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Investigation of the distribution of arabinose residues on the xylan backbone of water-soluble arabinoxylans from wheat flour

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Abstract

Homogeneous water-soluble non-feruloylated arabinoxylan fractions from wheat flour (cv. Soissons) have been studied for their fine structure. Arabinosyl residue distribution on arabinoxylan chains was investigated through periodate oxidation and enzymic hydrolysis methods. A double oxidation and reduction sequence was used to effect complete oxidation of arabinoxylans and an hydrolysis time of 180 min was used for Smith degradation with HCl 0.02 M at 100 °C. Periodate oxidation, reduction and mild acid hydrolysis produced xylosides containing up to six residues. Analysis by HPAEC of the xylosides liberated by mild acid hydrolysis revealed that an increase in arabinose to xylose ratio is expressed on the polymer by an increase in length of substituted regions and greater proportions of them. Enzymic hydrolysis with a xylanase from *Aspergillus aculeatus* of lowly and highly substituted fractions revealed great differences in degradation extent. Random distribution of arabinose residues along the xylan backbone was computed and compared to experimental results.

Keywords: Arabinoxylans; Smith degradation; Periodate oxidation; Endoxylanase treatment

1. Introduction

Arabinoxylans (AX) are non-starch polysaccharides that occur in cell walls of cereal grains (Fincher & Stone, 1986). They consist of a linear backbone of β-(1 → 4) linked D-xylopyranosyl units to which α-L-arabinofuranosyl residues are attached through O-2 and/or O-3; some ferulic acid residues may be esterified to arabinose residues at O-5. On average, an arabinose to xylose ratio (Ara/Xyl) of 0.6 is usually found in the water-soluble AX from wheat (Hoffmann, Roza, Maat, Karmeling, & Vliegenthart, 1991; Izydorczyk & Biliaderis, 1991), but large natural variations are observed (Cleemput et al., 1995; Dervilly, Saulnier, Roger, & Thibault, 2000; Izydorczyk & Biliaderis, 1995). AX from wheat endosperm are partly water-soluble and create highly viscous aqueous solutions. The high viscosity of water extracts from cereal grain has a positive effect in processes such as bread making (Biliaderis & Izydorczyk, 1995), but is generally detrimental for animal feed (Austin, Wiseman, & Chesson, 1999; Choct & Annison, 1992) and for brewing (Schwarz & Han, 1995; Viëtor, Voragen, Angelino, & Pilnik, 1991).

Previously (Dervilly-Pinel, Thibault, & Saulnier, 2001), we reported on the sugar composition, linkage analysis and macromolecular characterisation of many different homogeneous, desesterified AX fractions, with regard to the relations that might exist between structural and physicochemical properties. We have shown that variations in Ara/Xyl ratio do not influence the conformation or the behaviour of AX in solution. However, the extent to which the AX can be degraded, as well as other features, such as interaction with each other and/or with other cell wall components, might depend on the presence of arabinose residues and their distribution over the xylan backbone. Little information on this distribution is available. Periodate oxidation studies (Ewald & Perlin, 1959; Gruppen, Kormelink, & Voragen, 1993; Izydorczyk & Biliaderis, 1994) on water-soluble AX

Abbreviations: Ara, arabinose; AX, arabinoxylans; Ara/Xyl, arabinose to xylose ratio; DP, polymerisation degree; HPAEC, high performance anion exchange chromatography; [η], intrinsic viscosity; *I* polydispersity index; Mw, weight-average molar mass; NMR, nuclear magnetic resonance; PED, pulse electrochemical detector; PAD, pulsed amperometric detection; rG, radius of gyration; Xyl, xylose.

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from wheat flour indicated the presence of clusters of 1–4 contiguous substituted xylose residues. Previous studies on AX from wheat (Gruppen et al., 1993), barley (Viëtor, Kormeling, Angelino, & Voragen, 1994) and rye (Åman & Bengtsson, 1991) using xylanases, indicated a non-random distribution of arabinose residues along the xylan backbone.

The aim of this study was to elucidate, through periodate analysis and enzymic degradation the distribution of arabinose residues over the xylan backbone of homogeneous and well-characterised de-feruloylated AX fractions.

2. Experimental

2.1. Material

Isolation and characterisation of homogeneous AX fractions from wheat flour have been previously described (Dervilly-Pinel et al., 2001).

