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THE SUGARS OF GERMINATING CORN (Zea mays)[1]

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ABSTRACT

Raffinose, sucrose, glucose, fructose, myo-inositol, and glycerol were identified as being present in sound corn by isolation of the pure compounds and the preparation of crystalline derivatives. Maltose was found to appear after 48 hr. of germination.

The corn (43% moisture) was germinated at 16°C. for 12 days. The fate of the various sugars was determined by analyses at 48-hr. intervals. The glucose content remained essentially constant for 48 hr. and then increased rapidly. Sucrose showed a small but definite decrease for the first 2 days of germination and then increased rapidly. These two sugars comprised the largest part of the sugars studied. Fructose remained low and essentially constant. Maltose, which did not appear until the third day of germination, increased slowly thereafter. Alpha-amylase did not appear until the sixth day, a time which coincided with the rapid increase in reducing sugars, and beta-amylase was not detected during the test period.

Work in this laboratory (3,4) has shown that when corn is brought to a moisture content of 30% a very marked decrease in nonreducing sugars occurs. In contrast to wheat, this decrease is detectable within 24 hr. and prior to an increase in fat acidity or mold count or a decrease in seed viability. It appeared likely that at least a portion of the observed changes was the result of carbohydrate metabolism by the seed, initiated by the high moisture content involved.

Taufel *et al.* (16) recently reported on the levels of the simple sugars (raffinose, sucrose, glucose, maltose, and fructose) in germinating corn. Sucrose and maltose, identified by paper chromatography, were found to increase markedly during germination, and fructose to a much lesser degree. Maltose, not present in the ungerminated corn, appeared during germination, but the level remained considerably lower than that of glucose. Raffinose disappeared completely during the early stages of germination.

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MacLeod and her co-workers have published extensively on their investigations of carbohydrate metabolism in germinating barley (10–14). They concluded that sucrose forms the principal respiratory substrate during the early stages of growth initiation. They observed very pronounced increases in sucrose and fructose after 4 days of germination and more gradual increases in glucose and maltose. Xylose, arabinose, and possibly galactose appeared in trace amounts. Raffinose disappeared abruptly upon exposure of the steeped grain to the atmosphere.

The free sugars of corn have been only partially characterized. Peat *et al.* (15) identified fructose, glucose and sucrose in sweet corn and tentatively established the presence of maltose and raffinose.

The work presented here was concerned primarily with a positive identification of the simple sugars and similar small-molecular-weight compounds of corn. A study of the changes in these compounds during carefully controlled germination was also made.

Materials and Methods

Corn. No. 1 grade (98% germination) yellow dent corn was used throughout these studies.

Germination. Fifty-gram lots of the corn were steeped for 48 hr. in water at 16°C. The water was changed every 12 hr. Final moisture content of the grain was 43%. The corn was then germinated at 16°C. in air at 100% r.h. in a revolving drum-type germinator. A 50-g. lot was removed every 48 hr. for analyses.

Reducing and Nonreducing Sugars. These were determined by the alkaline ferricyanide procedure (1). Alpha-amylase was determined by measurement of the dextrinization activity in the presence of excess beta-amylase (1).

Chromatography. Quantitative paper chromatography of the various sugars, carried out as described by Koch *et al.* (8) and Dubois *et al.* (5), gave excellent reproducibility on chromatograms, using the solvent system pyridine, ethyl acetate, water, 4:12:3 (v/v) on Whatman No. 1 paper. Quantitative paper chromatography was performed using the same system. The sugars were visualized by means of the method described by Linko *et al.* (9). This involved dipping the chromatogram into an aqueous silver nitrate-acetone mixture followed by drying and dipping into 0.5*N* sodium hydroxide in aqueous methanol. Black spots developed immediately.

Isolation of Compounds. Finely ground, ungerminated corn (600 g.) was extracted three times with 1,500 ml. boiling 70% aqueous ethanol. The extracts were combined, filtered, reduced under vacuum to 200

ml., decolorized with charcoal (Darco G-60), and deionized by passage through an Amberlite MB-3 resin column.

Four hundred milligrams of the syrup were dissolved in 5 ml. distilled water and applied to a column (1,100 × 20 mm.) of Dowex 50-W (200–400 mesh, Li^+ form) (7). With distilled water as an eluent, 30-min. (2-ml.) fractions were collected using an automatic fraction collector. The fractions containing single and identical components as determined by paper chromatography were pooled. In this manner, three components (I, II, and III) were obtained in chromatographically pure form and were subsequently crystallized from aqueous methanol.

Several slower-moving compounds emerged from the column in a mixture. These compounds were separated as follows. Twenty grams of the original syrup were diluted with 20 ml. distilled water and applied to a column (65 × 5 cm.) of activated coconut charcoal (Fisher Scientific Co., 50–100 mesh) prepared as described by Barth and Timell (2). Elution with 2.5% aqueous ethanol resulted in the emergence, as a mixture, of compound III plus three additional compounds IV, V, and VI. These were successfully resolved on a column (600 × 35 mm.) of Whatman No. 1 cellulose powder to which were applied 500 mg. of the mixture. Elution was with pyridine, ethyl acetate, water, 4:12:3 (v/v). Compound IV was crystallized from aqueous methanol.

