Studies on the Permeability of Sugarbeet Tissue to Stored Sugar[1]

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Received for publication October 26, 1971

Introduction

In the sugarbeet (*Beta vulgaris* L.), sucrose accumulates in the root in concentrations up to 22% of the fresh weight (7), or even higher in some experimental varieties (E. L. Swift, personal communication). In some localities, beets are commonly stored for several months before processing and during this period various degenerative processes may occur (5, 6, 8).

In experiments in which permeability of beet tissue to the stored sugar was determined a wide range of values occurred. The possibilities were considered that permeability might be related to degeneration of beets in storage, the capabilities of beets to store well, or the ability of the beet to accumulate sugar. Before experiments testing these possibilities could be performed it was necessary to define the factors affecting permeability so that they could be suitably controlled. The present experiments describe the characteristics of diffusion from beet root tissue, the variability encountered, and the effects of environmental factors.

Some workers (1, 4) have studied the accumulation of sugars in small concentrations in plant tissue, but no work is available concerning sugar movement from cells which contain large sugar concentrations such as is normally present in mature beets.

Materials and Methods

Most of the sugarbeets used in these experiments were commercial cultivars grown on experiment station land and stored at 3 to 5°C in wood shavings moistened with a solution of Hyamine 3500[3]. Cultivars used were American 3S, American 3 Hybrid "A", American 2 Hybrid "B", American 3 Hybrid "Γ", and American 3 Hybrid "N" (American Crystal Sugar Company), IS 93 (Betaseed Inc.) and Zwaanpoly (Zwaanesse Inc.). Also used

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were fodder beets (Fort Collins lines 65-9702 and 67-9094) and mother beets (obtained from the American Crystal Sugar Company at East Grank Forks, Minn.). All beets used appeared healthy and firm.

A core 8.5 or 10 mm in diameter was removed from a slice of beet root by a cork borer and discs about 3 mm thick were cut, weighed and measured. The size of the discs was uniform for a particular experiment. Except in the short-term experiments, each disc was washed for 5.0 minutes in distilled water and placed in 100 to 250 ml of the experimental solution. Standard phosphate solutions were equimolar mixtures of KH_2PO_4 and K_2HPO_4. The pH was adjusted by varying the ratio of the two salts. When NaCl was used, the pH at the end of the experiment was 6.0 to 6.3. When other salts were used the pH was adjusted to 6.5 with HCl or KOH. Unless specified, solutions were at room temperature (24-26°C). In the temperature studies, the flasks containing the solutions and beet discs were placed in water baths at temperatures from 25 to 65°C. The series 25, 35, 45, and 55°C showed the relationships most satisfactorily.

The sugar which had diffused into the solution was determined from a 1-ml aliquot by the phenol-sulfuric acid method (2). When total sugar was determined, the discs were boiled for 30 min after the diffusion determinations and the extract analyzed for sugar. The sugar originally present in the beet tissue was the sum of the total sugar diffused from the tissue plus the extracted sugar.

Sugar concentrations determined by the phenol-sulfuric method agreed with determinations by polarimetry within the experimental error (± 3%); thus interference by non-sugars was nonsignificant. Because sugars other than sucrose are present in low concentrations in sugarbeet and react in the phenol-sulfuric acid method, the term sugar is used rather than sucrose. However, the effect of these other sugars is small. Glucose, fructose, and raffinose occur in measurable quantities in fresh beets and kestoses can also accumulate during storage (7). These sugars together generally comprise less than 1% of the dry weight but glucose can accumulate to over 1% if the temperature is high (6). Raffinose and kestose each can accumulate to over 2% under prolonged storage at temperatures slightly above freezing although they are usually much less (unpublished data). In the phenol-sulfuric reaction, glucose and fructose produce slightly higher absorbances and raffinose a slightly lower absorbance than sucrose (2). Thus the error due to non-sucrose sugars would usually be less than the experimental error also.

