Effects of Storage on Raffinose Content of Sugar Beets. I. Varietal Changes Occurring During Storage

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Raffinose is one of the many unwanted melassigenic substances, commonly called impurities, which interferes in the recovery and refining of sucrose. It is a dextrorotatory, trisaccharide compound with the chemical make up of $C_{18}H_{32}O_{16}.5H_2O$ and has very little commercial value. Like sucrose, it is precipitated by lime in the saccharate process and is mostly retained in the syrups because of continued recycling of the juices in the sugar factory. Zitkowski (9, 10. 11)[2] recognized the troublesome characteristics of raffinose and published methods of isolating it from beet sugar products. He used artificial mixtures of sucrose and raffinose and demonstrated by photomicrographs that small quantities of raffinose could interfere with the size and shape of the sucrose crystal. This is of importance to the beet sugar manufacturer whose aim it is to produce crystals as uniform as possible.

Breeding of sugar beets for low raffinose content was made possible by the development of the paper chromatography method of determination as described by Brown (2) and Serro and Brown (6). With this new technique it was possible to determine the raffinose content of individual beets very rapidly. Brown and Wood (3) also demonstrated that varieties differed in their raffinose content when grown under the same conditions and concluded that these differences were due to the genotype of the varieties. They also reported that the raffinose content of the same variety could vary from location to location, and that some increase in raffinose content was noticed during storage. Finkner and Bauserman (5) reported an approximate 30 percent increase in raffinose content from the same varieties which were harvested three weeks later in a date of harvest test. Progeny performance of roots which were selected from the same population for high and low raffinose content have been reported by Wood (7) and Finkner and Bauserman (5). Both papers concluded that the raffinose content of beets could be significantly increased or decreased by mass selection. Wood et al (8) studied the inheritance of raffinose production in sugar beets and reported the number of effective factor pairs for production of raffinose between the two parents used was about five and at least one is

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[2] Numbers in parentheses refer to literature cited.

non-isodirectional and all are equal in magnitude. In the crosses studied, neither dominance, heterosis, nor linkage appeared to be involved. Quantitatively, the factors for raffinose production in the two parents followed an arithmetic scale and consequently were additive. Correlation coefficients between raffinose and a number of other chemical characteristics of sugar beets have been calculated by several investigators (1) (4) (5). Most of them have reported a negative association of raffinose with sucrose percent, indicating some gain in sucrose should be made as raffinose is reduced by breeding methods. This also would be helpful from the processors standpoint.

The objectives of the present investigation were: To determine if raffinose content increased in topped beets during storage; to ascertain if all varieties would react similarly during storage, regardless of their original raffinose content.

Materials and Methods

The roots used in this storage study were taken from the border rows of the 1955 Chemical tests which have been reported by Finkner and Bauserman (5). The investigation was designed to have four varieties, four replications and four storage periods in a split plot design. Three of the four varieties used were selections from an elite stock of American No. 1. Strain 54-407 was the result of a random selection of mother beets from American No. 1, while 54-410 and 54-411 were selected for high and low raffinose content respectively from the same parental variety. The fourth variety was American No. 2 which is the commercial variety grown in the Rocky Ford, Colorado, area.

The original Chemical Test (5) had eight replications but beets from replications 1 and 2; 3 and 4; 5 and 6; 7 and 8, were bulked together to form the four replications of this storage test. A total of 35 beets of each variety in each of the four replications were topped and stored in the Rocky Ford root cellar on October 24, 1955. Seven beets of each variety within each replication were selected at random and stored together in crates, or a total of 28 beets per crate. The 16 crates, one crate per each replication and for four different storage dates, were randomized and stacked in the root cellar at a temperature of approximately 4 degrees Centigrade. This temperature varied from 2 to 6 degrees throughout the storage periods. The remaining beets, seven roots of each of the four varieties of each replication, were bulked together in large onion sacks and all four sacks, each containing a single replication, were buried in the storage pile at Manzanola, Colorado, on the evening of October 25, 1955.

After three weeks the beet samples in the storage pile were analyzed and compared with samples stored three weeks in the root cellar. Beet samples stored in the root cellar were analyzed at 3 week intervals. The non-stored check was in storage one day while the experiment was being set up.

Each beet in the root cellar was sampled twice by rasping and two determinations made on each pulp sample, making a total of four readings for every beet in storage. The raffinose content was determined by the paper chromatographic method and is reported as percent on dry substance.

