Physicochemical and Functional Properties of Rye Nonstarch Polysaccharides. VI. Variability in the Structure of Water-Unextractable Arabinoxylans

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ABSTRACT

A fraction rich in water-unextractable arabinoxylans was isolated from rye wholemeal. Arabinoxylans were solubilized by a sequence of alkaline extractions. Structural features of these arabinoxylans were investigated by methylation analysis and nuclear magnetic resonance spectroscopy. Three groups of water-unextractable arabinoxylans could be recognized, differing in extractability, solubility, and structure. The first group had an intermediate arabinose-to-xylose ratio (A/X) of 0.55–0.79 and was extractable either with saturated Ba(OH)2 containing 1% NaBH4 or with water after saturated Ba(OH)2 extraction. The polymers were soluble in water after neutralization of the alkaline extract, but they precipitated in saturated ammonium sulfate solution. They contained terminal arabinose residues and unsubstituted, 3-mono-, 2-mono-, and disubstituted xylose residues. A second group had an A/X of ~1.1 and was partially extractable with alkali; more could be extracted after delignification. This group was soluble in water and in saturated ammonium sulfate solution. Typical structural features in this arabinoxylan were: substituted arabinose residues (40% of arabinoses), terminal xylose residues (26% of xyloses), and terminal galactose residues. A third group of arabinoxylans was extractable with 1M KOH, had low water solubility after neutralization of the alkaline extract, and had an A/X of ~0.2. Besides arabinoxylan, β-glucan was present in large proportions in rye cell-wall material. Glucomannan and xyloglucan were also detected.

In the previous article of this series (Vinkx et al 1993), we described the isolation, fractionation, and structural variation of water-extractable rye arabinoxylans. However, the major part of the arabinoxylans in rye wholemeal is water-unextractable (Delcour et al 1989, Saini and Henry 1989). Water-unextractable arabinoxylans have an important (deleterious) influence on the breadmaking properties of rye flour (Meuser and Suckow 1986, Kühn and Grosch 1989, Weipert 1993). The use of pentosanase improves bread quality (Kühn and Grosch 1988). Because the degradability of arabinoxylans by endoxylanases depends on the presence of side groups (Düsterhöft et al 1993, Kormelink et al 1993, Viëtor et al 1994), insight in the structure of arabinoxylans is clearly relevant.

However, limited information is available on the structure of rye alkali-extracted arabinoxylans. A/X ratios of 0.1–1.1 were reported for alkali-extracted arabinoxylans from rye (Casier et al 1967, Holas et al 1972, Ali and D'Appolonia 1979, Hromádková and Ebringerová 1987, Saini and Henry 1989). Saini and Henry (1989) extracted the water-insoluble residue from rye wholemeal with 1.25M NaOH and subsequently with 3.75M NaOH. The authors found that, in percent of rye grain, the former alkaline extract contained 5.85% arabinoxylans (A/X 0.43), the latter alkaline extract contained 1.39% arabinoxylans (A/X 0.55), and the residue contained 1.05% arabinoxylans (A/X 1.02).

Casier et al (1973) reported a ratio of terminal arabinose to unsubstituted xylose of 1.5, as determined by methylation analysis. The structures of alkali-extractable arabinoxylans from rye bran were investigated in more detail by Hromádková and Ebringerová (1987), Hromádková et al (1987), and Ebringerová et al (1990). These authors divided the arabinoxylans into two groups. The first group contained soluble after extraction (0.3 M NH4OH) and neutralization, and represented arabinoxylans with A/X in the range of 0.5–1.1. For an arabinoxylan with A/X 0.78, methylation analysis revealed that 41% of the nonterminal xylose units were unsubstituted (dxyl), 28% were 3-mono-substituted (3mxyl), 5% were 2-mono-substituted (2mxyl), and 26% were disubstituted (dxyl). Also detected were: terminal xylose (16% of the total content of xylose), substituted arabinoses (22% of arabinoses), and a low proportion of terminal galactose. The second group of arabinoxylans was insoluble after neutralization of the alkaline (1.1M NaOH) extract and had a low A/X (0.1–0.3). Arabinose residues were linked at O-3 of xylose residues. The latter arabinoxylan group represented ~50% of the nonstarch and noncellulosic polysaccharides from rye bran.

It is clear that, to date, the structural features of only two arabinoxylan preparations from rye bran water-unextractables were reported in detail. As arabinoxylans are very heterogeneous, more information is required to describe the structural variability of water-unextractable arabinoxylans from rye wholemeal.

