Effect of Agronomic and Storage Practices on Raffinose, Reducing Sugar, and Amino Acid Content of Sugarbeet Varieties[1]

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Introduction

The decrease in bagged sucrose per ton of beets during storage results primarily from two factors. Sucrose is respired, evolving CO_2. The transformation of sucrose and other beet constituents into raffinose, reducing sugars, amino acids, etc., results in an accumulation of non-sucrose solutes in the thin juice and corresponding increased sucrose losses into the molasses. Reducing sugars, raffinose and amino acids account for a major portion of the fluctuation in impurities during storage (Wyse *et al.*, 1970). The purpose of this study was to determine the influence of harvest date, nitrogen fertilization and storage temperature on the content of these three impurities in several sugarbeet varieties.

Reducing Sugars

The predominant reducing sugars in the beet are glucose and fructose. Free galactose and arabinose are found only in trace amounts (Silin, 1964). Reducing sugars are presumably destroyed during lime defecation and occur in very small amounts in beet molasses (McGinnis, 1951; Silin, 1964). In this process the reducing sugars are degraded to acids (lactic, formic, acetic, saccharinic) (Carruthers *et al.*, 1959) which must be neutralized by the addition of sodium carbonate before evaporation to reduce soluble lime salts and to prevent sucrose inversion (Silin, 1964; McGinnis, 1951). As a result of the addition of sodium carbonate, molasses quantity and purity are increased resulting in increased sucrose losses (Silin, 1964).

The reducing sugar content of beets at harvest is normally very low and sodium carbonate addition usually is not required. However, during storage invert sugar may increase drastically, particularly if mold or rotting occurs (Stout, 1954; Walker *et al.*,

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1960). Even under storage conditions which prevent molding, sprouting, and wilting, reducing sugars may accumulate, particularly at storage temperatures above 10 C, because of changes in the metabolic balance in the beet root (Walker *et al.,* 1960). This accumulation is accelerated by storage conditions which allow desiccation of the beet root (Atterson *et al.,* 1963).

Raffinose

The major trisaccharide is raffinose which usually occurs as 0.3 to 0.5% of the sucrose present (McGinnis, 1951). The amount increases with prolonged cool periods during growth or storage. Raffinose is as chemically resistant to lime defecation as sucrose and therefore accumulates in the molasses. Raffinose is a particular problem in the Steffen process where it accumulates to high levels in the thick juice. A high raffinose content in the thick juice may produce distorted and elongated sucrose crystals as well as reducing the rate of crystallization (McGinnis, 1951).

The level of raffinose at harvest and the degree to which it accumulates during growth and storage are varietal characteristics. Several populations of mass selected beets were shown by Wood *et al.* (1956) to vary by 10-fold in raffinose content. Five varieties stored for 29 weeks at 4 C showed a significant variety-storage interaction.

The effect of temperature on raffinose accumulation was illustrated by storing beets at 1 C and 12 C (Walker *et al.,* 1960). At 1 C the raffinose content increased four-fold after 60 days and then declined with prolonged storage. At 12 C the raffinose content almost doubled but then remained essentially constant. The effect of temperature was found to be reversible since beets allowed to accumulate raffinose at 2 C for 120 days when shifted to 25 C storage for 25 days, declined almost to their harvest level (McCready and Goodwin, 1966).

Amino Acids

The amino acids contribute to lime salts in the factory and can cause a substantial pH drop during evaporation through deamination (Silin, 1964).

The amino acid content of the beet root is primarily a function of nitrogen fertilization (Haddock, *et al.,* 1956; Hac *et al.,* 1950). Glutamic acid, the dominant amino acid found in the beet root, appears to be a very sensitive indicator of the nitrogen fertility level and consequently has shown a high negative correlation with the sucrose content of the beet root (Woolley and Bennet, 1959; Walker *et al.,* 1950; Hac *et al.,* 1950). Excessive nitrogen fertilization also caused an increased accumulation of other non-sucrose substances resulting in a decreased purity (Henry *et al.,* 1961; Haddock *et al.,* 1956). Although a significant variety-nitrogen interaction is commonly found, the nitrogen effect is by far the most dominant in amino acid production (Finkner *et al.*, 1958; Henry *et al.*, 1961).

