Free Sugars in Developing Maize Grain

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ABSTRACT

The free sugar fraction of maize grain during development was studied by anion-exchange chromatography. Sucrose, glucose, fructose, maltose, cellobiose, and ribose were found in the embryo, and sucrose, glucose, fructose, maltose, galactose, ribose, mannose, cellobiose, and traces of xylose were detected in the endosperm. Two unknown orcinol-reacting compounds also were present both in the embryo and endosperm. Data related to quantitative variations in these sugars during seed development also are reported.

Maize seeds accumulate substantial quantities of carbohydrates during development. Of the carbohydrates reported to be present besides starch, the free sugar fraction has been studied frequently because of its relationship to the insoluble carbohydrates (Creech 1968). The free sugars consist mostly of sucrose, glucose, and fructose; minor quantities of maltose and raffinose have been described (Cerning 1970, Peat et al 1965, Jordan 1965). Uncertainties remain, however, concerning the quantity and kind of minor sugars in maize seed extracts. We report here data on the free sugars from developing maize seeds, separated, and determined quantitatively as negatively-charged borate derivatives by anion-exchange chromatography.

MATERIALS AND METHODS

Plants of the hybrid genotype B14A × B37 were grown in the summer of 1975. At flowering the plants were self-pollinated, and 15, 20, 25, 30, 35, 40, and 60 days later ears were harvested, dipped in liquid nitrogen until frozen and dehulled mechanically. The seed was stored at −20° C.

After removal of the pericarp, the seeds were separated into endosperms and embryos. A sample was freeze-dried, milled in a Udy cyclone mill, and used for sugar extraction.

Free sugars were extracted by the method of Ponte et al (1969), using a mixture of chloroform and methanol (1:1, v/v). A complete extraction was obtained by shaking 1 g of flour with 4 ml of the solvent mixture at room temperature for 20 min, adding 4 ml of water, centrifuging, and repeating this operation four times. The insoluble residue was then treated for 12 hr in a Dubnoff bath at 55° C with 200 ml of an aqueous solution of 90% dimethyl sulfoxide (DMSO). After centrifugation, the supernatant was diluted with water to 36% DMSO. Samples of 50 μl were then treated with 2.5 U of dialyzed glucoamylase (Boheringer, Mannheim, W. G.) in 0.1 *M* citrate buffer, pH 4.8, at a final volume of 200 μl for 60 min at 40° C. Starch was then determined as glucose × 0.9 by the method of Nelson (1944).

The free sugar containing supernatants were desalted by passing them through two columns (0.7 × 10 cm) fitted with anionic (Biorad AG1 × 2) and cationic (Biorad AG50w × 8) resins. The extracts were dried under vacuum on a rotary evaporator and the residues dissolved in 10 ml of 0.1 *M* borate buffer, pH 8.0.

Free sugars were analyzed by anion-exchange chromatography according to Catravas (1967) using a type S chromobead polystyrene resin (Technicon, Tarrytown, NY). For each extract, 25–100 μl aliquots were loaded on the column to give a quantitative estimate of sucrose. A second chromatogram was obtained by loading the column with 100–1500 μl aliquots of extract to evaluate glucose, fructose, and minor sugars. A third lot of samples were digested for 6 hr at 37° C with a large excess (20 U/1 mg of sucrose) of invertase (Boehringer, Mannheim, W.G.) to identify sugars, such as cellobiose, masked in the second type of chromatogram by the large sucrose peak.

The coefficients of regression obtained by plotting known

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amounts (10, 25, 50, 75, and 100 μg) of standard sugars against their peak heights were adopted for calculating quantitative values of each sugar present in the extracts. The identification of the free sugars was based on the retention time of standard sugars used in combination or individually. Qualitative sugar analysis also was confirmed by paper chromatography using (v/v) butanol, acetic acid, and water (120:30:50) and developing the spots with aniline-diphenylamine phosphate reagent (Cerning and Guilbot 1973).

RESULTS

Identification of Free Sugars

A typical chromatogram of a standard sugar solution is shown in Fig. 1A. In this solution raffinose was omitted because the mixture of sucrose, raffinose, and cellobiose was poorly resolved by our system due to the similarity of retention times of the three sugars. The retention time of raffinose was obtained independently and was intermediate between those of sucrose and cellobiose (dashed peak in Fig. 1A).

