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Water unextractable polysaccharides from three milling fractions of rye grain

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Water unextractable material from bran, an intermediate milling fraction and sieved flour of rye grain were sequentially extracted at room temperature with saturated barium hydroxide, water, 4 M potassium hydroxide and water followed by extraction with 2 M potassium hydroxide in a boiling water bath, giving repeatable recoveries of extracts and polysaccharide residue compositions in collected fractions. Total recoveries of polysaccharide residues in extracts and residue from the different water unextractable materials were 78–88%. Extracts in which 90–93% of the carbohydrates were arabinose and xylose residues were obtained by extraction with saturated barium hydroxide. Subsequent extraction with water yielded a fraction in which 64–68% of the carbohydrates were glucose residues. The extraction with hot alkali resulted in extracts in which 85–89% of the carbohydrates were arabinose and xylose residues. The ara/xyl ratio in the collected fractions ranged from 0.1–1.3, with the lowest ratios in fractions that precipitated after neutralisation of the 4 M potassium hydroxide extract and the highest ratios in the unextractable residues. Structural characterisation with ^{1}H-NMR spectroscopy revealed varying substitution patterns for arabinoxylans in the different extracts and that glucose residues in the extracts essentially originated from mixed-linked β-glucan. The proportion of disubstituted xylose residues was lower in barium hydroxide extracts compared to the other main extracts. A highly branched heteroxylan was extracted with hot alkali. The polysaccharides found in the corresponding extracts for all the starting materials had generally similar structural features, but the yield differed considerably. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Rye is a traditional dietary fibre- and starch-rich food cereal in Northern and Eastern Europe, mainly consumed as soft or crisp bread. The dietary fibre content has been reported to range between 13 and 17% (Graham *et al.*, 1987; Pettersson & Åman, 1987; Nilsson *et al.*, 1996) and the major dietary fibre constituent is arabinoxylan, of which 60–70% is water unextractable (Pettersson & Åman, 1987; Saini & Henry, 1989). Other important dietary fibre polysaccharides are mixed-linked (1→3),(1→4)-β-D-glucan (β-glucan) and cellulose. Dietary fibre polysaccharides have been shown to affect the baking performance of rye flour (Meuser & Suckow, 1986; Kühn & Grosch, 1988, 1989; Autio *et al.*, 1995) and also to influence the nutritional value of rye based diets for chickens (Antoniou *et al.*, 1981; Frigård *et al.*, 1994) and humans (Hagander *et al.*, 1987; Pettersson *et al.*, 1996). The fine structure and molecular weight of dietary fibre polysaccharides are important parameters since they will influence the physical properties of the polymers and therefore the technological performance and nutritional properties of the products. In this respect information on the occurrence and structure of arabinoxylan sidegroups are essential since they affect the enzymatic degradation of the polysaccharide (Düsterhöft *et al.*, 1993; Kormelink *et al.*, 1993; Vëtor *et al.*, 1994).

Water extractable arabinoxylans in rye have been shown to contain a backbone of 4-linked β-D-xylopyranosyl residues with side chains of terminal α-L-arabinofuranosyl residues essentially linked to C3 or to C2 and C3 but also to some extent to C2 (Åman & Bengtsson, 1991; Bengtsson *et al.*, 1992; Vinkx *et al.*, 1993, 1995a). Recently, an extraction technique that facilitates the fractionation and characterisation of cereal polysaccharides has been developed (Gruppen *et al.*, 1991; Vinkx *et al.*, 1995b). In this procedure aqueous saturated barium hydroxide preferentially extracts arabinoxylan from water unextractable mate-

rial of cereal grain, while most of the β-glucan remains insoluble. A large proportion of the β-glucan is released during the treatment with barium hydroxide and can subsequently be extracted with water. Only a few studies of water unextractable arabinoxylans in rye have been performed (Hromádková *et al.*, 1987; Saini & Henry, 1989; Ebringerová *et al.*, 1994; Vinkx *et al.*, 1995b). Water unextractable arabinoxylans have been divided in three groups differing in extractability, solubility and structure (Vinkx *et al.*, 1995b). One group was extractable in saturated barium hydroxide, or with water after the barium hydroxide treatment, and had an ara/xyl ratio of 0.55–0.79. A second, alkali extractable group, had an ara/xyl ratio of about 1.1. Typical structural features were substituted arabinose residues and terminal xylose and galactose residues. A third group that was extracted with 1 M potassium hydroxide and precipitated after neutralisation had an ara/xyl ratio of about 0.2. The water unextractability of arabinoxylans may be due to non-covalent links between unsubstituted xylose residues (Andrewartha *et al.*, 1979) or unsubstituted xylose residues and cellulose (McNeil *et al.*, 1975). Other suggested explanations are alkali labile diferulic acid cross-links (Markwalder & Neukom, 1976) and linkages between arabinoxylans and other cell wall components such as lignin (Jeffries, 1990).

Some structural features of β-glucan from rye were recently shown by studying oligosaccharides released by the action of lichenase on whole grain flour (Wood *et al.*, 1994). The ratio of 3-linked cellotriosyl to 3-linked cellotetraosyl units, which constituted 95% of the polysaccharide, was 3.1. Small proportions of mainly 3-liked cellopentaosyl, cellohexaosyl and cellononeosyl units were also quantified.

The aims of the present investigation were to study water unextractable polysaccharides in rye grain by applying the newly developed extraction procedure with saturated barium hydroxide to three different milling fractions and to characterise the polysaccharides in isolated fractions by sugar and ^{1}H-NMR analyses.

EXPERIMENTAL

Starting materials

Bran, an intermediate (D2-B3f) milling fraction and a sieved flour of rye, in the following referred to as bran, intermediate and flour, respectively, were obtained from a Swedish commercial mill (AB Nord mills, Malmö). Bran and intermediate were further ground in a Retsch laboratory mill using a 0.5 mm screen.