2.2. Periodate oxidation and Smith degradation

Periodate oxidation conditions were adapted from the literature (Åman & Bengtsson, 1991). Oxidation of arabinoxylan solutions (5 mg/ml in water) with 0.03 M $NaIO_4$, in the dark, at room temperature, was followed by measurement of the absorbance at 225 nm for 80 h. To stop the reaction, excess of periodate was destroyed by addition of ethylene glycol. The reaction mixtures were dialysed against distilled water for 48 h, concentrated to 10 ml, reduced with 0.8 M $NaBH_4$, dialysed against distilled water for 48 h and freeze-dried. The oxidation and reduction procedures were repeated twice to obtain full oxidation. The oxidised and reduced materials were dissolved in 0.02 M HCl (1 mg/ml) and heated at 100 °C. The kinetics of hydrolysis was monitored as follows, aliquots of reaction mixture were withdrawn between 10 min and 4 h and were neutralized with 0.2 M NaOH. Liberated oligomers were separated and quantified by HPAEC using a Dionex bioLC system equipped with a CarboPac PA-1 column (250×4 mm^2). Elution (1 ml/min, 25 °C) involved linear gradients of sodium acetate in 0.1 M NaOH: 0 M during 5 min, 0–0.05 M during 15 min, 0.05–0.1 M during 10 min, 0.1–0.2 M during 10 min, 0.2–0 M during 1 min and 0 M during 19 min. The effluent was monitored using a Dionex PED detector in the pulsed amperometric detection mode and a gold electrode. Identification of oligosaccharides and response factors were obtained using standard solutions of xylo-oligosaccharides (DP 1–6, Megazyme).

2.3. Enzymic degradation

An endoxylanase (EC 3.2.1.8.) from *Aspergillus aculeatus* purified in the laboratory (Dr E. Bonnin, personnal communication) was used for degradation of the AX. Five milligrams AX and 1 U endoxylanase in 1 ml buffer (20 mM acetate, pH 6) were incubated for 24 h at 40 °C. Enzyme activity was terminated by heating the mixture 10 min in a boiling water bath. AX oligosaccharides were fractionated according to size on a column (100×2.6 cm^2) of Bio-Gel P-2 and eluted with water (25 ml/h) at 50 °C. The eluent was collected in fractions of 3 ml and analysed for neutral sugars (Tollier & Robin, 1979).

2.4. Simulation of arabinose distribution

Simulation of the distribution of arabinosyl residues on the xylan chain was performed using two programs written in Fortran, running on a Silicon Graphics computer. A chain length of 10,000 xylosyl units was used.

The first program simulated a fully random distribution of arabinosyl residues on the xylan chain. The proportion of arabinosyl residues was set equal to the arabinose content, as determined by neutral sugar analysis (Dervilly-Pinel et al., 2001). Each xylosyl unit could be drawn by lot, a maximum of two times, in which case it would be di-substituted.

In the second program, proportions of the mono-substitutions at O-3 and di-substitutions at O-2 and O-3 were imposed. Those proportions were obtained from ^{1}H-NMR studies (Dervilly-Pinel et al., 2001). AX chains were then simulated by random assignment of mono- or di-substitutions to the backbone. So that neither pattern of substitution would be favoured, the program alternately took both, as long as both mono- and di-substituted patterns were left. The remaining pattern was then used to complete the substitution of the chain.

3. Results and discussion

3.1. Optimisation of periodate oxidation and Smith degradation

Conditions ranging from 18 h at 4 °C (Chaplin & Kennedy, 1986) to 70 h at room temperature have been reported for periodate oxidation of AX (Åman and Bengtsson, 1991; Painter & Larsen, 1970). We have measured the consumption of periodate during oxidation of two AX fractions (F30 and F60) to optimise this time. F30 and F60 have different levels of substitution (A/X = 0.38 and 0.82, respectively) and the kinetics of periodate consumption are shown in Fig. 1. After an initial, rapid consumption of periodate by the AX (0–5 h), the rate of oxidation diminished (5–45 h) and finally reached equilibrium (>45 h). After 72 h of oxidation, 4.27×10^{-6} and 4.11×10^{-6} mol/mg AX were consumed by F30 and F60, respectively. Those values, lower than the corresponding theoretical consumptions (5.82×10^{-6} and 5.67×10^{-6} mol/mg AX, respectively) attest the formation of hemiacetal linkages between the aldehyde groups of oxidised residues and hydroxyl groups on not-yet-oxidised

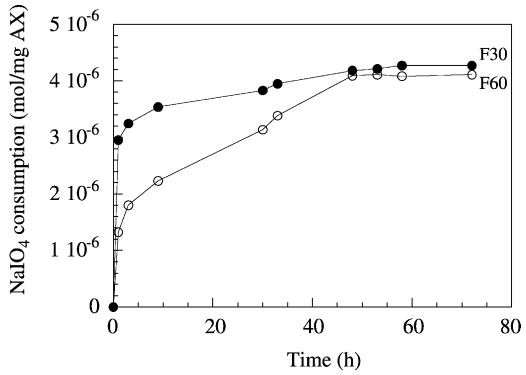

Fig. 1. Kinetics of arabinoxylan oxidation with 30 mM $NaIO_4$ during the first oxidation step.

residues (Åman and Bengtsson, 1991; Ishak & Painter, 1971; Painter & Larsen, 1970). For this first stage, the duration of oxidation has been set to 72 h at room temperature, in accordance with Åman and Bengtsson (1991). A second oxidation was performed, under the same conditions, to remove hemiacetal linkages. Consumption of periodate during this second step (1.55×10^{-6} mol/mg AX in both cases) was appropriate for full oxidation of the polymers.

