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Structural features of arabinoxylans from sorghum having good *roti*-making quality

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Abstract

Arabinoxylans (AX) from an Indian variety of sorghum (M354) having good *roti*(Indian flat bread)-making quality were isolated with barium hydroxide and 10% sodium hydroxide (Hemicellulose B). Arabinoxylans from both fractions were further purified by alcohol precipitation at acidic pH and glucoamylase digestion. Structural features of the purified arabinoxylans were studied by a combination of methylation analysis involving GC–MS, ^{13}C NMR, oxidation studies and FT-IR spectroscopy. The results indicated a xylan backbone in β-1,4 linkage, which is occasionally branched at O-3 or at O-2 and O-3. The branches contained arabinosyl residues in furanose form, linked mainly by α-1,3. Fully branched arabinosyl residues were also present.

Keywords: Sorghum; Arabinoxylans; Roti; Bread-making

1. Introduction

Sorghum is one of the important cereal crops of India, and is consumed mainly in the form *roti* (Indian flat bread). Nowadays it is being used in the brewing of lager type beers. Sorghum flours contain 5–6% (w/w) of cell wall material, which consists of non-starch polysaccharides (Verbruggen, Beldman, Voragen, & Hollemans, 1993). Arabinoxylans (AX) have been isolated from different cereals (Bengtsson & Aman, 1990; DuPont & Selvendran, 1987; Hoffmann, Roza, Maat, Kamerling, & Vliegenthart, 1991; Vietor, Angelino, & Voragen, 1992). The arabinoxylans are known to play an important role in maintaining water balance (Jelaca & Hlynka, 1971) and rheological properties of dough (Michniewicz, Biliaderis, & Bushuk, 1991), retrogradation of starch (Gudmundsson, Eliasson, Bengtsson, & Aman, 1991) and bread-making quality (Delcour, Vanhammel, & Hoseney, 1991). Since arabinoxylans are known to play an important role in the *roti*-making quality of sorghum, a systematic investigation was undertaken on the arabinoxylans of a sorghum variety (M354) having good *roti*-making quality (Chandrashekar & Desikachar, 1984).

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2. Materials and methods

2.1. Materials

Sorghum (*Sorghum bicolor*, M354 variety) was procured from the Agricultural College, Dharwad. The seeds were milled to flour of 100% extraction in a hammer mill (Kamas-Slagg) using a 0.8-mm sieve. Chemicals and reagents used were of analytical reagent grade.

2.2. Extraction of polysaccharides

Flour (100 g) was extracted with 70% ethanol (×3) to remove free sugars. The residue was suspended in water (1:100 w/v) and subjected to Termamyl (heat-stable α-amylase, Novo, Denmark) digestion in a boiling water bath and to glucoamylase (Sigma) digestion at 60 °C. The residue obtained after centrifugation was subjected to barium hydroxide extraction (containing 260 mM $NaBH_4$; Gruppen, Hamer, & Voragen, 1991), followed by extraction with sodium hydroxide solution (10%) (Salimath & Tharanathan, 1982). The barium hydroxide extract was dialysed thoroughly against sodium acetate buffer (0.1 M, pH 4.5) and distilled water, and lyophilised. The sodium hydroxide extract was centrifuged and the supernatant acidified to pH 5.0 with 50% (v/v) acetic acid in an ice bath to precipitate hemicellulose A. The supernatant was dialysed against water and

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lyophilised to obtain hemicellulose B. The alkali-insoluble residue was washed thoroughly to neutral pH and dried by solvent exchange (ethanol/diethyl ether).

2.3. Purification of arabinoxylans

The arabinoxylans were purified as described by Chanliaud, Saulnier, and Thibault (1995). The barium hydroxide extract (BE) and hemicellulose B (Hem B; 2.0 g each) were dispersed in 100 ml distilled water. The pH of the solution was adjusted to 3.0 with 1 M HCl. Polysaccharides were precipitated by adding two volumes of ethanol (95%) and leaving at 4 °C for 4 h. The suspension containing polysaccharides was centrifuged and the resulting precipitate was dissolved in acetate buffer (pH 4.5, 0.1 M) and subjected to glucoamylase digestion (100 mg). The polysaccharides were again precipitated by adding two volumes of ethanol and kept at 4 °C overnight. The residue obtained after centrifugation was uniformly dispersed and lyophilised.

