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Structural Features of Arabinoxylans from Bajra (Pearl Millet)

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Structures of arabinoxylans (AX) from bajra, from a variety known to have characteristic quality in the making of roti, an unleavened flat bread, were elucidated by a combination of methylation analysis, ^{13}C NMR, FT-IR, etc. Arabinoxylans isolated from barium hydroxide-extracted polysaccharides and hemicellulose B had a backbone of xylose residues with β-(1,4) linkages and were branched mainly through α-(1,3) linkages by arabinofuranosyl residues. Completely branched xylose residues were also present. The AX from bajra characteristically had large numbers of branches, and this may be one of the reasons for the crispiness of the rotis.

KEYWORDS: Arabinoxylans; bajra; pearl millet; roti

INTRODUCTION

Bajra is a drought-tolerant food crop and is consumed in India mostly in the form of roti, an unleavened flat bread (*1*). Roti made from bajra is crispy in nature and hence relished by consumers. Cereals such as wheat, sorghum, and bajra are known for their quality in the making of chapati/roti. The properties of chapati/roti vary with different varieties of cereals. Rotis made from sorghum and bajra have been found to correlate positively to uptake of water, cohesiveness of dough, starch damage, and roti-making quality (*2*). The bread-making quality of wheat has been attributed to the water balance of the dough (*3*), the rheological properties of the dough (*4*), and retrogradation of starch (*5*). The functional properties of bajra were previously studied in terms of water uptake, cohesiveness of dough, etc. (*6*). Since pentosans are known to play a vital role in bread-making quality, it is imperative to look into the structural features of arabinoxylans. Since bajra is known for its characteristic roti-making quality and has special attributes of crispiness and good keeping quality, the study of pentosans in bajra is of great interest. Earlier reports on bajra dealt with the isolation of pentosans and their composition (*7, 8*). Herein we report structural features of arabinoxylans in a barja variety having good roti-making quality (*9*).

MATERIALS AND METHODS

Extraction of Polysaccharides. Bajra (S203 variety) was milled by passing it through a Kamas Slagg Hammer Mill and sieved to 0.8 mm mesh size. Bajra flour was extracted with 70% alcohol to remove free sugars. The residue was cooked to gelatinize starch and subjected to Termamyl and glucoamylase digestion to hydrolyze starch, and the insoluble residue was collected after centrifugation (3000*g*). The residue was extracted with saturated barium hydroxide solution containing 250 mM sodium borohydride (×2) (*10*). The contents were centrifuged (3000*g*). The supernatant was lyophilized after being dialyzed against sodium acetate buffer and distilled water. The residue was extracted with 10% sodium hydroxide solution for 6 h under N_2 atmosphere (*11*). The contents were centrifuged (3000*g*). The supernatant was made to pH 4.5 with 50% acetic acid. The supernatant so obtained constitutes the hemicellulose B (Hem B) fraction that was dialyzed thoroughly against distilled water and then lyophilized.

Purification of Arabinoxylans (*12*). Purified arabinoxylans were isolated from barium hydroxide-extracted polysaccharide (BE) and Hem B by subjecting the solutions to alcohol precipitation (two volumes) at pH 3.0, followed by glucoamylase digestion and alcohol precipitation. The resulting precipitate was thoroughly dispersed in water and lyophilized.

Analytical Methods. Total sugars were estimated by the phenol−sulfuric acid method (*13*). Uronic acid was estimated by the carbazole method (*14*). To determine the sugar composition, polysaccharides (10 mg) were hydrolyzed with 2 N TFA at 100 °C for 6 h. The resulting monosaccharides were detected as alditol acetates by GLC on an OV-225 column (*15*). The column temperature was maintained at 200 °C, and the flow rate of nitrogen was 40 mL/min.

Methylation of arabinoxylans was conducted according to the method of Hakomori (*16*). Polysaccharides (5−10 mg) were dissolved in dry, distilled DMSO (0.5 mL) with stirring and/or occasional ultrasonication. Methyl sulfinyl carbanion was added to the above polysaccharide solution, followed by iodomethane at ice-cold temperature. Methylated polysaccharide was purified using a Sep-Pak C_{18} cartridge (*17*), and a solvent series of water, acetonitrile−water 3:17, acetonitrile−water 1:4, 100% acetonitrile, 100% methanol, and 95% ethanol was used. The fractions eluted with 100% acetonitrile and 100% methanol tested positive for carbohydrates were pooled and concentrated. The methylated polysaccharides were hydrolyzed (90% formic acid in boiling water and 2 N TFA at 100 °C for 2 h), reduced using $NaBD_4$ in D_2O, and acetylated. The partially methylated alditol acetates were analyzed using GLC-MS (Shimadzu model QP5000) with a fused silica capillary column (SP 2380, 30 m × 0.32 mm i.d.). The temperature gradient of 180−200 °C, at a rate of 4 °C/min, was maintained for the analysis. The ionization potential was 70 eV, and the mass range (*m*/*z*) was 40−400. Helium (1 mL/min) was used as the carrier gas.

