Isolation and Chromatographic Fractionation of Hemicelluloses from Wheat Flour[1]

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ABSTRACT

Hemicelluloses (insoluble pentosans) from the tailings fraction of wheat flour were solubilized with dilute base and fractionated on a diethylaminoethyl cellulose column (borate form) into five fractions. Xylose, arabinose, and glucose were found in the acid hydrolysates of all of the fractions, xylose and arabinose occurring to the greatest extent. Galactose was found in the hydrolysates of fractions 4 and 5. Fraction 5 was the only fraction which contained protein in addition to the other sugars.

The hemicellulosic "insoluble pentosans" of wheat flour are thought to originate from the cell walls of the endosperm (1). If flour which has been washed free of gluten is slurried in water and centrifuged, the hemicelluloses will be found in the gellike layer ("tailings") above the starch. They consist of alkali-soluble and alkali-insoluble hemicelluloses as well as occluded starch granules. These "tailings" become highly hydrated in water without going into solution, and markedly affect the water-absorption of the flour (2). Among those workers who have studied the chemical composition of these hemicelluloses are Montgomery and Smith, who isolated a fairly pure arabinoxylan from the "tailings" by acetylation and showed that it possessed a highly branched structure (3). Recently Perlin and Suzuki examined the starch-free "tailings" and isolated two new constituents, cellobiose and mannose, which had not been detected previously in this fraction (4).

To this author's knowledge, no work has been published on the fractionation or separation of the wheat flour-insoluble hemicelluloses by column chromatography. A logical approach to a study of this sort seemed to be through the use of diethylaminoethyl (DEAE) cellulose in the borate form as an adsorbent to separate the negatively charged borate complexes of these hemicelluloses. A similar chromatographic technique has been used by Kündig *et al.* (5) to fractionate the water-soluble glycoproteins of wheat flour. Stable solutions of the insoluble wheat flour hemicelluloses suitable for chromatographic analyses can be prepared if these polysaccharides are solubilized by alkaline extraction (3) followed by neutralization and dialysis of the extract. In this article a study is described in which these insoluble hemicelluloses have been freed of starch with the use of purified alpha-amylase, solubilized, and fractionated on a column of DEAE-cellulose in the borate form. Stepwise elutions were used to obtain five polysaccharide fractions differing from each other in composition. The quantitative analyses of these fractions are reported, and some of the properties of these fractionated polysaccharides are discussed.

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MATERIALS AND METHODS

Preparation of Hemicellulose

A hard red winter wheat flour (Montana), milled on a laboratory Buhler mill, was used as a source of the pentosans; the protein content of the flour was 13.54% and the ash content was 0.44% (dry basis). Gluten was washed from 500-g. quantities of the flour at one time, with distilled water. The gluten was discarded and the remaining solids were suspended in water and centrifuged. The "tailings" fraction above the starch layer was then removed mechanically and lyophilized. To remove the occluded starch, the "tailings" were suspended in phosphate buffer and treated with alpha-amylase (Porcine; Worthington Biochemicals) according to the procedure of Kündig *et al.* (5). This digestion mixture was dialyzed against phosphate buffer approximately 5 days at 25°–30°C. The crude hemicelluloses remaining after dialysis were removed by centrifugation, washed several times with distilled water, and lyophilized. Next they were extracted by stirring 2 g. of solid with 125 ml. of 0.5*N* sodium hydroxide under nitrogen for 1 hr. The mixture was centrifuged, and the supernatant was removed and adjusted to pH 7 with glacial acetic acid. Then the residue was extracted with 0.5*N* sodium hydroxide twice more in the manner just described; neutralization with acetic acid followed. The hemicelluloses were next precipitated from the pooled supernatants at 4°C. by adding ethanol (1 vol. of polysaccharide solution to 4 vol. absolute ethanol). The resulting neutral precipitate was removed, dissolved in water, treated with alpha-amylase, and dialyzed once more by the method described above to digest any residual starch. At the end of dialysis the solution was freed of enzyme (5) and concentrated in vacuum to a volume of 100 ml. All hemicellulose solutions prepared in this fashion gave a negative starch-iodine test.