Results

Short-term experiments. When discs of sugarbeet tissue were placed in distilled water or salt solutions, the rate of loss of sugar from the discs decreased from a high initial rate during the first minute to a rate which was relatively constant after 5 to 10 minutes (Figure 1). Data from 54 trials containing bets of several commercial varieties and with media of distilled water, 0.1 and 0.2 molar potassium phosphate solutions and 0.1 molar KCl, were pooled because the different treatments did not produce consistently different effects during this short period.

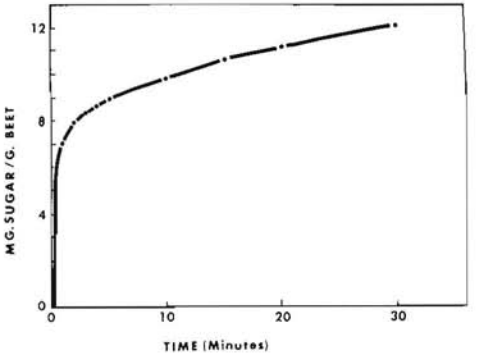

Figure 1. Loss of sugar from unwashed discs of sugarbeet root tissue showing time needed to remove sugar from cut and damaged cells. Average of 54 trials in salt solutions from 0 to 0.2 M.

Sugar from the cut and damaged cells of the surfaces of the discs can account for the rapid initial loss. Estimates of the sugar contained in the layer of injured cells agreed roughly with the amount of sugar accumulated in the solution during the first 5 minutes. Therefore, in the subsequent experiments, the discs were placed in distilled water for 5.0 minutes before being subjected to the experimental conditions.

Diffusion in distilled water. In distilled water, the rate of sugar loss decreased with time (Figure 2). The amount of sugar lost after 6 hr and the variability occurring among and within beets are given in Table 1. The coefficient of variation (CV) among beets of the same variety and cultural conditions varied from 55 to 98% and among discs from the same beet, from 31 to 35%.

The mother beets, which were selections for high sugar content, had a very high rate of sugar loss relative to the commercial beets. The fodder beets, which were inherently low in sugar, had a relatively low rate of loss. The low rate of sugar loss for the fodder beets was confirmed in later experiments.

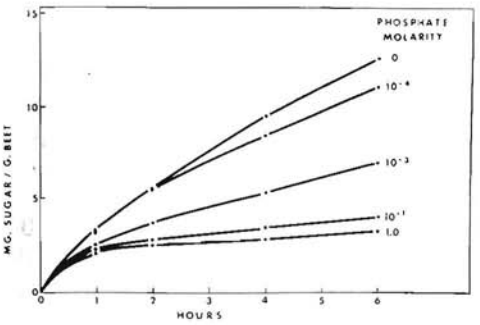

Figure 2. Diffusion of sugar from washed discs of sugarbeet root tissue at various phosphate concentrations.

Table 1.—Sugar losses from excised sugarbeet root tissue in distilled water after 6 hr. Group A is mother beets (sucrose content, 17 to 19%). Group B is commercial cultivars stored after harvest (sucrose content, 10 to 16%). Group C is fresh, immature beets (sucrose content, 7 to 13%). Group D is two cultivars of fodder beets (sucrose content, 6 to 7%).

Group	No. of beets	No. of samples	Mean	Mean square (beets)	Mean square (error)	Coefficient of variation among beets	Coefficient of variation within beets
			mg Sug/g beet				
A	8	29	41.3	517.2	168.3	55	31
B	40	223	10.0	95.4	9.73	98	31
C	9	24	11.4	88.4	16.2	82	35
D	2	6	4.8	3.18	1.67	67	35

Table 2 gives the analysis of variance for the loss of sugar after 6 hr from experiments in which 2 to 12 discs were cut from a single core and placed in distilled water. The CV among beets was 54%, within beets, 35% and within cores, 16%.

Table 2.—Analysis of variance for sugar losses after 6 hr from discs from cores of sugarbeet tissue (American 3 Hybrid "T"). Mean sugar loss was 8.43 mg sugar/g beet.

Source	DF	MS	CV	F
Beets	15	20.73	54	2.40
Cores	22	16.87	49	
Experimental Error (within beets)	7	8.62	35	4.59*
Sampling Error (within cores)	84	1.88	16	

* Significant at the 5% level.

