

Studies of Sugars and Sorbitol in Developing Corn Kernels¹

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ABSTRACT

Sugars and sorbitol were determined on corn (*Zea mays* L.) kernels harvested at various developmental stages, using *sugary* (*su*), *sugary-sugary enhancer* (*su se*), and *starchy* (*Su*) cultivars. In all cultivars tested, the sorbitol content increased from trace amounts in unpollinated ovules to a maximum at about the time that rapid starch synthesis was proceeding. Thereafter, sorbitol and sugars decreased continuously to the mature dry stage. Sorbitol in the *su se* kernels was higher than that of other cultivars from 28 days postpollination onwards; sucrose and maltose were higher from 21 days onwards. [¹⁴C]Sorbitol was recovered from kernel base, pedicel, and endosperm of IL677a (*su se*) kernels after allowing a flag leaf to fix ¹⁴CO₂ photosynthetically. No [¹⁴C]sorbitol was detected in the shank of the ear, and none was detected by the gas chromatograph. [¹⁴C]Sucrose was the predominant labeled substance recovered from the kernel base, pedicel, and endosperm tissues during the 10-h chase period, as well as from the shank of the ear, and nonradioactive sucrose was the predominant ethanol-soluble compound detected by the gas chromatograph. Hence, sorbitol appears not to be translocated from corn leaves as it is in certain woody plants of the rose family. The altered sugar profile of *su se* kernels may be related to reduced starch synthesis, but the biochemical mechanism is not yet known.

The *su*³ inbred, IL677a, has a recessive modifier (*se*) that causes increased sugar levels in developing seeds compared to other *su* varieties that lack *se* (9, 10, 13). Sucrose is approximately doubled, and maltose appears as the *se* seeds mature. IL677a kernels possess normal levels of phytyglycogen (8) in marked contrast to high sugar shrunken and brittle genotypes which lack phytyglycogen (3, 14).

Sorbitol is not widely recognized as a metabolite of corn, although there are reports that this compound is present in corn meal (24) and in immature seeds (20). Sorbitol was recently detected in mature dry kernels of IL677a which had higher levels of this hexitol than most other *su* varieties tested (5). However, the sorbitol content of IL677a was not different from other *su* varieties when kernels were analyzed at 21 d postpollination (6). The recent work with IL677a included a rigorous identification of sorbitol; the possibility that the corn compound was a hexitol other than sorbitol was ruled out (5).

The present study was undertaken to gain additional information about the *se* trait and the production of sorbitol in corn seeds. A survey was conducted to learn whether appreciable amounts of sorbitol occur in mature dry seeds of various starch deficient endosperm mutants and in starchy varieties. The pattern of sorbitol accumulation during seed development was also compared for several varieties to learn when *se* becomes different from the others. Additional *se* inbreds became available during the course of this work (19), so one of them (IL731a) was included in the developmental study. In addition, the ability of developing seeds to form sorbitol was assessed by use of photosynthetically fixed ¹⁴CO₂.

MATERIALS AND METHODS

Plant Material. The corn (*Zea mays* L.) seeds used in this study were all produced at Urbana, IL. Mature, dry seeds for the comparative study of *su*, *Su*, *sh*, and *sh2* genotypes were obtained from Dr. R. Lambert and Dr. A. M. Rhodes. These and other mature seeds were ground in a Wiley mill to pass a 20-mesh screen. Fresh ears were harvested for analysis at various developmental stages during the summer of 1981. Corn varieties were chosen to ensure diverse germplasm for this study. Included were two *su* lines (IL451b, Seneca Scout), two *su se* (IL677a, IL731a), and four *Su*. Two of the latter were dent corn (B73, B73XM017) and two were roasting ears bred for human consumption (TX17w and an inbred selection of TX17w). A hybrid of each genotype was included (except none of *su se* was available) to ensure production of adequate amounts of seed in case growing conditions were as poor as during the previous summer. Three self-pollinated ears of each variety were harvested weekly through 35 d postpollination. These ears were frozen immediately after harvest, and stored at -80°C until analysis. Mature dry ears were harvested also.