^{13}C NMR was carried out on D_2O (99%)-exchanged samples. Samples (50 mg) were dissolved in 1 mL of D_2O (99.96%) and their spectra recorded on a Bruker 400 MHz spectrometer (5 mm multi-

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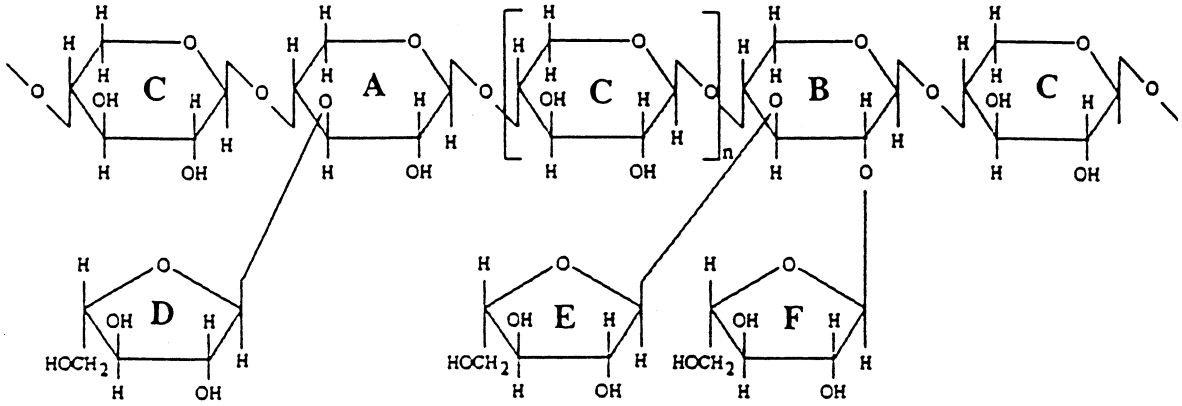

Figure 1. Partial (one of the probable) structure of arabinoxylans (identification of ^{13}C NMR signals is shown in **Table 3**).

Table 1. Carbohydrate Composition of Native and Purified Fractions of Barium Hydroxide-Extracted Polysaccharides (BE) and Hemicellulose B (Hem B) from Bajra Flour

fraction	total sugar (%)	uronic acid (%)	sugars identified (%)					A/X ratio
			Ara	Xyl	Man	Gal	Glc	
BE								
native	86.2	1.7	45.5	36.6		2.4	15.5	1.24
purified	84.8	6.9	46.3	43.1		4.9	5.7	1.07
Hem B								
native	85.4	2.1	44.4	41.7	0.6	4.1	9.2	1.06
purified	86.5	5.9	59.9	40.1			Tr	1.49

Table 2. Methylation Analysis of Arabinoxylans Purified from BE and Hem B of Bajra Flour

alditol acetates of	BE (%)	Hem B (%)
2,3,5-Me_3-Ara	28.8	39.2
2,3-Me_2-Ara	4.7	5.2
2,3,4-Me_3-Xyl	trace	3.7
2,3-Me_2-Xyl	27.3	26.0
2 -Me-Xyl	35.9	20.7
Xyl	3.3	5.2
[2,3-Me_2-Xyl]/[2-Me-Xyl] + [Xyl]	0.7	1.0
Xyl/2-Me-Xyl	0.1	0.3

nucleus probe) at 70 °C for 5 h, using a spectral width of 22 727 Hz with 6000 scans. Tetramethylsilane was used as the external standard.

FT-IR spectral analysis was done on a Perkin-Elmer 2000 system GC-IR operating at 4 cm^{-1} resolutions. Polysaccharide (1 mg) was blended thoroughly with Nuzol (liquid paraffin), and the homogeneous smear was used for the analysis.

Periodate consumption was measured according to the method of Fluery and Lange (*18*). Briefly, polysaccharide (5 mL, 0.1%) was mixed with 5 mL of sodium metaperiodate (5 mL, 20 mM), and the oxidation was continued at 4 °C in the dark. At different time intervals, 0.5 mL of the reaction mixture was withdrawn, and the amount of periodate consumed was measured by titrating against iodine solution. At the end of the reaction, formic acid was measured by titrating against 0.01 N sodium hydroxide (*19*). Optical rotation was measured using a 0.5−1% aqueous solution of the polysaccharide in a Perkin-Elmer model 243 polarimeter.

