Preparation and Storage of Beet Pulp Samples for Sucrose Analysis

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The practice of freezing sugar beet pulp and analyzing it later for sucrose content has facilitated greatly the harvesting of sugar beet plants in physiological studies. Cormany (2)[2] observed little to no change between fresh and frozen pulp stored in metal and paper containers for a period of 12 weeks at —15° F. and Price and Fife (3) found the same to be true for pulp stored in glass containers for 15 months at —5° to +8° F. In the present study their techniques have been modified to eliminate the mixing and weighing of frozen pulp, polyethylene bags were substituted for bulky metal and paper containers and dry ice was used for quick freezing. A relatively broad base for the test was provided by storing pulp ranging in sucrose concentration from 8.6 to 16.0%. Within these limits the storage of pulp at —2° F. for periods of 0, 3.5, 9, 12, 25 and 32 months had no significant effect upon the sucrose content of the frozen pulp samples. These results and their statistical evaluation are presented in this paper.

Methods and Procedures

The pulp samples for this study were obtained from the storage roots of sugar beet plants of the US 75 variety that had been grown outdoors in a uniformity trial as single beets per 5-gallon pot filled with vermiculite. The plants were watered daily with modified ½ strength Hoagland's solution (5) throughout the growing period from May 18 to October 26, 1954. At the time of harvest the beet roots were washed free of vermiculite, dried with a towel and a V-shaped wedge removed from the roots by means of a Kiel rasp. The pulp from an individual beet root was mixed thoroughly with a spatula and two 26.0 gram samples were weighed for analysis. Each 26.0 gm sample was placed immediately into a 4″ X 2″ X 4″ polyethylene bag of 1.5 mil thickness, sealed with a heat sealer and frozen immediately by placing the sealed bag in direct contact with dry ice. After the samples were quick frozen, they were placed into a deep freeze cabinet maintained at —2° F. for storage. Sucrose was determined by thoroughly mixing and digesting the frozen pulp sample with 179.1 ml of basic lead acetate for thirty minutes in a covered metal beaker set into a hot water bath at 80° C.

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[2] Numbers in parentheses refer to literature cited.

After cooling to 20° to 23° C, the mixture was shaken vigorously, filtered and the percent sucrose in the filtrate determined in a saccharimeter (1).

A total of 150 beets were sampled in duplicate as described above. The first member of each pair of samples was analyzed for sucrose within one week after the samples were frozen. The results of these analyses constitute the "initial" values. At the end of each storage period, including zero time, 25 samples were selected at random for the "final" sucrose analysis.

A sensitive measure of the effect of storage time on the sucrose concentration of the frozen beet pulp is the difference between the final analysis (X_f) and the initial analysis (X_i) for each pair of samples. These differences are reported in Table 1 for each sampling period and an analysis of variance of the differences due to storage is given in Table 2. The mean sucrose values, the range in sucrose concentrations, the regression equation for the initial and final analyses, the correlation coefficients, the coefficients of variation, and error variances for each group

Table 1.—Differences Between Final and Initial Sucrose Analysis ($X_f - X_i$)

Sample #	Storage Period in Months					
	0	3.5	9	12	25	32
1	0.0	+0.1	0.2	+1.1	-0.2	-0.2
2	-0.1	—0.1	0.1	—0.1	0.0	-0.1
3	-0.1	+0.1	0.0	+0.1	0.0	0.0
4	-0.1	—0.7	0.1	+0.1	0.0	-0.2
5	-0.1	+0.2	0.2	—0.6	0.1	-0.1
6	-0.2	+0.3	0.1	0.0	0.0	0.0
7	0.1	-0.1	0.0	0.0	0.1	0.0
8	0.2	0.2	0.1	-0.1	0.2	-0.1
9	0.0	0.2	—0.3	-0.1	0.0	0.0
10	-0.1	0.0	+0.2	—0.2	0.1	0.2
11	-0.1	+0.1	—0.1	—0.1	—0.1	—0.1
12	0.0	—0.2	0.0	—0.9	0.3	+0.1
13	0.1	0.0	0.2	—0.6	0.0	+0.3
14	—0.1	0.0	0.0	—1.1	0.1	+0.2
15	-0.1	0.1	0.0	-0.1	0.0	+0.2
16	-0.1	0.0	0.1	—0.1	0.2	0.0
17	-0.1	+0.3	0.3	—0.1	—1.2	0.3
18	0.0	+0.3	0.0	+0.1	—0.1	0.0
19	0.0	+0.1	0.3	—0.1	—0.3	0.0
20	+0.2	+0.2	—0.1	-0.1	—0.1	-0.1
21	+0.6	0.0	0.2	0.0	—0.1	-0.2
22	+0.2	+0.1	0.0	-0.1	+0.3	0.1
23	—0.1	+0.1	+0.1	-0.1	0.0	-0.1
24	-0.1	0.0	—0.1	-0.2	+0.3	-0.3
25	0.1	0.7	0.0	0.1	+0.1	-0.2
Mean	0.020	0.008	0.056	-0.128	0.076	0.024