Extraction and Analysis of Sugars and Sorbitol. These procedures were patterned after those used earlier (9, 10). A sample of frozen kernels (5–6 g) was taken from each ear and extracted in a Sorvall Omni-Mixer with boiling 80% (v/v) ethanol. All *su* varieties were homogenized with 25 ml ethanol, and the homogenate was clarified by centrifugation at 17,000g. Each pellet was rinsed three times with 25-ml portions of 80% ethanol. Rinses and initial extract were combined, and the final volume was adjusted to 100 ml. Frozen samples from the *Su* varieties were extracted into a final volume of 50 ml. Samples of meal (3.0 g) from the dry seeds were extracted with 50 ml (*Su* lines) or 100 ml of 80% ethanol (all other lines). Duplicate samples of each ethanol extract were dried, and the TMS-oxime derivatives were prepared and subjected to GC as described earlier (9). One μ l samples were injected into the 3% OV-17 column with Helium carrier gas set at 20 cc/min and initial oven temperature at 160°C. After 2 min, the oven temperature was increased 1°C/min for 5 or 6 min and then 10°C/min to 275°C. Data were analyzed statistically using a completely randomized design, and least significant difference values were calculated for the 1% and 5% levels of significance. These LSD values are given with the

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³ Abbreviations: *su*, *sugary*; *se*, *sugary enhancer*; *Su*, *starchy*; *sh*, *shrunken*; *sh2*, *shrunken2*.

Table 1. Sorbitol and Sugars in Corn Seeds of Various Genotypes
 Mature dry seeds were used; values are % of dry weight.

Cultivar	Genotype	Sorbitol	Fructose	Glucose	Sucrose	Maltose
				%		
IL677a	<i>su se</i>	0.37	0.76	0.89	9.46	3.41
IL451b	<i>su Se</i>	0.05	0.11	0.71	3.01	0.04
OH43	<i>sh2</i>	0.02	0.10	0.16	3.84	0.23
Candyman	<i>sh2</i>	0.00	0.06	0.05	3.25	0.08
B37	<i>Su</i>	<0.01	0.09	0.07	1.46	0.03
MO17	<i>Su</i>	0.01	0.04	0.04	1.50	0.04
WF9	<i>Su</i>	0.01	0.05	0.05	1.30	0.04
WF9 × M14	<i>Su</i>	0.01	0.04	0.04	1.23	0.02
TX17w	<i>Su</i>	0.01	0.04	0.05	1.38	0.05
PA405	<i>Su</i>	<0.01	0.02	0.03	1.50	0.10
M14	<i>Su</i>	<0.01	0.04	0.04	1.37	0.03
B73	<i>Su</i>	0.00	<0.01	0.03	1.39	0.03
OH43	<i>sh</i>	<0.01	<0.01	0.01	1.58	0.01
OH43	<i>Su</i>	0.00	0.01	0.02	1.21	0.02
Hy2	<i>Su</i>	0.00	<0.01	0.02	1.19	0.04
1% LSD		0.014	0.031	0.052	0.449	0.161
5% LSD		0.017	0.037	0.063	0.538	0.193

results.

¹⁴CO₂ Study. ¹⁴CO₂ was given to the ear leaf of an IL677a plant 21 d after it was self-pollinated. NaH¹⁴CO₃ (0.35 mCi, 56 mCi/mmol) was injected into acid in a closed system consisting of a clear lucite box connected to a pump and a flask containing the acid (8 N H₃PO₄). The leaf was allowed to fix ¹⁴CO₂ for 29 min, and atmospheric CO₂ was fixed thereafter. One-fourth of the ear was removed immediately and frozen. The remaining three quarters were removed and frozen 2, 5, and 10 h after the end of the pulse of ¹⁴CO₂. The shank connecting the ear to the plant was also removed at 10 h, and all were lyophilized after making slits in the tops of the kernels to facilitate drying (22). Nine to 11 dry kernels were removed from each of the four sections of the ear, and they were dissected into kernel base, pedicel, and endosperm (22). Similar plant parts were combined to give a sample representing each section of the ear. These samples and the shank were extracted as described earlier, except that the volume of ethanol was reduced to accommodate the smaller amounts of tissue, and tissue extraction was done with a Brinkmann Polytron homogenizer operated at top speed. Radioactive sugars were separated from each other and from hexitols on Whatman No. 1 paper developed descendingly with 2-butanone:glacial acetic acid:water saturated with boric acid (9:1:1, v/v). Standards were placed on both sides of the tracks (6.4 mm wide) that contained radioactive extracts. After development (50 h), the standards were cut away from the radioactive tracks and visualized with AgNO₃ reagent. The tracks were passed through a radiochromatogram scanner, but the level of radioactive sorbitol was too low to be detected. Therefore, the tracks were cut into pieces, placed in scintillation vials with 10 ml Permafluor V (Packard Co.) and counted at 71% efficiency. The section near the origin (first 4 or 5 cm) was cut into 0.5-cm pieces, and the remainder was cut into 1-cm pieces.