2.4. Analytical methods

Total sugars were estimated by the phenol-sulphuric acid method (McKelvy & Lee, 1969) and uronic acid by the carbazole method (Dische, 1947). Sugar composition in the fractions was determined by GC (Shimadzu, Model CR4A) after hydrolysing the samples and converting the hydrolysates to alditol acetates (Sawardekar, Slonekar, & Jeanes, 1967). High Performance size exclusion chromatography (HPSEC) was done on a Shimadzu (HIC-6A) model using an E-linear column (Waters Associates) and eluted with triple-distilled water (0.6 ml/min). The column was calibrated using dextran standards (T10, T20, T70, T500, T2000). IR-Spectra (Kacurakova, Ebringerova, Hirsch, & Hromadkova, 1994) of the samples were recorded after blending the samples with Nujol (liquid paraffin) so that a homogeneous smear is obtained on the window. The IR spectra were obtained with a FT-IR (Perkin-Elmer-2000 system GC-IR) instrument operating at 4 cm^{-1} resolution. Optical rotations were measured using a Perkin-Elmer (Model 243) polarimeter with 0.5–1.0% aqueous solutions of polysaccharides.

2.5. Permethylation analysis

Samples (5–10 mg) were methylated (Hakomori, 1964) using methyl sulphinyl carbanion and iodomethane. The products were purified by passing through a Sep-Pak C18 cartridge. After hydrolysis with formic acid-H_2SO_4, and reduction (using $NaBD_4$ in D_2O), partially methylated alditol acetates were analysed by GC–MS (Waeghe, Darvill, McNeil, & Albersheim, 1983). A Shimadzu (Model QP5000) GC–MS, fitted with SP-2380 fused silica capillary column, was used to analyse the permethylated samples. A temperature gradient of 180–200 °C, with an increase in 4 °C/min, was applied for the analysis. Chloroform was used as solvent and the carrier gas was helium.

2.6. ^{13}C NMR

^{13}C NMR spectroscopy was carried out on D_2O-exchanged samples (50 mg/ml D_2O) using a Bruker (AMX-400) 400 MHz spectrometer at 70 °C.

3. Results and discussion

3.1. Carbohydrate composition

The carbohydrate composition of native samples of BE and Hem B, and the AX isolated from them is given in Table 1. Native fractions of both BE and Hem B contain substantial amounts of glucose, likely to originate from starch as seen in the reduction of the amount of glucose after glucoamylase digestion and alcohol precipitation. The yields of AX from both BE and Hem B is around 40% of the native sample. The total sugar and uronic acid compositions are greater in AX from BE than Hem B. Both AX fractions exhibit a high arabinose/xylose ratio (A/X; 1.64). A high ratio of A/X in sorghum has been reported by previous workers.

Table 1
Carbohydrate composition (%) of native and purified AX from barium hydroxide-extracted polysaccharide (BE) and hemicellulose B (Hem B) of sorghum flour

Fraction	Total sugar[a]	Uronic acid	Arabinose	Xylose	Mannose	Galactose	Glucose	Arabinose/Xylose ratio
BE								
Native	92.0	2.5	63.4	26.3	–	–	10.3	2.41
Purified	94.1	4.8	58.5	35.8	1.0	1.4	3.3	1.63
Hem B								
Native	79.4	2.6	35.4	32.3	–	3.0	29.3	1.09
Purified	83.0	3.9	54.5	33.3	5.3	3.4	3.5	1.64

[a] Excluding uronic acids.

Verbruggen et al. (1993) reported an A/X ratio of around 1.0 in barium hydroxide extract, whereas Karim and Rooney (1972) reported an A/X ratio of 1.6 for alkali-soluble pentosans from sorghum.

3.2. HPSEC chromatography

HPSEC of AX from BE and Hem B resulted in elution of AX as a single major peak just before T2000 dextran which indicates its large molecular weight (>2 million). This kind of elution pattern is possibly due to aggregation. Overestimation of molecular weights of AX isolated from cereals, such as wheat, is reported by others (Gruppen, Hamer, & Voragen, 1992; Michniewicz, Biliaderis, & Bushuk, 1990).

3.3. Methylation analysis

Methylation analysis (Table 2) of AX from BE and Hem B revealed their highly branched nature. Around 50 and 45% of the xylose residues are monosubstituted in AX from BE and Hem B, respectively. Approximately 40% of the xylose residues on the xylan backbone are unsubstituted. The remaining xylose residues are doubly branched. Most of the arabinose residues are present as terminal sugars, the remainder being backbone and branched residues. Short arabinosyl chains are reported in rice bran arabinoxylans (Shibuya & Iwasaki, 1985). Fully branched arabinoses are present in AX fractions of both BE and Hem B to the extent of around 3.0%. The ratio of unbranched to branched xylose and that of doubly branched to singly branched xyloses is similar in both fractions. The general structure of AX from both fractions shows the presence of a xylan backbone to which arabinose residues are attached, either at O-3 or at O-2 and O-3 positions. Short chains of arabinosyl residues are also present. Contaminating polysaccharides, arising from unhydrolysed starch, are present, yielding 2,3,4,6-Me_4-Glc and 2,3,6-Me_3-Glc in minor amounts.