LSD at the 5% and 1% levels are equal to 0.189 and 0.200, respectively.

of 25 pairs of samples are reported in Table 3. The regression of sucrose observed for the 32-month storage period relative to the original values is given in Figure 1 while the sucrose changes in relation to storage time are presented graphically in Figure 2.

Table 2.—Analysis of Variance of Sucrose Differences (X_f - X_i)

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square[1]	Observed F	Required F 5%	Required F 1%
Total	149	9.7197				
Time	5	0.4781	0.0956	1.49	2.21	3.02
Error	144	9.2416	0.0642			

[1] The mean square for error is the pooled error variance of the differences between the final and initial values presented in Table 1 and is equivalent to $sp^2(4)$.

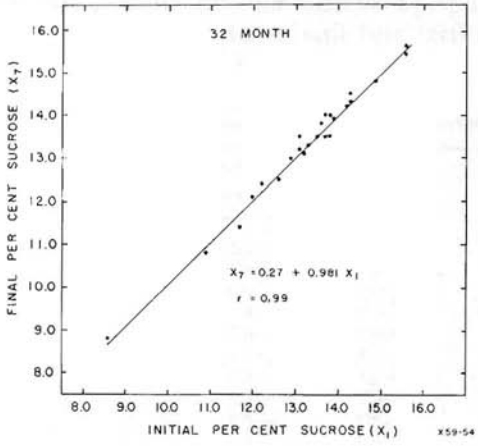

Figure 1.—Regression of initial and final sucrose percent.

Discussion of Results

The differences between the final and initial analyses for the storage periods of 3.5 to 32 months, recorded in Table 1, are in reality the combined effects of sampling and analytical variation (within a pair of duplicate samples) and the effect of storage time on the sucrose content of the pulp. An inspection of these differences for each storage period reveals that the values are relatively constant and differ very little from the results of the initial storage period at time "zero." At zero time the differences are primarily due to sampling and analytical variation and not due to time of storage. The relative constancy of these differences for all storage periods indicates that there is no

Table 3.—Summary of Results

Storage Time in Months	Sucrose Range Between Pairs %	Mean Sucrose Final %	Mean Sucrose Initial %	Mean Difference $X_f - X_i$	Regression Equations	Correlation Coefficient r	Standard Deviation s^1	Coefficient of Variation %
0	11.1-16.0	13.43	13.41	+0.02	$X_2=0.06+1.006X_1$	0.99	0.12	0.88
3½	10.6-15.9	13.17	13.18	—0.01	$X_3=0.17+0.988X_1$	0.98	0.19	1.43
9	11.5-15.7	13.60	13.66	—0.06	$X_4=0.34+0.971X_1$	0.99	0.10	0.73
12	11.5-14.5	13.20	13.32	—0.13	$X_5=0.84+0.928X_1$	0.88	0.28	2.11
25	10.4-16.0	13.49	13.57	—0.08	$X_6=0.31+0.971X_1$	0.97	0.20	1.46
32	8.6-15.6	13.33	13.32	+0.01	$X_7=0.27+0.981X_1$	0.99	0.13	0.96
All values	8.6-16.0	13.37	13.41	—0.04	$X_8=0.25+0.978X_1$	0.98	0.18	1.34

[1]s is the standard deviation of a single determination calculated from the initial and final sucrose values of the pulp samples (4). $2s^2=sp^2$ of Table 2. (4).

