The Carbohydrate Composition of Corn Cob Hemicelluloses[1]

B. J. DONNELLY, J. L. HELM, and H. A. LEE[2], Anheuser-Busch, Inc.,
St. Louis, Missouri 63118

ABSTRACT

Hemicelluloses A and B isolated from corn cobs of different synthetic genetic populations were analyzed for their carbohydrate composition. Isolation of the hemicelluloses involved delignification and fractionation into hemicelluloses A and B. The isolated hemicelluloses were acid hydrolyzed, and the carbohydrate composition of the hydrolysates determined by paper and gas chromatography. The major component was xylose, followed by arabinose and minor quantities of glucose and galactose. The quantities of these sugars varied, but there were consistently higher amounts of xylose in hemicellulose A than in B. With arabinose this situation was reversed. The xylose:arabinose ratios also showed variation throughout. Since populations differ in xylose:arabinose ratios, it appears possible to select genetic strains for specific cob hemicellulose composition.

Corn cobs contain a considerable reservoir of carbohydrates, most of which are of a polysaccharide nature. These polysaccharides consist principally of cell-wall cellulose and hemicellulose. The term hemicellulose denotes those polysaccharides extractable from plants by aqueous alkali. They are characterized by the type of sugar residue present; thus, D-xylan is a polymer of xylose residues, D-mannan of D-mannose residues, etc. However, the natural occurrence of homoglycans is considerably less than that of heteroglycans in present-day corn cobs. The heteroglycans usually contain two to four different types of sugar residues, for example, L-arabino-D-xylans and L-arabino-D-glucurono-D-xylans, and have branched structures. The hemicelluloses of corn cobs are mainly heteroxylans. The cell walls of the corn cob contain cellulose bundles which are embedded in an amorphous mass of lignin and polysaccharide material, thus giving a strong and rigid structure (1). Lignin interferes with the extraction of the hemicelluloses by alkali for two main reasons: a) it retards complete solution of the hemicellulose, and b) it dissolves in the extract, causing difficulty in the purification of the hemicellulose.

To overcome these problems, the corn cob can be delignified with sodium chlorite and acetic acid after the method of Whistler et al. (2), which leaves the polysaccharides in practically their original state. The residual cellulose and hemicellulose mixture is called holocellulose (3). Subsequent extraction of the holocellulose with alkali and acidification of the extract to pH 4.5 to 5.0 with acetic acid yields hemicellulose A. The mother liquor from the acid solution yields hemicellulose B upon addition of three times its volume of 95% ethanol (4).

Corn cob hemicellulose has long been recognized as a good source of xylose (5). Hemicellulose constitutes approximately 30% of the mature cob (6,7). Ehrenthal et al. (8) reported the hydrolysis products of methylated corn cob hemicellulose to have a xylan portion containing 83% xylose with 5.9% L-arabinose and 1.7% D-glucose. More recent work on the composition of the individual hemicellulose A and B fractions from the corn cob and stalk (9,10) has shown that xylose is the main component of each of these and that the major difference between them relates to the arabinose and uronic acid content. The hemicellulose A fraction

[1]Presented at the 57th Annual Meeting, Miami Beach, Oct.-Nov. 1972.
[2]Chemist, geneticist, and enzymologist, Corn Products Division, Anheuser-Busch, Inc.

contains less arabinose than the B fraction and more uronic acid (11).

Recently we have had occasion to investigate suitable economic sources of xylose for the possible production of glucose-isomerizing enzymes. Corn cobs were one of the sources investigated. We looked at cobs from a broad cross-section of corn belt populations to determine whether or not genetic variability exists for xylose content and for the xylose:arabinose ratio. This paper, then, reports our results obtained in the xylose and arabinose assay of these different corn cobs and their possible genetic implications.

MATERIALS AND METHODS

Material

Fifty random cobs were harvested from open-pollinated plots of ten Iowa-released synthetics grown at Mason City, Ill. in 1969. The corn cobs were dried and ground in a Wiley mill to pass through a 20-mesh screen.