The paper chromatography solvent system was chosen for its ability to separate sorbitol from sugars and other hexitols. The mobilities of mannitol and galactitol relative to sorbitol were 0.87 and 0.77, respectively. Consequently, sorbitol appeared as a separate spot when it was cochromatographed with these hexitols. After 50 h of development, the standards and their approximate distances from the origin (cm) were as follows: sorbitol (28), fructose (17), glucose (10), sucrose (3), and maltose (2). The solvent used to separate labeled sugars extracted from

Su corn kernels (butanol:acetic acid:water, 3:1:1; Ref. 21) was not satisfactory because sorbitol migrated midway between glucose and fructose and was not completely separated from either.

RESULTS

The diverse group of starchy and the several shrunken varieties tested all had significantly lower levels of sorbitol and sugars than did IL677a; in several cases, no sorbitol was detected (Table 1). This result emphasizes the uniqueness of IL677a and explains why sorbitol is not commonly recognized as a constituent of mature corn seeds. As expected (5, 9), IL677a contained significantly higher levels of the various sugars and sorbitol than did IL451b, the ordinary *su* variety included as a control. At the outset of this study, the temperature program of the gas chromatograph was modified from that used in earlier work (5, 9). After various trials, a 1°C/min temperature gradient was combined with a 10°C/min gradient in order to obtain an improved separation of sorbitol from fructose but still elute disaccharides in a reasonable time.

The study of maturing kernels revealed some distinctive developmental patterns concerning the accumulation of sorbitol and sugars (Fig. 1). Sorbitol and sucrose both increased from trace amounts in unpollinated ovules to maximum levels at approximately 21 to 28 d postpollination. Then both compounds declined until seeds were mature and dry (Fig. 1, A and B). Kernels of the ordinary *su* and the *Su* lines possessed similar sorbitol levels throughout development, but the two *su se* lines (IL677a and IL731a) had more sorbitol at 28 d than did the others. This difference persisted in the later stages of development. The sucrose content of the two *su se* lines became different from the other *su* lines at 21 d, and this difference also persisted. The maltose level in the two *su se* lines also became higher than that in the others from 21 d onwards (Fig. 1C). Maltose was not detected in starchy lines at 21 d; the two ordinary *su* averaged 0.014 mg and the *se* 0.15 mg/kernel at that time. This difference was caused by a continuous accumulation of maltose that occurred only in *su se* (Fig. 1C). The data in Figure 1 were expressed on a per kernel basis, a constant unit unlike dry weight which changes rapidly during kernel development. Expressing the data as a percentage of dry weight would not alter the trends noted above, because kernels of the various *su* lines had similar dry weights (23). However, sugars and sorbitol would appear to drop

