Sugar Transformations in Stored Sugar Beets

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In recent years American beet sugar factories in areas that have low night time temperatures in fall and winter have lengthened their operating season by storing factory beets for longer periods (6, 18).[2] These factory beets lose sugar as a result of at least three different processes. One is spoilage by microorganisms which use up sugar by respiration, and produce enzymes which convert sucrose to invert sugars and oligosaccharides. Since it has not been economical up to now to treat beets with fungicides, clean storage at low temperatures in ventilated piles is employed to minimize losses to microorganisms.

A second substantial source of sugar loss occurs through direct respiration by stored sugar beets. Live beets resist infection and maintain their structure. Estimates have placed direct respiration sugar losses up to 0.5 pound of sugar per ton of beets per day. Up to now chemical inhibition of beet respiration has not been commercially feasible, but storage of clean beets at low temperature in ventilated piles also reduces respiration losses.

A third source of sugar loss is through biochemical transformations of sucrose into invert sugar, raffinose and other carbohydrates. These enzymatic processes use up sucrose and also produce materials that cause inaccuracies in sucrose analysis, inhibit crystallization and cause other difficulties in beet sugar processing. Among the three processes causing sugar losses in beets, biochemical transformations have received the least attention.

It seemed worthwhile to store beets under controlled conditions of temperature where changes are known to occur and to learn if manipulation of temperature alone could restore the original carbohydrate composition of the beet. The experiments reported here show the effects of two storage temperatures on sucrose in sugar beets and transformation of sucrose to nonsucrose carbohydrates.

Methods and Materials

Beets and Storage Conditions.—Beet storage experiments began in November with factory beets (derived from variety U.S.

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[2] Numbers in parentheses refer to literature cited.

75) grown at Pleasanton, California. There was no frost or cold weather during growth or harvest. Sound washed beets approximately one kilo each were selected from the conveyor belt, mixed, taken to the laboratory and cooled rapidly in a tank of shaved ice. About twenty-four to twenty-seven cold beets (11, 15) were packed in wooden boxes with moist pine shavings (10) for each sample, and the boxes were stored at 2°C. Zero-time samples consisted of two boxes of 27 beets each. Each box was sliced into cossettes in the laboratory slicer and mixed thoroughly (17).

A sample of mixed cossettes was passed through a food chopper and the juice expressed through a nylon cloth with a hydraulic press. About 1 kg of juice was weighed immediately into a pot and boiled 1 minute to inactivate enzymes that might alter the composition of the juice during freezing and storage. The boiled juice was brought back to the original weight with distilled water and quickly frozen in plastic bottles for analysis.

Each whole box of beets was removed from cold storage and treated as described for analysis at appropriate time intervals up to 115 days storage. After 86 days of cold storage, 6 boxes of beets were unpacked and spread out overnight to warm to 25°C. These beets were repacked in moist pine shavings as before and held at 25°C. Each box of beets was sliced and prepared for analysis at time intervals of 2, 6, 9, 13, 16 and 22 days. Heat-treated press juice was prepared as described and preserved by freezing for analysis.

Juice samples were thawed quickly and processed into purified juice by the lime-phosphoric acid method of Carruthers and Oldfield (5) and preserved for analysis by quick freezing. Walker *et al.* (19) showed changes in the carbohydrate composition of beets under selected storage conditions while many other constituents such as nitrogen compounds, acids and others remained fairly constant. In the studies reported here, analyses were made only for total solids and carbohydrates in the juice.

Analysis for Sucrose, Invert and Solids.—Refractometer dry solids (RDS) were measured on pressed juice and purified juice with a Bausch and Lomb precision refractometer. Sucrose was run by polarization (Pol) on the purified juice. Apparent purity was calculated from RDS and Pol sucrose. Chemical sucrose was run by determining reducing sugar before and after treatment with yeast invertase by the ferricyanide-ceric sulfate method of Hassid (13, 14). A correction was made for sucrose by subtracting raffinose and 1-kestose (determined separately by paper chromatography) although both of these sugars, like sucrose, are hydrolyzed by invertase to reducing sugars.