Therefore, this article provides structural information on the various arabinoxylans present in that fraction representing the majority of the water-unextractable arabinoxylans from rye wholemeal. The arabinoxylans were extracted by sequential alkaline extraction. The structural information on the various arabinoxylans was obtained both by nuclear magnetic resonance and methylation analysis. The data obtained were compared with data on water-extractable rye arabinoxylans and on wheat arabinoxylans.

MATERIALS AND METHODS

Rye Wholemeal

Rye (cv. Halo, 1992 harvest, grown in Belgium) was milled with a Tecator sample mill to pass a 0.5-mm sieve. The meal was heated for 90 min at 130°C to inactivate enzymes. Defatting was accomplished by extraction with n-hexane (Soxhlet, 4 hr).

Enzymes

Bacillus licheniformis α-amylase (type XII-A, A-3403, 17,980 units/ml [one unit will liberate 1 mg of maltose from starch in 3 min at pH 6.9 at 20°C]) was from Sigma Chemical Co. (St Louis, MO). Streptomyces griseus pronase E (4,000,000 PU/g, activity measured with casein, pH 7.4, 10 min, Folin-Ciocalteu phenol reagent) was obtained from Merck (Darmstadt, Germany). Englyst Fibrezym Kits for nonstarch polysaccharide (NSP) analysis were obtained from Novo Nordisk Bioindustries (Farnham, Surrey, UK).

Fractionation of Rye Wholemeal (Isolation of WU)

A fractionation method for wheat flour published by Gruppen et al (1989) was modified. The fractionation was performed in duplicate. All centrifugations were for 35 min at 10,000 × g at 20°C.
Rye wholemeal (200 g) was extracted with 1,200 ml of distilled water for 90 min at room temperature by end-over-end rotation. The slurry was sieved over a 125-μm sieve, and the residue on the sieve was washed with 600 ml of water. The throughs were centrifuged and three fractions were obtained: a supernatant; an upper, brown tailing fraction; and an underlaying white starch fraction. The starch fraction was resuspended in 100 ml of water and centrifuged again, yielding three fractions that were pooled with the corresponding fractions from the former centrifugation. The starch fraction (ST) and the supernatants (WE) were lyophilized.

The fraction on the sieve and the tailing fraction were purified by amyloglucosidase and proteolysis. Each was suspended in 800 ml of buffer (0.05 M maleic acid, 0.05 M tris, 0.001 M CaCl₂, 0.05% NaN₃) and adjusted to pH 7.5 with diluted NaOH. The suspension was incubated with 20 mg of pronase E at 40°C. After 3 hr, a second 20-mg portion of pronase was added, and the pH was adjusted to 7.5 (final pH 7.1). After 6 hr, the suspension was acidified to pH 6.5 using diluted HCl. For amyloglucosidase, 1 ml of α-amylase solution was added, and the suspension was incubated at 70°C for 90 min. The suspension was tested for residual starch (iodine-iodide solution). Only the tailing fraction was incubated further with 0.5 ml of α-amylase solution for 30 min at 70°C. After cooling and adjusting to pH 7.5 using diluted NaOH, a second proteolysis step was performed on both fractions with 20 mg of pronase E (11 hr at 40°C). The suspensions were neutralized with diluted HCl and centrifuged. The supernatants and corresponding residues were SUP₁ and WU₁ (originating from the material on the sieve), and SUP₂ and WU₂ (originating from the tailings). All fractions were lyophilized.

**Alkali Extraction of Arabinoxylans from WU₁**

The extraction sequence (according to Gruppen et al 1992) was conducted in duplicate. All dialysis steps were performed in the cold room (6°C) for 48 hr against distilled water. WU₁ from fractionation A (3 g) was extracted for 16 hr at room temperature with 500 ml of saturated Ba(OH)₂ solution. After centrifugation, the residue was extracted again for 1 hr with 200 ml of the same solution. After centrifugation, the supernatants were combined, acidified to pH 5.0 with acetic acid, dialyzed, and lyophilized (BE1). To the residue, 100 ml of water was added, and the suspension was acidified to pH 5.0 using acetic acid and then extracted for 1 hr at room temperature. After centrifugation, the residue was extracted twice more with 100 ml of water. The supernatants were combined, dialyzed, and lyophilized (BE2). The residue was further extracted with 200 ml of 1 M KOH (containing 1% NaBH₄) for 16 hr at room temperature. After centrifugation, the residue was extracted again for 1 hr at room temperature with the same extractant and then centrifuged. The supernatants were combined, acidified to pH 5.0 with acetic acid, dialyzed, and lyophilized (1M). The residue was then extracted for 16 hr with 200 ml of 4 M NaOH (containing 1% NaBH₄). After centrifugation, the residue was extracted once more with 100 ml of the same solution for 1 hr. The residue was finally washed three times with 100 ml of water for 30 min and centrifuged. The supernatants obtained were combined, acidified to pH 5.0 with acetic acid, dialyzed, and lyophilized (4M). The residue was lyophilized (RES).