During development of the embryo, sucrose, glucose, fructose, maltose, and ribose are present. At maturity (Fig. 1B) the embryo extracts no longer contain fructose; in this tissue two unknown orcinol-reacting compounds (UK1 and UK2 of Fig. 1B) are detectable 20 days after pollination and also at later stages.

Sucrose, glucose, fructose, maltose, galactose, ribose, and mannose are found in the endosperm accompanied by the two unknowns UK1 and UK2 (Fig. 1C). In both embryo and endosperm, the chromatography of invertase-treated extracts reveals cellobiose. This is clearly shown for the endosperm extract in Fig. 1D where, along with the small sucrose peak (a minor fraction not hydrolyzed by invertase), another component showing the typical retention time of cellobiose is present. In the same chromatogram, trace amounts of xylose were noted between galactose and glucose.

A distinct raffinose peak was absent from our chromatogram, though this compound was previously reported in maize seed by authors working with paper chromatography (Cerning 1970); we therefore assayed our extracts by the last technique. R_f values were obtained for standard solutions of fructose, glucose, sucrose, maltose, and raffinose. Paper chromatography of embryo and endosperm extracts showed the major spots of sucrose, glucose, and fructose; the maltose spot also was clear, with its typical gray color. Minor spots were present but poorly resolved because of the large spots of the major sugars. A minor spot with a low R_f value also was found. In paper chromatograms of endosperm extracts and standard solutions, run side by side, this small spot migrated to a position definitely different from that of raffinose. We concluded that, at least with our method of extraction and analysis, raffinose was not detectable in the seeds of the maize genotype studied.

Quantitative Estimates of Free Sugars

Table I shows dry weight, starch, and sugars contents of the embryo. On the basis of dry matter, starch decreases during development. The level of sucrose falls slowly from 25 to 40 days after pollination but increases in the final part of the grain filling period reaching the relevant percentage of 6.94 at maturity. Glucose and fructose decrease rapidly from early to late stages of embryo development, and at maturity, this tissue is almost devoid of these two monoses. The three minor sugars, maltose, ribose, and cellobiose are detected only at later stages of development.

Table II reports dry weight, starch, and sugar contents of the endosperm. As expected, starch increases from 15 days after pollination until maturity, while the amounts of sucrose, glucose, and fructose generally decrease. At maturity the sucrose content (0.322%) is higher than that of glucose (0.049%) and fructose

