) were more rapidly degraded by the enzyme and yielded relatively high proportions of small fragments (DP $\leqslant$ 6). In contrast, the low-molecular-weight fractions (F70, F80, and F100) resisted enzymic hydrolysis and produced rather small amounts of DP $\leqslant$ 6 materials. Moreover, the rate of increase of individual fragments with increasing extent of hydrolysis (from 15 min to 48 h) was comparatively very slow for the low-molecular-weight fractions.

The amounts of individual oligosaccharides with DP $\leqslant$ 6 in the xylanase digests of arabinoxylan fractions, obtained after 30 min and 24 h of incubation, are given in Table 5. For all fractions, 30 min of incubation with xylanase resulted in digests in which very little material was present as oligosaccharides with DP $\leqslant$ 6. There were, however, substantial differences in the amounts of these small fragments, e.g. fraction F55 yielded *c.* 24% of material with DP $\leqslant$ 6, whereas F100 yielded only *c.* 9%. In the digests of F60, F70, F80, and

Table 5. Abundance[a] of mono- and oligosaccharides in xylanase digests of arabinoxylan fractions

	F55	F60	F70	F80	F100
30-min Hydrolysis					
X_1	1·4	—	—	—	—
X_2	4·1	4·1	2·8	2·8	2·6
X_3	1·6	0·9	0·7	—	—
X_4	0·8	0·7	0·7	0·7	0·6
X_5	12·4	3·0	2·8	1·9	1·4
X_6	4·0	2·5	3·5	4·0	4·0
Total DP ⩽ 6	24·3	11·2	10·5	9·4	8·6
24-h Hydrolysis					
X_1	4·2	3·6	2·5	1·9	1·9
X_2	8·0	6·1	3·6	3·4	3·0
X_3	—	—	—	—	—
X_4	3·3	1·8	1·0	0·9	0·9
X_5	20·1	8·0	5·0	2·7	2·7
X_6	9·0	6·0	6·0	7·5	7·8
Total DP ⩽ 6	44·6	25·5	18·1	16·4	16·3

[a]Expressed as percentage of total carbohydrate material.

F100, no monosaccharides were detected, and only fractions F55, F60, and F70 gave small amounts of trisaccharides. Moreover, the digests of F55 and F60 contained relatively high proportions of pentasaccharides, whereas, in the digests of F70, F80, and F100, hexasaccharides were the predominant species. A 24-h treatment of arabinoxylan fractions with xylanase substantially increased the amount of DP ⩽ 6 oligosaccharides in all fractions. The digest of F55 contained relatively high amounts of low-DP oligomeric products (44%). In the digests of the remaining fractions, however, rather small amounts of DP ⩽ 6 oligosaccharides were still obtained, which implies more densely substituted xylan backbones. After 24 h of incubation with the enzyme, monosaccharides were found in all fractions: the highest amount in the digest of F55, the lowest in that of F100. Relatively high amounts of disaccharides were found in F55 as compared with other fractions. In contrast to the 30-min digestion, no trisaccharides were detected in any of the 24-h digests, which suggests that this oligomer was eventually hydrolysed to smaller fragments. As in the 30-min incubation, pentasaccharides were the preponderant species in F55 and F60, whereas hexasaccharides predominated in the digests of F70, F80, and F100. This trend was evident not only after 30-min and 24-h digestions but also at other hydrolysis periods.

Figures 2 and 3 illustrate the entire profiles of xylanase digests (24 h) of two arabinoxylan fractions, obtained by fractionation of the digestion products on Bio-Gel P-2. In addition to differences in the amounts of DP ⩽ 6 fragments among the fractions, a few other points became apparent from these profiles. The higher-

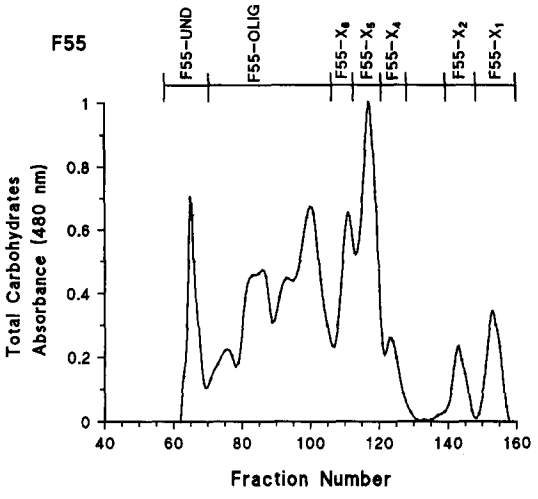


Fig. 2. Elution profile of arabinoxylan digest (fraction F55, 40 mg of polysaccharide incubated with 20 U xylanase for 24 h) on Bio-Gel P-2 (2·6 × 95 cm, eluted with water at 25 ml/h, 25°C). Several fractions, as designated, were pooled from this digest.