The hydrolysis step of Smith degradation is a difficult compromise between removal of oxidised and reduced residues and preservation of glycosidic linkages. Conditions usually reported in literature are 0.02 M HCl, at 100 °C for 20 min (Åman & Bengtsson, 1991; Aspinall & Ross, 1963; Gruppen et al., 1993; Izydorczyk & Biliaderis, 1994) and liberated glycerol-xylosides are measured. We have observed that, under these conditions, a portion of glycerol-xylosides were hydrolysed into their corresponding xylosides after only 10 min of hydrolysis, although some survive up to 120 min of hydrolysis (Fig. 2). At the same time, glycosidic linkages between sugar residues are hydrolysed quite extensively as shown in Fig. 3 by the fate of xylo-tetraose in 0.02 M HCl at 100 °C. In the absence of glycerol-xyloside standards for quantitative measurement by HPAEC, we have looked for conditions producing the highest amount of xyloside oligomers with DPs in the range of 1–6. Using a highly substituted AX fraction (F60), quantities of xylosides with DP in the range of 1–6 increased during the first 180 min of hydrolysis (Fig. 4). Consequently this hydrolysis time was used for Smith degradation in subsequent experiments.

3.2. Periodate analysis

Six monodisperse arabinoxylan fractions have been used. Their chemical, structural and physicochemical characteristics, previously determined (Dervilly-Pinel et al., 2001), are indicated in Table 1. Table 2 shows the relative amounts of xylosides obtained after periodate oxidation and subsequent Smith degradation. Xylosides with DP between

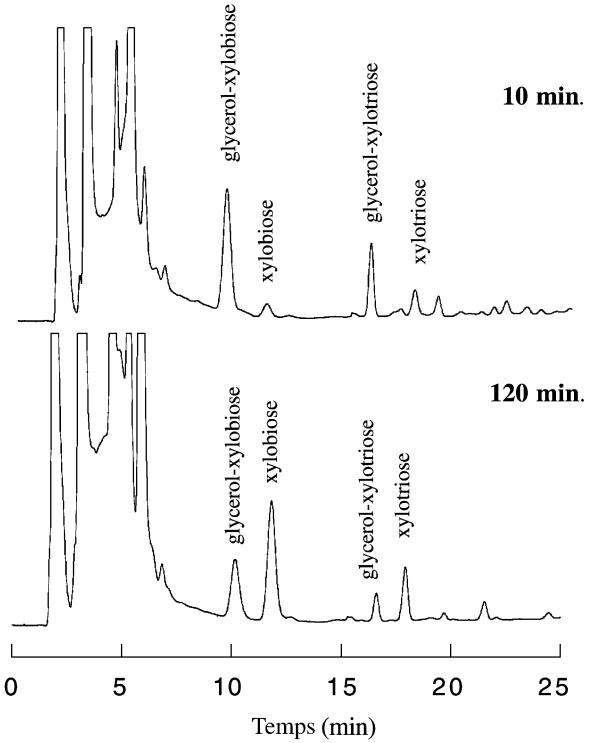

Fig. 2. Elution pattern (HPAEC) of Smith degradation products after 10 and 120 min of hydrolysis with HCL 0.02 M at 100 °C.

1 and 4 were liberated from fractions F30.3 and F30.9. Xylosides with DP between 1 and 5 were obtained from F40.4, and xylosides with DP of up to or higher 6 were observed for F50.3, F70.2 and F70.5. Xylosides were not quantified when their polymerisation degree was higher than 6. Although, the amount of each oligomer did not reflect their actual proportion in the oxidised polymer, we clearly observed that an increase in substitution degree of the AX was correlated to an increase in length of substituted regions and greater proportions of them.

