A Study of Changes in the Marc Content of the Sugarbeet During Storage[1]

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Introduction

The sugarbeet is at its peak for processing at harvest. However, most of the beets harvested cannot be processed immediately and must be stored in piles for varying lengths of time. During storage, respiration not only reduces sucrose and dry matter content, but molding, freezing or heating lead to deterioration of the beet root.

As a result of the deterioration in storage, soluble pectic substances increase in the factory diffusion juice reducing factory efficiency. These pectic substances result in insoluble calcium pectate gels during lime defecation which cause reduced filtration rates. The source of these objectionable compounds is the marc of the beet root.

Sugarbeet marc is the insoluble residue remaining after extraction of the beet with water (McGinnis, 1951). The marc averages approximately 5 percent of the fresh weight of the beet (Silin, 1964), but may range from 3 to 6 percent depending on variety (Owen *et al.*, 1954).

The marc is composed primarily of cellulose (25%), hemicellulose (25%) and pectic substances (25%) (McCready, 1966). Cellulose and hemicellulose are stable under conditions of processing. The pectic substances exist in two forms: water insoluble and water soluble. The water insoluble form is protopectin, the structure of which is unknown, but is thought to exist in a matrix complex of pectin, cellulose and hemicellulose (Kertesz, 1951; Joslyn, 1962). The breakdown of protopectin by water is highly temperature dependent. Protopectin when treated with hot water swells and gradually dissolves as pectin (Silin, 1964).

The water soluble forms of the pectic substances are pectin, pectic, and pectinic acids. In pectic and pectinic acid the carboxyls are free and can readily react with calcium to form insoluble calcium pectate gels (Joslyn, 1962; Kertesz, 1951).

Silin (1964) reported that microbial activity and sprouting increase the quantity of soluble pectins during storage. Walker

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et al. (1960) found no significant change in the pectin content of diffusion juice over a 48 day storage period when beets remained in good condition, but when stored for 161 days at 34 F the soluble pectin content decreased by one-third. If freezing occurs, pectin, which in a normal beet is a large macromolecule, will be degraded by enzyme action to a small and more soluble colloidal size (Claassen, 1943).

This paper reports the percentage marc in beets subjected to various storage practices after extraction at different temperatures. The effect of variety and several agronomic practices on marc stability in storage were evaluated.

Materials and Methods

In 1966 eight varieties of sugarbeets were grown near Saginaw, Michigan, and samples were machine harvested, washed and stored in heavy canvas bags for 140 days at 7 C. As a result of machine harvesting the roots were badly bruised.

Three varieties were grown near Sebewaing, Michigan in 1967, and harvested on October 6, October 26, and November 6. The beets were hand dug, washed and stored in 2 mil polyethylene bags at 3 C for 65 and 130 days. A small bag of wet wood chips was added to each sample to eliminate the problem of desiccation.

Beets grown near Saginaw, Michigan in 1968, were hand harvested on September 1, October 1, and November 1 and stored as in 1967 for 100 days. Three varieties were also harvested from a breeding nursery in East Lansing and stored at 3 or 10 C for 100 days.

Marc determinations were made using 50 grams of well mixed brei. The brei was placed in a 300 ml beaker and approximately 200-250 ml of water at the desired temperature was added. The beaker was then placed in a water bath at the same temperature for 20 minutes. After incubation the samples were quantitatively transferred to tared 9 cm two-piece plastic Buchner funnels containing a powdered cellulose pad. The residue was then extracted with distilled water at 25 C by repeated washing with ten 200 ml aliquots. The filters were redried at 105 C for 48 hours and the percent marc calculated as percent fresh weight. All determinations were corrected for weight loss in storage.

The dry matter content of the sugarbeet root was divided into the following fractions, based on our experimental conditions, to facilitate discussion:

Fraction I—Dry matter soluble in distilled water at 20 C.

Fraction II—Dry matter insoluble in distilled water at 20 C but soluble at 70 C.

Fraction III—Dry matter insoluble in distilled water at 70 C but soluble at 80 C.

Fraction IV—Dry matter insoluble in distilled water at 80 C.

The sucrose content of the roots was determined using the method of Dexter *et al.* (1967).

Sucrose - U-C^{14}, specific activity 5 mc/mM, was obtained from New England Nuclear Corporation. Radioactivity in the insoluble cell wall residue was determined using a modified Schöniger procedure (Kelly *et al.*, 1961).

Results and Discussion

Storage of beets in the canvas bags at 7 C in 1966 resulted in desiccation (12%), some sprouting and some crown rot. The percent marc was determined at an 80 C incubation temperature.

The loss of marc averaged 13.5% for the experiment but the differences between varieties was substantial (Table 1).

