

Communication

Changes in Soluble Carbohydrates during Seed Storage¹

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ABSTRACT

The soluble sugars present in the maize (*Zea mays* L.) embryo may serve as important components of protection or may contribute to the deteriorative changes occurring during seed storage. Examination of the changes in sugars during accelerated aging of maize seeds indicates that the decline in vigor is associated with a marked decline in monosaccharides and in raffinose. Sucrose content remains relatively stable. The depletion of raffinose may have special relevance to the decline in seed vigor.

The decline of seed quality during storage is expressed first as a decrease in the growth rate of the germinating axis (vigor) and subsequently as a loss of actual germinability. Changes in the soluble carbohydrate contents could contribute to both the declines of vigor and of germinability of the seeds. It is known that soluble carbohydrates generally decline with seed aging (15), and this decline might result in limited availability of respiratory substrates for germination (9). Another possibility is that depletions of disaccharides may lessen the protective effects of sugars on structural integrity of membranes (7) or may limit the ability of the seeds to maintain the vitrified state, a noncrystalline liquid state of high viscosity (3, 20). Finally, the presence of reducing sugars may lead to deterioration of protein components through Amadori and Maillard reactions. These are nonenzymatic carbonyl-amine reactions that take place preferentially in dry systems (11). They may contribute to seed deterioration (19).

In quiescent seeds, the main soluble embryonic carbohydrate reserves are sucrose, usually associated with lesser amounts of the oligosaccharides, raffinose, stachyose, and/or verbascose (1). Sucrose is exceptionally effective in protecting membrane integrity in dry systems (6), as well as being one of the best vitrifying sugars (12). Raffinose is known to enhance the protective effects of sucrose by limiting crystallization (5).

Interconversion of sugars may occur in dry seeds. It has been found that α -galactosidase activity was detectable in dry seeds of cotton (16). Invertase activity is also present in dry seeds, but its activity is at least 5 to 10 times less than that

shown by α -galactosidase. These facts reinforce the expectation that changes in sugars may occur during seed storage.

We have carried out experiments on the sugar contents of maize seeds as a function of accelerated aging, with the intent to examine the possible relation of carbohydrate changes to the deterioration of vigor, and then to the loss of germinability.

MATERIALS AND METHODS

Plant Material

Maize seeds (*Zea mays* L.) NYCO113XNYPA33 (C2) were obtained from the New York Seed Improvement Cooperative, Ithaca, NY. Before storage, the seeds were surface-sterilized with 3.5% sodium hypochlorite twice, for 3 min, and each time, they were rinsed with sterilized deionized water and blotted dry. All manipulations were done aseptically. The seeds were then placed in storage at 30°C and 75% RH, using a saturated NaCl solution to buffer the humidity. The term water activity is used to refer to a material that has been equilibrated in an atmosphere at a given proportion of saturation. Thus, these seeds were at a water activity of 0.75 during the storage treatment.

Germination and Vigor

Seeds were germinated in wet paper rolls (Anchor Paper Company, St. Paul, MN) in the dark at 25°C. Vigor was expressed as the length of the axis (coleoptile plus radicle) measured after 5 d of germination.

Sugar Determinations

After various periods of accelerated aging, maize embryos were excised, and three replicate samples of 75 to 100 mg were homogenized with a Polytron in 75% ethanol containing melezitose as internal standard. The homogenate was allowed to steep for 30 min, centrifuged at 5000g for 5 min at room temperature, and the pellet was washed with 75% ethanol and centrifuged again. The supernatants were pooled and dried under reduced pressure. The dry residue was resuspended in water and filtered through a column consisting of PVPP and a mixed bed of ion-exchange resins (2). The eluent was divided into two aliquots before lyophilization. One dried aliquot was dissolved in a mixture of 75% acetonitrile and 25% water, filtered through a 45 μ m pore size nylon filter, and analyzed by HPLC according to Koster and Leopold (13).

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The other dry aliquot was dissolved in 0.5 mL of *N*-trimethylsilylimidazole (Supelco) to form the trimethylsilyl-derivatives of sugars, and analyzed by GC. Derivatized samples were injected into a Hewlett-Packard 5890A gas chromatograph equipped with an Alltech SE-54 capillary column and flame-ionization detector. Chromatographic conditions were temperature programmed from 100 to 200°C at 8°C/min. Injection port and detector temperatures were set at 150 and 100°C, respectively. Response factors were determined utilizing a Hewlett-Packard model 3308S integrator. Standards of D-fructose, D-galactose, and D-glucose were derivatized as described above and analyzed to determine response factors, which were linear over the concentration ranges evaluated.