Paper Chromatography.—Galactinol, raffinose, and l-kestose were run by paper chromatography. There was no galactinol present in the juices prepared from the control beets or from any of the stored samples. Paper chromatography was run essentially as described by Bichsel and Johnson (2), using the organic layer of a mixture of l-butanol, glacial acetic acid, and deionized water (4:1:5 v/v) as developing solvent. One microliter of sample (about 10% solids) and separate standards of 1, 2, 3, and 4 micrograms of raffinose and l-kestose were placed on Schleicher and Schuell No. 2043-b paper sheets and developed descending for 20 hours. Air-dried papers were dipped in an indicator containing 1 ml of saturated silver nitrate in 200 ml of acetone, dried, and dipped in 0.5% solution of sodium hydroxide in ethanol. After air drying for about one hour, the sheets were dipped first into a saturated solution of sodium thiosulfate in 60% alcohol and then into 60% ethanol. The alcohol-washed chromatograms were dried in air.

The density of the developed spots on paper were determined with a Photovolt TLC Transmission Densitometer[1] equipped with a Varicord variable-response recorder. The area of each peak was determined by multiplying the peak height times the band width at half peak height. These standards yielded a straight-line relationship for raffinose and for l-kestose between concentration and peak area under these experimental conditions. Spots containing l-kestose developed with silver nitrate as described were only about half as dense as those from an equal amount of raffinose.

Determination of Raffinose with Galactose Oxidase.—The galactose oxidase method uses a coupled enzyme system for quantitative determination of either free or combined galactose. When the 6-OH group of galactose is enzymatically oxidized to an aldehyde, hydrogen peroxide is liberated in the reaction permitting a quantitative determination to be made.

The assay was conducted as described by Worthington Biochemical Corporation[2]. A series of standards 0.025, 0.050, 0.100, 0.200 and 0.300 mg/ml of raffinose were prepared. The procedure consisted of adding 1.0 ml of 0.1 M phosphate buffer pH 7.0 to 2.0 ml of galactose oxidase reagent, containing enzyme and chromogen, mixing and warming to 37° C for 5 minutes, adding 1.0 ml of standard raffinose or 0.25 ml of purified beet

[1] Model 530 and recorder model 42-B Photovolt Corporation, 1115 Broadway, New York 10, New York.

[2] Galactose oxidase was obtained from Worthington Biochemical Corporation, Freehold, New Jersey.

juice diluted to 1.0 ml, mixing, and incubating the reaction mixture for 1 hour at 37° C. After adding 6.0 ml of 0.25 M glycine buffer pH 9.7 to stop the reaction, the absorbance was read at 425 mμ as soon as possible. The plot of absorbance versus concentration was a straight line under the conditions of these experiments.

Results and Discussion

Analyses of the purified juice reported in Table 1 in these experiments show that true purity of beet juice endures under ideal storage conditions for more than two months. When the beets were returned to storage at 25° C, carbohydrates changed very rapidly. Dextran and levan were detected by the thin layer chromatographic method of Atterson *et al.* (1) after 60 days cold storage of beets and in the 25° C-stored samples. The concentration of these polymers appeared to increase rapidly as the cold-stored beets warmed to room temperature. These polysaccharides are synthesized by carbohydrate transferase enzymes at the expense of sucrose.

Figure 1 shows the slow drop in sucrose content with time in cold storage. When the 86-day cold-stored beets were warmed to 25° C and kept at that temperature, the sucrose content dropped rapidly.

Table 1.—Analyses of pressed and purified juice from sugar beets stored at 2° C.

	R D S %		Pol. %		
Days stored	Pressed juice	Purified juice	Purified juice	Apparent purity	True purity
0	17.31	13.35	12.00	89.9	94.5
4	17.25	13.28	12.04	90.7	93.5
8	17.25	13.48	12.19	90.4	96.0
11	17.21	13.18	12.00	91.0	95.2
14	15.69	12.65	11.37	89.9	94.1
17	16.57	13.21	12.03	91.1	92.8
21	16.66	13.77	12.62	91.7	97.3
24	16.57	13.25	11.76	88.8	92.2
30	16.60	12.71	11.68	91.9	91.3
36	16.66	13.44	12.35	91.9	87.4
39	16.70	12.61	11.21	88.9	93.9
46	16.34	12.41	11.17	90.0	93.4
60	16.41	13.44	12.25	91.1	88.9
86	16.47	12.85	11.35	83.3	86.1
115	15.99	12.25	10.53	86.0	80.8
	Beets after 86 days at 2° C returned to storage at 25° C.				
2 at 25°	17.80	14.14	11.96	84.6	83.9
6	17.58	13.51	11.28	83.5	84.5
9	17.34	11.03	8.56	77.6	69.6
13	16.92	11.91	9.18	77.1	83.9
16	15.37	12.08	9.45	78.3	84.6
21	14.85	11.00	6.83	62.1	69.1

