STORAGE OF SUGARBEETS: AGRONOMIC, PHYSIOLOGICAL AND QUALITY ASPECTS

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ABSTRACT

STORAGE OF SUGARBEETS: AGRONOMIC, PHYSIOLOGICAL AND QUALITY ASPECTS

By

Roger E. Wyse

A study was made of changes in the composition and integrity of beets during storage and to develop methods for evaluating beets for desirable storage and processing characteristics.

Experiments were conducted in 1967 and 1968 to determine the influence of nitrogen fertilization, harvest date, topping and preharvest sprays on recoverable sucrose losses in storage. The factors affecting sucrose losses included those related to the direct loss of sucrose (respiration and sugar conversions) and those affecting sucrose losses in the factory (clear juice purity, acid-base balance, melassigenic impurities). Samples were analyzed for percent sucrose, clear juice purity, raffinose, reducing sugar, amino acid, sodium, potassium and chloride. A study was also made of marc stability in storage. Included in the storage practices were temperatures, desiccation and modified atmospheres including carbon monoxide inhibition of respiration.

Methods are presented for the rapid determination of raffinose and reducing sugars and a technique for producing uniform storage samples by specific gravity sorting. This technique for sample preparation increased sample uniformity 400 percent.

Raffinose, reducing sugars, amino acids, sodium, potassium and chloride accounted for approximately 65 percent of the total impurities at harvest and in beets stored below 4 C. Changes in raffinose, reducing sugars and amino acids accounted for a large proportion of the changes in total impurities in storage below 4 C. Above 4 C other impurities not determined in this study accumulated.

A formula was derived to correct the polarimetric sucrose determination for the optical activity of raffinose and invert. The effect of major impurities accumulating during storage on sucrose losses to molasses are discussed. A relationship is developed whereby the acid-base balance of the clear juice can be used to evaluate stored beets on the basis of loss of recoverable sucrose due to sodium carbonate addition. The recoverable sugar per ton estimate, as modified for stored beets, was used to evaluate the influence of agronomic and storage practices on the loss of sucrose in several sugarbeet varieties during storage.

Slightly over 50 percent of the RSPT losses in storage were due to respiration and sugar conversion. The balance of the losses were due to increased factory losses

resulting from the accumulation of melassigenic substances in the clear juice of stored beets.

Other carbohydrates besides sucrose contributed substantial amounts of substrate for respiration. This was evident since the dry matter loss in storage was considerably greater than the loss of sucrose. Means of reducing respirational losses were studied as were the enzyme systems responsible for sucrose degradation. Respiration in the beet showed extreme sensitivity to regulation by high levels of carbon dioxide and inhibition by carbon monoxide. Three percent carbon monoxide inhibited respiration 20 percent in the intact beets. The enzymatic hydrolysis of sucrose was found to involve two enzymes, one with a pH optimum at 5, the other at pH 7. The significance of these two enzymes on the accumulation of reducing sugars in storage is discussed.

The marc content remained relatively constant during storage at 3 C, if molding and desiccation were prevented. The marc remaining after 20 C extraction declined during storage but the residue after 70 or 80 C extraction was very stable. The 70 C residue actually increased in some cases under ideal storage conditions.

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By

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INTRODUCTION

Sugarbeet acreage and yields, in the past 20 years, have increased greatly without a corresponding increase in daily factory processing capacity. As a result an increasing percentage of each year's crop must be stored for considerable time prior to processing. In a factory in Michigan no beets were piled between 1946 and 1959 and the processing campaign averaged 45 days. During the next five years enough beets were piled annually to operate the factory an extra 73 days. In 1969 over 75 percent of all beets processed were stored.

Storage losses of extractable sucrose can be divided into two general categories. One is the direct loss of sucrose due to respiration or sugar transformations; the other is the loss incurred during processing. These factory losses are dependent on the physical integrity and chemical composition of the root. Since the majority of beets processed are stored, the storage characteristics of varieties and the effect of storage practices on changes in the chemical composition of the beet have become of utmost importance.