This is the first time that contiguous blocks of five and six substituted xylosyl residues have been clearly identified for water-soluble AX from wheat. However, due to

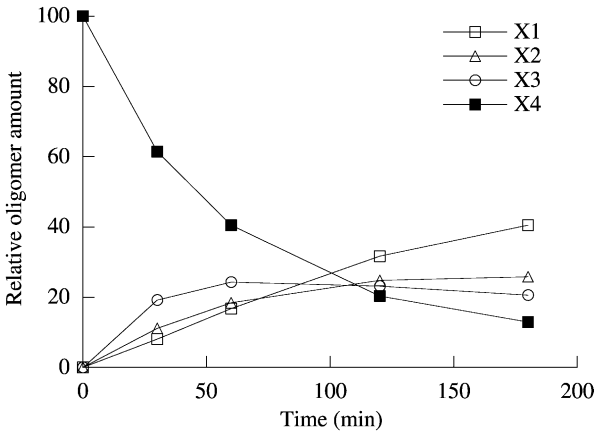

Fig. 3. Kinetics of hydrolysis of xylo-tetraose with 0.02 M HCl at 100 °C.

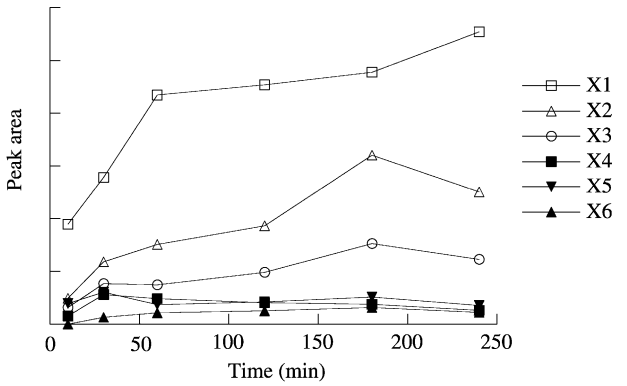

Fig. 4. Release of xylosides (DP 1–6) during Smith degradation of arabinoxylans as a function of time.

the hydrolysis conditions, the amounts of xyloside blocks of higher DP are undoubtedly underestimated. Contiguous blocks of up to three (Gruppen et al., 1993) and four (Izydorczyk & Biliaderis, 1994) substituted residues have been previously observed in wheat flour AX. Åman and Bengtsson (1991) had obtained blocks from water-soluble AX from rye that, according to NMR characterisation, contained 5–8 xylosyl residues.

3.3. Simulation of arabinose distributions on AX chains

Random distributions of arabinosyl residues were simulated for comparison with the experimental distributions. The first comparison was based on the number of mono-substitutions vs. the number of di-substitutions, which also afforded a comparison of the number of substituted residues and patterns of substitution. The second comparison was based on the pattern of substitution, given that the ratio of mono- to di-substitution was held constant.

The first program randomly distributes the arabinosyl units on the xylan backbone imposing the Ara/Xyl ratio that was experimentally determined for the different fractions (Dervilly-Pinel et al., 2001). Levels of mono- and di-substituted xylosyl units were then compared to experimental results (Fig. 5). The proportion of mono-substitution was significantly higher from simulation than from experiment, and consequently the proportion of di-substitution was lower than indicated by experiment. In both cases, di-substituted xylose levels clearly increased with the Ara/Xyl ratio. However, a random distribution of arabinosyl units along the xylan backbone gave a higher weight to mono-substitution than experimentally observed, which

Table 1
Characteristics of the six arabinoxylan populations studied

	Neutral sugars			Xylosyl residues		Physicochemical properties			
	Xyl (g/100 g)	Ara (g/100 g)	A/X	Unsubstituted[a]	Substituted[a]	Mw ($\times 10^3$ g/mol)	[η] (ml/g)	rG (nm)	I
F30.3	51.4	23.4	0.45	68.7	31.3	300	550	44	1.1
F30.9	63.6	23.9	0.37	74.4	25.6	115	280	28	1.2
F40.4	41.2	26.2	0.64	61.7	38.3	195	425	32	1.1
F50.3	44.1	32.6	0.74	58.9	41.1	300	560	41	1.1
F70.2	48.1	61.7	1.28	27.1	72.9	535	520	51	1.1
F70.5	43.7	51.5	1.18	33.4	66.6	155	220	25	1.0

Mw: molar mass, [η]: intrinsic viscosity, rG: radius of giration, I: polydispersity index.
[a] g/100 g xylose in AX.