Isolation of water unextractable material (WUM)

Samples (40 g) were refluxed twice with 200 ml 90% aqueous ethanol for 30 min in a boiling water bath and the insoluble residue isolated by centrifugation (3000 g, 15 min) (Fig. 1). The pellet was suspended in 75 ml water and treated in an ultraturrax for 20 s. The ultraturrax rod was washed with 46 ml water for 20 s and the slurries were combined. A mixture of 75 ml water, 56 mg $CaCl_2$ and 4.0 ml thermostable α-amylase (Termamyl 120 L, Novo Nordisk A/S, Copenhagen, Denmark) was pre-heated for 15 min in a boiling water bath. This α-amylase mixture was added to the combined slurry and treated in a boiling water bath for 90 min. The insoluble residue was isolated by centrifugation (8000 g, 15 min) and extracted in the same manner as the pellet from ethanol extraction but using half the amount of α-amylase. Remaining WUM was washed with 2 × 100 ml water, isolated by centrifugation and freeze-dried.

Fractionation of WUM

Duplicate samples (4 g) of WUM were extracted with sequential alkaline extraction essentially as described by Gruppen *et al.* (1991) and as outlined in Fig. 1. Except when otherwise stated, extractions were carried out by magnetic stirring at room temperature, dialysis was performed against running deionised water for 72 h and extracts and residues were isolated by centrifugation at 8000 g for 15 min.

WUM was extracted with 400 ml saturated aqueous barium hydroxide containing 0.1% (w/v) KBH_4 for 16 h. After centrifugation, the pellet was further extracted for 1 h with another 200 ml of the same solvent. The supernatants were combined, acidified with acetic acid to pH 5.0, dialysed and separated into a soluble (BE) and an insoluble (BP) fraction by centrifugation. To the insoluble material from the barium hydroxide extraction, 400 ml water was added and the slurry acidified to pH 5.0 with acetic acid. Extraction was carried out for 1 h and the pellet was re-extracted with 200 ml water for 1 h. The supernatants were combined and isolated after dialysis (BN).

The remaining pellet was extracted with 400 ml 4 M aqueous potassium hydroxide containing 0.1% (w/v) KBH_4 for 16 h. After centrifugation, the pellet was further extracted for 1 h with another 200 ml of the same solvent. The supernatants were combined, acidified with acetic acid to pH 5.0, dialysed and separated into a soluble (PE) and an insoluble (PP) fraction by centrifugation. To the remaining pellet from the potassium hydroxide extraction, 400 ml water was added and the slurry acidified to pH 5.0 with acetic acid followed by extraction for 1 h and centrifugation. The supernatant was dialysed and freeze-dried (PN-fraction), and the insoluble residue was freeze-dried.

Part of the residue (1.5 g) was suspended in 2 M potassium hydroxide and heated for 2.5 h in a boiling water bath. After centrifugation the pellet was washed

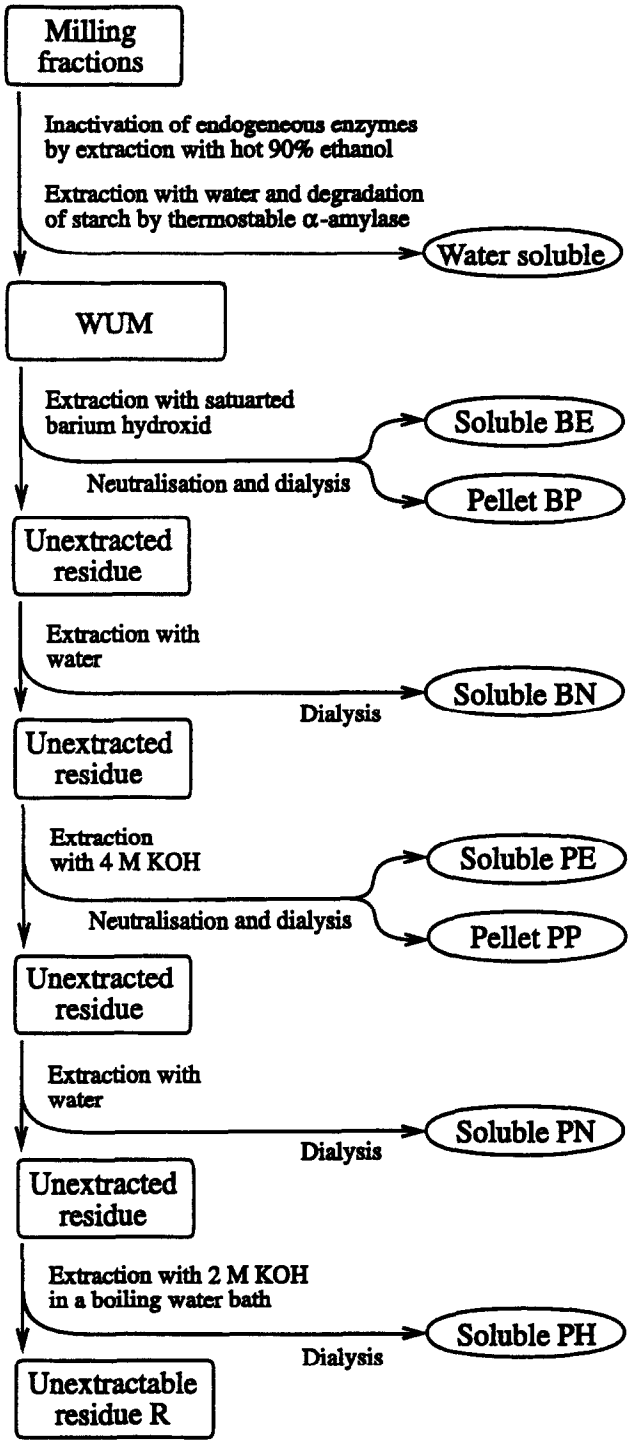

Fig. 1. Outline of the extraction procedure used for the isolation of water insoluble material (WUM), extracts (BE, BP, BN, PE, PP, PN and PH) and unextractable residue (R).

with 50 ml 2 M potassium hydroxide and thereafter with 50 ml water. The three supernatants were pooled and acidified to pH 5.0 with 8 M sulphuric acid. Supernatants were dialysed overnight, concentrated to about 50 ml in a rotary evaporator and freeze-dried. The dry supernatant was redissolved in water (10 ml), further desalted by chromatography on a Sephadex G50 column which was eluted with water, and finally freeze-dried (PH). The extraction residue (R) was washed with water (50 ml), dried by solvent exchange (95% ethanol and acetone) and further evaporated in a vacuum oven at 40°C for 48 h.