This study investigated factors affecting changes in the composition and integrity of the beet during storage and developed methods for evaluating beets for desirable storage and processing characteristics.

REVIEW OF LITERATURE

In 1924 A. D. Pack wrote a series of articles on the storage of sugarbeets in which he referred to storage work conducted in 1876 to determine the losses incurred.

Loss of Sucrose During Storage

Sugar Losses Due to Respiration

Loss of sucrose by respiration can be substantial particularly at higher storage temperatures (Silin, 1964; McGinnis, 1951).

Barr et al. (1940) determined the loss of sugar by respiration at various temperatures over a 47-day storage period and found that the amount of carbon dioxide evolved accounted for approximately 60 percent of the total apparent sucrose losses. The percentage of carbon dioxide evolved varied with temperature.

Stout and Smith (1950) stored beets in large drums at 20 C for 45 days and found approximately 80 percent of the sucrose loss was due to respiration and 20 percent to inversion.

The loss of sucrose by respiration has been estimated at between 0.3 and 0.5 pounds per ton per day at 21 C (Stout and Spikes, 1957). The early work of Barr et al.

(1940) indicated a very strong temperature effect where losses varied from 0.1 pound per ton per day at 3 C to 1.8 pound per ton per day at 35 C.

Dilley et al. (1969) found the respiration Q_{10} to be approximately 2 between 10 and 20 C. In the first few days after harvest the respiration rate was high but declined steadily after a few days in storage to a much lower and essentially constant rate. Bruising or other injury drastically increased the respiratory losses in the period immediately following harvest (Dilley et al., 1969; Stout and Smith, 1950) and also increased the susceptibility to mold invasion.

The rate of respiration of the sugarbeet is closely correlated to its surface area. This phenomenon was first observed by Stout (1954) and more recently a mathematical relationship has been developed to relate surface area to respiration rate (Vajna, 1960).

^{14}C labeled sugars introduced into harvested beet roots have shown that sucrose, in short term storage, is almost exclusively the substrate for respiration in the sugarbeet (Barbour and Wang, 1961; Wang and Barbour, 1961).

Several studies have been made to reduce respiration losses by modifying storage atmospheres. Reductions in storage losses of up to 65 percent have been found (Stout, 1954; Vajna, 1960). Oxygen levels below 5 percent and carbon dioxide above 15 percent were found detrimental (Vajna, 1960).

Indirect--Factory Losses

The decrease in recoverable sucrose during storage is not solely a result of sucrose lost as CO_2, but is also due to an increase in impurities in the factory thin juice (McGinnis, 1951; Silin, 1964; Carruthers et al., 1962; Dexter et al., 1965). Factory losses incurred in processing the sugarbeet can be predicted by using the purity of the clear juice in conjunction with a formula derived by the Great Western Sugar Company. From this formula a 1 percent loss in clear juice purity will result in approximately a 6 pound or 2 percent loss in recoverable sugar per ton of beets.

In an extensive examination of compositional changes in diffusion juice from stored beets, Walker et al. (1960) found a decrease in purity from 92.2 percent to 87.5 percent in 90 days at 10 C. The apparent impurities calculated from thin juice purity increased from 8,500 to 14,700 mg per 100 gms sugar. The only significant compositional change found was an increase in invert at the expense of sucrose with no loss in total sugars. The 6042 mg per 100 gm sugar increase in total impurities was approximately equal to the 6200 mg per 100 gms sugar increase in invert.

Carruthers et al. (1962) derived the relationship of $(3.5 \times Na) + (2.5 \times K) + (10 \times NH_2)$ which accounted for a high percentage of the total impurities in fresh beets. Dexter et al. (1966) using Carruther's factors for Na, K,

and αNH_2 in the clear juice of stored beets found that as the apparent impurities increased particularly at warm temperatures the percent of impurities unaccounted for by Na, K, and αNH_2-N increased sharply. If this relationship is applied to the data presented by Walker the same trend is noted. Dexter proposed that these unaccounted-for impurities may be weak acids with soluble calcium salts formed in the decomposition of sugars and/or cell wall constituents.