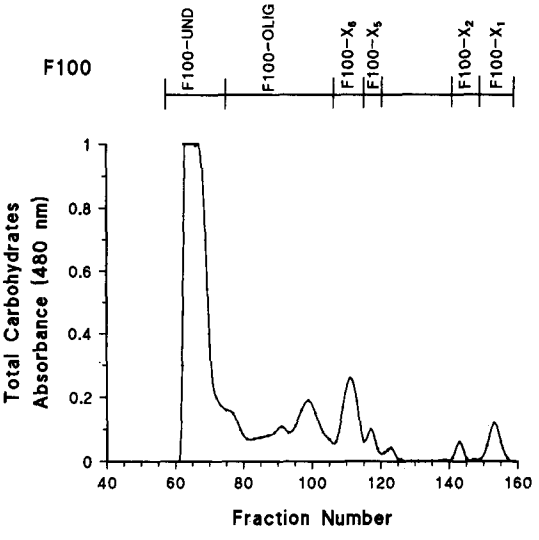


Fig. 3. Elution profile of arabinoxylan digest (fraction F100, 40 mg of polysaccharide incubated with 20 U xylanase for 24 h) on Bio-Gel P-2 (2·6 × 95 cm, eluted with water at 25 ml/h, 25°C). Several fractions, as designated, were pooled from this digest.

molecular-weight fractions F55 and F60 (not shown) yielded upon extensive enzyme hydrolysis relatively high amounts of higher oligosaccharides (in the case of F55 *c.* 40%), which eluted between the undigested polymeric material (V_o) and the DP = 6 fragment, and rather small amounts of the gel-excluded (void-volume) polymeric fraction (*c.* 15% for F55). The low-molecular-weight fraction, F100 (also F70 and F80, not shown) by contrast, was not easily degraded even after prolonged hydrolysis and consequently appeared mainly (75%) as an undigested polymeric fraction (eluted in the vicinity of the void volume).

Table 6. Monosaccharide residue and linkage distribution in fractions F55-UND, F100-UND, F55-OLIG, and F100-OLIG

Alditol acetate	Mode of linkage	F55-UND	F100-UND	F55-OLIG	F100-OLIG
2,3,5 Me_3Ara	Ara*f*-(1→	38·0	38·0	35·0	37·0
3,5 Me_2Ara	→2)-Ara*f*-(1→	—	5·1	—	4·0
2,5 Me_2Ara	→3)-Ara*f*-(1→	—	1·0	—	1·0
2,3 Me_2Ara	→5)-Ara*f*-(1→	2·2	4·7	1·6	5·5
2,3,4 Me_3Xyl	Xyl*p*-(1→	4·5	6·0	7·0	6·5
2,3 Me_2Xyl	→4)-Xyl*p*-(1→	27·0	18·0	28·7	20·0
2 MeXyl+ 3MeXyl	→3,4)-Xyl*p*-(1→ + →2,4)-Xyl*p*-(1→	12·3 (9·2)[a]	10·2 (2·1)	17·9 (tr)[b]	11·0 (tr)[b]
Xyl	→2,3,4)-Xyl*p*-(1→	16·0	17·0	9·8	16·0

[a] Numbers in parentheses are ratios of C-3 to C-2 monosubstituted Xyl*p*.
[b] Trace.

Glycosidic-linkage analysis of enzymic fragments

In order to elucidate further the differences in the primary structure of arabinoxylan fractions, in this section, only the two most extreme fractions (F55 and F100) are considered.

From the F55 and F100 hydrolysates, undigested (void-volume) materials (denoted as F55-UND and F100-UND) were pooled and analysed for glycosidic-linkage composition by methylation analysis (Table 6). In general, there were significant differences between F55 and F55-UND. In F55-UND as in F55, arabinose predominated as terminal furanosyl residues (2,3,5-Me_3-Ara); however, the gel-excluded, undigested fractions contained relatively higher amounts of this monosaccharide. Furthermore, the void fraction had a substantially lower content of unsubstituted (2,3-Me_2-Xyl) and singly (2-Me-Xyl + 3-Me-Xyl) substituted xyloses. Interestingly, the ratio of C-3 to C-2 monosubstituted xyloses in F55 was two-and-a-half-fold higher than that in F55-UND, which indicated a much higher content of C-2 monosubstituted xylose residues in the undigested material. Moreover, the void fraction was approximately two-fold enriched in xyloses carrying two substituent residues relative to the parent F55 fraction. In contrast, very few differences were detected between F100 and F100-UND. Quite understandably, the material of F100-UND was of lower molecular weight as indicated by the higher content of non-reducing end xyloses (2,3,4-Me_3-Xyl). In addition, F100-UND had a slightly lower amount of unsubstituted xylose residues than the parent. The most pronounced change was in the relative amounts of C-3 and C-2 monosubstituted Xyl*p*; in F100-UND, the ratio of C-3 to C-2 monosubstituted xylose residues was two-fold lower than in F100.