Table 2
Methylation analysis of AX from barium hydroxide-extracted polysaccharides (BE) and hemicellulose B (Hem B) of sorghum flour

Alditol acetates of[a]	BE (%)	Hem B (%)
2,3,5-Me_3-Ara	44.4	38.0
2,3-Me_2-Ara	4.9	10.0
Ara	2.5	3.0
2,3,4-Me_3-Xyl	Tr	1.8
2,3-Me_2-Xyl	17.5	18.6
2-Me-Xyl	24.2	21.8
Xyl	6.5	6.8
[2,3-Me_2-Xyl]	0.6	0.7
[2-Me-Xyl]+[Xyl]	0.3	0.3
[Xyl] [2-Me-Xyl]		

[a] 2,3,5-Me_3-Ara is 2,3,5-tri-O-methyl-1,4-di-O-acetyl-arabinitol, etc.

3.4. ^{13}C NMR spectroscopy

^{13}C NMR spectroscopy was carried out for AX from BE only (Fig. 1). Peaks were assigned, based on the partial structure given in Fig. 2. The spectra showed the prominent peaks assignable to anomeric carbons of arabinose and xylose residues. The signals were assigned, based on the data available in the literature (Brillouet & Joseleau, 1987; Joseleau, Chambat, Vignon, & Barnoud, 1977; Kovac, Hirsch, Shashkov, Usov & Yarotsky, 1980; Kovac & Hirsch, 1982). The resonances in the region of 109–110 ppm and 101–110.3 ppm were attributed to the anomeric carbon atoms of arabinofuranose (Araf) in α-linkage and xylopyranose (Xylp) in β-linkage, respectively (Table 3). The peaks at 110.3 and 109.6 ppm corresponded to C-1 of Araf residues linked to completely branched xylose substituted at O-2 and O-3, respectively. The signal at 109.3 corresponded to C-1 of α-L-Araf linked to xylose at O-3 only. The resonance due to C-1 of unsubstituted xylose

Table 3
Assignment of ^{13}C NMR signals of arabinoxylan purified from barium hydroxide-extracted polysaccharides of sorghum flour

Sugar	C_1	C_2	C_3	C_4	C_5
	Chemical shifts (ppm)				
A	102.936	74.466	78.978	75.477	64.516
B	101.619	72.939	–	–	64.516
C	103.500	73.714	75.477	78.534	64.516
D	109.299	82.536	78.978	86.446	63.045
E	109.662	82.809	–	85.926	63.045
F	110.304	83.168	–	85.926	63.045

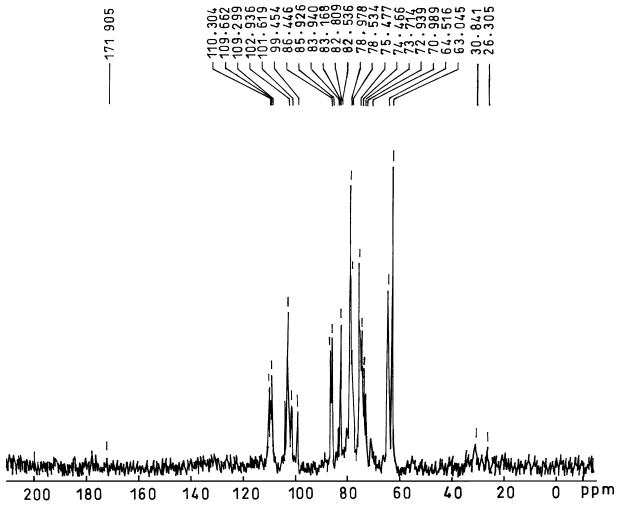

Fig. 1. ^{13}C NMR spectrum of arabinoxylan purified from barium hydroxide-extracted polysaccharides (BE) of sorghum flour.