Loss of Physical Integrity During Storage

One of the major problems in processing stored beets is the decrease in the filtration rate of limed diffusion juice which drastically reduces factory efficiency. The problem is caused by the formation of calcium pectate gels as a result of the solubilizing of cell wall pectin due to poor storage conditions (McGinnis, 1951; Silin, 1964).

Breakdown of Marc

Sugarbeet marc is the insoluble residue which remains after extraction of the beet with water (McGinnis, 1951). The marc is composed primarily of cellulose (~25%), hemicellulose (~25%), and pectic substances (~25%) (McCready, 1966). The pectic substances are stable only in cold water and swell and become soluble in hot water (Silin, 1964).

The marc averages approximately 5 percent of the fresh weight of the beet (Silin, 1964). In a test of 15

varieties, Owens et al. (1954) found the marc content to vary from 3 to 6 percent.

Pectic Substances as Sources of Impurities

The pectic substances are high molecular weight polymers, derivatives of pectic acid, and contain galacturonic acid residues. In pectic acid, the carboxyls are free and can readily react with calcium to form insoluble calcium pectate. In pectin and protopectin the carboxyls are esterified with methanol (Joslyn, 1962). In the sugarbeet the free carboxyls may be as high as 50 percent methylated (Goodban and McCready, 1965). From 6 percent (Kertesz, 1951) to 30 percent (Goodban and McCready, 1965) of the hydroxyls in the two and three positions may be acetylated.

The pectic substances exist in two forms: water soluble and water insoluble. The water insoluble form is protopectin, the structure of which is unknown, but which is thought to exist in a matrix complex of pectin, cellulose, and hemicellulose. Protopectin upon hydrolysis yields the water soluble pectic acid (Kertesz, 1951 and 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 and Owen et al., 1955). Silin (1931) extracted dried beet pulp and found only a slight increase in solubility up to 80 C., however, the amount of pectin extracted at 90 C was 30 times greater than at 80 C.

The soluble forms of the pectic substances are pectin, pectic and pectinic acids. Pectic and pectinic acids have insoluble calcium salts, but are highly esterified and yield methanol and soluble acetic acid salts when heated in alkaline solution (Kertesz, 1951).

Changes in the Pectic Substances During Storage

The soluble pectins after an initial increase in the early stages of growth, decrease steadily until harvest. The amount of total pectic substances decreased under desiccation. Fertilizer has no significant effect (Gaponenkov, 1943).

Silin (1964) reports that microbial activity and sprouting increase the quantity of soluble pectins during storage. Walker et al. (1960) found no significant change in the pectin content 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 during storage the pectin, which in a normal healthy beet is a large macromolecule, will be degraded by enzyme action to a smaller and more soluble colloidal size (Claassen, 1943).

Effect of Pectic Substances on Processing

The first noticeable effect of pectic substances in the factory is in the diffuser. Beets which have been exposed to conditions which weaken the protopectin stability must be diffused at lower temperatures to prevent the cossettes from losing their resilience. Loss of resilience will prevent the free flow of water through the diffuser. For frozen or spoiled beets the diffuser temperature should not be above 70 to 75 C. Silin (1964) found a 110 percent increase in pectic substances in diffusion juices by increasing the temperature from 75.5 to 85.1 C.

During defecation the pectinic acid present in the diffusion juice is de-esterified, splitting off methanol and acetic acid. The acetic acid forms soluble calcium acetate and the polygalacturonic acid residue forms an insoluble calcium pectate (Silin, 1964; Goodban and McCready, 1965).

Non-Sucrose Components of the Beet Root

Reducing Sugars

The predominant reducing sugars occurring 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 low amounts in beet molasses (McGinnis, 1951; Silin, 1964). In the process of alkali decomposition the reducing sugars from stable clear juices 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 prevent sucrose inversion (Silin, 1964; McGinnis, 1951). As a result of sodium addition molasses quantity and purity are increased resulting in increased sucrose losses (Silin, 1963; McCready, 1969).