Table 2
Distributions of substituted blocks of xylosyl residues in different arabinoxylan populations

	F30.9		F30.3		F40.4		F50.3		F60.2		F70.5		F70.2	
Ara/Xyl	0.37		0.45		0.64		0.74		0.89		1.18		1.28	
Subs. Xyl.[a]	25.6		31.3		38.3		41.1		50.4		66.6		72.9	
	Exp	Sim	Exp	Sim	Exp	Sim	Exp	Sim	Exp	Sim	Exp	Sim	Exp	Sim
X1[b]	68.8	55.9	74.7	47.2	81.3	38.3	73.5	34.7	71.1	24.5	61.1	11.5	59.9	7.6
X2	25.6	28.1	19.0	29.6	15.6	29.6	19.6	28.6	19.5	24.8	19.6	15.2	22.1	11.0
X3	4.8	10.9	4.5	13.9	2.6	16.7	5.2	17.6	6.4	18.8	10.8	15.3	9.6	12.1
X4	0.9	3.5	1.7	5.8	0.4	8.5	1.1	9.5	1.9	12.7	4.2	13.8	4.0	11.7
X5	0	1.2	0	2.3	0.2	3.9	0.5	4.8	0.8	7.9	2.7	11.1	2.5	10.7
X6	0	0.4	0	0.9	0	1.9	0.1	2.5	0.3	4.8	1.5	8.9	1.8	9.3
>X6[c]	0	0	0	0.5	0	1.0	0	2.3	0	6.4	NQ	24.2	NQ	37.6

Exp., sim.: experimental, simulated distribution (using random distribution of mono- and di-substitutions).
[a] Determined from Ara/Xyl and NMR characterisations (g/100 g xylose in the fraction).
[b] Grams of the oligomer for 100 g of detected oligomers.
[c] Xylosides of DP > 6 were detected in fractions F70.2 and F70.5 but not quantified (NQ).

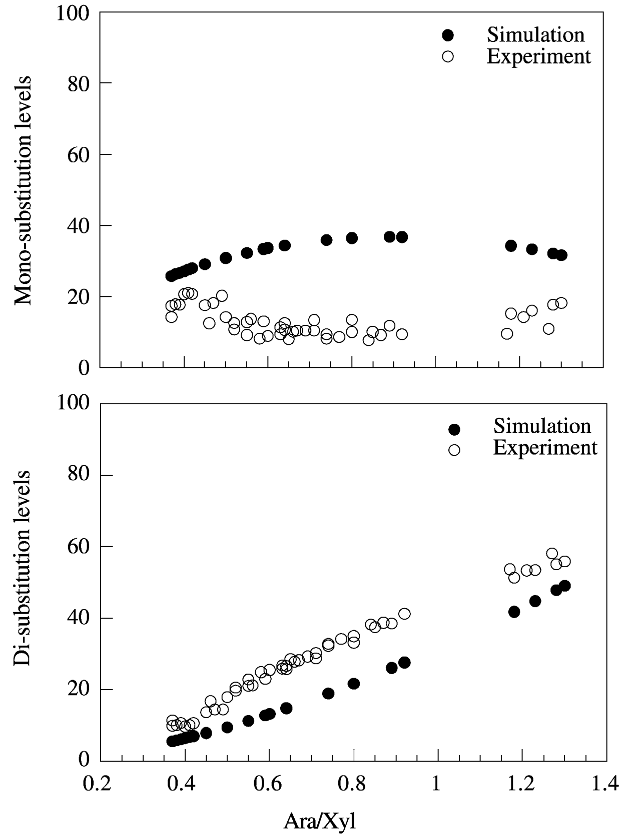

Fig. 5. Comparison of experimental and simulated distributions of mono- and di-substituted xylosyl residues in the polymer chain as a function of substitution degree (Ara/Xyl). Simulation was performed by random distribution of arabinosyl residues on the xylan backbone.

suggests that regulation occurred during the biosynthesis of the polymer. The mechanisms that govern the different patterns of substitution are, to our knowledge, unknown.

A second program computed the distribution of substituted blocks with imposed levels of mono- and di-substitution. Chains with a random distribution of the two types of substituted residues were then built. Distributions of blocks of substituted xylosyl units are given in Table 2. Representative segments from one simulation of their 10,000 units models for fractions F30.9 and F70.5 are shown in Fig. 6. These segments show that random substitution results in clusters of substituted residues, even in lightly substituted F30.9, or, in the case of heavily substituted F70.5, clusters of unsubstituted residues. Admittedly, simulation predicted larger blocks of substituted residues than found experimentally, especially for the highly substituted fractions. We feel that these discrepancies are mainly due to conditions of the Smith degradation, in which the glycosidic linkages were not totally preserved from hydrolysis. Therefore, the formation of xylose and oligomers with a low DP was favoured. As a matter of fact the level of substituted blocks of DP1 observed for fraction F70.5 and F70.2 is not compatible with the level of unsusbtituted residues in the chain (Table 2).