Periodate-oxidation analysis of the undigested fragments showed that, in F55-UND, the branched xylose residues were present as isolated units (46·2%) or as blocks of two (44·0%) or three (9·8%) contiguously substituted residues. In F100-UND, isolated xylose units constituted 29·1%, whereas blocks of two, three, and four residues made up 35·3, 23·4, and 12·2%, respectively, of the total substituted material.

Table 6 also lists the linkage compositions of the oligomeric fractions, F55-OLIG and F100-OLIG. Although the digest of F100 contained very little of the oligomeric material (7%), large amounts of digest were chromatographed, and the appropriate fractions were pooled and analysed. The bulk of F55-OLIG contained less terminal arabinofuranosyl and fewer doubly branched xylopyranosyl residues, but more singly branched xylopyranosyl residues than the F55-UND. The higher content of terminal non-reducing end xyloses (2,3,4-Me_3-Xyl) reflects the much lower molecular weight of F55-OLIG than of F55-UND. In contrast, fraction F100-OLIG resembled in its linkage composition the undigested fraction F100-UND, except for some differences in the molecular weight (as reflected by the amount of 2,3,4-Me_3-Xyl). Considering the resemblance of fraction F100-OLIG to F100-UND and the very small content of the former, it can be concluded that the digest of F100 was largely lacking the equivalent of F55-OLIG intermediate oligomeric material.

The structural data for F55-X_6, F55-X_5, F100-X_6, and F100-X_5 obtained from monosaccharide and methylation analyses are presented in Table 7. It is clear, on the basis of the evidence presented in this table, that the oligosaccharides F55-X_6 and F100-X_6 were a xylotetrasaccharide doubly substituted at C-2 and C-3 of an internal Xyl*p* by Ara*f* residues. Furthermore, the oligosaccharides F55-X_5 and F100-X_5 contained the same xylotetrasaccharide but were substituted at C-3 only of an internal Xyl*p* by a single Ara*f* residue.

^{1}H-NMR spectroscopy of small oligosaccharide fragments

In order to elucidate further the primary structure of small (DP $\leqslant$ 6) oligosaccharides, obtained upon diges-

Table 7. Monosaccharide residue and linkage distribution in fractions F55-X_6, F55-X_5, F100-X_6, and F100-X_5

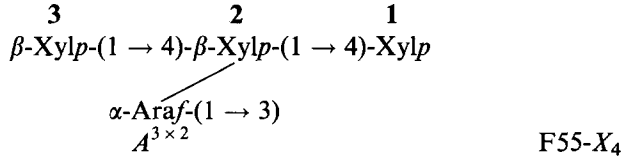
	F55-X_6	F55-X_5	F100-X_6	F100-X_5
Monosaccharide[a]				
Ara	2·0	1·0	2·0	1·0
Xyl	4·1	4·0	4·2	4·0
Alditol acetate[b]				
2,3,5 Me_3Ara	2·3	1·2	2·3	1·2
2,3,4 Me_3Xyl	1·1	1·2	1·2	1·2
2,3 Me_2Xyl	1·4	1·2	1·4	1·3
2 MeXyl	0·2	1·0[c]	0·1	1·0[c]
Xyl	1·0[c]	—	1·0[c]	—

[a] Expressed as molar ratio relative to Ara.
[b] Because of the relatively high volatility of alditol acetate of 1,2,3,5-Me_4-Xyl, only traces of this residue were found upon GLC of the methylated mixture.
[c] Taken as 1·0.

tion of fractions F55 and F100 with β-xylanase, the appropriate fractions were pooled and analysed by ^{1}H-NMR spectroscopy. All assignments were made on the basis of data published recently in the literature (Hoffmann *et al.*, 1991*b*, 1992*b*; Gruppen *et al.*, 1992). Based on these data, the ^{1}H-NMR spectrum of F55-X_1 (not shown) corresponds to signals representing a single xylose residue. The doublet at 5·18 ppm can be unequivocally assigned to H-1 of α-Xyl*p* and the doublet at 4·58 ppm to H-1 of β-Xyl*p*.