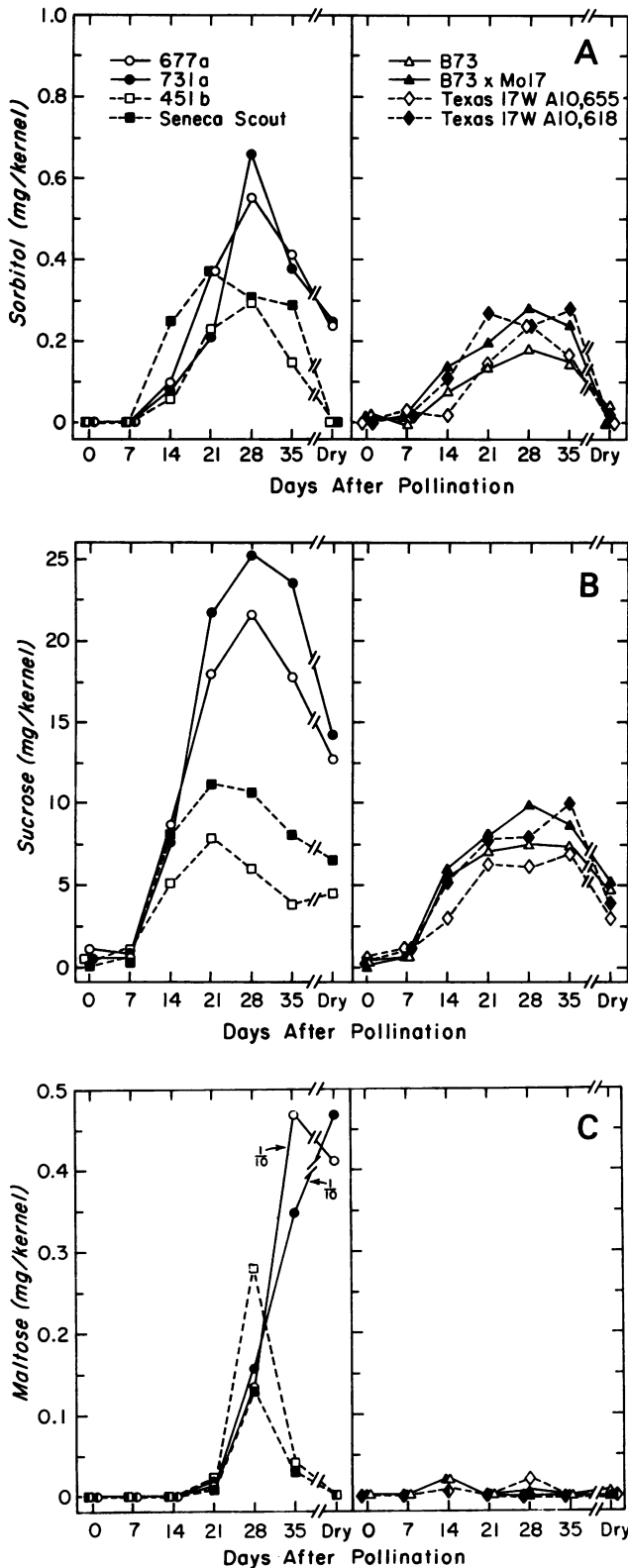


FIG. 1. Sorbitol, sucrose, and maltose in developing kernels of several corn varieties. IL677a and IL731a are *se* inbreds. IL451b and Seneca Scout are *su* inbred and hybrid, respectively. All other varieties are *Su* inbreds or hybrids. Each point is an average from four gas chromatograph injections. The values at zero d postpollination are from extracts of unpollinated ovules. A, Sorbitol (above). The 5% and 1% LSD values, respectively, for the various developmental stages are as follows: 14 d, 0.06 and 0.08; 21 d, 0.06 and 0.08; 28 d, 0.06 and 0.08; 35 d, 0.05 and