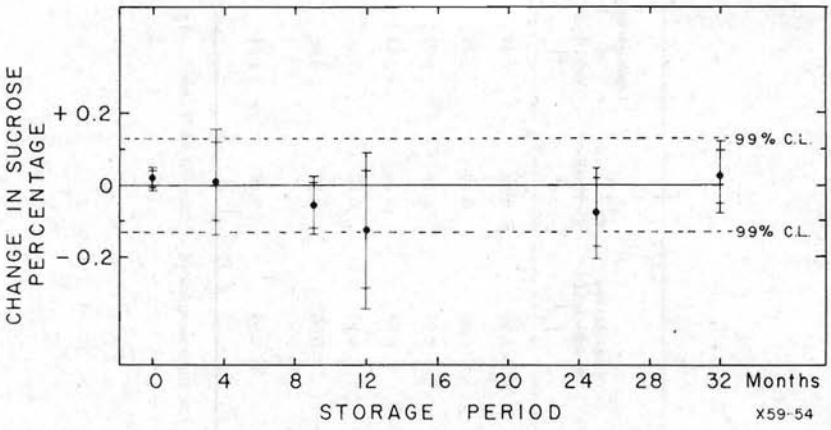

Figure 2.—Effect of storage on sucrose percentage of sugar beet pulp. The upper and lower limits of the 95% and 99% confidence limits for the mean differences from Table 1 are indicated for each storage period. The horizontal dotted lines are the overall 99% confidence limit about mean 0.

appreciable loss in sucrose by enzymatic hydrolysis nor an appreciable gain in sucrose from the hydrolysis of polysaccharides nor an apparent gain in sucrose from a loss of moisture during storage. Apparently the variations noted between the final and initial analyses for all storage periods are those due to random variations that are associated with minor differences in technique and not to storage time. An analysis of variance of the differences for the six storage periods supports this hypothesis (Table 2). The F-value for the storage periods given in Table 2 is non-significant and this fact indicates that the differences between the final and initial analyses for all storage periods (Table 1) are the same, i.e., the differences were drawn from a single uniform population of differences.

The correlation coefficients (r) for the six storage periods indicate that the degree of correlation between the initial and final sucrose determinations for the pulp samples is very good indeed (Table 3). In all instances the coefficients exceeded 0.96 except for the 12-month period. The decrease in correlation coefficient to 0.88 for the 12-month period indicates that either the time of storage has begun to affect some of the samples more than others or that a difference in technique affected the results or that the readings were in error. The two latter possibilities are most likely the cause of the decline in the correlation coefficient for the 12-month period, since the values for subsequent periods are again high (Table 3) and the differences between

the final and initial sucrose values (Table 1) do not increase. It is to be noted too that the decline in the correlation coefficient for the 12-month period is mainly associated with two large differences, +1.1 and -0.9, which are exceptionally large not only for the 12-month period but for all other storage periods as well. In retrospect an inadequate mixing of the pulp prior to sampling appears to have been the most likely cause for these large differences. Mechanical mixing of the pulp before sampling and a vigorous stirring of the slurry prior to filtration are now stressed in the procedure for determining sucrose in beet pulp samples.

The regression coefficients for the final and initial sucrose concentrations for all storage periods are approximately one except for the 12-month period, which dropped to a value of 0.928 (Table 3). This relatively large decrease in the regression coefficient is again associated with the two large differences +1.1 and -0.9 (Table 1). The finding that the slopes are approximately one indicates that the differences due to technique and in storage time are relatively constant over a range of 8.6 to 16.0 percent for a storage period of 32 months (Figure 1). The standard deviations for a single determination and the coefficients of variation are also relatively constant except for the 12-month period where again the two large differences of +1.1 and -0.9 increased these values (Table 3).

The differences for each storage period have been averaged and plotted about an assumed mean of zero and this is indicated by the solid horizontal line in Figure 2. If a normal distribution of means is assumed, then the 99% limits can be calculated as $0 \pm 2.58 \frac{s_D}{\sqrt{N}}$ which is equal in this instance to 0 ± 0.131 where $N = 25$, s_D equals 0.253, the square root of 0.0642, (Table 2) and 2.58 is the t-value. These limits, $\pm$ 0.131 are indicated as a dotted line. Whenever the means for any particular period lie within these limits, it is concluded that there is no significant effect of time on the percent sucrose content of the beet pulp at the 1% level of significance. Upon examining Figure 2, it is evident that there is some variation from the assumed mean at all storage periods and that the greatest deviation of -0.128% is again for the 12-month period. This deviation, as well as the others, is not significant at the 1% level and thus, the deviations from the assumed mean of zero may be attributed to the variability in sampling and analyzing and not to time of storage.

Summary

Procedures for storing sugar beet pulp samples prior to sucrose analysis and for evaluating the results statistically have been developed. Fresh beet pulp samples weighing 26.0 grams each were sealed in small polyethylene bags, frozen immediately and kept in storage at —2° F for periods up to 32 months. A statistical study of the differences between the final and initial values indicated that the length of storage had no significant effect on the sucrose content of the pulp. Mechanical mixing of the pulp before sampling and a vigorous stirring of the slurry before filtration are recommended as added steps in the procedure for determining sucrose in beet pulp samples.

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