F30.9 DP:634 Xylm:14.2% Xyld: 11.4% - Substitution degree 25.5

010000020001000000100022001100000100200000000000022002001001002000000020000020
0000000012010010000100000**212**000000000000**2010102002111111**00000000000000000010002
00**1102**000000000000000**11021**000010010100100010020000100220000000011002000000002
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0200000010001000100200000100100000001000002000000010020022000002001002001010
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01000**112**0000**20201**000**10102**00200001001001002002001020002000000000110000000000001
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F70.5 DP:537 Xylm:15.2% Xyld: 51.4% Substitution degree 66.4%

02220012010202212201102220222212**002000200**22022022120222020010202220222122212
20020220121020022200201222022222**000**22**000**2222020200102212222220020222121202 0
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102112222221

Fig. 6. Random distribution of substituted xylosyl residues for fraction F30.9 and F70.5. Clusters of lowly substituted and highly substituted areas are depicted in bold for F30.9 and F70.5, respectively. 0 = unsubstituted xylose, 1 = mono-substituted xylose, 2 = di-substituted xylose.

3.4. Enzymic degradation

To get further insight into the distribution of arabinose over the xylan backbone, the two extreme AX fractions F30.9 and F70.5 were degraded with an endoxylanase from *A. aculeatus*. Fig. 7 shows the chromatographic profiles of the xylanase digest of the fractions on Bio-Gel P-2. The relative

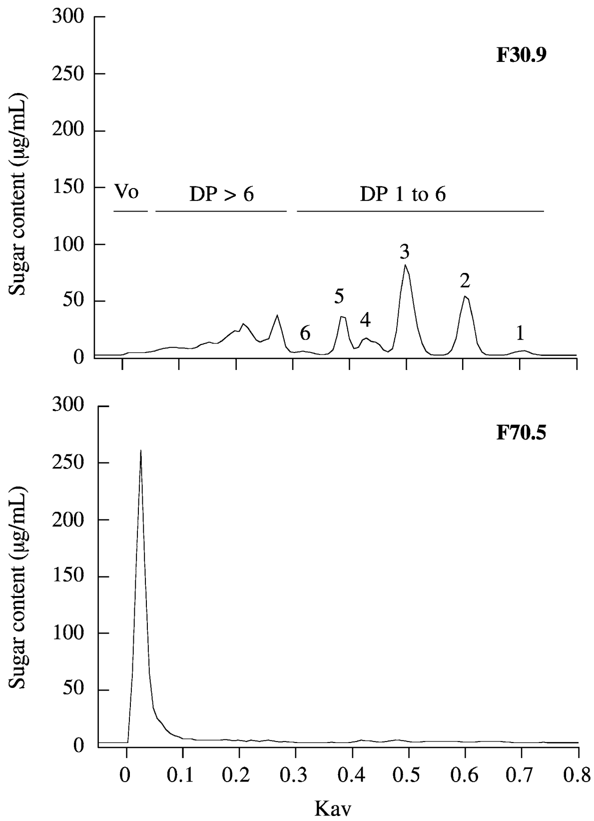

Fig. 7. Elution profiles of arabinoxylan digest (fractions F30.9 and F70.5, 5 mg of polysaccharide incubated with 1 U xylanase for 24 h) on Bio-Gel P-2 (100×2.6 cm^2 eluted with water at 25 ml/h at 50 °C).

Table 3
Proportions of peaks eluted from Bio-Gel P-2 column after degradation of fractions F30.9 and F70.5 by endoxylanase

	A/X	Vo[a]	DP > 6	DP1	DP2	DP3	DP4	DP5	DP6
F30.9	0.37	1.5	38.5	3.0	15.2	23.0	6.2	8.6	4.0
F70.5	1.18	89.0	7.0	0.1	0.4	0.6	1.2	1.7	0.1

[a] Percent of total carbohydrate material eluted from the column.

amounts of the different degradation products depended on the Ara/Xyl ratio, as already reported (Viëtor et al., 1994). The fraction with an Ara/Xyl ratio of 0.37 was almost totally degraded into small oligomers, whereas the fraction with an Ara/Xyl ratio of 1.18 showed much less degradation (Table 3), with the main part of the degradation being eluted at the void volume of the column, indicating a high DP. Four to seven sub-sites have been described for substrate binding on endoxylanases, and the specific bonds cleaved depend on the side-chain residues (Kulkarni, Shendye, & Rao, 1999). For example, depending on the type of arabinose linkage, a xylanase (endoI) from *Aspergillus awamori* (Kormelink, Gruppen, Viëtor, & Voragen, 1993) may need only one or two contiguous unsubstituted xylosyl residues to hydrolyse the xylan backbone, whereas another xylanase (endoIII) from the same organism needs at least three unsubstituted residues (Kormelink et al., 1993). According to the random distribution of arabinose shown in Fig. 6 for F70.5, the presence of clusters of unsubstituted xylose may explain that the endoxylanase has some action on this highly substituted AX. Similarly the small amount of small oligomers (DP $<$ 6) produced by the enzyme is in agreement with the high proportion of long blocks of substituted xylosyl units depicted in Fig. 6. However, Fig. 6 does not necessarily reflect the actual distribution, as the probability to find a mono-substitution or a di-substitution in contiguous xylosyl residues was set equal in our simulation, whereas the biosynthetic mechanism might favour specific contiguous distributions.