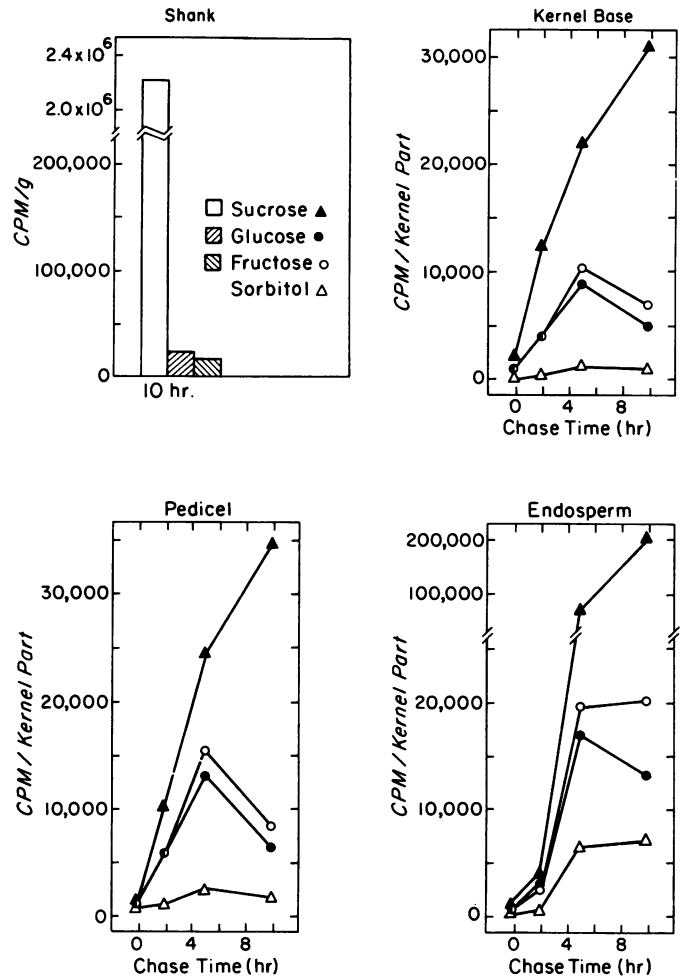


FIG. 2. Radioactive sugars and sorbitol in various parts of an IL677a ear. Elapsed time refers to the duration of the chase period after administration of ¹⁴CO₂ to the ear leaf.

more rapidly in maturing *Su* kernels due to their greater dry weight.

A radioactive compound corresponding to the sorbitol standard was detected when extracts of 21-d kernels were chromatographed after the ear leaf was allowed to fix ¹⁴CO₂ for 20 min and portions of the ear harvested 2, 5, and 10 h later. Radioactive sucrose, glucose, and fructose were present in all parts of the kernel in addition to a smaller amount of labeled sorbitol. The possibility that this radioactive substance was galactitol or mannitol was ruled out by their different chromatographic mobility (see "Materials and Methods"). Its identity was confirmed using extracts from the 10-h chase period. Chromatograms were developed in 2-butanone:acetic acid:water (9:1:1, v/v), and appropriate portions of these chromatograms were eluted with water. The combined eluates gave a single radioactive peak that corresponded to sorbitol upon being chromatographed in the same solvent and in a basic solvent (*n*-butanol:pyridine:water, 6:4:3, v/v).

0.07; dry, 0.06 and 0.09. B, Sucrose (middle). The 5% and 1% LSD values are as follows: ovules, 0.20 and 0.27; 7 d, 0.23 and 0.31; 14 d, 1.2 and 1.6; 21 d, 1.4 and 1.9; 28 d, 2.4 and 3.3; 35 d, 1.4 and 1.9; dry, 1.3 and 1.8. C, Maltose (below). Maltose values for the *su se* inbreds were all divided by 10 before being graphed. The 5% and 1% LSD values are as follows: 21 d, 0.08 and 0.11; 28 d, 0.32 and 0.43; 35 d, 0.18 and 0.25; dry, 1.0 and 1.4.

Table II. Sorbitol and Sugars in Labeled IL677a Kernels

Kernels were analyzed by GC, using the same ethanol extracts as used to determine radioactivity. Values for kernels are averages for the 5- and 10-h chase periods. The shank was analyzed at 10 h only.

Plant Part	Dry Wt	Sorbitol	Fructose	Glucose	Sucrose	Maltose
	mg/part			%		
Endosperm	67.0	0.58	1.3	0.7	18.4	0.08
Pedicle	4.7	0.84	5.2	4.4	26.8	0.00
Base of kernel	5.0	0.35	3.2	2.3	25.8	0.00
Shank ^a	21.4	0.00	3.0	4.4	19.5	0.00

^a Weight of the shank is given in grams.