**Ammonium Sulfate Fractionation**

Solutions of BE1a (200 mg in 40 ml of water) were saturated with ammonium sulfate, kept overnight at room temperature, and centrifuged. The fractions were dialyzed and lyophilized (BE1asup, BE1ares).

**Delignification and Further Extraction of the Residue Obtained After Sequential Alkaline Extraction**

RESa (300 mg) and RESb (300 mg) were pooled and delignified (Brillet and Mercier 1981, Düsterhöft et al 1991). To that end, 20 ml of a sodium chlorite solution (1 g of NaClO₂ + 0.25 ml of HOAc/50 ml of water) were added to 600 mg of RES. The mixture was incubated for 2 hr at 70°C. After centrifugation (15 min, 10,000 × g), the residue was extracted with 20 ml of 1 M KOH (1% NaBH₄) for 120 min and subsequently extracted with 20 ml of 4 M NaOH (1% NaBH₄) for 120 min at room temperature. The final residue was washed with 20 ml of water, and the washings were added to the 4 M extract. All extracts were acidified to pH 5.0 using acetic acid, dialyzed and lyophilized (RES1M, RES4M). The relationship between meal fractions, extracts, and fractions of extracts is expressed in Figure 1.

**Analysis of Meal and Meal Fractions**

Protein (N × 6.25) was measured using a Kjeldahl method (Bremner 1965). Ash and moisture were measured by standard methods (AACC 1983). NSP content and composition were measured by the method of Englyst and Cummings (1984). Total polysaccharides were measured by omitting the isolation step of NSP. For SUP, ST, and wholemeal, the arabinose, xylose, mannanose, and galactose residues were measured as NSP, whereas WE, WU₁, and WU₁ were measured as total polysaccharides. Uronic acids from NSP were measured colorimetrically with the dimethylphenol method according to Scott (1979).

**Analysis of Extracts**

Protein content was measured according to Lowry et al (1951) with bovine serum albumin as standard. Total monosaccharide composition and total uronic acids were measured as described above.

Methylation analysis was performed as described in Gruppen et al (1992). The fractions were methylated by a modification of the Hakomori method (Sanford and Conrad 1966), dialyzed, and dried in a stream of air. This procedure was repeated once to improve completeness of the reaction. Glycerol acetates were prepared (Englyst and Cummings 1984) after hydrolysis of the samples using 2 M trifluoroacetic acid (1 hr, 121°C). Samples were analyzed with gas chromatography (GC) with flame ionization detection and identities were confirmed by GC-mass spectrometry as described by Gruppen et al (1992). The 2- and 3-O-methylated xylosyl acetates coeluted. Their relative amounts were calculated from the relative abundance of the ions at the ratio molecular mass to charge m/z 117 and m/z 129, respectively.

For nuclear magnetic resonance (NMR) spectroscopy, 1H-NMR spectra were recorded with a Bruker AM-300 (300 MHz) apparatus at 85°C. Samples were dissolved in D₂O (99.8% D), freeze-dried, and dissolved again in D₂O. Pulse repetition time was 2 sec. Number of scans varied from 4,000 to 16,000. Acetonitrile was used as standard (62.23 ppm). Peak assignments were made on the basis of the data by Hoffmann et al (1977) for arabinins from Rosa glauca, and by Hoffmann et al (1992) for wheat flour water-extractable arabinoxylans.
Commercial barley β-glucan (Novo Industri, Copenhagen, Denmark) produced anomeric resonance peaks at 84.78, 84.75, 84.58, 84.57, 84.55, and 84.54 ppm. For α-dextrin at 90°C in D₂O, anomeric resonance peaks at 85.37 and 85.38 ppm were reported for internal unbranched residues (McIntyre and Vogel 1990).