4. Conclusion

Conditions for complete oxidation of arabinoxylans have been determined and employed. Because of the incomplete specificity of the Smith degradation, we did not succeed in carrying out a quantitative hydrolysis of the oxidised and reduced polymer into xylosides. Therefore the proportions of long blocks have probably been underestimated. Nevertheless, using homogeneous arabinoxylans, this is the first report of blocks containing up to six substituted xylosyl residues. Previous authors have used very polydisperse and structurally heterogeneous AX, in which the proportion of highly substituted populations such as F70.5 and 70.2 is very low, which explains why such long blocks were not detected.

Simulation indicated that the proportion of the two types of substitution is probably governed by biosynthetic mechanisms that favour di-substitution. When the two types of substitution were held at their experimental levels, a random distribution of arabinose gave clusters of substituted residues (Fig. 6). Although predicted blocks were larger than experimentally observed, such a distribution is compatible with a limited attack of endoxylanases as observed for F70.5.

We have isolated from wheat flours several populations of AX presenting A/X ratios varying from 0.4 up to 1.2 (Dervilly-Pinel et al., 2001). The question of the distribution of arabinosyl residues in these molecules is not easy to answer as periodate oxidation only allows determination of the distribution of substituted and unsubstituted residues without any idea of the distribution of mono- and di-substitution within substituted blocks. Using an endoxylanase, the distribution of mono- and di-substituted residues is theoretically possible to attain, but in practice enzymes have a limited access to the most substituted AX molecules. As a matter of fact, oligosaccharides presenting at most two contiguous substituted xylose residues have been isolated and identified from endoxylanases digests (Gruppen et al., 1992, 1993; Viëtor et al., 1994; Vinkx, Reynaert, Grobet, & Delcour, 1993), whereas periodate oxidation demonstrates that blocks made at least of six consecutive substituted xylosyl residues are present in wheat AX.

The results presented in this paper and by other authors (Gruppen et al., 1993; Viëtor et al., 1994) indicate that distribution of the type of substitution (mono- or di-substitution) on the xylan backbone is not the result of a random process. Biosynthetic mechanisms favour di-substitution and probably the arrangement of the type of substitution within contiguous substituted xylosyl residues. When only the distribution of substituted (irrespective of the substitution type) and unsubstituted residues is considered, then a random distribution, as shown in Fig. 6, is supported by our results of periodate oxidation and enzymatic degradation. Such a random distribution of substituted and unsubstituted residues is also compatible with the presence of clusters with high substitution patterns and low substitution patterns, as already described (Gruppen et al., 1993). In conclusion, the different models proposed for AX from wheat flour support an irregular distribution of arabinosyl residues along the chain. For wood glucuronoxylan, Jacobs, Larsson, and Dahlman (2001) found an irregular distribution of glucuronic acid in hardwood, and a regular distribution in softwood.

References

Åman, P., & Bengtsson, S. (1991). Periodate-oxidation and degradation studies on the major water-soluble arabinoxylan in rye grain. *Carbohydrate Polymers*, *15*(4), 405–414.

Aspinall, G. O., & Ross, K. M. (1963). The degradation of two periodate-oxidised arabinoxylans. *Journal of Chemical Society*, *2*, 1681–1686.

Austin, S. C., Wiseman, J., & Chesson, A. (1999). Influence of non-starch polysaccharides structure on the metabolisable energy of UK wheat fed to poultry. *Journal of Cereal Science*, *29*(1), 77–88.

Biliaderis, C. G., & Izydorczyk, M. S. (1995). Observations on retrogradation of starch polymers in the presence of wheat and rye arabinoxylans. In G. O. Phillips, P. A. Williams, & D. J. Wedlock (Eds.), *Gums and stabilisers for the food industry* (pp. 227–230). Oxford: IRL press.

Chaplin, M.F., & Kennedy, J.F. (1986) In *Carbohydrate analysis: A practical approach* (pp. 71–75). Oxford: IRL Press.

Choct, M., & Annison, G. (1992). Antinutritive effect of wheat pentosans in broiler-chickens—roles of viscosity and gut microflora. *British Poultry Science*, *33*(4), 821–834.