^{1}H-NMR

Proton nuclear magnetic resonance (^{1}H-NMR) spectra (400 MHz) were recorded at 85°C on a Varian VXR 400 instrument. Pulse repetition time was 3.75 s (~2000 pulses) and radio frequency pulse angle 45°. Dried samples (10–20 mg) were dissolved in D_2O and evaporated. This procedure was repeated 4–5 times, and finally samples were dissolved in 1 ml D_2O. The water signal was used as reference.

General analyses

The dry matter content was determined by drying at 105°C for 16 h. Starch, including traces of glucose, maltose, and malto-oligosaccharides, was determined enzymatically (Åman *et al.*, 1994). Crude protein ($N \times 6.25$) and ash were analysed according to standard methods (AOAC, 1984). Crude fat was determined gravimetrically after acid hydrolysis and petroleum ether extraction in a Soxtec HT6 (Tecator AB, Sweden) (Anon., 1971). β-Glucan was determined enzymatically (Åman & Graham, 1987).

Total dietary fibre in starting materials and WUM was determined as the sum of neutral and acidic dietary fibre polysaccharide residues and Klason lignin, according to the procedure of Theander *et al.* (1995). When analysing fractions BE, BP, BN, PE, PP, PN and PH for dietary fibre polysaccharides the starch removal step was omitted and 5–10 mg of fractions were weighed into a 25 ml tube. A mixture of 300 μl of 72% sulphuric acid and 8.4 ml water containing 1 mg myo-inositol as internal standard was added and the samples were autoclaved and analysed in the same manner as described in the dietary fibre method. Fraction R (5–10 mg) was also treated with 300 μl of 72% sulphuric acid at 30°C for 30 min as described in the dietary fibre method before addition of the 8.4 ml H_2O containing 1 mg myo-inositol. All analyses were carried out in duplicate and are reported on a dry matter basis.

RESULTS AND DISCUSSION

Starting materials

Bran, intermediate and flour were obtained from a commercial mill. Chemical analysis revealed, as expected, that the bran had the highest content of ash, crude protein, crude fat, dietary fibre and β-glucan, and the lowest content of starch (Table 1). The flour on the

Table 1. Chemical composition of bran, intermediate milling fraction and sieved flour from rye grain (% dry matter)

	Ash	Crude Protein	Crude Fat	Starch[a]	Dietary Fibre	
					Total[b]	β-Glucan
Bran	4.1	14.8	2.7	19.8	38.4	3.4
Intermediate	1.2	10.7	1.7	51.7	23.8	2.2
Flour	0.6	8.0	1.1	76.8	8.4	1.3

[a]Excluding enzyme resistant starch.
[b]Calculated as the sum of neutral and acidic dietary fibre polysaccharide residues, including enzyme resistant starch, and Klason lignin.

other hand had the highest content of starch and the lowest content of the other components analysed.

Characterisation of WUM

The starting materials were extracted with 90% hot ethanol, in order to inactivate endogenous enzymes and to remove soluble extractants, and further treated with α-amylase in water in order to remove starch and water-soluble materials (Fig. 1). The WUMs isolated constituted 50%, 25% and 9% of the bran, intermediate and flour, respectively and their levels of residual starch were less than 2% (Table 2). Total content of polysaccharide residues in WUMs varied from 43 to 64%, mainly arabinose, xylose and glucose. Small amounts of mannose, galactose and uronic acid residues as well as traces of rhamnose and fucose residues were also analysed. WUM derived from intermediate contained the highest amount of mannose residues and the ratio of arabinose to xylose residues was lowest in WUM from bran (0.49) and highest in WUM from flour (0.78). WUM from bran contained 7% Klason lignin and WUM from flour only 2%. On the other hand the crude protein content was highest in WUM from flour. The yield and chemical composition of WUMs were repeatable, as shown by the duplicate analyses presented. Similar water unextractable material has previously been isolated for whole grain rye and this material had a arabinose to xylose ratio of 0.52 (Vinkx *et al.*, 1995b).

Recovery of polysaccharide residues

WUM from the different starting materials was extracted in duplicate as outlined in Fig. 1. The percentage yield of dry matter as extracts and residue varied from 66 to 73% with the lowest percentage yield for the flour (Table 3). The relatively low recovery of dry matter indicates that losses occurred during the dialysis and precipitation procedures. BE was the main fraction for all WUMs with a percentage yield of dry matter varying from 22 to 30%.

Total recovery of polysaccharide residues ranged from 78 to 88% (Table 3). The main part of the polysaccharide residues was generally recovered in BE, BN, PE, PH and R. The recovery of polysaccharide residues was only 0.2–0.5% in BP and PN, respectively. For WUM derived from bran, about 50% of the polysaccharide residues were extracted in BE and BN while about 20% required 4 M potassium hydroxide at room temperature to be solubilized (PE, PN and PP) and about 6% hot 2 M potassium hydroxide (PH). About 11% of the polysaccharide residues were recovered in R. A larger proportion of the polysaccharide residues in WUMs derived from flour was extracted in BE and BN (60%) while smaller proportions were solubilized with 4 M potassium hydroxide treatment (8%), hot 2 M potassium hydroxide (4%) and left in R (7%). Total recovery of polysaccharide residues in extracts and R derived from intermediate were generally in between those of bran and flour.

Total recoveries of arabinose and xylose residues in the extracts and R were high (87–92%) and those of glucose and uronic acid residues varied between 73 and 89% (Table 3). Recoveries of mannose (23–29%) and galactose (46–60%) residues were substantially lower. The repeatability in the recovery of total as well as individual (the duplicate analysis not shown in the table) polysaccharide residues was generally very good.

