NOTE ON THE ISOLATION OF SORGHUM HUSK POLYSACCHARIDES AND FRACTIONATION OF HEMICELLULOSE B

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Cereal grains contain both hemicelluloses and nonstarchy water-soluble gums. The gums contain D-glucose, D-xylose, L-arabinose, and lesser amounts of D-galactose and D-mannose (1,2). The hemicelluloses (3) have backbones of 1,4-linked β-D-xylopyranose residues (4–6). L-Arabinofuranosyl and D-glucuronic acid units occur as side chains in cereal hemicelluloses (5,6).

The gums and hemicelluloses of barley (1,2,7–14), wheat (1,7,8,15), rye (1,7,10), oats (1,7,8,16,17), and maize (1,7,18,19) have been studied. Most investigations on the structure of cereal polysaccharides have been carried out on preparations judged to be pure by a single criterion. Only in isolated investigations, *e.g.* studies on barley β-glucans (11–14), have the polysaccharide preparations been separated into the component polymers.

This paper describes the separation procedures successfully developed for the fractionation of the hemicelluloses of the B group obtained from sorghum grain husk.

MATERIAL AND METHODS

General Methods

Extractions, effected on *ca.* 12.5% (w/v) suspensions of husk, were repeated until analysis for carbohydrate showed the extraction was complete.

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Hemicelluloses were fractionated on columns (30 × 1.9 cm) of DEAE cellulose (Whatman DE-52) and on columns (85 × 1.5 cm) of Bio-Gel A-15m, unless otherwise stated. Polysaccharides were analyzed for component sugars by hydrolyzing a portion of each in $1M$ H_2SO_4 followed by gas-liquid chromatographic (glc) analysis of the derived alditol acetates (20). Optical rotations were measured with a Bendix-NPL Type 143D Automatic Polarimeter for aqueous solutions at 20° C. Diffusion and sedimentation coefficients were measured on a Beckman Spinco Model E Analytical Ultracentrifuge. The molecular weight (mol wt) of hemicellulose B was determined according to the sedimentation equilibrium procedure (21).

Material

A random sample of Barnard Red sorghum grain of the 1972 harvest was

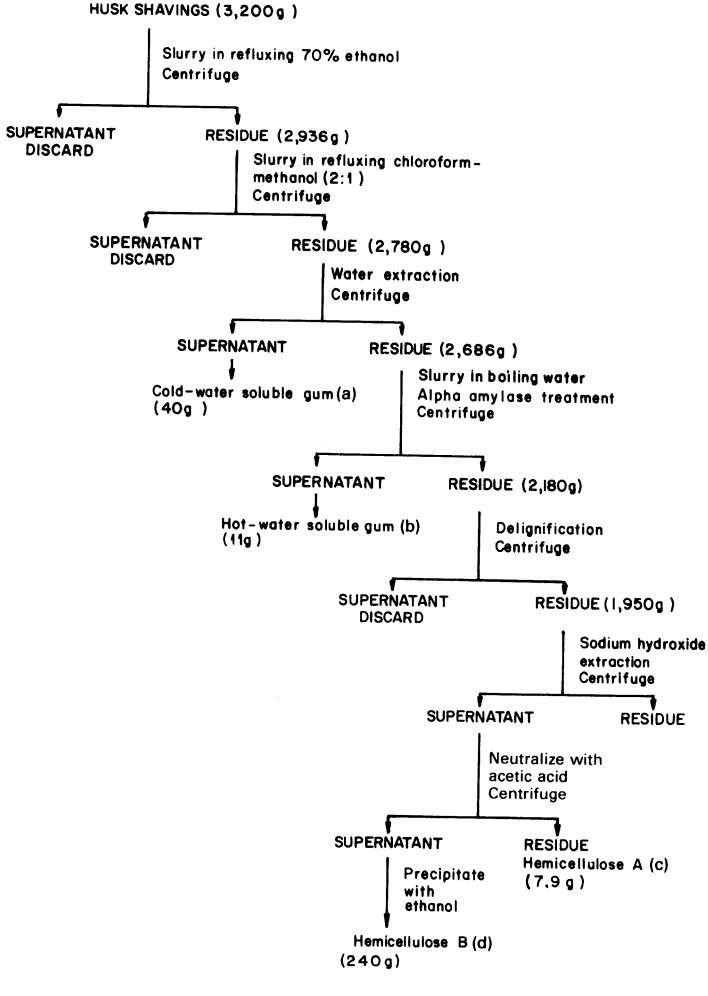

Fig. 1. Schematic diagram for the isolation of polysaccharides of sorghum grain husk.

passed through a Miag rice-polishing machine. The husk shavings (7.1% of total grain), shown by microscopic investigation to be free of adhering endosperm, were used for this study.