No radioactive sorbitol was found in the shank, in spite of an abundance of labeled fructose, glucose, and especially sucrose (Fig. 2). In all the kernel tissues tested, labeled sorbitol increased slowly during the first 2 h of the chase, more rapidly from 2 to 5 h, and then declined or else increased very slowly from 5 to 10 h (Fig. 2). This pattern closely resembles that observed for the labeled hexoses, so it is possible that one of the labeled hexoses acts as a sorbitol precursor. Labeled sucrose increased continuously in all parts of the kernels during the 10-h chase period and was the predominant labeled sugar at all times except immediately after the end of the pulse of ¹⁴CO₂.

Sorbitol and sugars were determined on the radioactive extracts representing the 5- and 10-h chase periods to make sure that the endosperm sugar profile was that of *se* and to learn whether sorbitol was present in tissues other than the endosperm. The endosperm did have typical *se* sugar levels, and sorbitol was present in the pedicel and kernel base (Table II). No sorbitol was detected in the shank, however, and sucrose was the predominant compound in all tissues analyzed. The 5- and 10-h values for the various kernel parts were averaged before being tabulated since, as expected, they were practically identical.

DISCUSSION

The initial increase of sorbitol in immature kernels and the subsequent decrease that accompanied maturation indicates that it is a metabolically active compound, but the metabolic role of sorbitol in the corn plant is not yet clear. Sorbitol is a translocated form of carbon in some plants of the rose family, being produced in chloroplasts with hexose phosphate as the likely precursor (2, 7). However, sucrose is reported to be the form in which carbon is translocated from leaf to ear of the corn plant (21, 22). The present study agrees with published work, since [¹⁴C]sucrose was the predominant labeled compound in stem tissue after ¹⁴CO₂ fixation by the leaf, and no labeled sorbitol was detected in the shank of the ear. Furthermore, no sorbitol was detected in the shank upon analysis with the gas chromatograph, and none was found earlier in photosynthesizing corn leaves (5). An NAD-specific enzyme from leaves of rosaceous plants converts glucose-6-P into sorbitol-6-P, and an NAD-specific enzyme from non-photosynthetic apple tissue produces fructose from sorbitol (2, 15, 16). There is no information yet whether these enzymes occur in corn, or how sorbitol production and utilization fit into the overall conversion of sucrose to reserve materials in the developing seeds. Nor is the role of sorbitol known for barley seeds or the liquid endosperm of coconut, where its presence has been reported (11, 12, 17). It is possible that the sorbitol observed in the endosperm and pedicel tissues was actually produced in the kernel base, but just as likely that hexitol production occurred within the cells of all three kernel tissues examined. Possible functions for sorbitol in the corn plant include intracellular carbon transport across a membrane such as that of the amyloplast or the temporary storage of carbon. Reports that sorbitol is a cryoprotectant in apple (18) and an osmoregulant in a salt marsh plant (1) seem irrelevant to the situation in corn.

A successful transfer of *se* from IL677a to IL731a was indicated by the similar sugar profiles of these two *su* inbreds, and the observation that they differed in several respects from the other *su* lines tested. Less starch accumulates in the kernels of IL677a than in those of ordinary *su* corn (8, 13), but it is not clear why this reduction occurs or how it might be related to increased sorbitol, sucrose, and maltose. Correlation coefficients for sorbitol and the various sugars in the developing kernels were calculated for each genotype over all stages of development (23), and the only coefficient significant for all genotypes was that between sorbitol and sucrose ($r = 0.74$ to 0.97). This result does not necessarily indicate a close metabolic relation between these two compounds, since independent factors that increase the level of one could increase the other. A survey of mature, dry *se* seed (4) showed that some seed lots did not have more sorbitol than *su*, so environmental factors may affect the rate and extent of decline in level of this compound during maturation. The lack of labeled maltose in IL677a endosperm at a time when sucrose is high and maltose is beginning to increase indicates that this compound is not an immediate product of the labeled sugars arriving at the endosperm. Phytoglycogen and starch would require some time to become highly radioactive, so production of appreciable labeled maltose from these substances might require a longer chase period or more ¹⁴CO₂ than was used here.

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