Fractionation on Cellulose Column

The DEAE-cellulose, a product of Bio-Rad Laboratories (exchange capacity 0.7 meq./g.), was prepared in the borate form according to the method of Neukom *et al.* (6). A glass column (3 × 56 cm.) was filled with 300 ml. of adsorbent, slurried in water, washed with water, and loaded with 25 ml. of hemicellulose solution containing 0.65 g. of polysaccharide. The elution was accomplished stepwise with distilled water, 0.008*M*, 0.1*M*, and 0.5*M* sodium borate, and with 0.2*N* sodium hydroxide. The eluate was collected automatically at a rate of 1.5 ml. per min. in 20-ml. quantities. The elution of polysaccharide fractions was followed colorimetrically with the phenol-sulfuric acid method of Dubois *et al.* (7), with xylose as a standard. After the positions of the individual fractions were located, the corresponding eluates were combined, evaporated *in vacuo* to a volume of 100 ml., dialyzed against distilled water, and lyophilized. Nitrogen determinations were made on each fraction by a micro-Kjeldahl method. Moistures were determined by drying the sample to constant weight at 100°C. *in vacuo*.

Preparation of Sephadex Gel

Sephadex G–200 was equilibrated in 0.1*M* sodium chloride for 2 days and then packed into a glass column (30 cm. × 1 cm.). The column void

volume was 10 ml. The amount of hemicellulose placed on the column contained 400 γ of sugar (based on xylose as standard).

Hydrolysis of Polysaccharide and Paper Chromatography

Approximately 40 mg. of each fraction was hydrolyzed with 1*N* sulfuric acid for 4 hr. at 100°C. The hydrolysate was neutralized with small amounts of Dowex-2 resin (HCO_3^- form) and chromatographed on Whatman No. 1 paper with ethyl acetate:pyridine:water (2:1:2) for elution solvent. The positions of sugars were located on guide strips with the alkaline silver nitrate dip (8). Arabinose, xylose, and glucose were eluted from the chromatograms with water and quantitatively determined with phenol-sulfuric acid. Galactose was measured directly on the neutralized hydrolysate with a Galactostat reagent (Worthington Biochemicals) which contained a coupled oxidase enzyme system suitable for the quantitative determination of galactose. All results were calculated as percentages of each anhydrosugar present in each fraction.

RESULTS AND DISCUSSION

About 80% of the hemicellulose solids left from the preliminary starch digestion could be solubilized with dilute base. No precipitate was formed in this extract upon neutralization. The small amount of soluble starch remaining in this extract was removed completely by another treatment with amylase. During both of these enzymatic digestions and dialyses, only glucose and/or polymers which could be hydrolyzed to glucose were detected in the dialysis medium. The neutral starch-free hemicellulose solution was fairly clear and highly viscous. After storage at 4°C. for several months, no precipitate or cloudiness had developed. Analyses of these dried hemicellulose solids were as follows: xylose, 54%; arabinose, 33%; glucose, 11%; galactose, 2%; and protein, 2%. The adsorbent, DEAE-cellulose (borate form), was effective in separating the hemicelluloses into fractions differing in composition (see Figs. 1 and 2). Other adsorbents such as

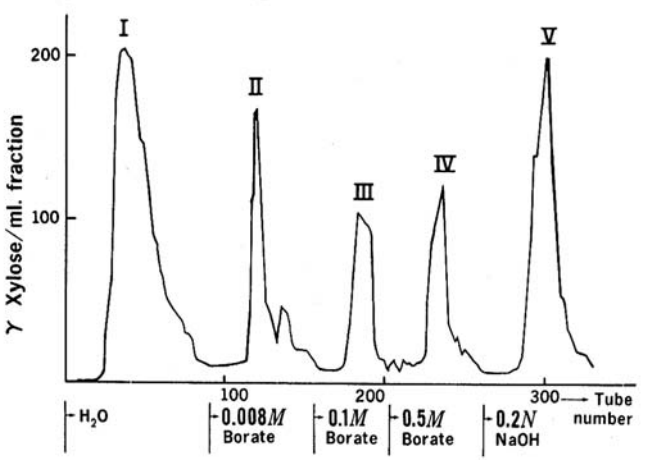

Fig. 1. Fractionation of hemicelluloses (250 mg.) from wheat flour on a DEAE-cellulose column (borate form). Volume collected in each tube: 20 ml.