Delignification and Isolation of Holocellulose

To a mixture of ground cobs (50 g.) and water (700 ml.) at 74°C., glacial acetic acid (2.5 ml.) and sodium chlorite (7.5 g.) were added, with stirring (2). Carbon dioxide was bubbled through the mixture, and the addition of acetic acid and sodium chlorite was repeated every 15 min. for a total of four additions. Excessive foam was controlled by the addition of 2 drops of octyl alcohol. The whole process was carried out in a well-ventilated hood. After 1 hr. the mixture was cooled to room temperature, filtered through a filter cloth, washed with water until free of acid, and finally washed with acetone. The dried product was almost white.

Isolation of Hemicellulose A

The holocellulose (30 g.) was extracted with 10% potassium hydroxide (500 ml.) by stirring overnight in an atmosphere of nitrogen; the extract was filtered on a filter cloth and washed with water. The clear filtrate was adjusted to pH 5.0 with 50% acetic acid solution and refrigerated overnight. Hemicellulose A was collected by centrifugation, and the supernatant liquid was removed and saved for isolation of the hemicellulose B. The hemicellulose A was washed with water three times and finally suspended in water and freeze-dried. The alkali solubilization and reprecipitation of the hemicellulose A was repeated twice. The final product was a white amorphous powder.

Isolation of Hemicellulose B

The supernatant liquid from the hemicellulose A extract was poured into three times its volume of 95% ethanol with rapid stirring. The precipitate was collected as described for hemicellulose A, except that the product was washed with 95% ethanol. The freeze-dried product was reprecipitated twice by redissolving in alkali, adjusting the pH to 5.0 with 50% acetic acid, and precipitating with 95% ethanol. The lyophilized product was an off-white powder.

Acid Hydrolysis of Hemicellulose

The hemicellulose (1 g.) in 1N sulfuric acid (25 ml.) was refluxed for 2 hr. The cooled solution was neutralized with saturated barium hydroxide solution. The precipitate was removed by centrifugation and the clear supernatant liquid collected. The final solution volume was made up to 200 ml.

Paper Chromatography

The acid-hydrolyzed hemicelluloses were chromatographed on Whatman No. 1 paper by descending chromatography. The developing solvent was ethyl acetate:pyridine:water (80:20:10). After 48 hr., the paper was dried and sprayed with *p*-anisidine hydrochloride (2 g.) in *n*-butanol:ethanol:water (40:10:10). Upon heating, the pentoses appear as pink spots and the hexoses as olive-colored spots (12). The R_{xylose} values were: L-arabinose, 0.74; D-glucose, 0.43; and D-galactose, 0.33.

Preparation of Alditol Acetates

An aliquot (10 ml.) of the hemicellulose hydrolysate, containing erythritol (37.5 mg.) as internal standard, was reduced with sodium borohydride (60 mg.). After 3 hr. the reduction was stopped by acidification to pH 5.8 to 6.3 with glacial acetic acid. The alditol solution (2 ml.) was freeze-dried and the residue acetylated with acetic anhydride (1 ml.) by heating in a Teflon-lined stoppered tube at 115°C. for 3 hr. The sample, on cooling, was ready for injection into the gas chromatograph. The flame detector response curves were calculated from data obtained with standard xylose and arabinose solutions, prepared as above, with erythritol as internal standard.

Gas Chromatography

For the quantitative determination of xylose and arabinose (as alditol acetates) the following gas chromatography conditions were used: gas chromatograph, Varian 1520; flame, 10; attenuation, 16; detector, 300°C.; injector, 275°C.; oven temperature program, 170°C., isothermal for 2 min., then 8°C./min. for 10 min.; He 30 ml./min.; H_2 30 ml./min.; air 300 ml./min.; chart speed, 1 in./min.; 6 ft. × 0.125 in. copper column with 7% QF-1 and 1.7% B.D.S. on Chromosorb W (100–120 mesh). The retention times relative to zero time injection were: erythritol tetraacetate, 4.0 min.; xylitol pentaacetate, 7.6 min.; and arabinitol pentaacetate, 6.6 min.