A high proportion of the arabinose (48–59%) and

Table 2. Percentage yield of water unextractable material from duplicate extractions of bran, intermediate milling fraction and sieved flour and their contents of dietary fibre constituents (polysaccharide residues and Klason lignin) and crude protein (% of dry matter)

	Yield	Dietary fibre constituents									
		Ara	Xyl	Man	Gal	Glc	Uronic acid	Klason Lignin	Sum[a]	Ara/Xyl[b]	Crude Protein
Bran	50.5	12.0	24.1	1.3	1.8	15.1	1.4	6.9	62.6	0.50	20.2
	49.1	12.1	25.0	1.3	1.8	15.0	1.4	7.0	63.6	0.48	20.5
Intermediate	25.0	13.9	20.2	3.1	1.8	16.7	1.0	5.0	61.6	0.69	26.0
	24.1	13.6	19.4	3.0	1.7	16.8	1.0	5.1	60.6	0.70	25.7
Flour	9.2	9.9	12.6	2.3	1.4	13.3	0.8	2.1	42.5	0.79	44.0
	9.2	10.2	13.3	2.5	1.4	13.9	0.8	1.8	43.8	0.77	44.3

[a]Including traces of rhamnose and fucose residues.
[b]The ratio of arabinose to xylose residues.

Table 3. Percentage yield (% dry matter) and recovery of polysaccharide residues (% of corresponding residue in WUM) in extracts (BE, BP, BN, PE, PP, PN and PH) and unextractable residues (R) after extraction of water insoluble material from bran, intermediate milling fraction and sieved flour. Percentage yield and total recovery of polysaccharide residues are presented in duplicate (1 and 2), while individual polysaccharide residues are presented as means of two extractions

			Polysaccharide residues							
	Percentage yield		Ara	Xyl	Man	Gal	Glc	Uronic acid	Total[a]	
Extraction	1	2							1	2
Bran										
BE	25.0	22.3	47.8	43.0	3.4	18.6	3.5	16.2	29.0	32.9
BP	2.7	3.0	0.3	0.1	0.5	0.7	0.2	0.0	0.2	0.2
BN	12.1	10.9	9.7	7.9	6.0	13.5	44.1	14.1	19.2	17.3
PE	11.4	10.4	8.0	14.6	5.4	7.5	8.9	18.7	13.0	9.7
PP	8.2	7.7	2.9	20.7	0.3	1.1	0.7	6.2	10.4	9.6
PN	0.5	0.5	0.5	0.3	0.3	0.4	0.1	1.1	0.4	0.3
PH	4.7	4.5	12.7	5.7	0.3	10.3	0.5	14.5	6.5	5.6
R	8.4	8.2	10.4	4.4	8.7	8.1	25.4	7.0	12.0	11.1
total	72.9	67.5	92.2	96.8	25.0	60.3	83.5	77.7	87.7	86.7
Intermediate										
BE	28.1	26.1	53.7	57.6	3.0	17.0	4.9	20.1	34.0	37.8
BP	3.9	3.9	0.4	0.3	0.2	0.8	0.1	0.0	0.3	0.3
BN	13.7	12.8	10.7	9.6	10.2	11.8	43.7	19.7	20.4	20.0
PE	6.1	6.7	5.7	6.7	7.7	7.9	8.4	18.6	7.8	6.7
PP	6.4	6.7	1.4	8.1	0.3	1.6	0.7	2.1	3.8	3.3
PN	0.7	0.7	0.7	0.5	0.4	0.9	0.2	2.6	0.5	0.5
PH	4.7	4.7	10.1	6.8	0.1	10.7	0.7	16.7	5.9	5.6
R	8.1	6.9	7.4	4.5	6.9	7.6	22.7	9.7	11.9	10.1
total	71.7	68.5	90.0	94.1	28.8	58.2	81.4	89.5	84.3	84.3
Flour										
BE	29.1	29.7	58.6	67.9	3.7	20.7	07.0	27.4	40.6	38.0
BP	9.7	9.3	0.4	0.3	0.4	1.0	0.2	0.0	0.3	0.3
BN	10.0	10.0	10.7	8.9	9.7	9.7	43.0	22.2	20.9	20.8
PE	4.7	5.1	4.4	5.3	6.1	5.2	7.3	14.1	5.3	6.6
PP	6.2	6.3	1.3	2.3	0.3	1.7	2.0	4.9	1.9	1.8
PN	0.3	0.2	0.3	0.2	0.2	0.3	0.2	0.9	0.2	0.3
PH	2.2	2.0	7.8	5.5	0.1	5.3	0.3	9.3	4.4	3.8
R	3.5	3.2	3.8	2.3	2.5	2.4	14.0	5.0	7.1	6.2
total	65.7	65.8	87.3	92.8	23.1	46.3	73.9	83.9	80.7	77.8

[a]Including traces of Rhamnose and Fucose.

xylose (43–68%) residues was recovered in BE (extracted with barium hydroxide) for all WUMs and significant amounts also in BN, PE, PH and R (Table 3). The recovery of arabinose and xylose residues in BE was substantially higher for the flour WUMs than for the intermediate and bran WUMs. A larger proportion of the arabinose and xylose residues in the latter two WUMs required stronger alkali for extraction or were not extracted at all. The recovery of xylose residues in PP from bran WUM (20%) was much higher than those extracted from intermediate and flour WUMs and indicated the presence of a specific xylan in the bran. Glucose residues were mainly recovered in BN (43–44%) and R (14–25%). The recovered mannose residues were mainly found in BN, PE and R and recovered galactose residues were present in the same extracts as well as in BE and PH. The low recovery of mannose and galactose residues indicates that a large proportion of these residues was present as smaller fragments that were released during the extraction and subsequently lost in the dialysis.

Similar extraction procedures to the one used in this study have been applied to wheat and barley. In those studies it was also shown that arabinoxylan was enriched in BE and β-glucan in BN and PE (Gruppen *et al.*, 1992; Viëtor *et al.*, 1992).