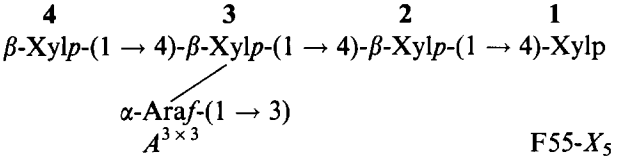
The spectrum of fraction F55-X_2 was typical of xylobiose. In addition to the two doublets corresponding to the anomeric protons of the reducing end xylose (Xyl*p*-1 α and β anomeric forms), the doublet at ~4·46 ppm is due to anomeric protons of the non-reducing end xylose: β-Xyl*p*-(1 → 4)-Xyl*p*-α (δ 4·47), β-Xyl*p*-(1 → 4)-Xyl*p*-β (δ 4·45).

The intensities of the signals for anomeric protons in the ^{1}H-NMR spectrum of F55-X_4 indicated the presence of a single arabinosylxylotriose. The H-1 resonance of α-Ara*f* ($A^{3\times2}$) is responsible for the most downfield signal at 5·39 ppm. The other signals originate from the three xylose residues: H-1 of α-Xyl*p*-1 (doublet at δ 5·18), H-1 of β-Xyl*p*-1 (doublet at δ 4·58), H-1 of β-Xyl*p*-2 (doublet at δ 4·50), and H-1 of β-Xyl*p*-3 (doublet at δ 4·42).



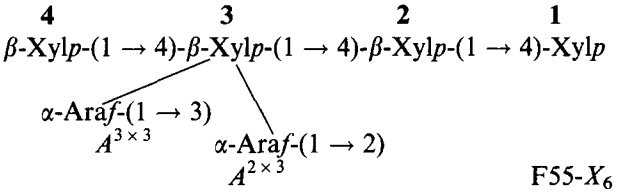
F55-X_4

The intensities of the H-1 signals for F55-X_5 matched the ^{1}H-NMR spectrum of an arabinosylxylotetraose. The signal at 5·39 ppm is assigned to H-1 of α-Ara*f* ($A^{3\times3}$). The anomeric protons of the four xyloses were responsible for the following signals: α-Xyl*p*-1 (doublet at δ 5·18), H-1 of β-Xyl*p*-1 (doublet at δ 4·58), β-Xyl*p*-3 (doublet at δ 4·52), β-Xyl*p*-2 (doublet at 4·47), β-Xyl*p*-4 (doublet at δ 4·42).



F55-X_5

The arabinose anomeric signals for F55-X_6 clearly indicated the presence of a diarabinosylxylotetraose fragment. Two signals at δ 5·27 and δ 5·22 can be assigned to anomeric protons of two arabinose residues, α-Ara*f* ($A^{3\times3}$) and α-Ara*f* ($A^{2\times3}$), respectively, linked to the same xylose residue. A low signal at δ 5·39 indicated the presence of a very small quantity (the integration value was very small compared with the signals of other arabinoses) of arabinosylxylotetraose (structure F55-X_5). The anomeric protons of the four xyloses were responsible for the following signals: α-Xyl*p*-1 (doublet at δ 5·18), H-1 of β-Xyl*p*-1 (doublet at δ 4·58), β-Xyl*p*-3 (doublet at δ 4·63), β-Xyl*p*-2 (doublet at 4·46), β-Xyl*p*-4 (doublet at δ 4·42).



F55-X_6

The spectra for F100-X_1, F100-X_2, F100-X_5, and F100-X_6 indicated the presence of xylose, xylobiose, arabinosyltetraose, and diarabinosyltetraose, respectively. The chemical-shift data matched exactly those of F55-X_1, F55-X_2, F55-X_5, and F55-X_6, which are discussed above.

Detailed analyses of the small oligosaccharide fragments obtained after digestion of arabinoxylan fractions with β-xylanase from *Trichoderma viride* seems to confirm the fact that this enzyme requires at least three contiguously unsubstituted xylose residues for its hydrolytic action. This conclusion is based on the fact that, in most arabinosylxylo-oligosaccharides detected in the digests, the substituted xylose (singly or doubly) was preceded by one unsubstituted residue from the non-reducing end and followed by two unsubstituted residues from the reducing end (as in fragments F55-X_5, F55-X_6, F100-X_5, F100-X_6). The presence of fragments such as F55-X_4 with only one unsubstituted xylose on the both sides of the branched residue suggests that only two contiguously unsubstituted xyloses are needed between branched residues for the enzyme to act. The fact, however, that the number of fragments such as F55-X_4 was very low in relation to fragments such as F55-X_5 (and others mentioned above) prompts the conclusion that F55-X_4 originated from regions containing a relatively high amount of contiguously unsubstituted xylose clusters in the immediate vicinity of F55-X_4 (e.g. [Xyl-Xyl(Ara)-Xyl]-[Xyl_{2-3}]). The