A 1H-decoupled 13C-NMR spectrum was recorded with a Bruker AM-300 apparatus (75 MHz) at 85°C. The sample was dissolved (30 mg/ml) in D₂O (99.8% D). Pulse repetition time was 2 sec, and the number of scans was 10,000. Dioxane was used as standard (667.4 ppm). Peak assignments were made on the basis of assignments in the literature (McIntyre and Vogel 1990).

The eluent was 0.3% NaCl (0.4 ml/min). Fractions (150 sec) were collected and analyzed (0.8 ml) for total carbohydrates with the phenol-sulfuric acid method according to Dubois et al (1956). The variability of structures within the BE 1 fraction was demonstrated by monosaccharide analysis of fractions obtained by ammonium sulfate fractionation. Saturated ammonium sulfate yielded a supernatant fraction with A/X 1.06 and a residue with 48 and 17% in WU₁ and WU₂, respectively and in the SUP fractions (22%). A low amount was extracted with cold water (13%). The NSP glucose content of wholemeal (3.61%) probably included β-glucan, cellulose, glucomannan, and xylol glucan. The content of soluble NSP glucose, predominantly β-glucans, was 0.48% in rye wholemeal (dmb) in accordance with the 0.3–1.2% range reported in literature data (Bengtsson et al 1992).

Mannose was present in low amounts in WU and WE fractions. The mannose detected in the WE fraction may have resulted from sucrose and fructans. Indeed, wholemeal contains sucrose and fructans (Henry 1985, Bengtsson and Åman 1990). Fructos e was detected as a mixture of glucose and mannose. Glucomannans probably were present in the WU fractions (Gruppen et al 1989, 1992).

Water-extractable arabinoxylans have been reported before for rye (Meuser et al 1986) and could explain the presence of galactose in the WE fractions.

Uronic acids were only detected in low proportions (0.32% in meal). They were concentrated by the fractionation in WU₁ (1.17%, or 38% of total uronic acids) and in WU₂ (0.89%, or 17% of total recovered uronic acids). Uronic acids and galactose in the WU fractions may be structural moieties of rye bran heteroxylans (Ebringerová et al 1990).

### Table 1

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<th>Fractionation A</th>
<th>Yield</th>
<th>Moisture</th>
<th>Ash</th>
<th>Protein</th>
<th>Ara</th>
<th>Xyl</th>
<th>Man</th>
<th>G1c</th>
<th>GLc</th>
<th>NSP-Glc</th>
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<th>Xyl</th>
<th>Man</th>
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<th>GLc</th>
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<td>65</td>
<td>(3.61)</td>
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</table>

*Expressed as weight percent of fraction (as is basis); yield as weight percent of rye wholemeal. Ara = arabinose, Xyl = xylose, Man = manno se, Gal = galactose, G1c = glucose, NSP-Glc = non-starch polysaccharide (in parentheses), UA = uronic acids, A + X = arabinose and xylose, A/X = arabinose to xylose ratio. WE, ST, WU₁, and SUP₁ = water-extractable, starch, water-unextractable arabinoxylans, and the supernatants of amylolysis and proteolysis treatment of water-unextractable arabinoxylans, respectively, from rye wholemeal.

*Not detected.
*Not determined.
*Trace.
a WU = Water-unextractable arabinoxylans from rye wholemeal. Expressed as weight percent of fraction (as is basis); yield as weight percent of unfractionated sample. Ara = arabinose, Xyl = xylose, Man = mannose, Gal = galactose, Glc = glucose, UA = uronic acids, A + X = arabinose and xylose, A/X = arabinose to xylose ratio. BElares, BE2, IM, and 4M = extracts obtained by sequential alkaline extraction from WU. RES = nonalkaline-extractable residue from WU.

Not detected.

- Trace.

Not determined.

A/X 0.55 (Table III).

Water extraction after Ba(OH)₂/NaBH₄ extraction yielded the BE2 extract. The BE2 fraction contained β-glucan (as shown by 1H-NMR) (Fig. 2b), 62% arabinoxylan, and only 3% protein. The A/X (0.56) was lower than that of BE1 (0.65). BE2 contained 17% of all arabinoxylans recovered from WU.