Materials and Methods

1967 Experiments

The beets in 1967 were grown in replicated plots near Sebewaing, Michigan.[3] In the fall of 1966, 375 pounds of 0-0-60 was broadcast and plowed down. Just prior to planting, 900 pounds of 0-20-0 was applied broadcast and incorporated with a field cultivator. On May 3, 1967, the beets were planted in 28-inch rows with 200 pounds of 12-6-6 applied as starter fertilizer. After thinning to 120 beets per 100 feet of row, the beets designated as high nitrogen were side-dressed with 125 pounds of nitrogen per acre for a total of 150 pounds of applied nitrogen per acre. The beets with low nitrogen received only the 24 pounds per acre of nitrogen applied as a row fertilizer at the time of planting.

The five varieties and their quality ratings based on a combination of sucrose, clear juice purity (CJP), and yield are given below:

Variety number	Variety	Type	Quality rating
1	SP5481-0	Multigerm	Average
2	SP63194-0	Monogerm	Poor
3	02 Clone	Multigerm	Good
4	SP6322-0	Multigerm	Excellent
5	USH20	Current Commercial Hybrid Monogerm	Excellent

Harvests were made on October 6, October 26 and November 6, 1967. All five varieties were harvested on October 26, but only varieties 2, 3, and 5 were harvested on the other two dates. Beets from six field replications were pooled at harvest and lightly topped to remove the terminal bud. The samples were transported to the Michigan Sugarbeet Research Laboratory in Saginaw for analysis and storage.

1968 Experiments

Beets of variety 5 were grown near St. Charles, Michigan, in 1968. The only fertilizer applied was 500 pounds of 6-24-12 at planting. The beets were thinned to 120 per 100 feet of

[3] These beets and the yield data at harvest were obtained with the cooperation of Dr. Richard C. Zielke.

row, four weeks after planting. They were harvested on September 1, October 1, and November 1.

Additional beets of the three varieties shown below were harvested on November 10, 1968, from the breeding nursery at the Michigan Agricultural Experiment Station.

Variety number	Variety
5	US H20
6	SP 6721-01 ms
7	SL129 × UI4661 × UI4661 ms

After washing with high pressure cold water, the pooled field samples were sorted by specific gravity (Dexter, *et al.*, 1969). After sorting, the beets were rinsed to remove any adhering sodium chloride and, after surface drying, were made into 10 beet samples of uniform weight for storage or immediate analysis. All samples were stored in sealed 10" × 14" × 28" polyethylene bags. Each sample bag contained a one-pint perforated polyethylene bag containing wet wood chips to saturate the atmosphere and thus prevent wilting. All treatments except temperature were replicated four times.

At the time of each analysis, clarified juice was prepared by the method of Dexter *et al.*, (1967). The concentration of raffinose, reducing sugars, and amino acids in the clear juice were determined using galactose oxidase, 3.5 dinitrosalicylic acid, and ninhydrin, respectively, as reported previously (Dexter *et al.*, 1969; Dexter *et al.*, 1970a).

Results and Discussion

Raffinose

The raffinose content of beets during storage was very temperature sensitive. In 1967 variety 5 was stored at four temperatures ranging from 2 to 12.8 C for 35, 70, 105, and 145 days. At 4.5 C the raffinose content remained essentially constant and declined at all higher temperatures (Figure 1). To avoid raffinose accumulation, the optimum storage temperature for this variety appeared to be slightly above 4 C.

Five varieties were stored in 1967 to determine the variety effect on raffinose. At harvest there was a small but significant difference between varieties (Figure 2); however, after 65 days of storage at 3 C, the range between varieties increased to 1400 mg/kg. Varieties 2 and 4 accumulated considerably higher levels in the first 65 days of storage than the other three varieties. Raffinose declined in the last 65-day period for all varieties except variety 3 in which the raffinose increased linearly over the entire storage period.