presence of arabinosylxylo-oligosaccharides, such as F55-X_5, with one and two unsubstituted xylose residues on both sides of the substituted xylose residue and the detection of unsubstituted xylotriose fragments (Table 5) in the arabinoxylan digests, suggest that clusters of at least six contiguously unsubstituted xylose units are possible in some regions of the arabinoxylan molecule (the occurrence of longer unsubstituted clusters cannot be excluded).

Structural models for arabinoxylan fractions

On the basis of the results presented so far, it can be concluded that significant differences in the distribution of arabinoses along the xylan backbone exist between the two structurally extreme arabinoxylan fractions F55 and F100. Fraction F55 seems to be built up of three regions differing in the distribution of arabinoses on the xylan core. Region I_{55}, corresponding to the undigested fraction (F55-UND), contains high amounts of terminal arabinoses. Almost all of these arabinoses are linked to xylose residues doubly substituted at C-2 and C-3, i.e. region I_{55} contains relatively more doubly than singly substituted xyloses. Periodate-oxidation studies also suggest that the xylose residues carrying arabinose substituents are isolated, in pairs, or even as three contiguously substituted residues; however, the occurrence of the former arrangement is the most frequent and the latter the least. Although no direct evidence is available from these studies, owing to steric hindrance, clusters of three contiguous substituted xylose residues are unlikely to contain all xyloses doubly substituted with arabinose residues. Blocks of two neighbouring disubstituted xylose residues have been reported (Hoffmann *et al.*, 1992*b*). Fragments corresponding to region I_{55} are of relatively high molecular weight and are inaccessible to the xylanase because of the high frequency of arabinose branches. Region I_{55} constitutes approximately 15% of the fraction F55. Region II_{55}, corresponding to the oligomeric fraction (F55-OLIG), still contains relatively high amounts of terminal arabinoses, but the majority of them are linked to xylose residues at C-3 only, rather than at both C-2 and C-3 positions as in F55-UND. This region contains fragments much shorter than F55-UND and constitutes approximately 40% of the F55 fraction. Finally, region III_{55} (45%), the most smooth, i.e., containing high amounts of contiguous (at least up to six, but possibly more) unsubstituted xylose residues, is the most accessible to the xylanase hydrolytic action. Consequently, this region yields, upon hydrolysis, small oligosaccharide fragments: xylose, xylobiose, xylotriose, arabinosylxylotriose (F55-X_4), arabinosylxylotetraose (F55-X_5), and diarabinosylxylotetraose (F55-X_6). Among the arabinosylxylo-oligosaccharides, the pentasaccharide (F55-X_5) preponderates. Fragments of the type III_{55} are probably located between the two more highly substituted regions I_{55} and II_{55}. The above structural domains of arabinoxylan fraction F55 are depicted in Fig. 4.