Isolation of Water-Soluble Gums

Figure 1 shows the scheme used for the isolation of the polysaccharides from the husk sample.

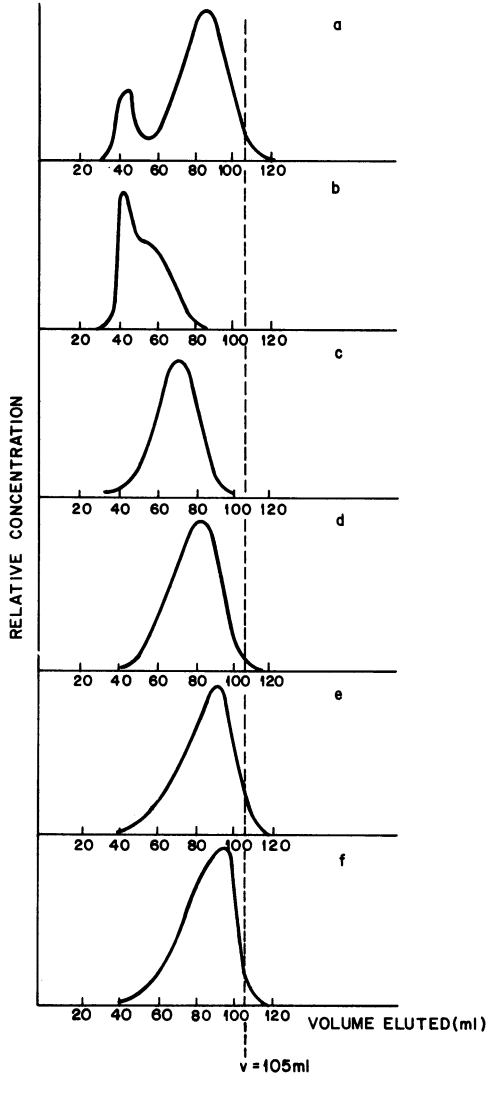

Fig. 2. Bio-Gel A-15m elution patterns of hemicellulose B (a) and Bio-Gel A-15m fractions (b–f). Samples *ca.* 4 mg eluted with 0.5*M* sodium chloride; flow rate, 10 ml/hr.

The husk (3200 g) was refluxed in 70% aqueous ethanol for 2 hr to denature enzymes and remove low-mol-wt materials. The residue, recovered by centrifugation, was suspended in chloroform-methanol (2:1) and refluxed for 4 hr. The residue was extracted by stirring with distilled water at 20°C, and recovered by centrifugation. The aqueous supernatant was concentrated (2000 ml), dialyzed, filtered, and freeze-dried. The product (40 g) is the cold-water soluble gum (a) referred to in Fig. 1.

An aqueous slurry of the residue obtained after cold-water extraction was boiled to gelatinize the starch. The solution was cooled to 55°C and α-amylase (6 g in 50 ml water) was added. The supernatant, recovered by centrifugation, was concentrated (4000 ml), dialyzed, and filtered. The filtrate was concentrated (400 ml), precipitated with ethanol, dissolved in water, and freeze-dried. This product (11 g) is the hot-water soluble gum (b) shown in Fig. 1.

The residue from the α-amylase treatment (2180 g) was suspended in water (6000 ml) at 65°C, and delignified with glacial acetic acid (1500 ml) and sodium chlorite (800 g) (22). The holocellulose (1950 g) was washed with water until free of acid.

Isolation of Hemicelluloses A and B

The holocellulose was extracted four times with 4% aqueous sodium hydroxide. Each extraction was carried out for 16 hr in a nitrogen atmosphere. The supernatant, recovered after centrifugation, was adjusted to pH 5.0 with acetic acid. Hemicellulose A was collected by centrifugation and washed three times with 5% acetic acid; then it was dissolved in 4% sodium hydroxide and reprecipitated with acetic acid. The polysaccharide was dissolved in water, precipitated with ethanol, redissolved, dialyzed, and freeze-dried. This polysaccharide (7.9 g) is hemicellulose A, shown as fraction (c) in Fig. 1.