RESULTS AND DISCUSSION

Earlier, we reported on the carbohydrate composition of bajra (S203 variety) and its isolated fractions (*20*). The carbohydrate compositions of native and purified arabinoxylans from barium hydroxide-extracted polysaccharides (BE) and hemicellulose B (Hem B) are given **Table 1**. Both native and purified fractions were rich in carbohydrates. The content of arabinoxylans isolated in BE was 9.5%, and that in Hem B was 1.7%. The yields of the purified fractions from both of them were around 40%. The native fractions had a substantial amount of glucose, which may come from undigested starch molecules. This amount decreased substantially in purified fractions. In AX from BE, arabinose and xylose residues constituted around 90% of the total sugars. The remaining 10% was contributed by galactose and glucose. Galactose may come from galactoarabinoxylans, the presence of which has been reported in the members of graminae (*21*). Arabinoxylans from Hem B had predominantly arabinose and xylose residues as constituent sugars, with trace amounts of glucose. The uronic acid content was around 6−7% in both AX fractions. The A/X ratio was higher (1.5) in arabinoxylans from Hem B than in those from BE.

Methylation Analysis. Arabinoxylans from both BE and Hem B were subjected to methylation by the method of Hakomori (*16*). Methylation analysis revealed xylose residues in the main chain with 1,4 linkages (**Table 2**). The backbone was substituted by arabinofuranosyl residues at the O-3 position, as indicated by higher amounts of 2-Me-Xyl. Singly substituted xyloses were present in higher amounts in arabinoxylans from BE than in those from Hem B, as indicated by the presence of higher amounts of 2-Me-Xyl. Fully substituted xylose residues were present at the levels of 3 and 5% in arabinoxylans from BE and Hem B, respectively. Doubly substituted xyloses have been reported in arabinoxylans from wheat (*22*), barley (*23*), rice (*24*), and sorghum (*24*). Most of the arabinosyl residues were present as terminal sugars. Branched arabinosyl residues were observed which were present to the extent of 5%. Short arabinosyl chains have been reported in arabinoxylans from rice bran (*25*), wheat bran (*26*), and wheat flour (*26*).

The ratio of unbranched to branched xyloses was higher in arabinoxylans from Hem B than in those from BE, thus indicating that AX from BE is more branched than that from Hem B. The ratio of doubly to singly substituted xylose was, however, higher in AX from Hem B than in that from BE.

^{13}C NMR. ^{13}C NMR spectroscopy was carried out on D_2O-exchanged samples of arabinoxylans from both BE and Hem B. The peaks were assigned on the basis of the partial structure given in **Figure 1**. and the assignment of signals is given in **Table 3**. The NMR spectrum of AX from BE is given in **Figure 2**. Peaks were assigned on the basis of the data available in the literature (*27*−*30*). Signals due to main-chain xylose residues, monosubstituted xyloses, and arabinosyl residues substituted at O-3 of xylose residues were predominant. Anomeric signals due to the xylan backbone were observed at 102.2 ppm in AX from both BE and Hem B, characteristic of β-linked xylopyranose. Multiplicity of signals in the anomeric region of xyloses could