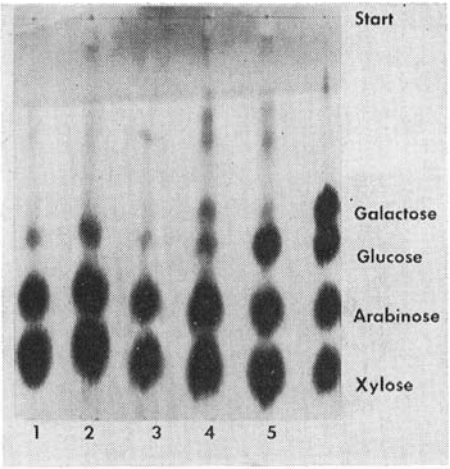

Fig. 2. Paper chromatogram of hydrolysate of wheat flour pentosans showing fractions 1 to 5 eluted from DEAE-cellulose column (borate form).

Sephadex G–200 (9) were ineffective. When a quantity of hemicellulose containing 400 γ of sugar (based on xylose) was placed on the column of Sephadex, 375 γ of xylose was eluted in the void volume (10 ml.). Thus the hemicelluloses were excluded on Sephadex 200, indicating that, under the elution conditions used, these polysaccharides were either highly associated or had molecular weights exceeding 2×10^5. The presence of such high-molecular-weight polysaccharides in these hemicellulose preparations also indicates that a minimum of degradation has occurred during solubilization with dilute alkali. If appreciable degradation had occurred, one would expect to find lower-molecular-weight fragments present. Not all of the hemicelluloses placed on the DEAE-cellulose column could be eluted with the solvents used. About one-fourth of the total solids was not removed, even by prolonged elution with 0.2*N* sodium hydroxide. A large quantity of polysaccharide which did not complex with borate was eluted with water (fraction 1; see Table I). Fractions 1 to 4, inclusive, contained negligible amounts of nitrogen; however, over one-third of fraction 5 was protein.

TABLE I
ANALYSES OF PENTOSAN FRACTIONS

FRACTION NO.	PERCENT OF TOTAL WEIGHT ELUTED	ARA[a]	XYL[a]	GLU[a]	GAL[a]	TOTAL
	%					
1	42	27	66	1	....	94
2	15	32	66	5	....	103
3	10	53	53	trace	....	106
4	11	45	50	3	1	99
5	22	20	35	13	trace	68[b]

[a] Abbreviations for sugars are as follows: ARA, arabinose; XYL, xylose; GLU, glucose; GAL, galactose. (Units are reported in percent anhydro sugar present.)

[b] The remainder of solid in this sample is mostly protein. This fraction contained 6% nitrogen.

This fraction may be similar to the glycoproteins in the water-soluble portion of wheat flour, as described by Kündig and co-workers (5). Also, recently Upton and Hester have demonstrated the presence of glycoproteins in the "tailings" fraction (10). Glucose occurred in sizable amount in the hydrolysates of some of the fractions, particularly No. 5. The presence of this sugar is of interest because starch, which was the main contributor of glucose, was removed from these pentosan preparations prior to chromatographic separation. Whether glucose is linked covalently to the pentosan molecule or whether these glucose-containing fractions are mixtures of glucosans and pentosans is yet to be determined. Another sugar detected in fractions 4 and 5 was galactose. The galactose content (2%) of these alkali-soluble hemicelluloses is lower than that reported for insoluble cell-wall materials by other workers (8). Under the conditions of this experiment most of the galactose-containing polymer was retained on the column. Other reducing substances were detected in very small amounts in the hydrolysates of the fractions (see Fig. 2, fractions 4 and 5, slow-moving spots on the chromatogram); their R_f values were similar to those of uronic acids. However, the hydrolysates did not give positive uronic acid tests with naphthoresorcinol and only a faint positive test with carbazole (11). Thus few if any carboxyl groups are attached to these polysaccharide molecules. Also, the lack of appreciable amounts of carboxyl groups provides further evidence that no extensive oxidation of the hemicelluloses occurred during treatment with alkali.

In summary, insoluble pentosans from wheat flour were separated on a DEAE-cellulose column into fractions which differ quantitatively in their monosaccharide contents. However, to determine fully the extent of homogeneity of the various fractions, further physical measurements will have to be made.

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