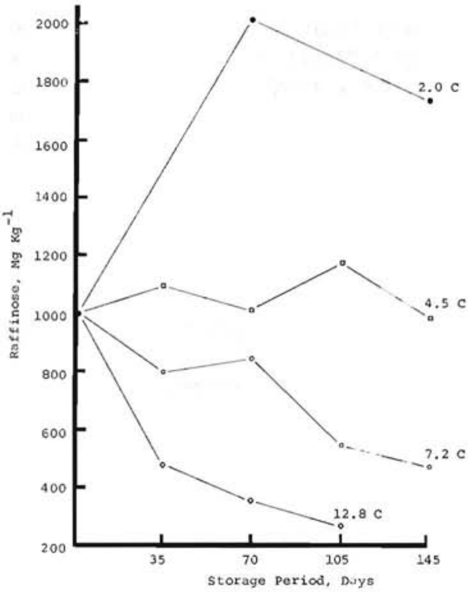

Figure 1.—Effect of storage temperature on the raffinose content of variety 5 stored up to 145 days in 1967.

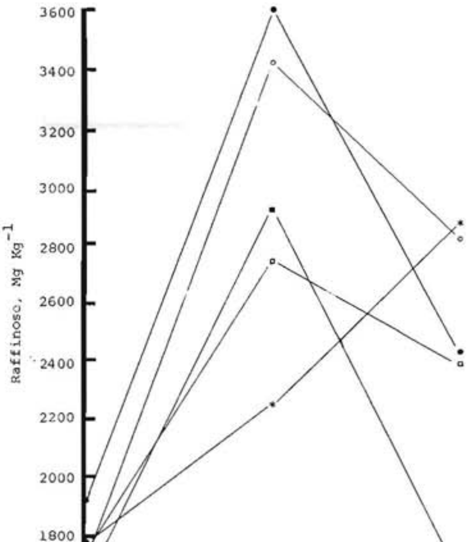

Figure 2.—The raffinose content of five varieties during storage at 3 C in 1967. All beets were harvested on Oct. 26. Variety: 1, □; 2, O; 3, ◆; 4, ●; 5, ★.

Raffinose was very high at harvest in 1967, apparently as a result of cool, damp weather during October. Prior to harvest on November 6, a severe frost caused visible freezing injury to some of the beet crowns. The average raffinose content of three varieties (2,3,5) increased 35% from 1300 to 1850 mg/kg between the October 6 and the November 6 harvests (Figure 3). During storage at 3 C beets from all harvests reached approximately the same level after 65 days.

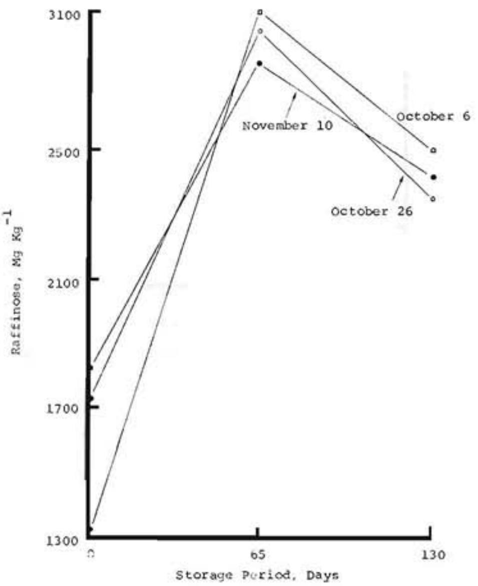

Figure 3.—The effect of harvest date on the accumulation of raffinose during storage at 3 C in 1967. The data for each harvest date is the average of varieties 2, 3 and 5.

The very high raffinose levels at harvest and during storage in 1967 caused a considerable error in the percent of sucrose and in the CJP determinations resulting in an overestimation of the recoverable sucrose per ton (RSPT). These results indicated the need for correcting the percent sucrose and CJP determinations of stored beets, and of harvested beets for some years (Dexter, *et al.,* 1969b).

Nitrogen fertilization had no effect on the raffinose content of the roots either at harvest or after storage (Table 1).