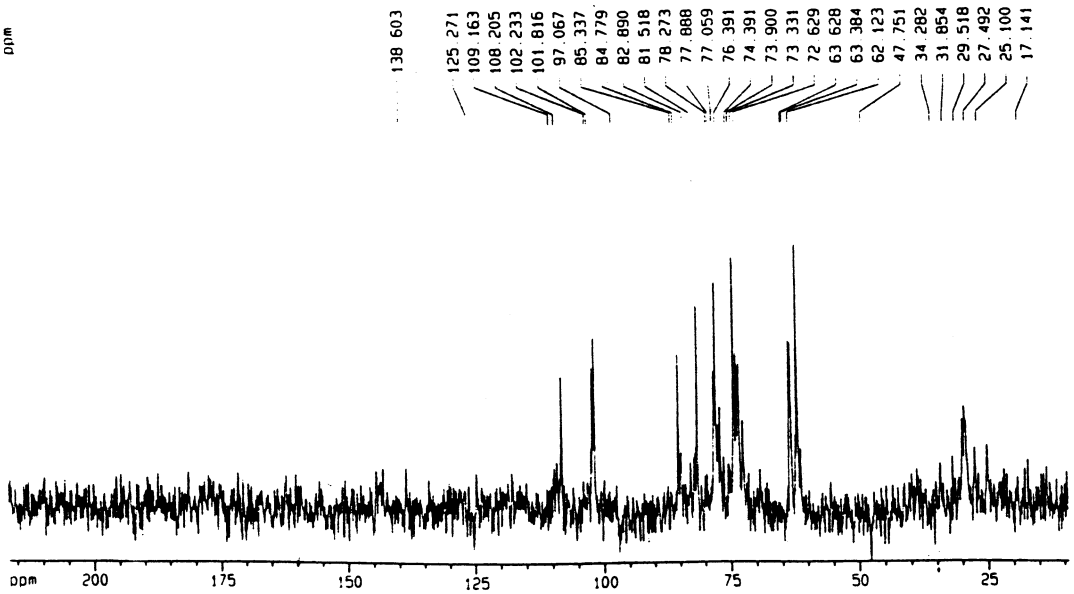

Figure 2. NMR spectrum of AX from BE.

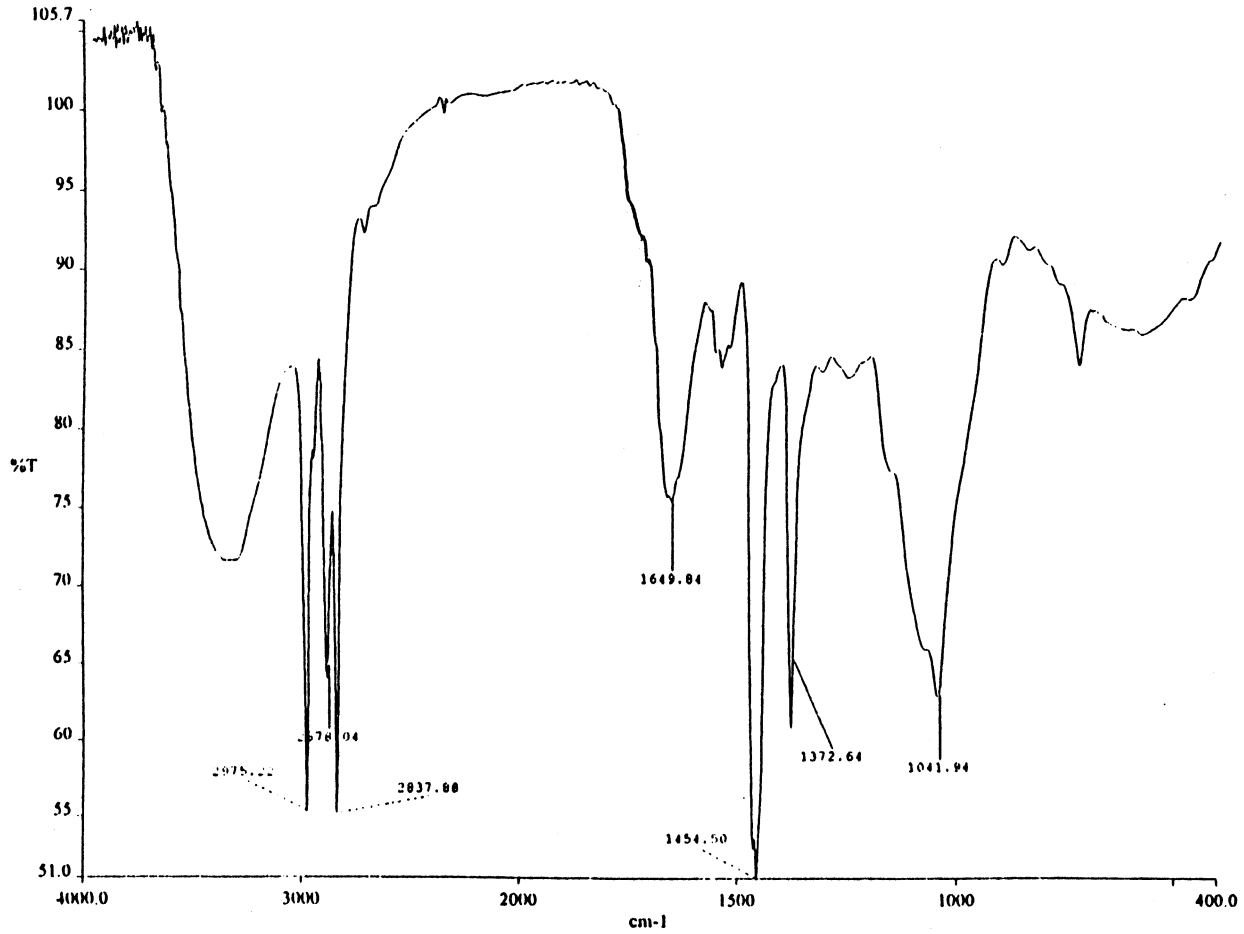

Figure 3. IR spectrum of AX from BE.

be observed, indicating the presence of xyloses having different branching modes. The presence of signals from arabinosyl residues at 108.2 ppm indicates the presence of arabinose residues in α-linkages. Since AX are complex molecules, the shifts due to ring carbon atoms in the region of 73.4 and 74.7 ppm are not fully resolved (*31*).