Compounds I to IV gave positive Molisch and ammoniacal silver nitrate tests. Two additional compounds (V and VI) were obtained from the cellulose column which gave positive tests with ammoniacal silver nitrate but negative Molisch tests. These compounds were obtained in pure form by taking advantage of the observation that the mixed-bed resin Rexyn IRG-501 (H^+ and OH^- form) retained reducing sugars considerably more strongly than nonreducing sugars. Ten grams of the original syrup were applied to a column (550 × 35 mm.) of this resin. Elution was made with water and the material in those fractions which gave a positive test with ammoniacal silver nitrate but a negative Molisch test were pooled and the water removed under vacuum. Compound V crystallized readily from a mixture of V and VI in aqueous methanol. Compound VI was then obtained as a chromatographically pure viscous liquid by elution after chromatographing on Whatman 3MM paper using the solvent system pyridine-ethyl acetate-water as before.

An additional compound (VII) appeared after 4 days of germination. It migrated, on paper and column, very close to compound II and was partially obscured by the considerably larger amounts of the latter compound, later shown to be sucrose. Accordingly a portion of the original syrup was treated with invertase and compound VII

was eluted from a coconut charcoal column by 10% aqueous ethanol after other material had been washed out with 2.5% aqueous methanol. Compound VII was then crystallized from aqueous methanol.

Results

The compounds isolated as described were identified as follows:

Compound I: Raffinose. Crystallized from aqueous methanol, m.p and mixed m.p. 80°C. The raffinose hendecaacetate was prepared using sodium acetate and acetic anhydride, heating the mixture to 130°C. m.p.; mixed m.p. was 99°–101°C., $[\alpha]_D^{26}$ + 105.0 (C, 3.0, water), partially hydrolyzed in 0.1*N* sulfuric acid to give four compounds chromatographically identical to glucose, galactose, fructose, and melibiose.

Compound II: Sucrose. Crystallized from aqueous ethanol, m.p. and mixed m.p. 188°C., $[\alpha]_D^{26}$ + 66 (C, 5.0, water). The polymorphic sucrose octaacetate was prepared using sodium acetate and acetic anhydride, m.p. and mixed m.p. 89°C. Hydrolysis of the original compound with 0.1*N* sulfuric acid gave only two substances which were chromatographically identical to glucose and fructose respectively.

Compound III: Glucose. Crystallized from aqueous methanol, m.p. and mixed m.p. 146°C., $[\alpha]_D^{26}$ + 52.0 (equilibrium value, C, 5.0, water). Acetylated in pyridine-acetic anhydride to alpha-D-glucose pentaacetate, m.p. and mixed m.p. 114°C.

Compound IV: Fructose. Crystallized from aqueous methanol, m.p. and mixed m.p. 101°C., $[\alpha]_D^{26}$ − 94 (equilibrium value, C, 2.0, water). Reduction with sodium borohydride gave crystalline mannitol, m.p. and mixed m.p. 166°C. Treatment of the mannitol with sodium acetate and acetic anhydride gave mannitol hexaacetate, m.p. and mixed m.p. 123°–124°C. The anomeric sorbitol did not crystallize. It was acetylated by treatment of the reaction mixture supernatant with sodium acetate-acetic anhydride to give sorbitol hexaacetate, m.p. and mixed m.p. 98°–99°C.

Compound V: Myo-inositol. Crystallized from aqueous methanol, m.p. and mixed m.p. 225°C. Acetylation by treatment with sodium acetate-acetic anhydride gave the hexaacetate, m.p. and mixed m.p. 219°–220°C.

Compound VI: Glycerol. Identified by treatment with *p*-nitrobenzoyl chloride in pyridine to give glycerol tri-*p*-nitrobenzoate, m.p. and mixed m.p. 194°–195°C.

Compound VII: Maltose. Crystallized from aqueous methanol as the monohydrate of beta-maltose, m.p. and mixed m.p. 160°–165°C., $[\alpha]_D^{26}$ + 131 (equilibrium value, C, 2.0, water). Treatment with sodium

acetate-acetic anhydride gave beta-maltose octaacetate, m.p. and mixed m.p. 159°–160°C.

The changes observed in reducing and nonreducing sugars during the 14-day period are shown in Fig. 1. Reducing sugars, expressed as maltose, remained constant during the steep period, after which they rose rapidly to a final value 14 times that of the original. Nonreducing sugars, expressed as sucrose, decreased by 50% during the first 4 days, after which they rose to a final value twice that of the original.