Determination of D-glucose and D-galactose

The amounts of D-glucose and D-galactose present in the hemicellulose hydrolysates were determined with the Glucostat and Galactostat reagents, respectively, as supplied by Worthington Biochemical Co.

RESULTS AND DISCUSSION

Percent holocellulose and hemicellulose of the cobs is shown in Table I. The range in holocellulose content, 80.0 to 87.6%, is as expected. Hemicellulose content ranged from 18.0 to 22.0%, and is significantly lower than previously reported values of about 30% (6). We think this is due to our repeated reprecipitation which lowered yield, but increased purity. The ratio of holo- to hemicellulose is important. It identifies those populations (gene pools) that have genetic potential to produce more or less hemicellulose. Our ratios of 3.6 to 4.5 indicate genetic variability exists. Thus, Iowa Corn Borer Synthetic No. 4 with the lowest ratio has the highest percent hemicellulose as opposed to Iowa High Oil No. 1 with a low hemicellulose content and thus a high ratio. The divergence of the two populations for hemicellulose content probably was a random event, but one should not overlook the possibility of linkages to genes for the traits selected (high oil or

TABLE I. HOLOCELLULOSE AND HEMICELLULOSE CONTENT OF CORN COBS

Sample	Holo-cellulose[a]	Hemi-cellulose[a]	Holocellulose: Hemicellulose	Hemi-cellulose A[b]	Hemi-cellulose B[b]
Iowa Long Ear Synthetic	87.6	19.5	4.5	6.8	15.4
Iowa Stiff Stalk No. 2	87.0	21.0	4.1	8.6	15.5
Iowa Corn Borer Synthetic No. 3	84.6	21.7	3.9	15.2	10.4
Iowa Elite Line Synthetic	84.0	21.9	3.8	14.5	11.6
Iowa High Oil No. 1	83.4	18.9	4.4	12.6	10.0
Iowa Super Stiff Stalk	82.4	19.5	4.2	14.0	9.7
Iowa Two Ear Synthetic	82.4	20.0	4.1	12.4	11.9
Tuxlan Synthetic	82.0	19.0	4.3	11.4	11.8
Iowa Corn Borer Synthetic No. 5	80.6	18.9	4.3	11.3	12.1
Iowa Corn Borer Synthetic No. 4	80.0	22.0	3.6	12.5	15.0

[a]Percent of corn cob.
[b]Percent of corn cob holocellulose.

resistance to corn borer). These results indicate that one should be able to select for high or low hemicellulose content in corn belt corn.

The hemicellulose A and hemicellulose B fractions of the hemicellulose expressed as percent of holocellulose are shown in Table I. Here one can see greater variability than for total hemicellulose content. Hydrolysis of the two hemicellulosic fractions with 1N sulfuric acid liberated the component sugars. Since we were mainly concerned with the xylose and arabinose content, no attempt was made to isolate, identify, and quantitate the uronic acids present. Paper chromatography of the hydrolysates indicated xylose to be the major sugar present. Arabinose was the next most prominent sugar, with glucose and galactose present in trace to minor amounts.

The quantitative determinations of the sugars are shown in Table II. These results agree with previous results (11), and show xylose to be more predominant in

TABLE II. XYLOSE-ARABINOSE CONTENT OF CORN COB HEMICELLULOSE[a]

Sample	Hemicellulose	Xylose	Arabinose	Xylose:Arabinose
Iowa Long Ear Synthetic	A	87.0	2.5	35.0
	B	66.0	12.5	5.3
Iowa Stiff Stalk No. 2	A	95.0	3.0	31.6
	B	60.0	8.5	7.0
Iowa Corn Borer Synthetic No. 3	A	75.0	6.0	12.5
	B	60.0	12.0	5.0
Iowa Elite Line Synthetic	A	87.0	2.5	35.0
	B	66.0	12.5	5.3
Iowa High Oil No. 1	A	95.0	2.5	38.0
	B	65.0	10.0	6.5
Iowa Super Stiff Stalk	A	76.0	6.0	12.7
	B	66.0	12.5	5.3
Iowa Two Ear Synthetic	A	87.0	2.5	35.0
	B	62.5	12.5	5.0
Tuxlan Synthetic	A	87.5	,2.5	35.0
	B	60.0	12.5	4.8
Iowa Corn Borer Synthetic No. 5	A	90.0	2.5	36.0
	B	72.0	12.5	5.8
Iowa Corn Borer Synthetic No. 4	A	95.0	4.0	24.0
	B	65.0	12.5	5.2