Results and Discussion

On October 24 all beets for the storage test were harvested and kept in the root cellar at Rocky Ford, Colorado. The next day all roots were crated and stored according to the random design, and one set of beets was buried in the beet storage pile at Manzanola, Colorado. The first group was rasped on October 26th and it was considered as the non-treated or non-stored group as it was in the root cellar for only one day.

Three weeks later the group of roots stored in the Manzanola storage pile was taken out of the pile and sampled. During the process of removing these beets the drag-line tore open one sack and all the beets from the first replication were lost. The same day, November 15th, a similar group of roots was removed from the root cellar at Rocky Ford and they also were sampled.

These two groups of beets were analyzed in a split plot design with three replications and the analysis of variance is given in Table 1.

Table 1.—Analysis of Variance of Four Varieties and Two Different Types of Storage.

Source of Variation	D.F.	M.S.	F. Value
Total	671		
Replications	2	.3237	
Varieties	3	1.5624	10.76[1]
Error A (Rep. x Var.)	6	.1450	
Storage	1	.1357	0.41
Variety x Storage	3	.2996	0.91
Error B	8	.3307	
Beets within plots	144	.0650	
Rasping from same beet	168	.0141	
Determinations	336	.0113	

[1] Significant at the one percent level.

No significant differences were detected for raffinose content between the roots stored in a commercial storage pile and the roots stored in the root cellar. Therefore, either place could be used for testing different varieties and consecutive storage periods. This experiment was designed to utilize the root cellar.

The means of the four varieties under both types of storage conditions and the general means of each storage type are shown in Table 2.

Highly significant differences were detected for varieties. This was expected since selection pressure was applied in opposite directions in two of the varieties for raffinose content. It also had been shown previously by progeny tests (5) that selections 54-410 and 54-411 contained significantly different amounts of raffinose.

The two remaining sets of beets in the root cellar were rasped on December 7 and December 28. The four variety means and the storage means are shown in Table 3 and Figure 1. These means are from four replications while the means in Table 2 are from only three replications.

Table 2.—Variety and Storage Means for Raffinose as Percent on Dry Substance.

	Storages		
Varieties	Root Cellar	Storage Pile	Variety Means
American No. 2	.504	.377	.441
54-410	.617	.547	.582
54-407	.389	.426	.408
54-411	.334	.380	.357
Storage Means	.461	.433	

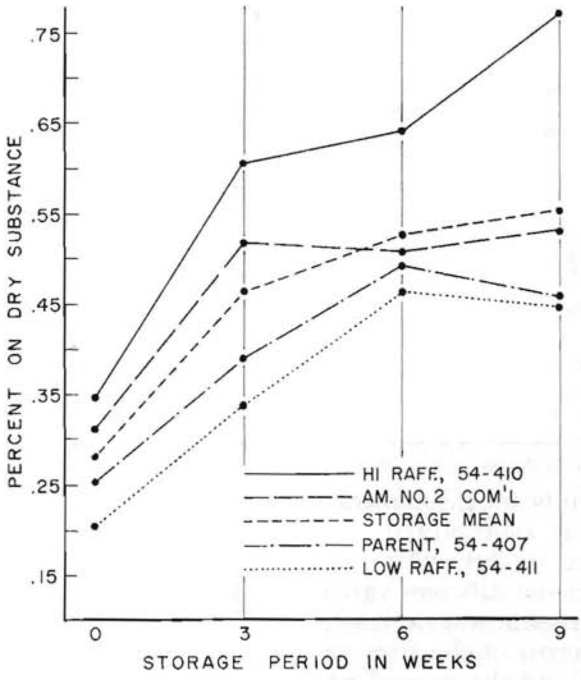

Figure 1.—The reaction of four varieties in storage and the general mean.

Table 3.—Variety and Storage Period Means for Raffinose as Percent on Dry Substance.

	Storage Period in Weeks				
Variety	0	3	6	9	Variety Means
American No. 2	.312	.520	.507	.529	.467
54-410	.350	.608	.643	.773	.594
54-407	.255	.387	.492	.455	.397
54-411	.205	.338	.464	.448	.364
Storage Means	.281	.463	.526	.551	

Each variety mean at each storage period was the result of rasping seven beets from each replication twice, and running two determinations on each pulp sample from each rasping. Therefore a total of 112 raffinose determinations were made to estimate the mean of a single variety for any one storage period. The storage means for each period were estimated by using these 448 raffinose determinations. The analysis of variance of these four varieties and the four storage periods are shown in Table 4.

Table 4.—Analysis of Variance of Four Varieties and Four Storage Periods.