The effect of harvest date was again studied in 1968. The weather during the fall was warm and dry resulting in a lower raffinose content at harvest compared to the previous year (Figure 4). However, the raffinose content attained in storage was comparable in the two years. As in 1967 the less mature beets showed the greatest increase in raffinose during storage. The gen-

Table 1.—Nitrogen × removal interaction for raffinose accumulation in storage in 1967. Data are averages of 3 varieties and three harvest dates.

Nitrogen	At harvest	Storage period, days		Average
		65	130	
lbs/A		Mg Kg^{-1}		
24	1749	3017	2127	2298 ns
150	1778	2954	2675	2469 ns

ns—non-significant at the 5% level

eral decline with prolonged storage observed in 1967 occurred only in the case of the late harvested beets in 1968.

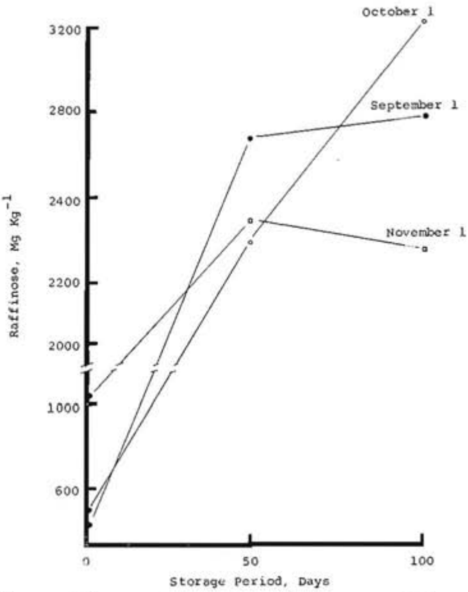

Figure 4.—Effect of harvest date on the accumulation of raffinose in variety 5 during storage at 3 C in 1968.

Three varieties were stored in 1968 at temperatures above and below the presumed threshold level of 4.5 C (based on variety 5, 1967; Figure 1) for raffinose production. Varieties 6 and 7 in 3 C storage increased 2800 and 3200 mg/kg respectively while variety 5 increased only 1600 mg/kg (Table 2). This correlates well with the varietal differences found in 1967.

Table 2.—Influence of temperature in the production of raffinose by three varieties during storage for 100 days at 3 and 10 C (1968).

Variety	At harvest	Storage temperature, C	
		3	10
		Mg Kg^{-1}	
5	1286	2966	890
6	2212	5014	2700
7	1694	4865	1798

LSD $_{.05}$ among means—281 Mg Kg^{-1}

The raffinose content of variety 5 at 10 C declined while varieties 6 and 7 both increased. The response of variety 5 was consistent with the 1967 experiment which gave a threshold of 4.5° for this variety. In this experiment the threshold for variety 5 may have been a few degrees higher than in 1967 since the raffinose content did not decline to the low levels found for the 7-12 C temperature range in the previous year. However, the threshold temperature appeared to be a rather consistent varietal characteristic. Varieties 6 and 7 had a much higher threshold temperature, possibly as high as 15 C for variety 6. The very high levels of raffinose accumulated in storage would make varieties 6 and 7 questionable candidates for commercial use.

The theoretical sucrose loss resulting from the production of raffinose is not as great as that for reducing sugars but is still substantial. One pound of raffinose is derived from 1.36 pounds of sucrose, assuming the galactose is produced originally from sucrose. This pound of raffinose will also carry 1.67 pounds of sucrose into the molasses, assuming a 62.5% molasses purity, for a total loss of 3 pounds.