[a]Percent of hemicellulose.

the A fraction. Conversely, the arabinose content of the B fraction is greater than in the A fraction. The B fraction contains 5 to 6% glucose and 3% galactose, whereas the A fraction contains only trace quantities of these sugars, 0.5% or less. The xylose:arabinose ratios were quite uniform in the B fraction, but varied widely in the A fraction. From a genetic standpoint this is advantageous, since the A fraction contains the greatest amount of xylose.

The results obtained in this investigation of corn belt synthetic populations indicate that genetic variability exists for several traits related to corn cob hemicellulose. This will allow the geneticist in a xylose production program to select for these desirable traits while developing inbred lines from particular corn belt populations. Likewise, a survey of the inbreds presently being used in commercial hybrids may identify two or more inbreds that could be used to produce agronomically acceptable hybrids whose cobs could be used for xylose production. One will probably find that lines with the highest hemicellulose yields will not have the highest xylose contents, and many generations of selection will be required to find the correct combination of genes to give high yields of xylose with only minor amounts of other sugars. This will mean a shift from mostly heteroxylans to hemicelluloses with mostly homoxylans, something the corn plant has evolved away from in nature.

Acknowledgments

We thank I. Ehrenthal for helpful suggestions and Jo Ann Rogers for technical assistance.

Literature Cited

1. HIRST, E. L. Some problems in the chemistry of the hemicelluloses. J. Chem. Soc. 1955: 2974.
2. WHISTLER, R. L., BACHRACH, J., and BOWMAN, D. R. Preparation and properties of corn cob holocelluloses. Arch. Biochem. 19: 25 (1948).
3. RITTER, G. J., and KURTH, E. F. Holocellulose, total carbohydrate fraction of extractive-free maple wood. Ind. Eng. Chem. 25: 1250 (1933).
4. ADAMS, G. A. Xylans. Arabinoglycuronoxylan, arabinoxylan, and xylan; purification using a copper complex and purification by fractional precipitation of acetates. V. General polysaccharides. In: Methods in carbohydrate chemistry, ed. by R. L. Whistler, Vol. V, p. 170. Academic Press: New York (1965).
5. PREECE, I. A. Studies on hemicelluloses. II. The hemicelluloses of maize cobs. Biochem. J. 24: 973 (1930).
6. WHISTLER, R. L., and LAUTERBACH, G. E. Hydrolysis products from methylated arabinoxyloglycan and arabinogalacto-mono-O-methylglucuronoxyloglycan of corn cobs. J. Amer. Chem. Soc. 80: 1987 (1958).
7. WHISTLER, R. L. Xylan. Advan. Carbohyd. Chem. 5: 269 (1950).
8. EHRENTHAL, I., MONTGOMERY, R., and SMITH, F. The carbohydrates of Gramineae. II. The constitution of the hemicelluloses of wheat straw and corn cobs. J. Amer. Chem. Soc. 76: 5509 (1954).
9. WHISTLER, R. L., and GAILLARD, B. D. E. Comparison of xylans from several annual plants. Arch. Biochem. Biophys. 93: 332 (1961).
10. GAILLARD, B. D. E. Comparison of the hemicelluloses from plants belonging to two different plant families. Phytochemistry 4: 631 (1965).
11. PIGMAN, W., and HORTON, D. The carbohydrates, Vol. II A , p. 447. Academic Press: New York and London (1970).
12. HOUGH, L., JONES, J. K. N., and WADMAN, W. H. Quantitative analysis of mixtures of sugars by the method of partition chromatography. Part V. Improved methods for the separation and detection of the sugars and their methylated derivatives on the paper chromatogram. J. Chem. Soc. 1950: 1702.

[Received December 20, 1972. Accepted March 20, 1973]