Fraction F100, by contrast, appears to be made up

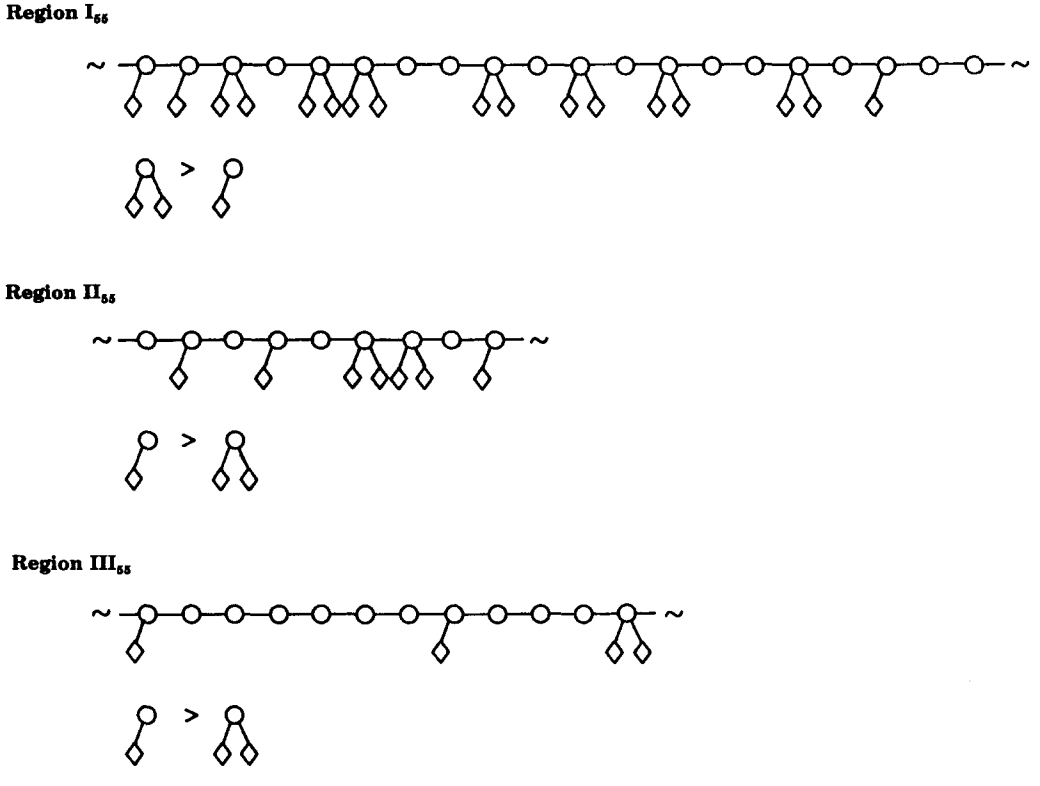


Fig. 4. Tentative model for arabinoxylan fraction F55.

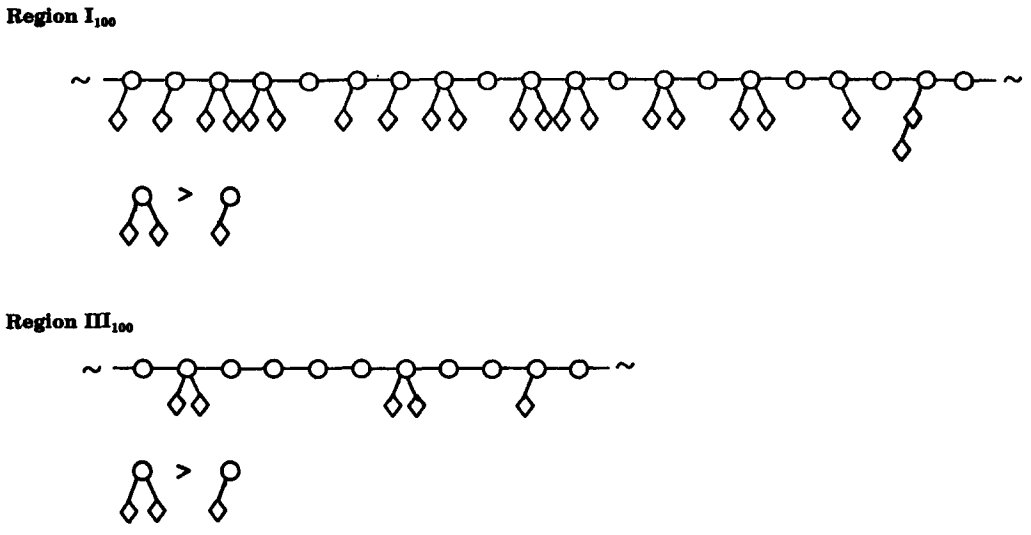


Fig. 5. Tentative model for arabinoxylan fraction F100.

mainly (75%) from the most highly substituted region, I_{100}. This region is similar to the region I_{55}. It contains high amounts of terminal arabinoses linked to xylose residues at C-2 and C-3. Unlike region I_{55}, it contains relatively high amounts of C-2 monosubstituted xyloses as well as short arabinose side chains. Furthermore, in this region, the presence of four contiguously substituted xylose residues is highly possible, although most of the substituted xyloses seem to exist in small isolated clusters of one, two, and three residues, as evidenced by the periodate-oxidation data. The equivalent of region II_{55} seems to be absent in fraction F100. Region III_{100} amounts to only 18% of the entire F100 and, similarly to III_{55} is highly susceptible to enzymic hydrolysis, which yields small oligosaccharides (DP $\leqslant$ 6). However, among these oligomers, the hexasaccharide (F100-X_6) with one internal doubly substituted xylose residue predominates over the pentasaccharide (F100-X_5) segment with one singly substituted xylose found more frequently in III_{55}. The domains of F100 are depicted in Fig. 5.