Polysaccharide content and composition in collected fractions

Total content of polysaccharide residues in BE varied between 55 and 75% with the lowest content in BE from flour (Table 4). All BE fractions proved to be mostly arabinoxylan since the sum of arabinose and xylose residues accounted for more than 90% of polysaccharide residues. The ara/xyl ratio in BE from intermediate and flour was approximately 0.65 while that of bran was only 0.54, indicating a lower degree of

Table 4. Polysaccharide content (% dry matter) and relative composition of polysaccharide residues in extracts (BE; BP; BN; PE; PP; PN and PH) and unextractable residue (R) from WIM derived from bran, intermediate milling fraction and sieved flour. Results are presented as means of two extractions

	Polysaccharide content	Ara	Xyl	Man	Gal	Glc	Uronic acid	Ara/Xyl[a]
Bran								
BE	74.5	32.9	60.4	0.3	1.9	3.1	1.3	0.54
BP	4.1	29.4	27.3	5.9	11.2	21.8	0.0	1.07
BN	89.4	11.3	18.8	0.8	2.4	64.4	1.9	0.60
PE	58.4	15.1	56.1	1.1	2.1	21.1	4.0	0.27
PP	71.4	6.1	89.9	0.1	0.4	1.9	1.5	0.07
PN	37.8	33.7	41.6	2.3	4.3	9.3	8.0	0.81
PH	73.0	44.8	41.5	0.1	5.5	2.2	5.9	1.08
R	78.8	19.1	16.7	1.8	2.3	58.7	1.5	1.15
Intermediate								
BE	74.8	36.4	56.6	0.4	1.4	4.0	1.0	0.64
BP	3.8	32.5	33.7	4.8	8.8	16.4	0.0	0.97
BN	86.1	12.8	16.7	2.7	1.8	64.0	1.7	0.77
PE	64.5	19.1	32.4	5.7	3.3	34.3	4.6	0.59
PP	30.6	9.3	81.0	0.5	1.4	6.2	1.1	0.12
PN	39.0	32.6	36.2	4.3	5.2	11.8	9.2	0.90
PH	68.9	43.3	42.0	0.1	5.7	3.7	5.3	1.03
R	81.5	16.6	14.6	3.4	2.1	61.7	1.6	1.14
Flour								
BE	55.2	36.2	54.0	0.6	1.8	5.8	1.4	0.67
BP	1.3	31.0	26.9	8.5	10.8	17.6	0.0	1.15
BN	86.6	12.4	13.4	2.7	1.6	67.6	2.1	0.93
PE	50.0	17.7	27.9	5.9	2.9	40.1	4.6	0.63
PP	12.2	16.9	38.1	1.0	3.1	34.9	5.2	0.44
PN	41.1	29.4	30.0	5.4	3.6	24.1	7.3	0.98
PH	76.9	46.5	42.2	0.2	4.4	2.3	4.5	1.10
R	80.3	14.2	11.2	2.2	1.2	69.8	1.5	1.27

[a]The ratio of arabinose to xylose residues.

branching of the arabinoxylan in BE from bran. With barium hydroxide extraction of WUM from whole grain rye an arabinoxylan was isolated with a similar ara/xyl ratio but at a much lower recovery (Vinkx *et al.*, 1995b).

Arabinose, xylose and glucose residues were the dominating polysaccharide constituents in all BP. However, the total content of polysaccharide residues was less than 4% in these extracts. Significant proportions of galactose residues were also detected. These residues are probably present as glycoprotein constituents (Fincher & Stone, 1986).

The total polysaccharide content in BN (86–89%) was quite similar for all WUMs (Table 4). These fractions consisted of about 65% glucose residues and 25–30% arabinoxylan. The ara/xyl ratio increased from bran BN (0.60) to flour BN (0.93). Vinkx *et al.* (1995b) recovered a fraction consisting of 62% arabinoxylan and 22% β-glucan with the subsequent water extraction of the barium hydroxide extracted residue from whole grain rye.

The content of polysaccharide residues was lower (50–65%) in PE compared to that in the other major extracts (Table 4). These extracts consisted mainly of arabinose, xylose and glucose residues with the highest proportion of arabinose and xylose residues in the bran PE. The low ara/xyl ratio of bran PE indicates the presence of an arabinoxylan with a low degree of branching. Such an arabinoxylan might form strong inter-molecular interactions by hydrogen bonding and therefore be more difficult to extract, which could explain why it was not extracted under the weaker alkaline conditions provided by the barium hydroxide (Andrewartha *et al.*, 1979). The content of polysaccharide residues in PP decreased from 71% when extracted from bran to only 12% when extracted from flour. The proportion of xylose residues was very high in bran PP (90%) and intermediate PP (80%), and these extracts had very low ara/xyl ratios (about 0.1).

All PN contained about 40% polysaccharides, of which 59–95% were arabinose and xylose residues (Table 4). The ara/xyl ratio increased from 0.81 in bran PN to 0.98 in flour PN.

After alkaline extraction at room temperature with barium hydroxide and potassium hydroxide a residue remained which still contained about 25% of the arabinoxylans originally present in WUM. The ara/xyl ratio was close to 1 in this residue, indicating a highly branched structure. Highly branched arabinoxylans have been previously extracted with hot alkali from maize