Cleemput, G., Van Oort, M., Hessing, M., Bergmans, M. E. F., Gruppen, H., Grobet, P. J., & Delcour, J. A. (1995). Variation in the degree of D-xylose substitution in arabinoxylans extracted from a european wheat-flour. *Journal of Cereal Science*, *22*(1), 73–84.

Dervilly, G., Saulnier, L., Roger, P., & Thibault, J.-F. (2000). Isolation of homogeneous fractions from wheat water-soluble arabinoxylans. Influence of the structure on their macromolecular characteristics. *Journal of Agricultural and Food Chemistry*, *48*(2), 270–278.

Dervilly-Pinel, G., Thibault, J.-F., & Saulnier, L. (2001). Experimental evidence for a semi-flexible conformation for arabinoxylans. *Carbohydrate Research*, *330*(3), 365–372.

Ewald, C. M., & Perlin, A. S. (1959). The arrangement of branching in an arabinoxylan of wheat flour. *Canadian Journal of Chemistry*, *37*, 1254–1259.

Fincher, G. B., & Stone, B. A. (1986). Cell walls and their components in cereal grain technology. In Y. Pomeranz (Ed.), *Advances in cereal science and technology* (pp. 207–295). American Association of Cereal Chemists.

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

Gruppen, H., Kormelink, F. J. M., & Voragen, A. G. J. (1993). Water-unextractable cell-wall material from wheat-flour. 3. A structural model for arabinoxylans. *Journal of Cereal Science*, *18*(2), 111–128.

Hoffmann, R. A., Roza, M., Maat, J., Karmeling, J. P., & Vliegenthart, J. F. G. (1991). Structural characteristics of the cold-water-soluble arabinoxylans from the white flour of the soft wheat variety Kadet. *Carbohydrate Polymers*, *15*(4), 415–430.

Ishak, M. F., & Painter, T. (1971). Formation of inter-residue hemiacetals during the oxidation of polysaccharides by periodate ion. *Acta Chemica Scandinavica*, *25*, 3875–3877.

Izydorczyk, M. S., & Biliaderis, C. G. (1991). Comparison of the structure and composition of water-soluble pentosans from different wheat-varieties. *Cereal Chemistry*, *68*(2), 139–144.

Izydorczyk, M. S., & Biliaderis, C. G. (1994). Studies on the structure of wheat-endosperm arabinoxylans. *Carbohydrate Polymers*, *24*(1), 61–71.

Izydorczyk, M. S., & Biliaderis, C. G. (1995). Cereal arabinoxylans: advances in structure and physicochemical properties. *Carbohydrate Polymers*, *28*(1), 33–48.

Jacobs, A., Larsson, P. T., & Dahlman, O. (2001). Distribution of uronic acids in xylans from various species of soft- and hardwood as determined by MALDI mass spectrometry. *Biomacromolecules*, *2*(3), 979–990.

Kormelink, F. J. M., Gruppen, H., Viëtor, R. J., & Voragen, A. G. J. (1993). Mode of action of the xylan-degrading enzymes from *Aspergillus awamori* on alkali-extractable cereal arabinoxylans. *Carbohydrate Research*, *249*(2), 355–367.

Kulkarni, N., Shendye, A., & Rao, M. (1999). Molecular and biotechnological aspects of xylanases. *FEMS Microbiology Reviews*, *23*, 411–456.

Painter, T., & Larsen, B. (1970). Transient hemiacetal structures formed during the periodate oxidation of xylan. *Acta Chemica Scandinavica*, *24*, 2366–2378.

Schwarz, P. B., & Han, J. Y. (1995). Arabinoxylan content of commercial beers. *Journal of the American Society of Brewing Chemists*, *53*(4), 157–159.

Tollier, M. T., & Robin, J.-P. (1979). Adaptation de la méthode à l'orcinol sulfurique au dosage automatique des glucides neutres totaux: conditions d'application aux extraits d'origine végétale. *Annales de Technologie Agricole*, *28*, 1–15.

Viëtor, R. J., Kormeling, F. J. M., Angelino, S. A. G. F., & Voragen, A. G. J. (1994). Substitution patterns of water-unextractable arabinoxylans from barley and malt. *Carbohydrate Polymers*, *24*(2), 113–118.

Viëtor, R. J., Voragen, A. G. J., Angelino, S. A. G. F., & Pilnik, W. (1991). Non-starch polysaccharides in barley: a mass balance of flour fractionation. *Journal of Cereal Science*, *14*, 73–83.

Vinkx, C. J. A., Reynaert, H. R., Grobet, P. J., & Delcour, J. A. (1993). Physicochemical and functional-properties of rye non-starch polysaccharides. 5. Variability in the structure of water-soluble arabinoxylans. *Cereal Chemistry*, *70*(3), 311–317.