residues was observed at 103.5 ppm. The signal at 102.9 ppm was attributed to C-1 of Xylp, substituted at O-3 by arabinofuranose. The signal at 83.1 ppm was due to C-2 and that observed at 85.9 ppm was due to C-4 of an Araf residue linked at O-2 of a doubly substituted xylose residue. The signals at 82.5 ppm and 85.9 ppm could be assigned to C-2 and C-4 of Araf, linked at O-3 of a doubly substituted xylose residue. The signals at 82.5 and 86.4 ppm were assigned to C-2 and C-4 of an Araf linked to xylose at O-3. The other signals present were 63.0 ppm, due to C-5 of Araf (linked to xylose at O-3, and O-2 and O-3), 73.7, 75.4, 64.5 due to C-2, C-3 and C-5, respectively, of unsubstituted xylose residues, and 74.4 due to C-2 of monosubstituted xylose. The multiplicity of resonances at 109–110 ppm reflects the branched structure of arabinose residues (Joseleau, Chambat, Vignon, & Barnoud, 1977). The signal at 99.4 ppm could be due to C-1 of glucuronic acid, linked to the xylose residues at O-2 (Swamy & Salimath, 1990).

3.5. FT-IR

The FT-IR spectra of AX from BE and Hem B were similar; the spectrum of AX from BE is shown in Fig. 3. The band in the region of 3356 cm^{-1} is due to the hydroxyl-stretching vibrations of polysaccharides and

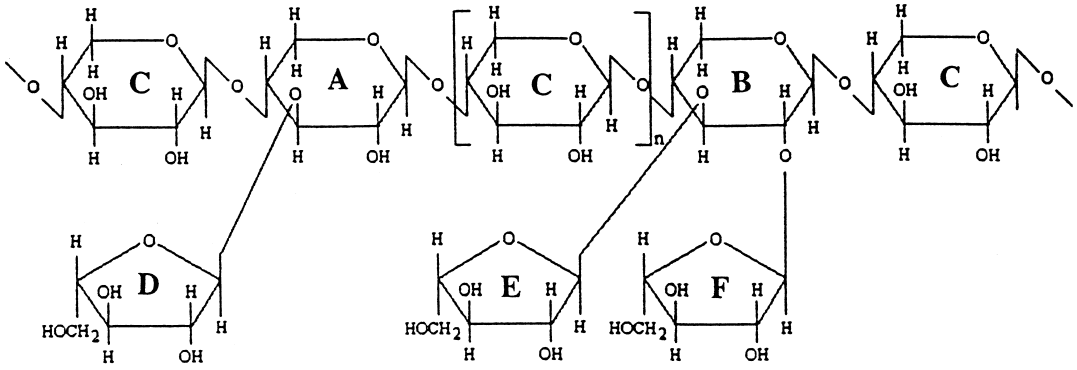

Fig. 2. Partial (one of the probable) structure of arabinoxylans (identification of ^{13}C NMR signals are shown in Table 3).