In conclusion, the data of the present study on various polymeric fractions of water-soluble arabinoxylans extracted from CWRS wheat class (cv. Laura) pointed to a more complex structural organization and diversity of these cell-wall polysaccharides than were suggested previously (Goldschmid & Perlin, 1963; Hoffmann *et al.*, 1992*a*). A range of polymeric structures, varying between the two extreme models of F55 and F100, seems to constitute the water-soluble arabinoxylan present in wheat endosperm. Variations in structures are very likely due to differences in the ratios of region I:region II:region III as well as in the compositions of regions I and III. The model proposed here for the low-molecular-weight, highly branched fraction F100 shows some resemblance to the model presented by Gruppen (1992) for alkali-extractable wheat arabinoxylans. In both, two-region models are proposed, where highly branched regions are enriched in C-2,3 di- and C-2 monosubstituted xyloses, with the latter units absent or considerably diminished in the less-branched regions.

ACKNOWLEDGMENT

The support from the Natural Sciences and Engineering Research Council of Canada is gratefully acknowledged.

REFERENCES

Aman, P. & Bengtsson, S. (1991). Periodate oxidation and degradation studies on the major water-soluble arabinoxylan in rye grain. *Carbohydr. Polym.*, **15**, 405–14.

Aman, P., Westerlund, E. & Bengtsson, S. (1990). Studies on pentosans in flour, dough and bread fractions. In *Dietary Fibre: Chemical and Biological Aspects*, ed. D.A.T. Southgate, K. Waldron, I.T. Johnson, and G.R. Fenwick. Royal Society of Chemistry. Cambridge, UK, pp. 56–60.

Biliaderis, C.G. & Izydorczyk, M.S. (1992). Observation on retrogradation of starch polymers in the presence of wheat and rye arabinoxylans. In *Gums and Stabilisers for the Food Industry*, eds G.O. Phillips, P.A. Williams & D.J. Wedlock. IRL Press, Oxford, UK, pp. 227–30.

Brillouet, J.-M. & Joseleau, J.-P. (1987). Investigation of the structure of a heteroxylan from the outer pericarp (beeswing bran) of wheat kernel. *Carbohydr. Res.*, **159**, 109–26.

Carpita, N.C. & Shea, E.M. (1989). Linkage structure of carbohydrates by gas chromatography–mass spectrometry (GC–MS) of partially methylated alditol. In *Analysis of Carbohydrates by GLC and MS*, eds C.J. Biermann & G.D. MacGinnis. CRL Press, Boca Raton, FL, USA, pp. 157–216.

Ciucanu, I. & Kerek, F. (1984). A simple and rapid method for the permethylation of carbohydrates. *Carbohydr. Res.*, **131**, 209–17.

Delcour, J.A., Vanhamel, S. & Hoseney, R.C. (1991). Physicochemical and functional properties of rye nonstarch polysaccharides. II: Impact of a fraction containing water-soluble pentosans and proteins on gluten-starch loaf volumes. *Cereal Chem.*, **68,** 72–6.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Anal. Chem.*, **28,** 350–6.

Ebringerova, A., Hromadkova, Z., Petrakova, E. & Hricovini, M. (1990). Structural features of a water-soluble L-arabino-D-xylan from rye bran. *Carbohydr. Res.*, **198,** 57–66.

Ebringerova, A., Hromadkova, Z., Alfoldi, J. & Berth, G. (1992). Structural and solution properties of corn cob heteroxylans. *Carbohydr. Polym.*, **19,** 99–105.

Englyst, H.N., Wiggins, M.S. & Cummings, J.H. (1982). *Analyst*, **107,** 307–18.

Ewald, C.M. & Perlin, A.S. (1959). The arrangement of branching in an arabinoxylan from wheat flour. *Can. J. Chem.*, **37,** 1254–9.

Fry, S.C. (1982). Phenolic components of the primary cell wall. *Biochem. J.*, **203,** 493–6.

Goldschmid, H.R. & Perlin, A.S. (1963). Interbranch sequences in the wheat arabino-xylan. *Can. J. Chem.*, **41,** 2272–7.

Gruppen, H. (1992). Structural characteristics of wheat flour arabinoxylans. Thesis, Wageningen Agricultural University, The Netherlands.

Gruppen, H., Hoffmann, R.A., Kormelink, F.J.M., Voragen, A.G.J., Kamerling, J.P. & Vliegenthart, J.F.G. (1992). Characterisation by ^{1}H-NMR spectroscopy of enzymically derived oligosaccharides from alkali-extractable wheat-flour arabinoxylan. *Carbohydr. Res.*, **233,** 45–64.

Gudmundsson, M., Eliasson, A.-C., Bengtsson, S. & Aman, P. (1991). The effects of water soluble arabinoxylan on gelatinization and retrogradation of starch. *Starke*, **43,** 5–10.