bran, with good yields and without degradation of the polysaccharide backbone (Chanliaud *et al.*, 1995). Hot alkali extraction released 50–55% of the remaining arabinoxylans in the residue after extraction with alkali at room temperature. The polysaccharide content of these fractions ranged from 69–75% and more than 85% of the carbohydrates were arabinose and xylose residues, with an ara/xyl ratio of 1.03–1.10, indicating the presence of a xylan with a very high degree of branching (Table 4). Galactose and uronic acid residues were also detected in these extracts and the polysaccharide composition was similar to the composition of the arabinoxylan-protein complex isolated from rye bran by Ebringerovà *et al.* (1994). A similar polysaccharide fraction has been obtained from whole grain rye after chlorite extraction but with a lower yield by Vinkx *et al.* (1995b). Actually, preliminary experiments have shown that hot alkali extraction of the residue obtained after alkali treatment at room temperature released twice the amount of arabinoxylans compared to chlorite extraction. Subsequent extraction of the chlorite treated residue by hot alkali released some arabinoxylan but the combined extraction (chlorite + hot alkali) did not give higher yields than extraction with hot alkali alone. Chlorite extraction after hot alkali treatment released only minor amounts of arabinoxylan. The extract obtained by chlorite extraction exhibited similar composition as the hot alkali extract, the main difference being a lower molecular weight in the former, as determined by gel permeation chromatography. There was also a difference in colour, as the hot alkali extracts were dark brown and the chlorite treated extracts bleached to off-white. It has previously been reported that chlorite pre-treatment of rye bran did not change the alkaline extractability of arabinoxylan (Hromádková *et al.*, 1987). In the extraction of maize bran, hot alkali was also shown to be much more efficient than chlorite to extract arabinoxylans (Saulnier *et al.*, 1995).

The polysaccharide content in R was around 80% and accounted for about 7–12% of the polysaccharides in the WUMs (Table 4). The major polysaccharide residue in R was glucose (59–70%), probably present as cellulose. Arabinose and xylose residues constituted 25–36% of the polysaccharide residues and could be present as an arabinoxylan with a very high degree of branching, as indicated by the very high ara/xyl ratio (1.14–1.27).

NMR studies

Signals at 5.4, 5.3 and 5.2 ppm in the ^{1}H-NMR spectra have been assigned to anomeric protons of the α-L-arabinofuranosyl residues of arabinoxylan (Bengtsson & Åman, 1990). The signal at 5.4 ppm was assigned to terminal arabinose residues linked to O–3 of monosubstituted xylose residues, and the signals at 5.2 and 5.3 to the terminal arabinose residues linked to O–2 and O-3 of disubstituted xylose residues. Split signals at 5.24 and 5.26 ppm together with split signals at 5.31 and 5.33 ppm originate from terminal arabinose residues attached to two consecutive di-substituted xylose residues (Hoffman *et al.*, 1992). The anomeric signals from the un-, mono- and disubstituted xylose residues occur at 4.4–4.7 ppm. Signal clusters around 4.8 and 4.6 ppm originate from β-glucan (Bock & Duus, 1991).

^{1}H-NMR spectra of the main extracts (BE, BN, PE and PH) showed that the polysaccharides in those extracts were mainly arabinoxylan and β-glucan (Figs 2–4). It is notable that the corresponding extracts from the three different WUMs showed such similar spectra. In BE, the major arabinose signals in the anomeric region were assigned to terminal residues linked to mono- and disubstituted xylose residues. In BN anomeric signals from clusters of the 3-and 4-linked residues of β-glucan dominated together with anomeric signals for the arabinoxylan. In this case larger arabinose signals from disubstituted and consecutive disubstituted xylose residues were found as was also indicated

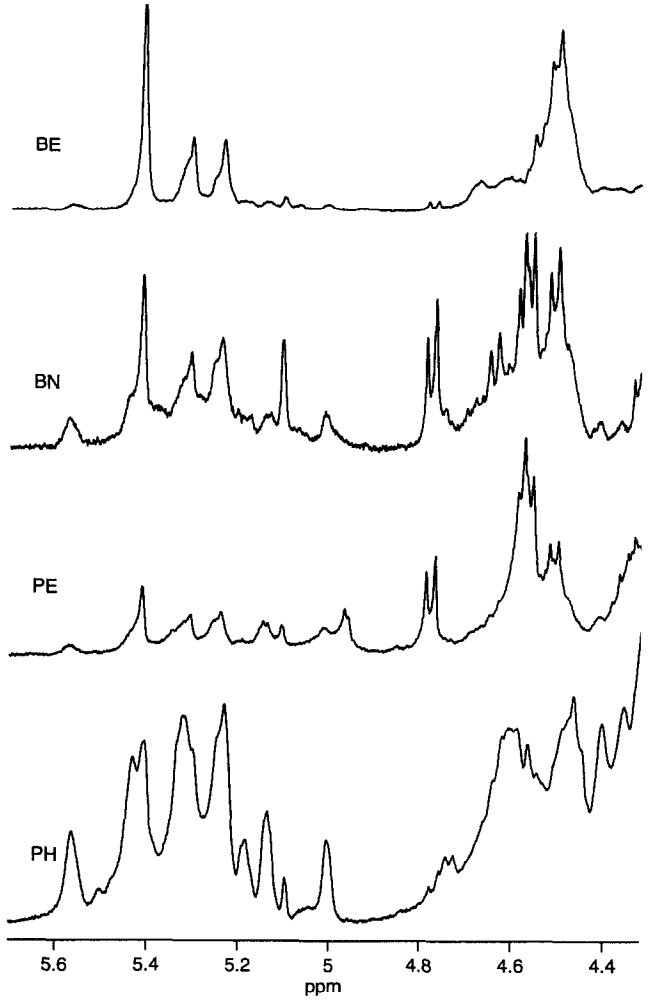

Fig. 2. ^{1}H-NMR spectra of extracts BE, BN, PE and PH of water insoluble material derived from bran.