Sources of Variation	D.F.	M.S.	F. Value
Total	1791		
Replications	3	.8649	
Varieties	3	4.6281	16.11[2]
Error A (Rep. x Var.)	9	.2872	
Storage	3	6.7044	61.96[2]
Varieties x Storage	9	.2812	2.60[1]
Error B	36	.1082	
Beets within plots	384	.1156	
Rasping from same beet	448	.0347	
Determinations	896	.0149	

[1] Significant at the five percent level.

[2] Significant at the one percent level.

Highly significant differences were detected for both storage periods and varieties, and a significant variety by storage period interaction was encountered. As shown in Table 3 and Figure 1 there was a very definite increase in raffinose content of all varieties tested. The varieties were not, however, responding entirely consistently throughout the storage periods. All varieties showed a significant increase during the first three weeks in storage. After that American No. 2 leveled off and remained constant. The high raffinose selection, 54-410, made a slight but non significant increase in raffinose content during the third to the sixth week of storage. Selections 54-407 and 54-411 both made a significant increase in raffinose content during the same period. From the sixth to the ninth week only 54-410 made a significant increase in raffinose while 54-407 and 54-411 showed a slight (not significant) decrease. Although some of these selections showed only a slight variation from one storage period to the next the combined effect of the inconsistent responses was great enough to make a significant ($P = .05$) interaction. The interaction effect was, however, very small as compared to the effect of the varieties or storage periods.

The percent increase of raffinose of the two extreme varieties, 54-410 and 54-411, the high and the low raffinose selections respectively, was approximately the same. Selection 54--410 showed an increase of 121.2 percent over the non--stored beets while 54-411 showed a 119.1 percent increase. American No. 2 had the lowest percentage increase of 69.7 percent and 54-407 was very similar with a percent increase of 78.7 percent.

In selecting beets for low raffinose content the plant breeder would obtain a greater spread in his material if he would store the roots for several weeks before they were sampled. Although the increase of raffinose content may be approximately equal on a percentage basis, this additional spread will make the selection of low raffinose beets easier and more accurate.

The curve shown in Figure 1, based on the means for each storage period, resembles a portion of a sigmoid curve. It shows a very rapid increase in raffinose accumulation during the early part of storage and then levels off which is typical for a wide variety of growth phenomena. Oftentimes, these response curves can be adequately approximated, within the limits under investigation, by a simple polynomial. Since the observations during storage were taken at equally spaced intervals the response can be easily characterized by the use of orthogonal polynominals. A linear response was first tested and was found to be significant but the deviations from linearity also were significant. The quadratic component was found to be significant and deviations from the quadratic were non significant. Therefore, this response curve within the limits of interest can be satisfactorily approximated by a second degree polynomial indicating that the rate of raffinose accumulation decreased as the beets remain in storage.

The fact that the raffinose content of topped beets in storage increased indicated that this phenomena is probably tied up with respiration system of the beets. It certainly could not have been associated with the photosynthesis mechanism. The exact mechanism responsible for raffinose increase is not known and additional investigations are needed to determine it.

One possible explanation or hypothesis could be as follows; as the temperature decreased to near freezing an enzyme may be activated which would convert certain respiratory products into raffinose. Therefore, raffinose is mainly produced during cold weather and when the respiration rate of the beet exceeds the rate of photosynthesis.

As early as 1911 Zitkowski (11) quoted Mr. Kelton, Superintendent of the West Bay City Sugar Company of Michigan, as follows: "All this indicates that raffinose is formed by the action of frost, in this case after the beets were harvested." Zitkowski also reported in the same paper that a "Professor Herzfeld as early as 1889 expressed the opinion that raffinose was formed in larger quantities when beets which had been exposed to freezing weather take on a new growth in which case raffinose was formed from dissolved pectic substances." In this same paper Mr. Zitkowski suggested the possibility of enzymatic action on other carbohydrates which would eventually form raffinose and "one of these conditions was undoubtedly the action of frost."

Perhaps the results of this study have done nothing more than to confirm what Mr. Kelton and Professor Herzfeld knew many years ago. However, in addition the findings have shown that, if a beet goes into storage with a genetically lower raffinose content it will remain lower throughout the storage season.

Summary

The raffinose content of topped beets stored in the root cellar at Rocky Ford, Colorado, showed a significant increase as they remained in storage.

No significant differences for raffinose content were found after a three week period between beets stored in a regular storage pile and those stored in the root cellar.

All varieties showed a significant increase in raffinose content during storage, however, they kept the same ranking, i.e., the varieties which had high and low raffinose content to start with also were the high and low varieties at the end of the experiment.

The quadratic component for storage means was found to be significant, indicating that the rate of raffinose accumulation decreased as the beets remained in storage.

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