Reducing Sugar

In 1967 beets of variety 5 were stored at three temperatures (4.5, 7.2, and 12.8) for up to 145 days. In the first 70 days of storage there was no significant change in the reducing sugar content at the three temperatures (Figure 5). Beets stored at 12.8 C began to sprout after 70 days, and this may account for the rapid increase in reducing sugars. Beets stored at 4.5 C had some small spots of mold which developed in the cut surface of the crown area as a result of excessive moisture condensation. This would account for the rapid rise in reducing sugars after 105 days of storage. The beets stored at 7.2 C remained in

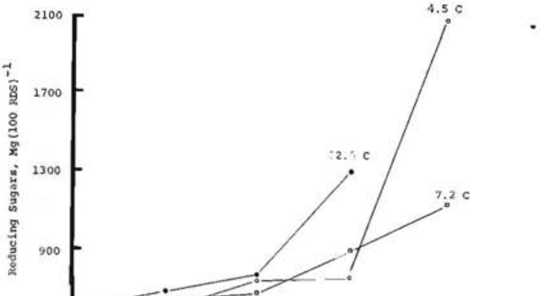

Figure 5.—Effect of temperature on the accumulation of reducing sugars in beets stored for up to 145 days in 1967.

excellent condition with neither mold nor sprouting, and the increase in reducing sugars was correspondingly small.

Five varieties stored at 3 C for 65 and 130 days in 1967 showed substantial accumulations of reducing sugars in only 65 days (Table 3). However, the occurence of small areas of mold was common in the cut area of the crown around the crown cavity. Although these areas were very small, they were extremely high in reducing sugars and thus increased the average reducing sugar content of the entire root. Although substantial differences were found between varieties, the variability introduced into the experiment by the small amounts of mold made it impossible to draw conclusions concerning varietal characteristics.

Table 3.—Accumulation of reducing sugar in five varieties stored for 65 and 130 days at 3 C in 1967.

Variety	At harvest	Storage period, days	
		65	130
		Mg Kg^{-1}	
1	660	1040	3711
2	869	1513	2649
3	641	799	2736
4	656	1144	2607
5	783	2394	2260
Ave.	721	1378	2793

LSD.05 for removal means—819 Mg Kg^{-1}

The reducing sugar content of the beets decreased slightly during the 1967 harvest period. During storage, the increase in reducing sugars was highly significant (Table 4). The occurrence of very high levels of reducing sugar after 130 days reflects the mold situation discussed previously. In general the beets harvested early, although higher in reducing sugars at harvest, accumulated reducing sugars only moderately in storage at 3 C. The same trend was observed in 1968 when no mold developed in storage (Figure 6).

Table 4.—Harvest × removal interaction for reducing sugar accumulation (1967) for beets stored at 3 C. Data are the averages of 3 varieties and 2 N levels.

Harvest date	At harvest	Storage period, days	
		65	130
		Mg Kg^{-1}	
October 6	771	944	1175
October 26	765	1203	2549
November 6	695	1084	2994

LSD.05 among means—303 Mg Kg^{-1}

Nitrogen fertilization had no effect on the reducing sugar content of the roots at harvest or after storage.

A highly significant variety-temperature interaction for reducing sugar accumulation occurred in 1968 when samples of

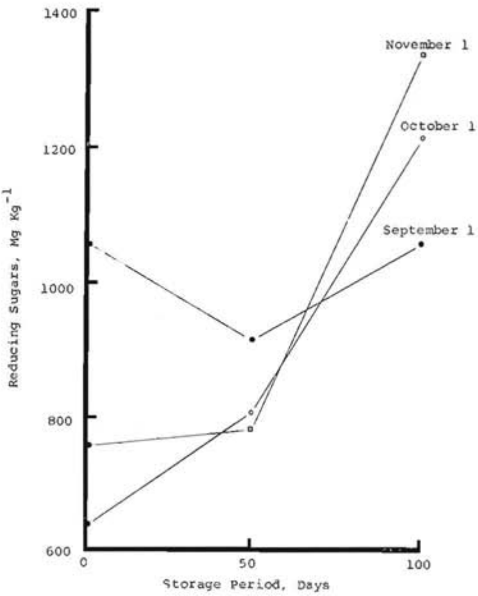

Figure 6.—Effect of harvest date on the accumulation of reducing sugars in variety 5 during storage at 3 C in 1968.

three varieties were stored at 3 and 10 C (Table 5). No molding occurred at either temperature. The accumulation of reducing sugars was very small for all varieties stored at 3 C, but they increased substantially in all varieties at 10 C. The sensitivity of the varieties to 10 C storage was different. Variety 5 doubled in concentration of reducing sugar, whereas variety 7 increased only 35%.