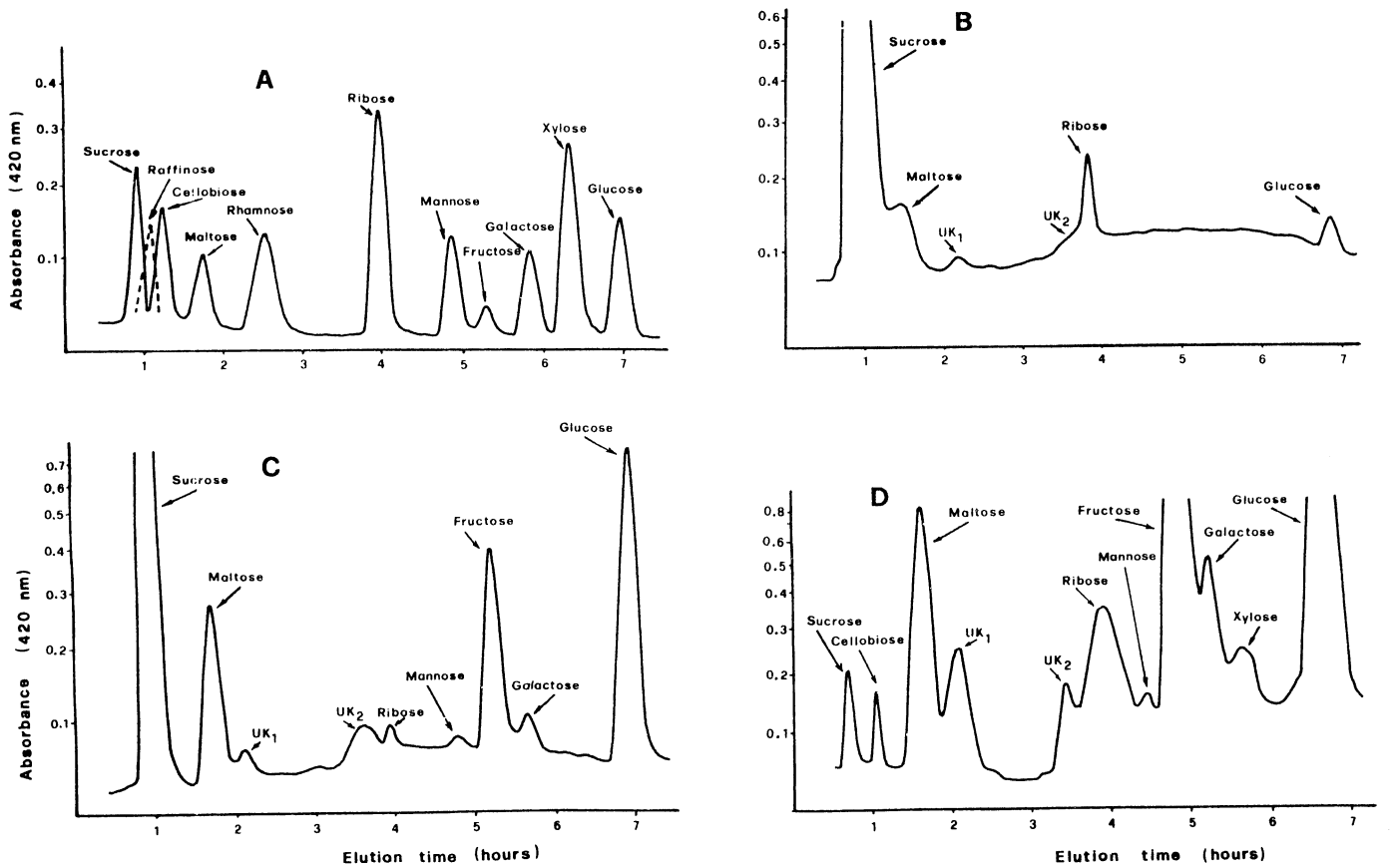

Fig. 1. Anion-exchange chromatography of orcinol-reacting compounds. A, retention times of standard sugars; the column was loaded with 25 μg of each sugar. B, chromatogram of an embryo extract (60 days after pollination). C, chromatogram of an endosperm extract (30 days after pollination). D, chromatogram of an invertase treated extract of the endosperm (30 days after pollination).

TABLE I
Dry Weight, Starch, and Free Sugar Content in Developing B37 × B14 Maize Embryos

Days After Pollination	Dry Weight (mg)	(mg/g, dry weight)[a] Starch	Sucrose	Glucose	Fructose	Maltose	Ribose	Cellobiose
25	4.0	189.6	48.64	9.04	6.84	...	...	...
30	8.6	159.5	51.38	5.06	6.03	t	...	...
35	14.4	157.1	47.23	3.37	2.58	t	...	t
40	19.3	112.4	43.48	2.37	1.56	0.01	...	0.05
60	57.3	72.5	69.49	0.16	...	0.81	0.20	...

[a]t = trace.

TABLE II
Dry Weight, Starch, and Free Sugar Content in Developing B37 × B14 Maize Endosperm

Days After Pollination	Dry Weight (mg)	(mg/g, dry weight)[a] Starch	Sucrose	Glucose	Fructose	Maltose	Galactose	Cellobiose	Ribose	Mannose
15	11.7	83.6	134.21	80.03	71.04	1.40	...	...	...	...
20	41.2	610.1	51.87	17.16	20.67	2.19	0.15	0.09	t	t
25	79.2	736.6	33.13	6.03	4.52	2.08	0.18	0.13	0.02	0.004
30	124.3	751.7	19.04	3.08	2.78	1.33	0.15	0.11	0.02	0.004
35	151.8	756.6	16.22	1.54	1.82	0.93	0.24	0.12	0.03	0.001
40	188.1	839.3	8.46	0.82	1.45	0.52	0.23	0.06	t	0.001
60	275.1	841.1	3.22	0.49	0.32	0.30	0.05	0.12	...	...