The supernatant and the acetic-acid washings from the hemicellulose A extract were poured into 5 vol of 95% ethanol. The precipitate was dissolved in 4% sodium hydroxide, the pH adjusted to 5.0 with acetic acid, and the clear solution poured into 5 vol of 95% ethanol. The precipitate was dissolved in water, dialyzed, and freeze-dried. The product (240 g) is hemicellulose B, shown as fraction (d) in Fig. 1.

Bio-Gel A-15m Chromatography of Hemicellulose B

Hemicellulose B (1.1 g) was eluted in 3-ml fractions from a column (5 × 70 cm) of Bio-Gel A-15m with 0.5*M* sodium chloride (Fig. 2a). Five fractions (tubes 101

TABLE I
Data on Polysaccharides from Sorghum Grain Husk

Polysaccharide	Recovery g	Ratio ARAB:XYL:GLU	$[\alpha]_D$
Cold-water soluble gum	40	1.3:1.0: 7.2	+150°
Hot-water soluble gum	11	1.3:1.0:20.5	+119°
Hemicellulose A	7.9	1.1:1.0: 0.7	− 50°
Hemicellulose B	240	1.2:1.0: 0.1	−106°

to 200, 201 to 300, 301 to 334, 335 to 368, and 369 to 430), each representing 20% of the area under the elution curve, were dialyzed and freeze-dried. Samples of each of these fractions were rechromatographed on Bio-Gel A-15m (Fig. 2b–f).

Fractionation of Hemicellulose B on DEAE Cellulose

Hemicellulose B (10 g) was applied to a column of DEAE cellulose, prepared in its phosphate form with 0.01*M* sodium phosphate buffer (pH 6.8), and eluted with 0.01*M* sodium phosphate buffer (pH 6.8). The hemicellulose present in this eluate (4.9 g) was labelled hemicellulose B(A). Hemicellulose B(B) (3.0 g) was eluted with 0.01*M* sodium phosphate buffer (pH 6.8) containing 0.3*M* sodium chloride. A noncarbohydrate fraction (210 mg) was eluted with 0.5*M* sodium hydroxide. Hemicellulose B(B) was further fractionated on DEAE cellulose (phosphate form) into five polymers using a stepwise increasing gradient of sodium chloride in 0.01*M* sodium phosphate buffer (pH 6.8).

After DEAE cellulose was converted to its borate form with 0.2*M* sodium borate buffer (pH 9.0), hemicellulose B(A) (4.4 g) was rechromatographed. The column was eluted with 0.01*M* sodium borate buffer (pH 9.0) to yield

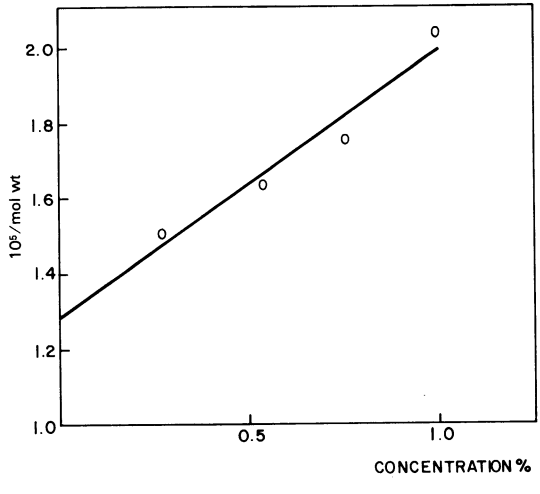

Fig. 3. Concentration dependence of molecular weight (mol wt) of hemicellulose B.

TABLE II
Data on Bio-Gel A-15m Hemicellulose B Fractions

Fraction	Recovery mg	Ratio ARAB:XYL:GLU	$[\alpha]_D$
1	121	1.4:1.0:0.2	−39°
2	298	1.2:1.0:0.1	−81°
3	171	1.0:1.0:0.1	−97°
4	174	1.0:1.0:0.1	−94°
5	284	1.0:1.0:0.1	−84°

hemicellulose $B(A)_1$ (2.7 g), and then with 0.01*M* sodium borate buffer (pH 9.0) containing 0.3*M* sodium chloride to give hemicellulose $B(A)_2$ (1.2 g). Hemicellulose $B(A)_1$ was further fractionated on DEAE cellulose (borate form) by eluting with water, 0.0025*M* sodium borate, and 0.01*M* sodium borate buffers. Hemicellulose $B(A)_2$ was fractionated into five polymers on DEAE cellulose (borate form) using a stepwise increasing gradient of sodium chloride in 0.01*M* sodium borate buffer (pH 9.0).