RESULTS

Seeds of maize were given an accelerated aging treatment, at 30°C and 75% RH, to follow the decline in vigor with time and its possible relationship to changes in the soluble sugars. As shown in Table I, the vigor of germination declined steadily during storage under these conditions, and the germinability declined abruptly after 90 d.

Measurements of the soluble carbohydrate contents of the seed embryos over the same period of time are shown in Figures 1 and 2. The predominant sugar was sucrose, representing about 140 mg/g dry weight of the axis. During the aging period, the sucrose level held fairly constant for about 35 d (Fig. 1), but then declined to 100 mg/g at 90 d. Raffinose was the other major soluble sugar, and it was largely depleted after 50 d of aging. The monosaccharides fructose, glucose, and galactose were present in very small amounts (less than 3 mg/g), and declined to less than 1.5 mg/g within 30 d of aging (Fig. 2).

The incremental decline in vigor, as measured by the relative growth of the coleoptile and radicle (Table I), was loosely associated with a decline in raffinose content (Fig. 1). At 90 d, there was a further decline in vigor, and a decrease in germinability; at this point, raffinose content was fairly depleted (Fig. 3).

DISCUSSION

The passage of time under conditions of slightly accelerated aging causes corn seeds first to lose vigor, such that less growth is achieved during germination. Loss of germinability occurs at a later period of storage. Our experiments were undertaken to determine whether the declines of vigor and germinability were correlated with changes in substrates readily accessible in the embryos: specifically, the soluble carbohydrates.

The decline in vigor was associated with declines in several soluble carbohydrates. The very small amounts of monosaccharides initially present in the embryo were largely depleted in the first 30 d. The largest sugar component, sucrose, remained constant through about 35 d, then showed subsequent decline. The raffinose content declined steadily during the aging treatment. The pattern of raffinose decline had some similarity to the decline in vigor (Fig. 3).

It is probable that during storage, the raffinose was being hydrolyzed by α -galactosidase. This enzyme is present in dry corn seeds (I. Bernal-Lugo, A.C. Leopold, unpublished data),

Table I. Effect of Accelerated Aging on Germination and Vigor of Corn Seeds

Time of Storage at 35°C, 75% RH	Germination ^a	Vigor ^b
d	%	cm
0	95.00 ± 5.00	16.59 ± 0.58
10	96.60 ± 5.80	14.43 ± 0.75
35	95.00 ± 5.00	11.90 ± 0.66
50	95.00 ± 8.70	9.69 ± 0.58
90	74.00 ± 1.00	4.02 ± 0.84

^a Germination at 72 h in three different replicates. ^b Length of seedlings; differences are significant at 0.01 level.

and is active at water activities as low as 0.50 (8), well below the water activity at which the seeds were stored ($A_w = 0.75$. Note that water activity, A_w , is the equilibrated RH $\times 10^{-2}$.) If the rapid disappearance of raffinose illustrated in Figure 1 was due to hydrolysis by α -galactosidase, there should have been a substantial increase in galactose and sucrose, or an increase in galactose, glucose, and fructose. The nonaccumulation of galactose and the disappearance of each of the other simple sugars could possibly be accounted for by their participation in the Amadori reaction, and its successor, the Maillard reaction (11). These protein-degrading reactions are known to occur in seeds (19). Galactose is among the most reactive of the hexose sugars with regard to the Amadori reaction. These nonenzymatic reactions would be expected to result in lowered activity of enzymic components of the embryo.

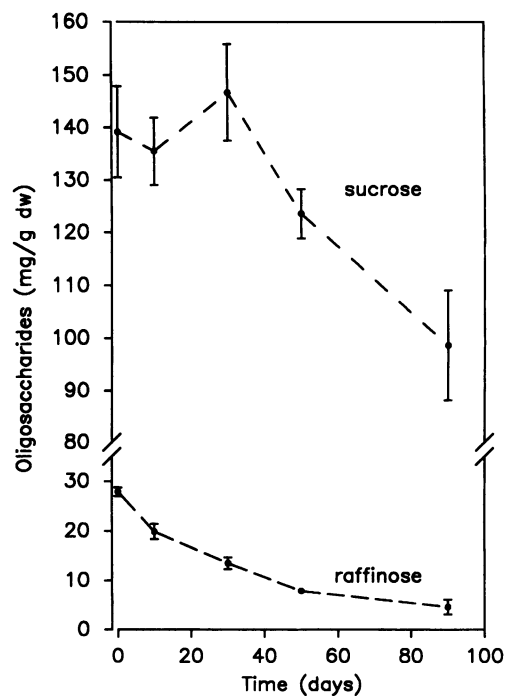


Figure 1. The sucrose and raffinose contents of corn embryos during storage for various times at 30°C and 75% RH.