Table 3 shows the analysis of variance for samples and sub samples from three radial locations of the beet. Two adjacent discs were cut from cores taken from the inner, middle, and outer portions of beets. Differences among the samples from the various locations were significant at the 5% level. Mean values for the inner, middle, and outer cores were, respectively, 14.8, 12.7, and

8.5 mg sugar per g of beet and 12.0 mg per g for all samples. The CV of the 2 adjacent discs was 8%.

Table 3.—Analysis of variance of amounts of loss of sugar after 5 hr from discs of beet tissue taken from cores at 3 locations within beets (American 3 Hybrid "N"). Inner location was ¼ distance from center to exterior of beet; middle location was ½ the distance; and the outer location was ¾ the distance. Two adjacent discs were used from each core. Mean sugar loss was 12.0 mg sugar/g beet. Sugar content of beets was 12 to 15%.

Source	DF	MS	CV	F
Location within beets	2	291.04	142	11.25**
Among beets	13	181.09	112	
Beets x Location	26	25.88	42	28.98**
Sampling Error (with cores)	42	.893	8	

** Significant at the 1% level.

Salt concentrations. Loss of sugar from beet discs in several phosphate concentrations is shown in Figure 2. Each point represents the mean of 11 samples from different beets. Similar results were obtained in NaCl, $MgSO_4$, KCl, and H_3BO_3. Extrapolation of the linear portion of the curves for 10^{-3} to 1 M concentrations in Figure 2 to zero time gives about 2.2 mg sugar per g beet for sugar which diffused in the first stage. This amount is about 13% of the sugar originally present in the beet.

Figure 3 shows the sugar loss after 6 hr in phosphate solutions up to 1.0 M phosphate. The mother beets (upper curve of Figure 3) showed consistently higher rates of diffusion than the commercial cultivars (lower curve). The sugar loss after 6 hr was progressively less with increasing salt concentrations up to about 0.2 M. At higher concentrations, sugar loss was highly variable among beets. Measurements of weight loss or gain by discs placed in various salt solutions showed that a solution of 0.2 M was approximately isotonic to sugarbeet root tissue.

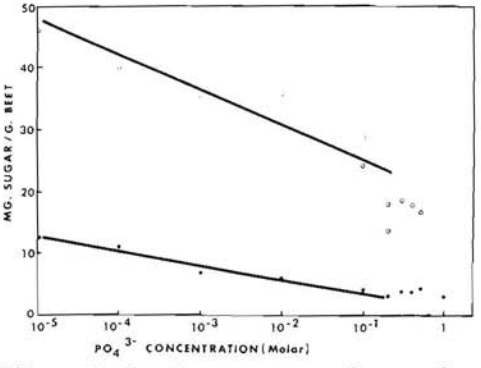

Figure 3. Effect of phosphate concentration on loss of sugar from washed discs of sugarbeet root tissue after 6 hr o Mother beets; • American 3 Hybrid "N".

pH. When discs of beet tissue were placed in phosphate-buffer solutions of various pH's, the rate of diffusion of sugar was least at 6.5. Figure 4 shows the amount of diffusion after 6 hr for 3 groups of beets: four American Crystal cultivars, which showed similar diffusion characteristics (American 2 Hybrid "B", American 3 Hybrid "N", American 3 Hybrid "T", and American 3S), IS 93, and Zwaanpoly. The first group of beets exhibited definite and large variations in diffusion rates with extremes in pH; cultivar IS 93 exhibited a slight variation; and Zwaanpoly displayed only a slight increase in diffusion rate at high pH. At the extremes of pH, the CV of sugar loss after 6 hr was about 50% higher than for the values near 6.5. Figure 5 shows diffusion with respect to time at pH 4.5, 5.5, 6.5 and 9.0 for the American Crystal cultivars. These data show that the higher amounts of sugar loss at 6 hr in Figure 4 resulted from higher diffusion rates through-