Table 5.—Interaction of variety with temperature in the accumulation of reducing sugars after 100 days of storage at 3 and 10 C (1968).

Variety	At harvest	Storage temperature, C	
		3	10
		Mg Kg-1	
5	656	658	1297
6	916	1095	1530
7	831	965	1115
Average	801	906	1314

$LSD_{.05}$ among means—227 Mg Kg^{-1}

The high levels of reducing sugar in the stored beet appeared to be largely the result of mold. Temperature effects were primarily manifested as secondary through sprouting and mold invasion. However, the results in Table 5 indicate that variety may be important when mold is eliminated. Variety 5 remained low in reducing sugars both in 1967 and 1968 when molds were absent (See Figure 5 and Table 5). It may be possible to select varieties which can tolerate storage above 5 C without a significant production of reducing sugars in the absence of molding.

Reducing sugars, in the absence of molds, can be produced by two enzymes, invertase or sucrose synthetase. Invertase activity in the beet is normally very low, but the activity is sufficient to support respiration (Wyse, 1969). Sucrose synthetase in the root is also capable of producing reducing sugars from sucrose. Sucrose synthetase has a K_{eq} of approximately 1.3 which indicates that it is easily reversible and may be an important source of sugar nucleotides and reducing sugars in the root (Milner, 1964; Wyse, 1970).

Invert sugars are degraded to acids during lime defecation and as a result, are particularly important in the study of storage losses. Many of these acids have soluble calcium salts which require neutralization with sodium carbonate. If sufficient sodium carbonate is added to neutralize all the acid produced from one pound of invert, 4 pounds of sucrose will be lost. This is a result of a 1 lb loss by inversion and a 3 lb loss to molasses resulting from sodium addition (Dexter, *et al.*, 1970b).

Amino Acids

Beets grown with 24 lb/A of applied nitrogen became nitrogen deficient (light green leaf color) several weeks prior to the October 6 harvest in 1967. Plants which received high nitrogen, 150 lb/A, were still succulent and green at the first harvest date, October 6. The free amino acid content of the roots of plants on low nitrogen decreased almost linearly during the harvest period (Figure 7, dotted lines). The free amino

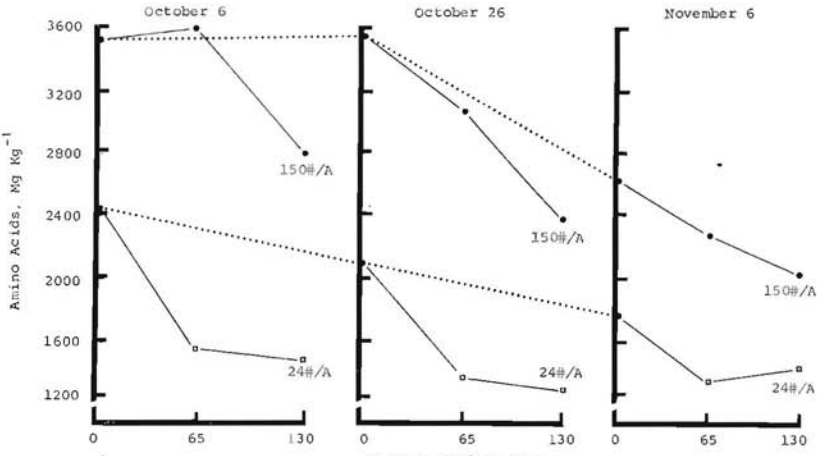

Figure 7.—Harvest × nitrogen × removal interaction for amino acid content in beets stored at 3 C for 65 and 130 days (average of varieties 2, 3 and 5).

acid pool in the root was apparently being depleted as a result of the nitrogen deficiency. Beets grown on high nitrogen remained high in free amino acids until October 26 after which they declined rapidly. On November 6 the beets grown with high nitrogen had the same amino acid content as the beets grown with low nitrogen and harvested on October 6.