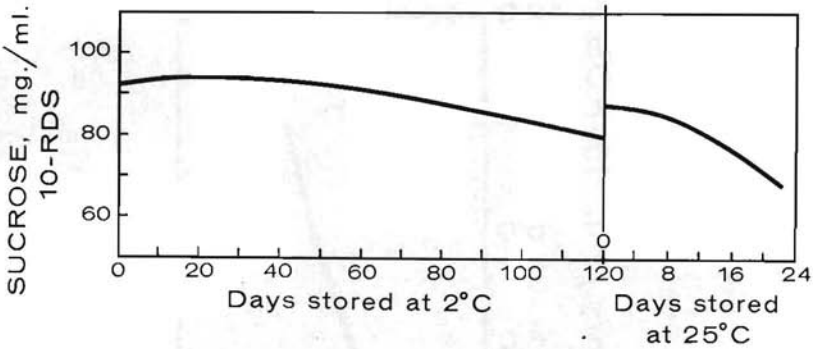

Figure 1.—The loss of sucrose in beet juice during storage of beets at 2° C for 120 days and after warming cold stored beets for 86 days to 25° C for 21 days.

Figure 2 shows the increase in reducing sugars during cold storage. After 86 days storage, reducing sugars had more than doubled, but their concentration was still low, and would only slightly impair processing of the juice to sugar.

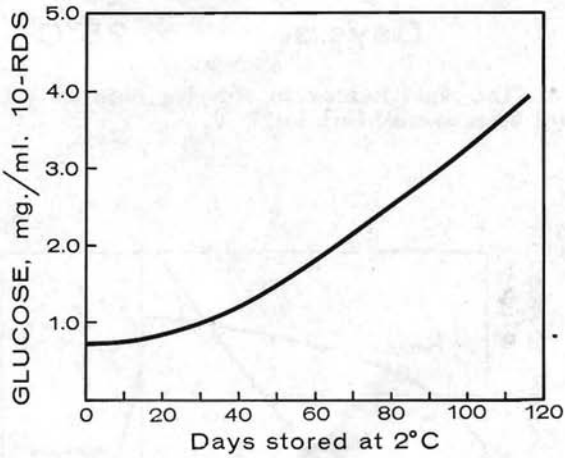

Figure 2.—The rise in reducing sugars in beet juice during storage of beets at 2° C.

Figure 3 shows how the reducing sugar increased after cold storage for 86 days and warming to 25° C. Reducing sugars rose rapidly, and in less than two weeks exceeded 1% of the sucrose. One percent reducing sugars would cause difficulties in processing beets to white sugar. Invert sugar in sugar beets is produced both by invertase present in invasive microorganisms

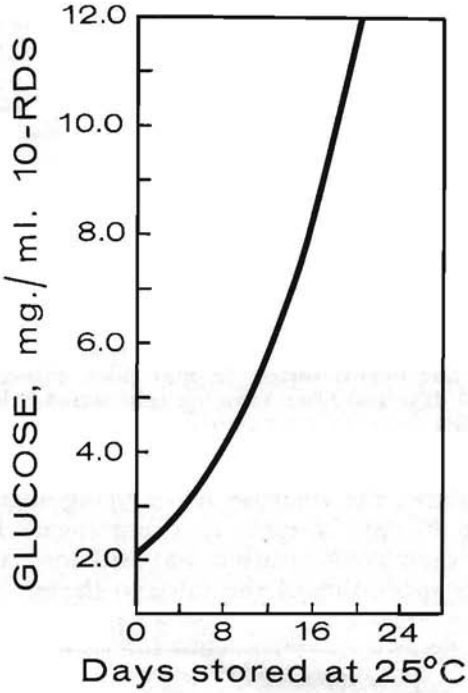

Figure 3.—The rapid increase in reducing sugar in beet juice from the cold-stored beets warmed back to 25° C.