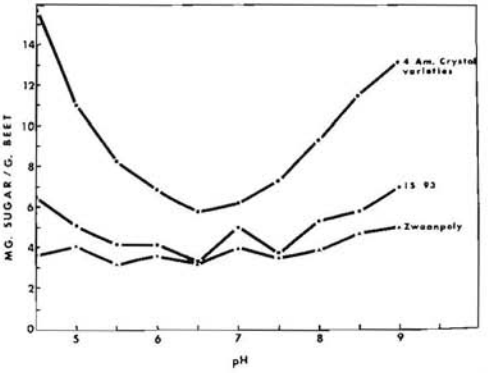

Figure 4. Effect of pH on loss of sugar from washed discs of sugarbeet tissue after 6 hr in 0.1 M phosphate buffer.

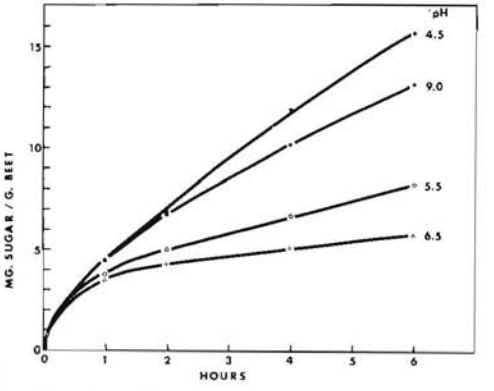

Figure 5. Diffusion of sugar from washed discs of sugarbeet tissue in 0.1 M phosphate buffers of given pH.

out the experimental period rather than increases in the rates during the experiment. Extrapolation of the linear portions of the curves in Fig. 5 to zero time gives about 3 mg sugar per gram of beet or about 18% of the total sugar.

Temperature. The interaction of temperature and salt concentration on the diffusion of sugar is illustrated in Figures 6 and 7. NaCl was used in these experiments to avoid possible phosphate precipitations at the higher temperatures. The data are averages from one experimental group, except for curves 55 C (b) in Figure 6 and 0.5 M (b) in Figure 7 which are taken from another experiment conducted at a higher temperature range. A comparison of curves 0.5 M (b), where complete extraction in 0.5 M NaCl was obtained at 60°C, and 0.5 M (a), in Figure 7 shows that some variation occurs among beets in the critical temperature range. Other results at narrower temperature ranges showed similar patterns to the data illustrated and were used to

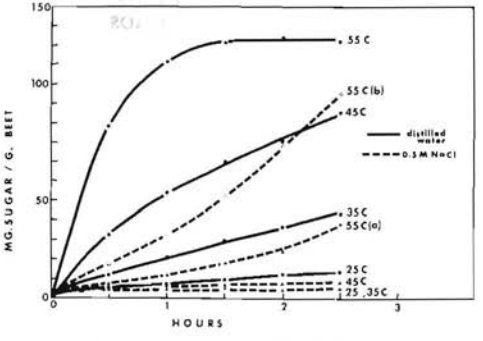

Figure 6. Effect of temperature and NaCl concentration on diffusion of sugar from washed discs of sugarbeet root tissue. 55 C (b) is from a different trial than the rest of the data.

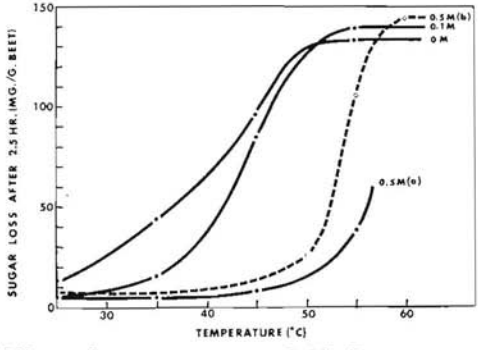

Figure 7. Effect of temperature and NaCl concentration on loss of sugar from washed discs of sugarbeet tissue after 2.5 hr. 0.5 M (b) (- - - -) is from a different trial than the rest of the data.

interpret the shape of the curves in Figure 7, e.g., total extraction of sugar was found to occur at 50°C in distilled water within a 2.5 hour period. The horizontal portions of the curves indicate complete extraction at these temperatures.