The 1M fraction contained arabinoxylans with a very low A/X (0.21), representing a considerable proportion (32%) of the arabinoxylans of WU. The uronic acid content of 1M (1.2%) was slightly higher than that of BE1 and BE2 (0.5 and 0.9%, respectively). A high content of glucose (24%) was measured (β-glucan as shown by 1H-NMR). As for wheat flour (Gruppen et al. 1992), most of the β-glucan was found in the 1M fraction (and BE2).

The 4M extract contained almost all the extracted mannose and only a minor proportion of the extracted arabinoxylans, as was the case for wheat flour (Gruppen et al. 1992), wheat bran (Brillouet and Mercier 1981), and rye bran (Hromádová and Ebringrová 1987). The A/X (0.32) was comparable to the 0.20–0.38 figures from Hromádová and Ebringrová (1987) but lower than the 0.55 reported by Saini and Henry (1989).

The residue of WU after alkaline extraction (RES) had a high A/X (1.10). For rye wholemeal, it was reported that 13% of the water-unextractable arabinoxylans remained in the residue after alkaline (up to 3.75M NaOH) extraction, and this fraction had an A/X of 1.02 (Saini and Henry 1989). In this study, 18% of the arabinoxylans of WU were not extracted. Therefore, we delignified RES and extracted the obtained residue with alkali.

After delignification, only part (33%) of the thus far alkali-unextractable arabinoxylans were recovered in alkaline extracts. All fractions had high A/X (Table III). The results were in agreement with data reported for wheat bran. Indeed, Brillouet and Mercier (1981) reported that a total delignification was not obtained, and that the residue contained a high level of hemicellulosic material firmly bound to the lignin-cellulose complex (representing 19.8% of original arabinoxylans). However, Hromádová and Ebringrová (1987) reported that the arabinoxylan that remained in the residue after delignification and alkaline extraction of rye bran had an A/X of 0.35.

Methylation Analysis and NMR Investigation of Arabinoxylan Structures

Intermediate A/X arabinoxylans. The spectrum of BElares (Fig. 2a) revealed that the structural features of water-extractable arabinoxylans were also present in alkali-extractable rye arabinoxylans. Methylation analysis revealed that this arabinoxylan, in molar percent of total xylose residues, consisted of 58% xyul, 25% 3mxyl, 2% 2mxyl, and 9% dxyl. A proportion of the xylose residues (5%) occurred as nonreducing terminal units (Tables IV and V).

The spectrum of BE2 (Fig. 2b) was similar to that of BElares. However, β-glucan and a minor amount of α-dextrin were also present, as well as minor peaks similar to those in BE1asup. The presence of β-glucan in BE2 was confirmed by methylation analysis that detected both 3-linked and 4-linked glucose (Table IV). 1H-NMR spectroscopy and methylation analysis showed that BE2 contained less 3-monosubstituted xyloses than BElares. Substituted arabinoses and terminal xyloses were measured in higher proportions. The content of 2-monosubstituted xylose residues was low for all samples (Tables IV and V), which is in accord with results obtained by Ebringrová et al. (1990) for rye alkali-extractable arabinoxylans.

The majority of the arabinoxylans in BE1 and BE2 extracts from WU can be considered to belong to a group of arabinoxylans with intermediate A/X, which are soluble in water after alkaline extraction.
extraction and precipitate in a saturated ammonium sulfate solution.

High A/X arabinoxylans. Apart from the known arabinose peaks, the spectrum of BE1asup (Fig. 3a) contained a variety of unidentified peaks, which revealed the complexity of the material. Some resonances in the range of 85.0–5.4 ppm possibly originated from substituted arabinoses (Joseleau et al 1977). The $^1$H-NMR spectra of the fractions released after delignification of the alkali-unextractable residue (RES1M and RES4M) much resembled the spectra of the ammonium sulfate-soluble fraction of the BE1 extract (BE1asup), as shown in Figure 3. This suggested that saturated barium hydroxide (containing 1% NaBH$_4$) can release a proportion of arabinoxylans that seems to be associated with lignin. Peak broadening occurred and peaks of paired substituted xylose units could not be recognized (Fig. 3).