Hoffmann, R.A., Roza, M., Maat, J., Kamerling, J.P. & Vliegenthart, J.F.G. (1991*a*). Structural characteristics of the cold-water-soluble arabinoxylans from the white flour of the soft wheat variety Kadet. *Carbohydr. Polym.*, **15,** 415–30.

Hoffmann, R.A., Leeflang, B.R., Debarse, M.M.J., Kamerling, J.P. & Vliegenthart, J.F.G. (1991*b*). Characterisation by ^{1}H-n.m.r. spectroscopy of oligosaccharides, derived from arabinoxylan of white endosperm of wheat, that contain the elements → 4)[α-L-Ara*f*-(1 → 3)]-β-D-Xyl*p*-(1 → or → 4)[α-L-Ara*f*-(1 → 2)][α-L-Ara*f*-(1 → 3)]-β-D-Xyl*p*-(1 →. *Carbohydr. Res.*, **221,** 63–81.

Hoffmann, R.A., Kamerling, J.P. & Vliegenthart, J.F.G. (1992*a*). Structural features of a water-soluble arabinoxylan from the endosperm of wheat. *Carbohydr. Res.*, **226,** 303–11.

Hoffmann, R.A., Geijtenbeek, T., Kamerling, J.P. & Vliegenthart, J.F.G. (1992*b*). ^{1}H-n.m.r. study of enzymically generated wheat-endosperm arabinoxylan oligosaccharides: structure of hepta- to tetradeca-saccharides containing two- or three-branched xylose residues. *Carbohydr. Res.*, **223,** 19–44.

Izydorczyk, M.S. & Biliaderis, C.G. (1992). Influence of structure on the physicochemical properties of wheat arabinoxylan. *Carbohydr. Polym.*, **17,** 237–47.

Izydorczyk, M.S. & Biliaderis, C.G. (1993). Structural heterogeneity of wheat endosperm arabinoxylans. *Cereal Chem.*, **70,** 641–6.

Izydorczyk, M.S., Biliaderis, C.G. & Bushuk, W. (1990). Oxidative gelation studies of water-soluble pentosans from wheat. *J. Cereal Sci.*, **11,** 153–69.

Izydorczyk, M.S., Biliaderis, C.G. & Bushuk, W. (1991). Physical properties of water-soluble pentosans from different wheat varieties. *Cereal Chem.*, **68,** 145–50.

Jelaca, S.L. & Hlynka, I. (1971). Water-binding capacity of wheat flour crude pentosans and their relation to mixing characteristics of dough. *Cereal Chem.*, **48,** 211–22.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, **193,** 265–75.

Meuser, F. & Suckow, P. (1986). Non-starch polysaccharides. In *Chemistry and Physics of Baking*, eds J.M.V. Blanshard, P.J. Frazier & T. Galliard. Royal Society of Chemistry, London, pp. 46–62.

Michniewicz, J., Biliaderis, C.G. & Bushuk, W. (1991). Effect of added pentosans on some physical and technological characteristics of dough and gluten. *Cereal Chem.*, **68,** 252–8.

Nelson, N. (1944). A photometric adaptation of the Somogyi method for the determination of glucose. *J. Biol. Chem.*, **153,** 375–80.

Patil, S.K., Tsen, C.C. & Lineback, D.R. (1975). Water-soluble pentosans of wheat flour. I: Viscosity properties and molecular weights estimated by gel filtration. *Cereal Chem.*, **52,** 44–56.

Perlin, A.S. (1951). Structure of the soluble pentosans of wheat flours. *Cereal Chem.*, **28,** 382–93.

Somogyi, N. (1952). Determination of blood sugar. *J. Biol. Chem.*, **195,** 19–23.

Sweet, D.P., Shapiro, R.H. & Albersheim, P. (1975). Quantitative analysis by various G.L.C. response-factor theories for partially methylated and partially ethylated alditol acetates. *Carbohydr. Res.*, **40,** 217.

Vietor, R.J., Angelino, S.A.G.F. & Voragen, A.G.J. (1992). Structural features of arabinoxylans from barley and malt cell wall material. *J. Cereal Sci.*, **15,** 213–22.

Vinkx, C.J.A., Reynaert, H.R., Grobet, P.J. & Delcour, J.A. (1993). Physicochemical and functional properties of rye nonstarch polysaccharides. V: Variability in the structure of water-soluble arabinoxylans. *Cereal Chem.*, **70,** 311–17.

Westerlund, E., Andersson, R., Aman, P. & Theander, O. (1990). Effects of baking on water-soluble non-starch polysaccharides in white bread fractions. *J. Cereal Sci.*, **12,** 33–42.