Of the various sugars in the corn embryo, the disappearance of raffinose is the most closely associated with the decline in subsequent vigor of the germinating seed (Fig. 3). It is possible for raffinose to participate in the Maillard reaction (10), but the glucosidic bond active in this reaction would be the α -1,2, which would lead to the formation of cellobiose. Our assays did not detect any cellobiose, and consequently it appears that raffinose was probably not itself participating in the Maillard reaction.

The decline in raffinose would be expected to lead to a loss of desiccation tolerance, according to the proposal of Leopold and Vertucci (14) and confirmed by Caffrey *et al.* (5) that raffinose can protect sucrose from crystallization in the dry state, and thus maintain the protective effects of the sucrose on membranes.

A decline in sucrose concentration occurs later than the decrease in raffinose. The apparent maintenance of sucrose levels during the first 30 d of storage may be a consequence of a balance between a loss of sucrose due to hydrolysis by invertase, and the formation of sucrose due to the hydrolysis of raffinose.

The changes in carbohydrates in the corn embryo, as recorded here, may be presumed to be occurring without respiratory metabolism (4), because maize seeds below about 20% water content (dry weight basis) do not show measurable oxygen uptake (18). Equilibration of the seeds to a water potential of 0.75 should bring the embryo up to a water content of about 16% (dry weight basis, [5]). At this moisture content, invertase (β -fructosidase) may be functioning in corn embryos, for Silver and Karel (17) have shown that invertase can hydrolyze sucrose at water potentials as low as 0.58.

Our data are less instructive about factors concerned with losses of germinability because only the end of the experimental aging period was associated with such loss. The one sampling time at which germination was altered was associated

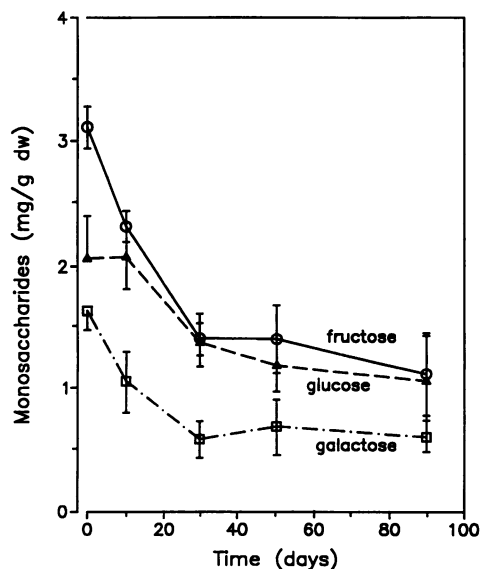


Figure 2. Monosaccharide contents in the corn embryos when stored for various periods as in Figure 1.

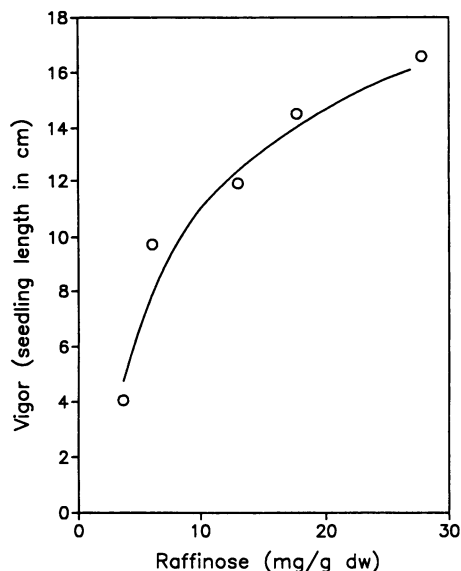


Figure 3. An apparent relationship between the vigor of seedling growth and the raffinose content of the embryos consequent to storage of corn seeds for five periods at 30°C and 75% RH.

with a considerable loss of sucrose, which could have limited the ability of the cell cytoplasm to become vitrified, but from the phase diagram of corn embryo vitrification provided by Williams and Leopold (20), the embryo would not have been vitrified at the 75% RH even at the start of the aging treatment. The resulting 16% water content would have restricted vitrification to temperature substantially below freezing.

In conclusion, we have shown that the decline in vigor of maize seeds under conditions of slightly accelerated aging may be related to the decline of content of several sugars in the embryo, and in particular, a decline in raffinose. The raffinose decline may be interpreted as leading to the hydrolytic formation of galactose, and that, in addition to the other reducing sugars, may be abetting the Amadori and Maillard reactions, by which enzymic effectiveness of the seed may be depressed.

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