Table 1.—Changes in the marc content of seven experimental varieties during 140 days of storage at 7 C in 1966.*

Variety**	At harvest	After storage	Loss as percent of original
	Percent		
A	4.24	3.69	13
B	4.17	3.32	20
C	3.94	3.36	15
D	4.01	3.61	10
E	4.43	3.98	10
F	3.84	3.36	13
G	4.42	3.82	14
Mean	4.15	3.59	13.5
LSD .05	.34	.30	4

* Desiccation, sprouting and small amounts of crown rot were prevalent. Incubation temperature was 80 C.

** A. SL(129 × 133) MS × SP(5822-0)
B. (SP 6121 × FL 31) MS × (SP 5822-0)
C. (SL 129 × SP 6121) MS × (SP 6428-0)
D. (SL 126 × SP 6121) MS × (SP 6428-0)
E. SP 5822-0
F. SP 6322-0
G. 02 Clone

Varieties D and E had relatively stable marcs compared to variety B which lost twice as much. The other varieties were intermediate to these extremes. The poor storage conditions coupled with the high extraction temperature apparently caused these relatively large losses.

Beets were stored in 1967 with very small amounts of resultant mold and rot, and desiccation was reduced to less than 2%. The incubation temperature for marc determination was decreased to 70 C.

Table 2 shows the average percent marc of three varieties averaged over three harvest dates in 1967. The results were very different from those of 1966, since the percent marc in-

Table 2.—Effect of variety on the percent marc at harvest and after storage at 3 C in 1967.*

Variety**	At Harvest	Days stored 65	Days stored 130
	%	%	%
1	4.19	4.40	4.32
2	4.16	4.20	4.38
3	3.67	4.02	3.96
Storage Means	4.01	4.21	4.22

** Variety 1—SP 63194-0 2—02—Clone 3—US H20
* Each value is the average of 36 determinations (3 harvest dates, 2 nitrogen levels, 3 reps, duplicate determinations). Incubation temperature was 70 C.
LSD .05 among varieties at harvest — .16%
LSD .05 among removals — .09%

creased 0.2% on the average for all varieties during storage.

During the harvest period of October 6 to November 6 the marc incubated at 70 C generally increased (Table 3). The

Table 3.—Effect of harvest date on the percent marc at harvest and after storage at 3 C in 1967.*

Harvest date	At harvest	Stored, days 65	Stored, days 130
Oct. 6	4.01	4.09	4.16
Oct. 26	3.86	4.33	4.25
Nov. 6	4.15	4.21	4.25
Ave.	4.01	4.21	4.22

* Each value is an average of 3 varieties (1, 2, 3), 2 nitrogen levels, 3 reps and duplicate determinations.
LSD .05 among harvest means — .09%

decline in percent marc on October 26 may have been due to water uptake as a result of a heavy rainfall several days prior to harvest. As a result the percent marc declined but total yield of marc per acre increased.

In storage the percent marc increased 0.2% on the average for all harvest dates. The greater increase for the October 26 harvest may have been a result of a conversion of more soluble forms of the pectic substances caused by the moist conditions at harvest to more insoluble forms under ideal storage conditions.

The percent marc in 1968 was determined at three incubation temperatures, 20, 70 and 80 C in an attempt to determine the source of the increased marc content found in the previous year. The percent marc at harvest varied some 25% between

varieties when extracted at 20 C (Table 4). Thus if extracted at 20 C variety 4 would yield 25% more pulp than variety 5. However, fraction III was two to three times more for variety 4 than for varieties 3 and 5 indicating a much greater instability under high extraction temperatures. Variety 4 had an extremely coarse, fibrous texture with vascular bundles which appeared to be highly lignified and convoluted throughout the entire root.

Table 4.—Effect of extraction temperature on the percent marc of three varieties at harvest in 1968.*

Variety	Extraction temperature, C			Content of fractions	
	20	70	80	II	III
			Percent of fresh weight		
3	4.06	3.78	3.65	0.28	0.13
4	4.97	4.72	4.45	0.25	0.27
5	4.10	3.90	3.81	0.20	0.09

* Each value is the average of 4 replications and duplicate determinations.

The three varieties were stored for 100 days at 3 and 10 C and the marc analysis repeated (Table 5). Variety 3 had the most unstable marc in storage and actually lost more marc at 3 C than the other varieties at 10 C. Variety 5 was the most stable of the varieties having negligible losses at either storage temperature.

Table 5.—Changes in marc content of three varieties stored 100 days at 3 and 10 C (1968).