The $^{13}$C-NMR spectrum (Fig. 4) revealed more information about the structure of RES1M. About the same proportion of xylose residues containing one or two arabinose residues were present, but the structure was more complex; there were additional peaks in the anomeric region and in the remaining part of the spectrum. The peaks at 699.7 ppm and 665.8 ppm were well resolved and intense. They can be attributed to terminal xylose units (Kovac et al 1980, Brillouet and Joseleau 1987, Ebringerová et al 1990, Ebringerová et al 1992). The presence of 2-linked or 5-linked arabinose, which can be recognized by resonances at 689.8 ppm and 667.1 ppm, respectively (Ebringerová et al 1992), could not be deduced from the spectrum (Fig. 4). Unassigned resonances could originate from other substituted arabinose residues or from galactose and glucose residues, which were also present in low proportions in the sample (Table IV).

The resemblance between BE1asup and RES1M observed using $^1$H-NMR (Fig. 3) was confirmed by methylation analysis (Table IV). While $^1$H-NMR revealed only the complexity of the polymer, methylation analysis showed substituted arabinoxyl residues (2-, 3-, 5-, and 2,3-linked arabinose) up to 40% of total arabinose. Terminal xylose, already detected by $^{13}$C-NMR (Fig. 4), made up 26% of the xyloses. Terminal galactose was also detected. All these observations indicated the presence of oligomer side chains. Indeed, terminal xylose and terminal galactose units can be linked through arabinose residues to the xylan chain (Wilkie 1979). The ratio of terminal galactose to substituted arabinose was ~0.23, and the ratio of terminal xylose to substituted arabinose was ~0.59 (samples from Table IV; a correction for xyloglucan had to be made in 4M). Substituted arabinoses were consistent with the observation of unknown peaks in the $^1$H-NMR spectra. According to Ebringerová et al (1990), 20% of the arabinose moieties were linked 2-, 3-, and 5- in some rye bran arabinoxylans. Substituted arabinoses, terminal xylose, and terminal galactose have been reported as features in arabinoxylans from rye bran (Ebringerová et al 1990); from wheat bran (Brillouet et al 1982, Brillouet and Joseleau 1987, DuPont and Selvendran 1987, Shiba et al 1993); from rice bran (Shibuya and Iwasaki, 1985); from maize kernels unextractable residue.

**TABLE IV**

Methylation Analysis of Water-Unextractable Arabinoxylan Fractions

<table>
<thead>
<tr>
<th>Methylated Compound</th>
<th>Linkage Mode</th>
<th>Fraction (Mol %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>BE1asup</td>
</tr>
<tr>
<td>2,3,5-Me$_2$-Ara</td>
<td>t-Araf</td>
<td>29.3</td>
</tr>
<tr>
<td>3,5-Me$_2$-Ara</td>
<td>2-Araf</td>
<td>4.9</td>
</tr>
<tr>
<td>2,5-Me$_2$-Ara</td>
<td>3-Araf</td>
<td>7.7</td>
</tr>
<tr>
<td>2,3-Me$_2$-Ara</td>
<td>5-Araf</td>
<td>3.3</td>
</tr>
<tr>
<td>5-Me-Ara</td>
<td>2,3-Araf</td>
<td>3.2</td>
</tr>
<tr>
<td>2,3,4-Me$_2$-Xyl</td>
<td>t-Xylp</td>
<td>11.5</td>
</tr>
<tr>
<td>2,3-Me$_2$-Xyl</td>
<td>4-Xylp</td>
<td>7.4</td>
</tr>
<tr>
<td>2-Me-Xyl</td>
<td>3,4-Xylp</td>
<td>13.5</td>
</tr>
<tr>
<td>3-Me-Xyl</td>
<td>2,4-Xylp</td>
<td>(7.6)$^c$</td>
</tr>
<tr>
<td>Xyl</td>
<td>2,3,4,6-Me$_2$-Xyl</td>
<td>11.6</td>
</tr>
<tr>
<td></td>
<td>t-Galp</td>
<td>4.3</td>
</tr>
<tr>
<td>2,4-Me-Gal</td>
<td>3,6-Galp</td>
<td>1.1</td>
</tr>
<tr>
<td>2,4,6-Me$_2$-Glc</td>
<td>3-Glcp</td>
<td>1.0</td>
</tr>
<tr>
<td>2,3,6-Me$_2$-Glc</td>
<td>4-Glcp</td>
<td>0.9</td>
</tr>
<tr>
<td>2,3,6-Me$_2$-Man</td>
<td>4,6-Glcp</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>TR</td>
<td>...</td>
</tr>
</tbody>
</table>

$^a$BE1asup, BE1ares = ammonium sulfate fractionation fractions from the WU$_1$ Ba(OH)$_2$ extract (BE1). BE2a, 1Ma, 4Ma = extracts obtained by sequential alkaline extraction from WU$_1$. RES1M = extract obtained after delignification of the alkaline-unextractable residue from WU$_1$. WU$_1$ = water unextractable arabinoxylans from rye wholemeal.