[a]t = trace.

(0.032%). Maltose is present at every stage of development and reaches maximum content 20 days after pollination. Galactose and cellobiose are absent 15 days after pollination but then show almost constant levels, though that of galactose drops at maturity. Ribose and mannose are found only at intermediate stages of endosperm development.

DISCUSSION

Eight and six free sugars, respectively, have been identified in the endosperm and in the embryo of developing maize seeds. In addition, two unknown orcinol reacting compounds were noted in both tissues.

Sucrose, fructose, and glucose were known to be present in the endosperm (Creech 1968, Whistler et al 1957). Jordan (1965) described maltose in the endosperm in normal corn and Peat et al (1965) in sweet corn. The latter authors also found raffinose in endosperm extracts of sweet corn, which was confirmed by Whistler et al (1957), working with the double genotype $su_1\ su_2$ and by Cerning (1970) for normal maize at advanced stages of endosperm development. Raffinose was not, however, present in the maize we analyzed. On the contrary, in the endosperm extracts we found galactose, cellobiose, ribose, mannose, and traces of xylose, all sugars not previously detected.

The free sugars of the maize embryo correspond to those of the endosperm, except that galactose, mannose, and xylose are not present in the embryo. The differences between embryo and endosperm are much more evident when the quantitative accumulation of sugars or their interconversion to starch are considered. In the endosperm, the starch content increases during development, while sucrose, glucose, fructose, and maltose decrease (Creech 1968, Shannon and Dougherty 1972, Tsai et al (1970). In the embryo, on the other hand, the percentage of starch decreases from 25 to 60 days after pollination; sucrose, the more important free sugar, is higher at all stages of development and reaches its maximum level at maturity. As in the endosperm, the embryo glucose and fructose decrease rapidly during development. The sucrose content of the mature embryo is of particular interest and may even be related to the metabolism of fatty acids, a class of compounds abundant in this tissue.

LITERATURE CITED

CATRAVAS, G. N. 1967. New developments in automatic sugar chromatography. In: Automation in Analytical Chemistry, p. 397. Mediad: New York.

CERNING, J. 1970. Contribution à l'étude de l'évolution de la composition glucidique des grains de céréales au cours de leur maturation: mais, blé, orge. These de doctorat d'Université, Lille, France.

CERNING, J., and GUILBOT, A. 1973. Changes in the carbohydrate composition during development and maturation of the wheat and barley kernel. Cereal Chem. 50:220-232.

CREECH, R. G. 1968. Carbohydrate synthesis in maize. Adv. Agron. 20:275-322.

JORDAN, W. S. 1965. Chromatographic analysis of the alcohol-soluble sugars isolated from kernel of maize endosperm mutants. PhD thesis, The Pennsylvania State University, Univ. Park, PA.

NELSON, N. 1944. A photometric adaptation of the Somogy method for the determination of glucose. J. Biol. Chem. 153:375-380.

PEAT, S., WHELAN, W. J., and TURVEY, I. R. 1965. The soluble polyglucose of sweet corn. J. Chem. Soc. 2317-2322.

PONTE, J. G., Jr., DeSTEFANIS, V. A., and TITCOMB, S. T. 1969. Application of thin-layer chromatography to sugar analysis in cereal based products. Cereal Sci. Today 14:100.

SHANNON, J. C., and DOUGHERTY, C. T. 1972. Movement of ^{14}C-labeled assimilates into kernels of *Zea mays* L. II. Invertase activity of the pedicel and placento-chalazal tissues. Plant. Physiol. 49:203-206.

TSAI, C. Y., SALAMINI, F., and NELSON, O. E. 1970. Enzymes of carbohydrate metabolism in the developing endosperm of maize. Plant Physiol. 46:299-306.

WHISTLER, R. L., KRAMER, H. H., and SMITH, R. D. 1957. Effect of certain genetic factors on the sugars produced in corn kernels at different stages of development. Arch. Biochem. Biophys. 66:374-380.

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