RESULTS AND DISCUSSION

A procedure has been described for the isolation of polysaccharides from the husk shavings of the Barnard Red variety of sorghum grain. Hemicellulose B was fractionated on DEAE cellulose into thirteen component polysaccharides.

Table I shows the analytical data for the cold- and hot-water soluble gums, and hemicelluloses A and B. Glucose is the predominant sugar in the water-soluble gums, while arabinose and xylose are present in lesser amounts. Hemicelluloses A and B contain arabinose and xylose as principal component sugars. These hemicelluloses differ from each other in that hemicellulose A also contains glucose as a major component sugar, compared with a low glucose content in hemicellulose B. The arabinose-to-xylose ratios for hemicelluloses A and B are 1.1:1 and 1.2:1, respectively. The hemicelluloses from sorghum husk contain considerably more arabinose than the arabinoxylans isolated from other cereal grains. The arabinose-to-xylose ratio for barley husk hemicelluloses is 1:6 (9), which is typical for many of the cereals studied.

The mol wt of hemicellulose B was found to be 77,500 at zero concentration. The dependence of mol wt on concentration is shown diagrammatically in Fig. 3.

TABLE III
Data on DEAE Cellulose Hemicellulose B Fractions

Polysaccharide		Eluting Buffer	Recovery from 80 g Hem. B g	Ratio ARAB:XYL:GLU	$[\alpha]_D$
$B(A)_1$			23.5	1.1:1.0:0.1	− 89°
	H-1	Water	4.9	1.0:1.0:0.7	− 53°
	H-2	0.0025*M* Borate	3.7	1.7:1.0	− 86°
	H-3	0.01*M* Borate	10.4	1.0:1.0	−106°
$B(A)_2$			12.7	1.1:1.0:0.1	− 86°
	H-4	0.025*M* NaCl	7.8	1.7:1.0:0.1	−101°
	H-5	0.05*M* NaCl	1.8	1.1:1.0:0.1	− 75°
	H-6	0.075*M* NaCl	0.9	1.2:1.0:0.1	− 61°
	H-7	0.1*M* NaCl	0.5	1.4:1.0:0.1	− 27°
	H-8	0.3*M* NaCl	0.5	1.5:1.0:0.3	+ 19°
B(B)			29.0	1.0:1.0	− 92°
	H-9	0.025*M* NaCl	16.0	1.0:1.0	− 98°
	H-10	0.05*M* NaCl	1.9	1.0:1.0	− 73°
	H-11	0.075*M* NaCl	1.3	1.0:1.0	− 70°
	H-12	0.1*M* NaCl	2.1	1.0:1.0	− 68°
	H-13	0.3*M* NaCl	2.6	1.0:1.0	− 44°

Table II shows the analytical data for the five fractions obtained on Bio-Gel A-15m chromatography of hemicellulose B. The elution diagrams (Fig. 2b–f) of these fractions demonstrate the polymolecular nature of this hemicellulose preparation.

Table III shows the analytical data for the polysaccharides obtained on DEAE cellulose chromatography of hemicellulose B. The three hemicellulose $B(A)_1$ subfractions are structurally different from each other. The polymer eluted with water contains arabinose, xylose, and glucose as principal component sugars; those eluted with $0.0025M$ and $0.01M$ sodium borate buffers are arabinoxylans. The high arabinose-to-xylose ratios indicate that these subfractions are highly branched.

The hemicellulose $B(A)_2$ subfractions are essentially arabinoxylans with varying arabinose-to-xylose ratios. The high proportions of arabinose indicate that these polymers are also highly branched. Glucose is present in these polysaccharides as a minor component sugar. Structural differences among these subfractions are indicated by the trend in optical rotation, which increases from a high negative value for the polymer eluted with $0.025M$ sodium chloride to a low positive value for that eluted with $0.3M$ sodium chloride.

The hemicellulose B(B) subfractions are arabinoxylans with 1:1 ratios of arabinose to xylose. They contain no glucose, and show a narrower range in optical rotation compared with the hemicelluloses of the $B(A)_2$ series. These observations indicate that the five fractions of hemicellulose B(B) not only differ from each other but that each differs from the corresponding fraction of hemicellulose $B(A)_2$.

This study has shown that hemicellulose B isolated from the husk shavings of sorghum grain is both polymolecular and polydisperse. Structural studies on the various hemicellulose B subfractions will be reported elsewhere.

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