The rate of diffusion of sugar into distilled water increased rapidly with increasing temperature but the rate of diffusion into 0.5 M NaCl remained relatively constant and low until the temperature exceeded 45°C. The results in 0.1 M NaCl were intermediate. Thus, an increasing salt concentration was increasingly effective in preventing a rise in the diffusion rate with increasing temperature until a critical temperature range was reached. In the critical range, the diffusion rate increased rapidly with increasing temparature.

Inhibitors and auxins. Several metabolically active componds, 3-indoleacetic acid (10 to 1000 ppm.), maleic hydrazide, (10 to 1000 ppm), dinitrophenol (1 to 100 ppm), and sodium fluoride (10 to 1000 ppm) dissolved in 0.01 M phosphate buffer (pH 7.1) had no apparent effect on diffusion. However, low amounts of diffusion and high variability may have obscured small effects.

Discussion

Sugar loss from cut pieces of sugarbeet tissue occurred in three stages. The first loss was from cut cells on the surface and was completed in about 5 min. In the second stage, the rate of loss decreased within an hour from a relatively rapid rate to the lower, constant rate, of the third stage. In Figures 2 and 5, when the linear parts of the curves are extrapolated to zero time, a relatively constant value is obtained. This value may be a measure of the sugar present in the "apparent free space" (3) and averaged about 15% of the total sugar in the data presented.

Salt concentration had a clear and reproducible effect on the diffusion of sugar. In isotonic solutions (approx. 0.2 M) the rate of diffusion from the beet tissue was very low after the initial stage. In lower salt concentrations there was a progressive increase in the rate of sugar loss. In higher salt concentrations loss of sugar from the tissue was not significantly different from the loss at 0.2 M, but the data were more variable. The regularity of the increase and the uniformity of the effect with several salts suggest that the increased rate of diffusion may have been due to the increased turgor pressure in the cells. The higher rate of sugar loss from the mother beets may have been due to effects of the storage environment or could have been related to their capacity for a higher sugar content. More recent studies have also indicated that mother beets bred for high sugar content have rates of sugar loss which are higher than that for commercial

beets (unpublished data). The fodder beets, which are normally low in sugar content, had low rates of sugar loss.

When the sugar lost after a specified time was plotted against temperature, a sigmoid curve was obtained (Figure 7). Increasing salt concentration causes a decrease in the rate of sugar loss at the lower temperature. A rapid rise in the rate of sugar loss is indicative of thermal damage to the cells.

The tests with enzyme inhibitors and auxins gave no evidence of metabolic factors preventing or promoting diffusion of sugar.

The differences in permeability among beets are of interest for several reasons. First, permeability may be indicative of quality of the beet in storage, and, if there is a progressive deterioration of the protoplast in non-ideal conditions of storage, permeability could be an indication of the deterioration. Secondly, if differences in permeability result from some physiological condition in the beet, these differences may be a measure of storage capability of the beets. Thirdly, permeability may be related to the ability of the beet to accumulate sugar. There is a suggestion of this last relationship in the data for fodder beets, commercial beets, and the mother beets. Experiments are in progress to test these possible relationships.

Summary

Diffusion of sugar from excised tissue of sugarbeet root (*Beta vulgaris* L.) was studied as a function of salt concentration from 10^{-5} to 1.0 M, of pH from 4.5 to 9.0, and of temperatures from 24-65° C. Rates of diffusion varied considerably among beets and methods of selecting samples for minimum variation among treatments are given. Rates of diffusion of sugar from washed tissue were inversely related to salt concentration up to about 0.2 M and were minimal at a pH of about 6.5. Rate of diffusion increased with increasing temperature until extraction was complete. The rates were progressively lower with higher salt concentrations until thermal damage to the cells occurred. Sugar loss from intact cells could be separated into a stage attributable to loss from the apparent free space and a stage attributable to diffusion through the protoplasmic membrane. Sugar in the apparent free space constituted 13-18% of the total sugar in the tissue. The significance of permeability for studies on sugar accumulation and the storage of beets is mentioned.

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