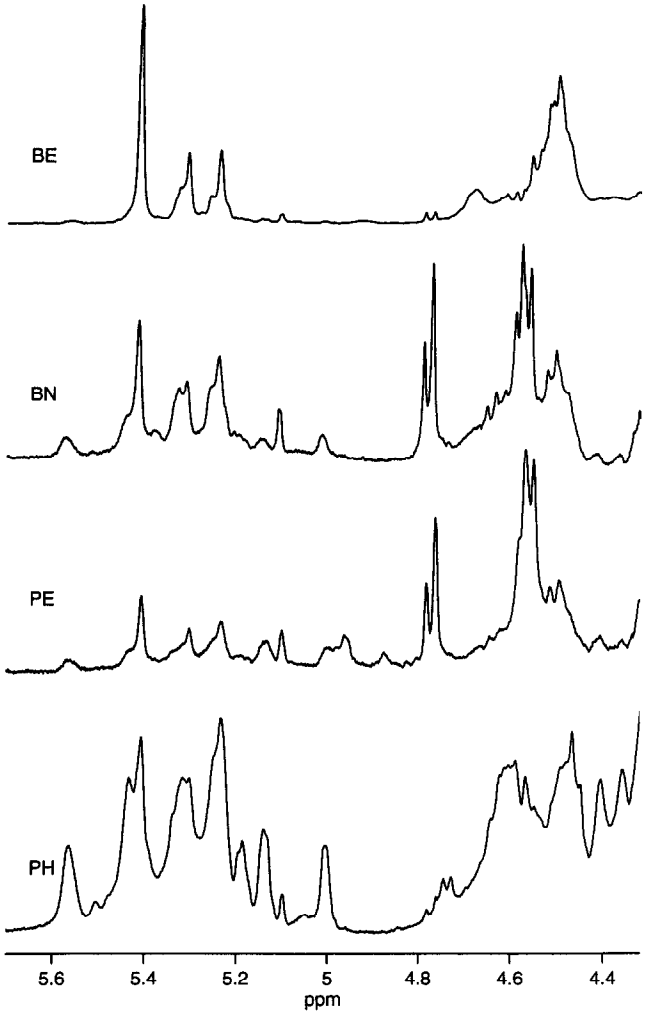

Fig. 3. ^{1}H-NMR spectra of extracts BE, BN, PE and PH of water insoluble material derived from intermediate milling fraction.

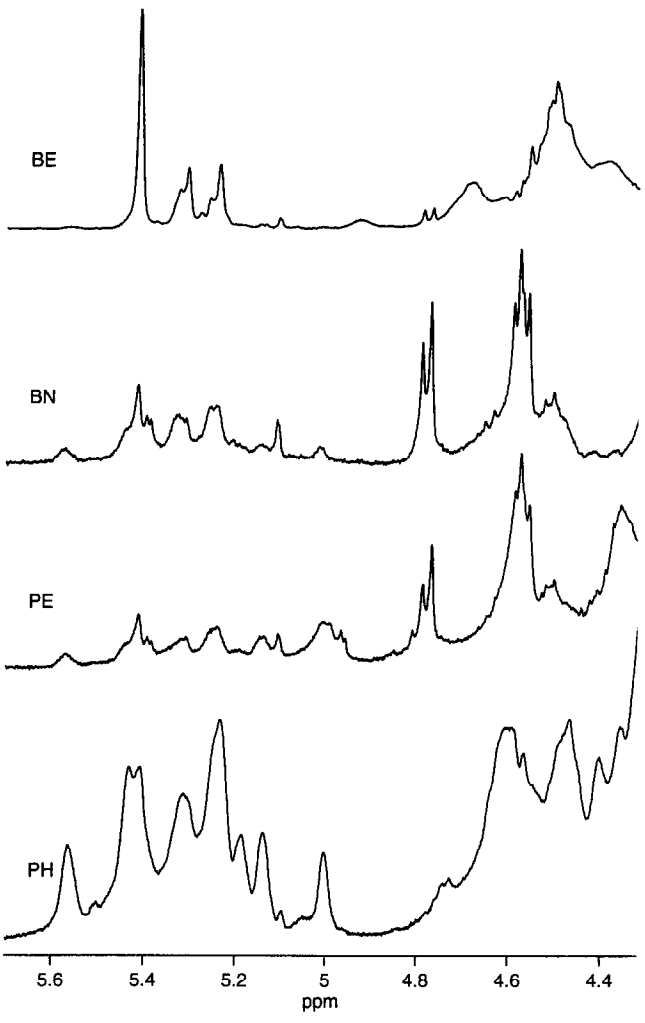

Fig. 4. ^{1}H-NMR spectra of extracts BE, BN, PE and PH of water insoluble material derived from sieved flour.

by the higher ara/xyl ratio of this fraction. Spectra of the anomeric region of polysaccharides in PE also revealed high contents of β-glucan and arabinoxylan with a more complex structure. The anomeric region of spectra from PH showed many signals indicating a very complex structure of the polysaccharides or a mixture of different types of polysaccharides. When comparing the anomeric signals in the different extracts it is evident that all signals are present in all extracts. It is only the relative distribution which differs. This indicates that no pure polysaccharide with a single structure is present in any of the extracts. However certain structures are highly concentrated in the different extracts.

In the study by Vinkx *et al.* (1995b) on whole grain rye it was shown that about 25% of the polysaccharides isolated by barium hydroxide extraction had a structure similar to a polysaccharide isolated after chlorite treatment of their residue after sequential alkaline extraction and to the one which in this study was isolated in PH. Glycosyl linkage analysis of the xylan isolated by Vinkx *et al.* (1995b) revealed that it contained substituted arabinose and, terminal xylose and galactose residues. The remaining 75% of the polysaccharides in the barium extraction of Vinkx *et al.* (1995b) was shown to be an arabinoxylan with a similar spectrum to the ones obtained in BE in the present study. The reason for this difference in extraction behaviour may be that Vinkx *et al.* (1995b) used a protease treatment on their water unextractable material before the alkaline extraction.

CONCLUSIONS

In this study, a series of repeatable extractions of water unextractable material were performed, which extracted different types of arabinoxylan and β-glucan from bran, intermediate and flour from rye. A selective fractionation of these polysaccharides was obtained, even though traces of all polysaccharide structures were found in all extracts. Further studies are in progress to evaluate the fine structure of the different polysaccharides in the extracts.

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REFERENCES

Åman, P. & Bengtsson, S. (1991). *Carbohydr. Polym,* **15,** 405–414.

Åman, P. & Graham, H. (1987). *J. Agric. Food Chem,* **35,** 704–709.

Åman, P., Westerlund, E. & Theander, O. (1994). In *Methods in Carbohydrate Chemistry.*

Andrewartha, K.A., Phillips, D.R. & Stone, B.A. (1979). *Carbohydr. Res,* **77,** 191–204.