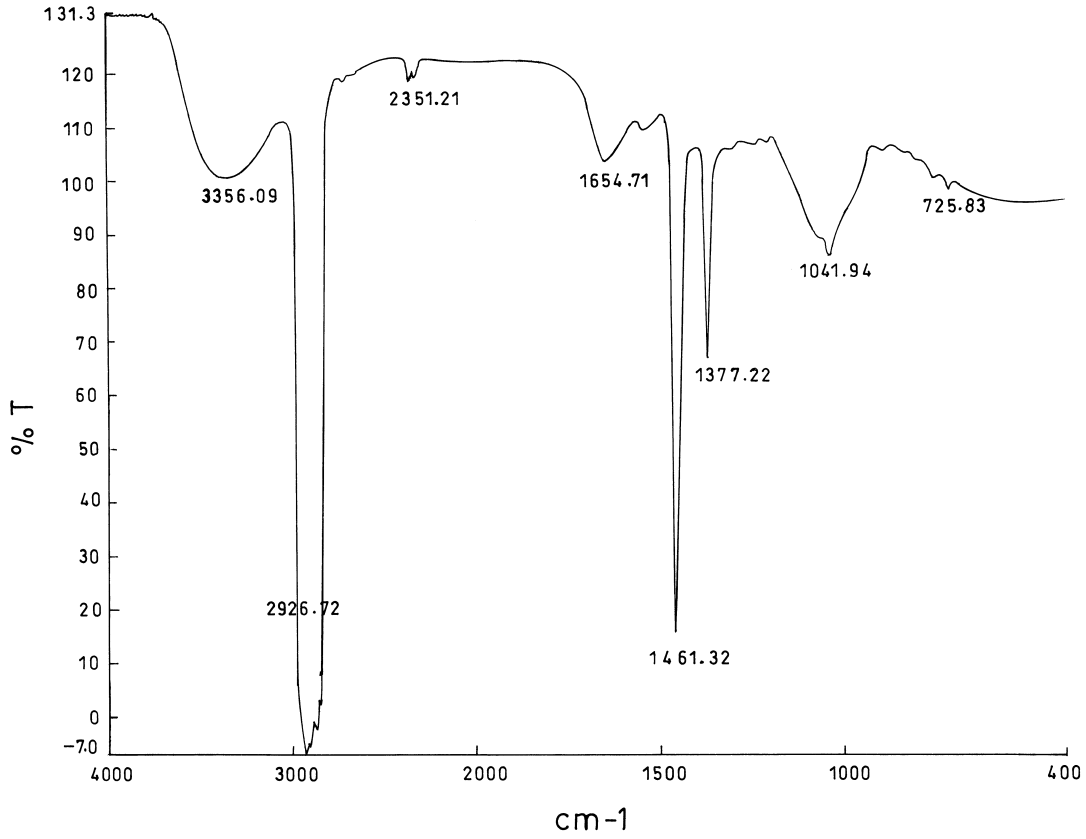

Fig. 3. FT–IR spectrum of arabinoxylan purified from barium hydroxide extract.

water involved in hydrogen-bonding (Fringant, Tvaroska, Mazeau, Rinaudo, & Desbrieres, 1995). The bands in the region of 2800–3000 cm^{-1} are due to C–H stretching vibrations and the band in the region of 1654 cm^{-1} is due to associated water (Kacurakova, Belton, Wilson, Hirsch, & Ebringerova, 1998). Deformation modes of $–CH_2$ are observed in the region between 1461 and 1377 cm^{-1}. Absorptions in the region between 800–1500 cm^{-1} are due to C–C, C–O, C–OH and C–O–C stretching modes of the polymer backbone. This spectral region has the characteristic pattern of xylooligosaccharides (Kacurakova, Ebringerova, Hirsch, & Hromadkova, 1994). The changes in the finger print are associated with the substitution of arabinosyl residues at the O-3 position of xylose and are accompanied by a decrease in transmittance intensity (1164 and 895 cm^{-1}) and a loss of peak intensity (1120–1000 cm^{-1}). The intensity of peak at 990 cm^{-1} is due to the arabinose residue attached only at O-3 of xylose residues (Ebringerova, Hromadkova, Alfoldi, & Berth, 1992). The spectral region between 800 and 1500 cm^{-1} appears as a smooth peak, which indicates its highly branched nature.

3.6. Optical rotation

The specific rotations were -70° for AX from BE and -81° for AX from Hem B and are in agreement with reported values (Woolard, Rathbone, & Novellie, 1976).

4. Discussion

These studies indicate that the AX from BE and Hem B have a xylan backbone with β-1,4 linkages. The backbone is branched, mainly at O-3, and contains a few completely substituted xylosyl residues. Arabinosyl residues, in furanose form, are α-linked and a few completely substituted arabinosyl residues are also present. The branches are terminated by arabinofuranosyl residues. Glucuronoarabinoxylans are reported from sorghum husk, which consist of arabinose, xylose, glucuronic acid (GlcA) and 4-O-methyl glucuronic acid (4-OMe-GlcA) (Woolard, Rathbone, & Novellie, 1976, 1977). GlcA and 4-O-Me-GlcA are attached to O-2 of xylopyranose. Glucuronoarabinoxylans (GAX) are selectively extracted with barium hydroxide from sorghum endosperm cell walls (Verbruggen, Beldman, & Voragen, 1995). The GAX were shown to be highly substituted with arabinose residues and all contain considerable amounts of glucuronic acid and minor amounts of galacturonic acid and 4-O-methyl glucuronic acid.

Arabinoxylans play an important role in determining bread-making quality. The contents of arabinose and xylose were shown to be higher in the varieties of wheat that have good tandoori *roti*-making quality (Saxena, Salimath, & Rao, 2000). Differences in the molecular features of AX, which include the degree of branching, spatial arrangement of arabinosyl substituents along the xylan backbone and the ferulic acid content, can affect the viscoelastic properties of gels. Although AX from various cereals have the same basic chemical structure, they differ in the substitution pattern of the xylan backbone. This leads to changes in their conformation and the capacity of AX molecules to interact with each other and with other polysaccharides. Since AX have the capacity to retain water, flat breads made out of these doughs would consequently have better palatability and pliability. AX also have the ability to retain gas in the dough, which is particularly important during *roti* making because the dough is left for at least half an hour to swell. Water acts as a plasticizer and lowers the rigidity of the products. *Rotis* which are made from sorghum are crispier than *chapati* made out of wheat flour. One of the reasons could be the highly branched nature of sorghum AX, which forms an inflexible matrix.

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