$^b$Not detected.

$^c$2-Me-Xyl and 3-Me-Xyl coeluted, the ratio of 2-Me-Xyl to 3-Me-Xyl was measured using MS and presented within parentheses.

$^d$Trace.

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(Saulnier et al 1993); and from sorghum husk (Woolard et al 1977). Quantitatively, the results of methylation analysis of \( BE_{\text{lasiup}} \) and \( RES\text{IM} \) corresponded quite well with results described for wheat beeeswing bran (Brillouet and Joseleau 1987, DuPont and Selvendran 1987).

In rye water-extractables, arabinoxylan fractions with high A/X (1.09, 1.34, and 1.42) were isolated (Vinkx et al 1993, 1995). However, they contained only trace levels of terminal xylose (up to 2% of xyloses) and substituted arabinoses (up to 2% of arabinoses). Therefore, they were not comparable to high A/X alkali-extractable arabinoxylans. In the rye water-extractables, trace amounts of substituted arabinose and terminal xylose are ascribed to contamination with bran polymers of the class of high A/X arabinoxylans. In wheat flour water-extractables, fractions in which up to 10% of arabinoses were substituted were reported by Izydorczyk et al (1993).

Low A/X arabinoxylans. The presence of a third group consisting of low branched xylans, was deduced from the data for 1M (Table IV). Hromádková et al (1987) extracted from rye bran an arabinoxylan with A/X 0.14 with 1.1 M NaOH and precipitated it by acidification of the alkaline extract. This arabinoxylan was water-insoluble and was substituted with single arabinose units at position 3 of every sixth or seventh xylosyl residue. It represented ~50% of the nonstarch and noncellulosic polysaccharides in rye bran (Hromádková et al 1987). In 1M, apart from terminal arabinose and unsubstituted and monosubstituted xylose residues, minor amounts of disubstituted xyloses were measured. Because Hromádková et al (1987) detected no disubstituted xyloses in rye arabinoxylans with a low degree of branching and 3% dxyl was measured in 1M, possibly 1M was not a pure arabinoxylan of this class (the A/X of 1M was 0.21). The isolation procedure included no separation between the water-soluble and water-insoluble fraction of the 1M extract. It was observed, however, that 1M had a low solubility.

The presence of both 4-linked glucose and 4-linked mannose in 4M (Table IV) was an indication of glucomannans (Fincher and Stone 1986, Gruppen et al 1992). The detection of 4-linked glucose also in 4M was an indication of xylglucans, \( \beta-(1-4)\)-glucan with terminal \( \alpha\)-xylose units linked at O-6 of glucose residues, which are known to solubilize in 4.3 M KOH but not in 0.7 M KOH (Hayashi 1989).

**Arabinoxylan Classes and Literature Data**

A strong similarity between the alkali-extractable arabinoxylans from rye and wheat was found when examining literature data. Three different groups of alkali-extractable arabinoxylans were described for wheat bran. The first group precipitated upon neutralization of the alkaline extract (hemicellulose A). This group represented about 28% of the arabinoxylan in industrial wheat bran (Brillouet and Mercier 1981). It had an A/X of 0.18–0.25 (Brillouet and Mercier 1981, DuPont and Selvendran 1987) and was probably located in the aleurone (Brillouet and Joseleau 1987). The water-soluble alkali-extracted arabinoxylans were divided into two groups. The intermediate branched arabinoxylans contained the usual structural elements (terminal arabinose, 4-, 3-, and 2,3,4-linked xylose). The main bran arabinoxylan was highly branched (A/X 0.9–1.23) and apart from the usual elements, also contained substantial amounts of substituted arabinoses (2-, 3-, 5-, or 2,3,5-linked), terminal xylose, terminal galactose, and terminal (4-O-methyl-)glucuronic acids (Brillouet et al 1982, Brillouet and Joseleau 1987, DuPont and Selvendran 1987, Shiba et al 1993).