The amino acid content declined in storage at 3 C. This has been reported previously (Dexter *et al.*, 1966; Silin, 1964) and is apparently a result of the synthesis of new enzymes and other proteins. The beets grown with low nitrogen declined rapidly in amino acid content and then appeared to reach an equilibrium level at 1200-1500 mg/kg. The beets with high nitrogen declined almost linearly in amino acids throughout the storage period. The five varieties stored for 65 and 130 days showed neither a significant variety-storage nor a variety-nitrogen-storage interaction. However, the variety-nitrogen interaction at harvest was highly significant (Table 6). On the average, the increased application of nitrogen caused a near doubling of the amino acid content in the root. Variety 5 appeared to be highly sensitive to nitrogen fertilization since its amino acid content increased the most with the higher nitrogen applications. This sensitively was also apparent in reduced RSPA with high levels of applied nitrogen (Wyse, *et al.*, 1970).

Table 6.—Variety × nitrogen interaction in amino acid content of five varieties harvested on Oct. 26, 1967.

Variety	Nitrogen applied, lbs/A	
	24	150
	Mg Kg⁻¹	
1	1643	2153
2	1454	2493
3	1609	3036
4	1322	2443
5	1541	3564
Average	1514	2783

LSD.05 among means—355 Mg Kg⁻¹

In 1968 three varieties stored at 3 and 10 C reacted very differently to temperature in terms of their amino acid content (Table 7). Variety 6 was extremely high in amino acids at harvest and accumulated substantial quantities at both storage temperatures. The high level of amino acids in this variety would apparently make it undesirable for commercial use. At 3 C variety 5 declined as it did in previous experiments (Dexter, *et al.*, 1966). However, the amino acid content of variety 5 was very sensitive to high temperature storage. Variety 7 was low in amino acids at harvest and did not accumulate significantly at either temperature.

Table 7.—The effect of storage temperature on the amino acid content of beets stored for 100 days in 1968.

Variety	Harvest	Storage temperature, C	
		3	10
		Mg Kg[-1]	
5	1241	1128 (113)[a]	1705 (+464)[a]
6	2267	2567 (+300)	2749 (+482)
7	808	864 (+56)	969 (+161)
Average	1439	1520	2117

[a] Parentheses indicate change during storage.
$LSD_{.05}$ among means—388 Mg Kg[-1]

Summary

The accumulation of raffinose in sugarbeet roots during growth and storage was primarily a function of temperature, increasing with lowered temperature either in the field or during storage. The threshold temperature for raffinose accumulation in roots during storage was approximately 5 C, but this varied somewhat between years and greatly between varieties. Beets harvested in September and early October were lower in raffinose content than beets harvested late, but the beets harvested early accumulated more raffinose in storage. Nitrogen fertilization had no effect on raffinose content at harvest or after storage.

Reducing sugar accumulation in roots was accentuated by mold. However, the variety-temperature interaction for reducing sugar accumulation was highly significant when mold and sprouting were eliminated. In the absence of mold, reducing sugar increased more in higher temperature (3 vs 10 C) storage.

The amino acid content of the beets was primarily a function of applied nitrogen. The variety-nitrogen interaction was highly significant at harvest but not after storage. Most varieties stored at 3 C generally declined in free amino acids. All varieties accumulated approximately 30% more amino acids when stored at 10 C than when stored at 3 C.

Varietal consistency from year to year could not be demonstrated conclusively because only one variety was studied in more than one year. However, this variety did show very consistent trends for all three major impurities.

With the exception of the amino acid content, less than ideal storage practices were found to have a much greater effect on the chemical composition of beets than did agronomic practices. In storing beets at various temperatures, varieties differed considerably with respect to the level of raffinose, reducing sugars, and amino acids in the clarified juice. Since these compounds make up the major proportion of the non-sucrose constituents of the clear juice, it would appear that the level of these impurities could be controlled through variety improvement and

proper agronomic and storage practices. The development of a variety which would tolerate storage temperatures in the 7 - 10 C range without the accumulation of reducing sugars or amino acids, assuming that sprouting is prevented, appears to be feasible and would greatly reduce the problem of storage temperature control.

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