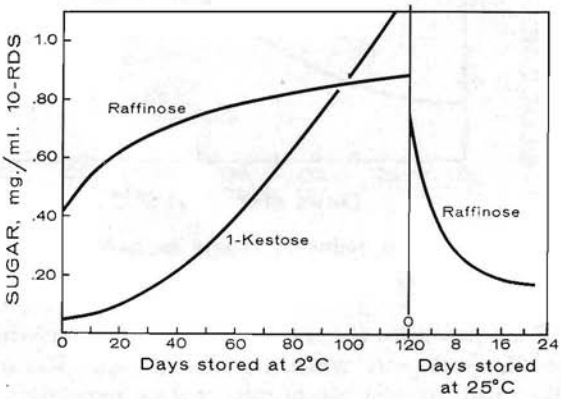

Figure 4.—The increase in raffinose and l-kestose in juice from beets stored at 2° C, and the drop in raffinose when the beets are warmed back to 25° C.

and by a reversal of the sucrose synthetase reactions with uridine-diphosphoglucopyranosyl transferase (16).

Figure 4 shows the increase of raffinose and l-kestose in the low-temperature storage and the decrease of raffinose when the cold beets were warmed to 25° C. It was not possible to determine l-kestose on the beets warmed to 25° C because an interfering spot appeared that overlapped the spot of l-kestose. This interfering spot was not identified. The qualitative appearance of the paper chromatograms indicated that l-kestose and other unidentified sugars increased during 25° C storage.

De Whalley suggested that l-kestose is probably formed by a β-fructofuranoside transferase enzyme produced from sucrose in the beets by microorganisms (8). To our knowledge raffinose has not been synthesized by sugar beet enzymes *in vitro*, but it has become apparent that sucrose, raffinose, and galactinol synthesis and much of the carbohydrate metabolism in plants involve nucleoside diphosphate derivatives (9).

Next to sucrose, raffinose is the most abundant oligosaccharide found in plants. The biosynthesis of raffinose *in vitro* has been achieved by allowing sucrose to react with *a*-D-galactopyranosides in the presence of *a*-D-galactoside galactohydrolase (7), but it seems more reasonable that sugar beet raffinose comes from a nucleoside diphosphate-D-galactose derivative such as that described by Bourne *et al.* in 1962 (3). More recently Bourne *et al.* (4) synthesized raffinose by reacting uridine triphosphate (UTP) and *a*-D-galactose-1-phosphate with radioactive sucrose in the presence of a bean enzyme system from a dormant seed preparation of *Vicia faba*. Uridine diphospho-galactose (UDP-galactose) appears to be the galactose donor to sucrose to form raffinose in this reaction. UDP-galactose was also the galactose donor to myoinositol to form galactinol in extracts from unripe peas (12).

In Figure 4 the raffinose concentration fell off rapidly during storage at 25° C, but it was not hydrolyzed to melibiose and fructose. If so the galactose oxidase results for raffinose would not have shown a decrease for raffinose because galactose-containing melibiose, like raffinose, reacts with the galactose oxidase reagents. Furthermore no melibiose was present on the paper chromatograms. These results show that raffinose broke down under these conditions by reversal of the synthetase mechanism. UDP-galactose and sucrose are produced initially but UDP-galactose is probably converted immediately by UDP-galactose-4-epimerase to UDP-glucose so free galactose never accumulates

in the tissue. The UDP-glucose went into the metabolic pool or was converted to glucose and other products.

It was hoped that this study would show that cold-stored beets high in raffinose might be warmed for a few days to decrease the raffinose content prior to processing. Actual results show that at the same time, too much sucrose would be hydrolyzed or otherwise converted to invert sugars. Proper temperature control might strike a balance between reduction of raffinose in high raffinose beets and accumulation of invert sugar but this would require further study. An alternative solution to the problem would be to find means to inhibit the nucleoside transferase activity that leads to raffinose formation under cold storage conditions.

Summary

Sugar beets were stored under ideal conditions at 2° C for 115 days. During this period beets were sampled, processed into purified juice, and analyzed for sucrose, reducing sugars and oligosaccharides. Beets stored at 2° C for 86 days were warmed to 25° C and sampled over 21 more days. Analyses of the purified juice showed changes in sucrose, reducing sugars, 1-kestose and raffinose. No galactinol was detected by the paper chromatographic method used for analysis of oligosaccharides.

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Reference to a company or product name does not imply approval or recommendation of the product by the U. S. Department of Agriculture to the exclusion of others that may be suitable.

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