The isolation procedure of Saini and Henry (1989) did not permit a distinction between different arabinoxylan classes in the alkaline extracts from rye grain. Ebringerová et al (1990) probably described a mixture of rye bran high and intermediate A/X arabinoxylans. The authors reported A/X and proportions of substituted arabinoses that were in between the results for the intermediate and the high A/X arabinoxylans described in this work. Only two different groups of arabinoxylans were recognized in rye bran (Hromádková et al 1987, Ebringerová et al 1990), while in this work three groups are described, much as what has been reported before for wheat bran.

**Molecular Weight Distributions**

Apparent molecular weights of solubilized rye water-unextractable arabinoxylans were in the same range (Fig. 5) as those

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**TABLE V**

<table>
<thead>
<tr>
<th>Relative Proportions of the Partially Methylated Xylose Residues Calculated from Methylation Analysis Data*</th>
</tr>
</thead>
<tbody>
<tr>
<td>( BE_{\text{lasiup}} )</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>% ( \text{uxyl} )</td>
</tr>
<tr>
<td>% ( \text{3mxyl} )</td>
</tr>
<tr>
<td>% ( \text{2mxyl} )</td>
</tr>
<tr>
<td>% ( \text{dxyl} )</td>
</tr>
<tr>
<td>% ( \text{txyl} )</td>
</tr>
</tbody>
</table>

*\( BE_{\text{lasiup}}, BE_{\text{lares}} = \) ammonium sulfate fractionation fractions from the \( WU_{1}\, \text{Ba(OH)}_{2}\) extract (\( BE_{1} \)), \( BE_{2a}, 1\text{Ma}, 4\text{Ma} = \) extracts obtained by sequential alkaline extraction from \( WU_{1}, RES\text{IM} = \) extract obtained after delignification of the alkaline-unextractable residue from \( WU_{1}, WU_{1} = \) water unextractable arabinoxylans from rye wholemeal, \( \text{uxyl} = \) xylose residue unsubstituted with arabinose, 3\( \text{mxyl} = \) 3-monosubstituted xylose, 2\( \text{mxyl} = \) 2-monosubstituted xylose, dxyl = disubstituted xylose, txyl = terminal xylose.

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**Fig. 3.** Diagnostic regions of the \(^1\text{H}-\)nuclear magnetic resonance spectra (\( D_{2}O, 85^\circ C, 300 \text{ MHz} \)) of rye alkali-extracted arabinoxylan fractions \( BE_{\text{lasiup}} \) (a) and \( RES\text{IM} \) (b).

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**Fig. 4.** \(^1\text{C}-\)nuclear magnetic resonance spectrum (\( D_{2}O, 85^\circ C, 75 \text{ MHz} \)) of the rye arabinoxylan fraction with high arabinose to xylose ratio (\( RES\text{IM} \)): arabinose anomeric region (1); xylose anomeric region (2); terminal xylose (3).
reported for water-extractable rye arabinoxylans (Vinkx et al. 1993). No molecular weight distribution was obtained for 1M and 4M fractions because of the low solubility of these preparations.

Extractability of Arabinoxylans

The low A/X of the arabinoxylans in 1M possibly explains why this fraction was neither water-extractable nor water-soluble after alkali-extraction. Andrewartha et al. (1979) found that wheat arabinoxylans with A/X <0.43 were water-insoluble because of noncovalent bonds between unsubstituted xyloses. In vitro noncovalent bonds between arabinoxylans (A/X 0.44) and cellulose and among arabinoxylans have been reported for barley aleurone (McNeil et al. 1975). The authors found that arabinoxylans with a high A/X (1.38) did not bind to cellulose in vitro. However, the observation that arabinoxylans that could not be solubilized from wheat flour water-unextractables by endoxylanase had a high A/X was interpreted as an indication that in vivo, a high degree of substitution does not hinder binding to cellulose (Gruppen et al. 1993).

The other two arabinoxylan classes were water-soluble after alkaline extraction. Extraction involves cleavage of the native bonds in the cell wall matrix. The extent to which these bonds occur causes differences in the extractability of the different arabinoxylans (Hromadkóva and Ebringérová 1992). Also, Gruppen et al. (1992) concluded that the difference in extractability between water-extractable and Ba(OH)₂-extractable wheat flour arabinoxylans was not caused by structural differences, but by differences in covalent or noncovalent bonds, while structural differences might have influenced the differences in extractability in different alkaline media. Markwalder and Neukom (1976) found evidence that alkaline-labile diferulic acid cross-links reduced the extractability of the intermediate A/X arabinoxylans. A release of the arabinoxylans then occurred when the β-glucans solubilized.

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LITERATURE CITED


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