FT-IR. FT-IR spectra were recorded for AX isolated from BE and Hem B.The spectra were characteristic of arabinoxylans (*32*). The IR spectrum for AX from BE is given in **Figure 3**. A band due to associated water was observed at 1649 cm^{-1} (*33*). Bands due to $-CH_2$ stretching vibrations were observed around 1454 and 1441 cm^{-1} in AX from BE and Hem B, respectively. The bands which appeared between 3000 and 2500 cm^{-1} represented the C−H stretching modes. The prominent band around 3300 cm^{-1} is attributed to the hydroxyl stretching vibrations of the polysaccharides and water involved in hydrogen bonding (*33*). One more prominent band was observed at 1041 cm^{-1}, which was attributed to C−O, C−C, and C−O−H

Table 3. Assignment of ^{13}C NMR Signals of AX from BE and Hem B

	chemical shifts (ppm)									
	C_1		C_2		C_3		C_4		C_5	
residue	BE	Hem B	BE	Hem B	BE	Hem B	BE	Hem B	BE	Hem B
A	102.2	101.9	73.9	73.8	78.3	77.9	77.1	77.2	63.4	63.2
B	101.8	101.9	72.6	72.7			76.4	77.2	63.4	63.2
C	102.2	102.3	73.3		74.4	74.4	77.9	77.2	63.6	63.2
D	108.2	108.2	81.5	81.6	78.3	77.9	77.0	85.4	62.1	62.1
E	109.2	108.2		81.6			84.8	85.0	62.1	62.1
F	110.0	108.2	82.9	82.9			84.8	83.4	62.1	62.1

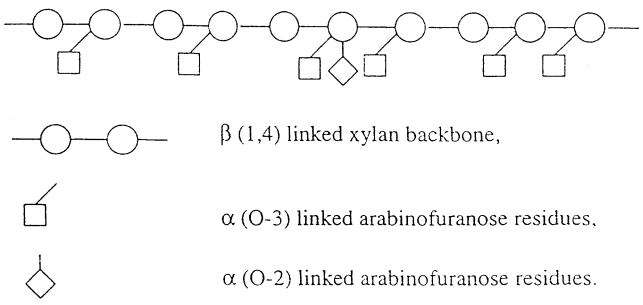

Figure 4. Proposed structure of arabinoxylans from bajra.

bending vibrations. This band shows variation in spectral shape depending on the branches at the O-2 and O-3 positions. With an increase in the number of branches, a decrease in the intensity at 1164−895 cm^{-1}, coupled with a loss of peak multiplicity in the region of 1120−1000 cm^{-1}, is reported to occur (*32*). From the spectral shape, it can be inferred that AX from BE is more branched than AX from Hem B because the spectral shape at 1041 cm^{-1} is relatively smoother for AX from BE than that for AX from Hem B, which substantiates the methylation analysis.

AX from BE and Hem B consumed around 0.6 and 0.7 mol of periodate, respectively, indicating that about 60−70% of the sugar residue had adjacent hydroxyl groups. These data agree well with the methylation analysis. Formic acid was released in trace amounts. The optical rotations observed were −54.5° and −81.8°, respectively, which is in agreement with the values reported in the literature for arabinoxylans (*34*).

The structural features of AX from bajra as elucidated by methylation, ^{13}C NMR, FT-IR, periodate consumption, and optical rotation measurements revealed that AX from bajra are highly branched (**Figure 4**). AX consists of a xylan backbone in which xylose residues are in the pyranose form, linked by β-(1,4) linkages. Arabinose residues in the furanose form were branched through O-3 and O-2 and O-3 of xylose residues. They were present in α-linkages. Small amounts of 1,5-linked arabinoses were also observed. Slight variations in branching patterns were observed in AX from BE and Hem B. AX from BE was more branched than that from Hem B, whereas the ratio of doubly branched to singly branched xyloses was higher in AX from Hem B than in that from BE. Thus, different extractants extract AX varying in branching pattern. The AX from bajra was more branched compared to the AX studied from sorghum (*35*) or wheat (*20*). Higher branching combined with the branching pattern of AX may be one reason for the observed differences in functionality. Ferulic acid substitution may also be one of the contributing factors.

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