Variety	Storage Temp, C	Extraction Temperature, C 20	70	80
			Percent	
3	10	—0.56	—0.24	—0.15
	3	—0.31	—0.07	—
4	10	—0.07	—	—
	3	—0.21	—0.17	—0.08
5	10	—0.10	—0.12	—
	3	—	—	+0.23

The increase of 0.23% in the marc extracted at 80 C was significant. This increase coupled with the generally very small losses in the marc determined at 60 and 80 C indicate that these fractions are very stable under good storage conditions. Although the marcs determined at 70 and 80 C were very stable in storage, the amount of soluble pectic substances dissolved into the "diffusion juice" is much greater at the higher extraction temperatures.

The marc content at all extraction temperatures remained very constant during the September 1 and November 1 harvest period (Table 6). The marc extracted at 70 C showed a tendency

to increase slightly as it did in 1967. Harvest date had no effect on the loss of marc in storage except for the marc extracted at 80 C on October 1 which lost slightly more than either that of September 1 or November 1.

Table 6.—Percent marc at three harvest dates and the loss in marc during 100 days of storage at 3 C in 1968.*

	Extraction temperature, C			Fraction
	20	70	80	III
	Percent			
Sept. 1				
At harvest	4.32	4.14	3.95	.19
After storage	3.88	3.79	3.79	
Loss	—0.44	—0.35	—0.16	
Oct. 1				
At harvest	4.39	4.16	4.01	.15
After storage	3.92	3.77	3.72	.05
Loss	—0.47	—0.39	—0.29	
Nov. 1				
At harvest	4.34	4.23	3.95	.28
After storage	3.97	3.81	3.82	—.01
Loss	—0.37	—0.42	—0.13	
Average				
At harvest	4.35	4.18	3.97	.21
After storage	3.92	3.79	3.78	.01
Loss	— .43	— .39	— .19	.20

* Each value is the average of 4 replications and duplicate determinations.

The losses in marc extracted at either 20 or 70 C were essentially equal while the loss at 80 C was only half as much. As a result, after storage the amount of residue remaining after extraction at 70 and 80 C is the same. (Fraction III decreased to essentially zero.)

In the post-harvest storage of many commodities, the cell wall tends to break down as senescence or ripening occurs. However, the sugarbeet, a biennial, does not go through a ripening phase and a very stable cell wall is maintained under good storage conditions. From the relatively stable nature of the sugarbeet marc, it was hypothesized that cell wall materials must be actively resynthesized during storage.

To determine the degree of this possible cell wall resynthesis, C^{14}-sucrose was added to beet roots and the incorporation of label into the various fractions of the alcohol insoluble residue were determined. The label was introduced by taking a .75 cm $\times$ 8 cm plug from a topped root vertically through the central core with a sterilized cork borer. Four milliliters of sterilized 15% sucrose containing 0.1 μc of sucrose-U-C^{14} was added and the opening sealed with paraffin. The beets were then stored in a polyethylene bag to prevent desiccation. After appropriate

time intervals (3-4 wks), the beets were sectioned and approximately a 300 gm cross-sectional sample was taken through the area containing the core. This sample was immediately homogenized in boiling 80% ethanol in a Waring blender. The insoluble residue was washed exhaustively with 80% ethanol and dried at 50 C. The dried residue was weighed and the radioactivity measured by the combustion technique (Wang and Willis, 1965). The remaining residue was then used for determining the localization of label.

Each experiment consisted of three beets. The alcohol insoluble residue from each beet was divided in half and all subsequent determinations were run in duplicate.

In all experiments substantial labeling occurred in the alcohol insoluble residue indicating that cell wall synthesis was taking place with sucrose acting as the substrate (Table 7). A

Table 7.—Solubilization of incorporated label from 80% alcohol insoluble fraction by extraction with distilled water at 20, 70 and 80 C.

Extraction temperature	Counts extracted	Wgt extracted	Percent of* total counts in alcohol residue	Specific activity
(°C)	(cpm)	gm		cpm/gm
20	6263	3.16	32	1981
70	7751	3.81	40	2035
80	9081	4.07	46	2231
Fraction I	6263	3.16	32	1981
Fraction II	1488	0.65	7.5	2290
Fraction III	1330	0.26	6.7	5120
Fraction IV	10680	15.62	54	684

Total counts in Alcohol insoluble Residue—19,760 CPM.

precise estimate of the percent incorporation into the alcohol insoluble fraction could not be made since only a portion of the beet was utilized and respiration losses were not measured. However, assuming no loss of label, a minimum estimate of percent incorporation can be made. To each beet 0.1 μc or 2.2×10^5 dpm of sucrose-^{14}C was added. Approximately 2×10^4 dpm or 10% were recovered in the 80% alcohol insoluble fraction. These results correspond very well with the 20% incorporation noted by Barbour and Wang (1961) when the entire root was sampled.