Anon (1971). *Off. J. Eur. Comm. L,* **297,** 995–997.

Antoniou, T., Marquardt, R.R. & Cansfield, P.E. (1981). *J. Agric. Food Chem,* **29,** 1240–1247.

AOAC (1984). *Official Methods of Analysis.* (14th ed.). Association of Official Analytical Chemists, Washington DC, USA.

Autio, K., Härkönen H., Åman, P., Parkkonen, T., Frigård, T., Siika-aho, M. & Poutanen, K. (1996). *Lebensmit.-Wiss. Technol.,* **29,** 18–27.

Bengtsson, S. & Åman, P. (1990). *Carbohydr. Polym.,* **12,** 267–277.

Bengtsson, S., Åman, P. & Andersson, R.E. (1992). *Carbohydr. Polym.,* **17,** 277–284.

Bock, K. & Duus, J.Ø. (1991). *Carbohydr. Res.,* **211,** 219–233.

Chanliaud, E., Saulnier, L. & Thibault, J.-F. (1995). *J. Cereal Sci.,* **21,** 195–203.

Düsterhöft, E-M., Verbruggen, M.A., Gruppen, H. Kormelink, F.J.M. & Voragen, A.G.J. (1993). In *Enzymes in animal nutrition.* eds C. Wenk & M. Boessinger. ETH Zürich, pp. 29–33.

Ebringerová, A., Hromádková, Z., Burchard, W., Dolega, R. & Vorwerg, W. (1994). *Carbohydr. Polym.,* **24,** 161–169.

Fengler, A.I. & Marquardt, R.R. (1988). *Cereal Chem,* **65,** 298–302.

Fincher, G.B. & Stone, B.A. (1986). *Adv. Cereal Sci. Technol.,* **8,** 207–295.

Frigård, T., Pettersson, D. & Åman, P. (1994). *J. Nutr.,* **124,** 2422–2430.

Graham, H., Åman, P. & Pettersson, D. (1987). *Proc. 23rd. Nordic Cereal Congress* 87–93.

Gruppen, H., Hamer, R.J. & Voragen, A.G.J. (1992). *J. Cereal Sci.,* **16,** 41–51.

Gruppen, H., Hamer, R.J. & Voragen, A.G.J. (1992). *J. Cereal Sci.,* **13,** 275–290.

Gruppen, H., Kormelink, F.J.M. & Voragen, A.G.J. (1993). *J. Cereal Sci.,* **18,** 111–128.

Hagander, B., Björk, I., Asp, N.-G., Efendic, S., Holm, J., Nilsson-Ehle, P., Lundquist, I. & Scherstén, B. (1987). *Diabetes Res. Clin. Pract.,* **3,** 85–96.

Hoffmann, R.A., Geijtenbeek, T., Kamerling, J.P. & Vliegenhart, J.F.G. (1992). *Carbohydr. Res.,* **223,** 19–44.

Hromádková, Z., Ebringerová, A. & Petráková, E. (1987). *Carbohydr. Res.,* **163,** 73–79.

Jeffries, T.W. (1990). *Biodegradation,* **1,** 163–176.

Kormelink, F.J.M., Gruppen, H., Viëtor, R.J. & Voragen, A.G.J. (1993). *Carbohydr. Res.,* **249,** 355–359.

Kühn, M.C. & Grosch, W. (1988). *J. Food Sci.,* **53,** 889–895.

Kühn, M.C. & Grosch, W. (1989). *Cereal Chem.,* **66,** 149–154.

Markwalder, H.U. & Neukom, H. (1976). *Phytochemistry,* **15,** 836–837.

McNeil, M., Alberheim, P., Taiz, L. & Jones, R.L. (1975). *Plant. Physiol.,* **55,** 64–68.

Meuser, F. & Suckow, P. (1986). In *Chemistry and Physics of Baking,* eds J.M.V. Blanchard, P.J. Frazier & T. Galliard, Royal Society of Chemistry, London, England, pp. 42–61.

Nilsson, M., Åman, P., Härkönen, H., Hallmans, G., Bach Knudsen, K.E., Mazur, W. & Adlercreutz, H. (1996). Submitted.

Pettersson, D. & Åman, P. (1987). *Acta Agric. Scand.,* **37,** 20–26.

Pettersson, D., Åman, P., Bach Knudsen, K.E., Lundin, E., Zhang, J-X., Hallmans, G., Härkönen, H. & Adlercreutz, H. (1996). *J. Nutr.,* **126,** 1594–1600.

Saini, H.S. & Henry, R.J. (1989). *Cereal Chem.,* **66,** 11–14.

Saulnier, L., Marot, C., Chanliaud, E. & Thibault, J.-F. (1995). *Carbohydr. Polym.,* **26,** 279–287.

Theander, O., Åman, P., Westerlund, E., Andersson, R. & Pettersson, D. (1995). AOAC method 994.13. *Official Methods of Analysis,* 16th edition, 1st supplement.

Viëtor, R.J., Angelino, S.A.G.F. & Voragen, A.G.J.(1992). *J. Cereal Sci.,* **15,** 213–222.

Viëtor, R.J., Hoffman, R.A., Angelino, S.A.G.F., Voragen, A.G.J., Kamerling, J.P. & Vliegenhart, J.F.G. (1994). *Carbohydr. Res.,* **254,** 245–255.

Vinkx, C.J.A., Reynart, H.R., Grobet, P.J. & Delcour, J.A. (1993). *Cereal Chem.,* **70,** 311–317.

Vinkx, C.J.A., Delcour, J.A., Verbruggen, M.A. & Gruppen, H. (1995). *Cereal Chem.,* **72,** 227–229.

Vinkx, C.J.A., Stevens, I., Gruppen, H., Grobet, P.J. & Delcour, J.A. (1995). *Cereal Chem.,* **72,** 411–418.

Wood, P.J., Weisz, J. & Blackwell, B.A. (1994). *Cereal Chem.,* **71,** 301–307.