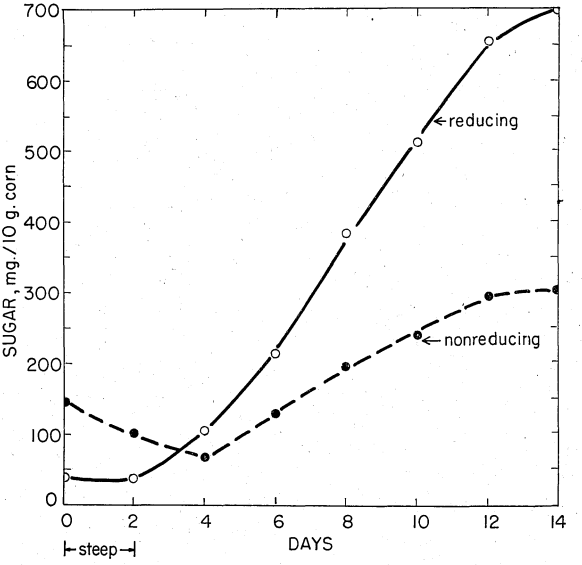

Fig. 1. Changes in reducing and nonreducing substances during steeping and germination of corn at 16°C.

The quantitative changes occurring in raffinose, sucrose, maltose, glucose, and fructose during a 2-day steep period and a 12-day germination period were observed, with the results shown in Fig. 2.

The most marked changes occurred in the sucrose and glucose contents. Sucrose decreased during the 2-day steep period and the first 2 days of germination. It increased rapidly thereafter to a final value four times that of the original. Glucose remained constant during the steep period and immediately began to rise to reach a final value almost as great as sucrose and 54 times that in the original corn.

Raffinose completely disappeared within 24 hr. after removal from the steepwater. Fructose, virtually absent originally, rose slightly and remained low and constant throughout. Maltose, absent initally,

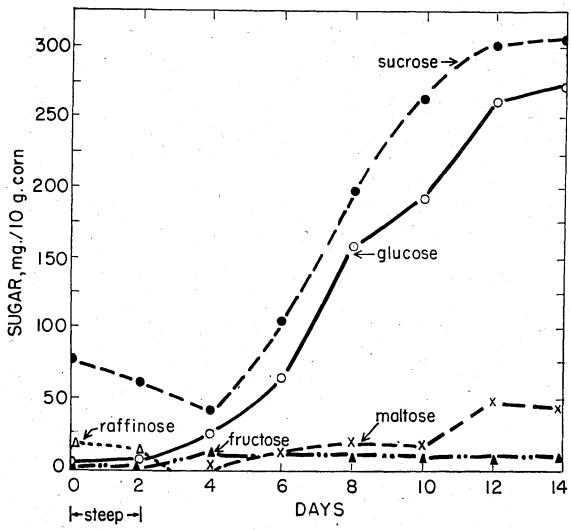

Fig. 2. Changes in individual sugars during the steeping and germination of corn at 16°C.

exhibited a somewhat greater rise, and its concentration fluctuated throughout the test period.

The apparent discrepancy between Figs. 1 and 2 in which much more reducing sugar is present than is represented by the total of glucose, maltose, and raffinose is, of course, the result of expressing the reducing sugars as maltose. For this work it would be much more meaningful to express reducing sugars as glucose.

Alpha-amylase appeared on the sixth day and rose to a final value of 192 maltose equivalents as shown below. Beta-amylase was absent throughout the period studied.

Alpha-Amylase Content of Germinating Corn

Days	*Alpha-amylase* maltose equiv.	*Days*	*Alpha-amylase* maltose equiv.
0	..	8	97
2	..	10	142
4	..	12	182
6	14	14	192

With relatively minor exceptions, the net result of carbohydrate metabolism in germinating corn was the synthesis of glucose and sucrose. However, a very dynamic system is present here, and the quantities of sugars observed give no indication of the quantities actually involved. A total of 4.7% of the dry weight of the corn dis-

appeared during the course of the 14-day trial period and if, as MacLeod suggests for barley, sucrose was the major respiratory substrate, this would mean that approximately 470 mg. sucrose per 10 g. of corn were utilized. These results are in good agreement with those of Taufel *et al.* (16). Edelman *et al.* (6) have presented evidence that sucrose is synthesized in the scutellum of cereal seedlings by a mechanism involving uridine diphosphoglucose. If this is the case with corn, then approximately 760 mg. of glucose per 10 g. corn was converted to sucrose during the germination period; glucose presumably arose from starch as a result of enzymatic hydrolysis.

The rapid disappearance of raffinose, also observed by MacLeod in barley, appears to be the result of a system, or systems, requiring oxygen. Preliminary experiments have shown that the addition of raffinose to an aqueous extract of germinated corn results in its disappearance, accompanied by the appearance of both sucrose and melibiose. A somewhat complicated system is thereby indicated for the metabolism of this oligosaccharide. In this connection trace amounts of a compound identified, by paper chromatography only, as galactose appeared during the period of raffinose disappearance. The amounts observed were very much less than would be expected from the quantity of raffinose involved.

Carbohydrate metabolism in germinating corn thus appears to resemble, in a general way, that of other cereals which have been studied. The precise nature and mechanisms of the gross changes observed remain to be established.

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