In the first experiment the alcohol insoluble residue was extracted with 20, 70 and 80 C distilled water as outlined in the procedure for the determination of marc. Of the 20,000 cpm in the alcohol insoluble fraction, 32% were soluble in fraction I. An additional 14% was removed when the extraction temperature was increased to 80 C (Table 7). This indicates that approximately one-half of the cell wall resynthesis occurred in the heat stable fractions of cellulose, hemicellulose and possibly

to a limited degree protopectin. The high specific activity of fraction III indicates preferential incorporation into the higher molecular weight, relatively heat stable polysaccharides.

In a second experiment the alcohol insoluble residue was treated with pectinase at pH 5 and room temperature for 6 hours. A control minus pectinase was also run. After 6 hours the residue was collected on a Buchner funnel and washed exhaustively with 25 C distilled water.

Pectinase treatment solubilized 4.2 additional grams of dry matter all of which was presumably pectin in nature (Table 8).[4] However this material contained a very low specific activity (317 cpm/gm) compared to the material solubilized by water alone (2485 cpm/gm). This indicates that the majority of incorporation of label is not into the heat stable pectin fractions but rather into the cellulose, hemicellulose and pectinase resistant protopectin portions of the cell wall.

Table 8.—Solubilization of the alcohol insoluble fraction by pectinase digestion at pH5 for 6 hours at room temperature.

	Grams extracted	CPM extracted	Specific activity
Pectinase treated	7.14	8734	1223
Control	2.98	7415	2485
Fraction extracted due to enzyme	4.16	1319	317

These results substantiate the increase in marc found during 1967 and 1968. Cell wall polysaccharides were apparently synthesized during storage with the highest specific activity occurring in fractions II and III. However 54% of the incorporated label was found in fraction IV. The newly synthesized polysaccharides in fraction II were apparently of short chain length and therefore did not contribute to the overall stability of the residue remaining after extraction at 20 C. However the label incorporated into the more heat stable fraction III and particularly fraction IV appeared to contribute considerably to the stability of these fractions. In some cases a net increase in total cell wall material relative to total dry matter occurred causing an increase in the percent marc determined at 80 C.

The instability of fraction II would presumably contribute to the impurity of the diffusion juice. However this may not be the case. Table 9 compares the loss of dry matter to the loss of sucrose during 65 and 130 days of storage in 1967. In all cases the loss of dry matter was greater than the loss of sucrose. These results indicate that nonsucrose substances are acting as substrates for respiration. This is not in agreement

[4] The pectinase used was a crude preparation and no effort was made to identify the products of the relatively short 6 hour digestion.

with the findings of some other workers. (Barr, 1940; Barbour and Wang, 1961). However we have noted this phenomenon in almost all of our storage work over a three year period. In studies where CO_2 evolution, dry matter loss, and changes in sucrose, raffinose and reducing sugars were monitored over a 102 day storage period, sugar loss accounted for only 60-80% of the CO_2 evolved (Wyse and Dexter, 1970). Part of the non-sucrose dry matter lost as a result of respiration may be derived through the decrease in fraction II.

Table 9.—Average loss of dry matter and sucrose after 65 and 130 days of storage at 3 C in 1967.*

Day stored	Sucrose loss	Dry matter loss
	pounds/ton	
65	— 8	—12
130	—12	—22

* Each Value is the Average of 54 Samples. (3 Varieties, 2 Nitrogen levels, 3 Harvest dates, and 3 Replications.)

Summary

Appreciable cell wall degradation during sugarbeet storage occurred only when desiccation, sprouting and rot were prevalent. Under ideal storage conditions at 3 C in saturated atmospheres the percent marc actually increased in some cases. Harvest date had no effect on marc stability in storage but variety appeared to play a definite role.

^{14}C labeling with sucrose indicated that when the beet root is maintained in good physical condition substantial resynthesis occurred in the 80% alcohol residue.

The dry matter content of the beet root was divided into four fractions based on water solubility at various temperatures and storage stability.

Fraction I was readily soluble in 20 C water and increased substantially in storage at the expense of fraction II. This fraction comprises approximately 95% of the dry matter content of the beet root.

Fraction II was insoluble in water at 20 C but soluble at 70 C. This fraction was composed of the unstable cell wall materials which degraded to a significant extent in storage.

Fraction III was insoluble at 70 but soluble at 80 C and remained relatively stable in storage. Resynthesis occurred preferentially in this fraction. However under less than ideal storage conditions this fraction may undergo considerable degradation.

Fraction IV was insoluble at 80 C and remained stable in storage apparently as a result of the inherent stability of its polysaccharide content and also substantial resynthesis. Although resynthesis does occur, desiccation and mold invasion will completely mask this beneficial effect by causing massive cell wall degradation.

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