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

In a beet devoid of microbial activity the degradation of sucrose to reducing sugars can occur via the action of two enzymes: invertase and the reversal of sucrose synthetase (Milner and Aulgad, 1964). The invertase activity of the beet root is very low and has been considered absent by many workers (Vaughn and MacDonald, 1967b). Invertase activity has been reported to increase during storage (Khelemskii, 1963), but no correlation between invertase

activity, respiration and reducing sugar accumulation was made.

The existence of a sucrose synthesizing enzyme in the beet root was first discovered by Dutton et al. (1961). In the recent work of Avigad (1966) the possible role of sucrose synthetase in the breakdown of sucrose in the root was studied. The K_{eq} for sucrose synthetase is 1.3 $\pm$ 0.2 at pH 7.2 and estimated to be 0.2 - 0.5 at pH 6.0 and is therefore easily reversible. The sucrose cleavage activity in root homogenates by sucrose synthetase was found to be much greater than that by invertase. Therefore sucrose synthetase is capable of playing an important role in the formation of reducing sugars in storage (Avigad, 1966).

Raffinose

The major trisaccharide occurring in the sugarbeet is raffinose which usually occurs as 0.3 to 0.5 percent of the sucrose present (McGinnis, 1951). The amount in beets 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. Since raffinose forms insoluble calcium saccharates as does sucrose, it causes problems by greatly increasing the raffinose content of Steffen molasses. A high raffinose content in the thick juice produces distorted and elongated sucrose crystals (McGinnis, 1951).

The level of raffinose, occurring naturally in the beet and the degree to which it accumulates during growth and storage, is a varietal characteristic. Several populations of mass selected beets were shown by Wood et al. (1956) to vary over a 10 fold range in raffinose content. Five varieties stored for 29 weeks at 4 C showed a significant variety x 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 times 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 declined almost to their harvest level after 25 days (McCready and Goodwin, 1966).

Atterson et al. (1963) found that not only temperature but also wilting affected the raffinose content of the beet root. Raffinose decreased markedly in beets stored under conditions permitting weight loss.

The biochemical pathway of raffinose synthesis and degradation in the sugarbeet has not been elucidated. Pridham and Hassid (1965) found that raffinose is synthesized in the broad bean (Vicia faba) by the transfer of UDP-galactose onto sucrose. Other workers found transgalactosidase activity between raffinose and sucrose in

wheat germ which did not require nucleotides as coenzymes. More recently Avigad (1968) found UDP-glucose-4-epimerase activity in sugarbeet root. Since sucrose degradation may occur via sucrose synthetase to yield UDP-glucose this enzyme may have particular significance in the elucidation of the synthetic pathway in the beet.

Effect of Agronomic Practices on Beet Root Composition and Storage Characteristics

Nitrogen Fertilization

The early work of Ulrich in the 1950's (Ulrich, 1954; Ulrich, 1955) on factors controlling the accumulation of sucrose in the beet root pointed out the very dominant effect of nitrogen fertilization. Sucrose accumulation in the root is inhibited by high levels of nitrogen (Haddock et al., 1959; Odgen et al., 1958; Haddock et al., 1959; Rounds et al, 1958; Haddock et al., 1956; Finkner et al, 1958; Finkner et al., 1964). This effect appears to be partially due to the decreased transport of sucrose from the leaf to the root. This is no doubt a result of continued vegetative growth late into the fall harvest season under high nitrogen regimes (Snyder and Tolbert, 1966).

Excessive nitrogen fertilization also causes an increased accumulation of non-sucrose substances resulting in a decreased purity (Henry et al., 1961; Haddock et al., 1956). 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).

Nitrogen not only increases the glutamic acid content of the beet but all of the other nitrogen containing components. Although a significant variety x nitrogen interaction is commonly found the nitrogen effect is by far the most dominant (Finkner et al., 1958; Henry et al., 1961).

Excessive nitrogen fertilization also increases the ash content primarily sodium, calcium, and to a lesser extent potassium (Henry et al., 1961; Finkner et al., 1958). Nitrogen fertilization has little effect on the raffinose or galactinol content (Rounds et al., 1958; Finkner et al., 1958). Due to the strong melassignic properties of sodium, potassium, and the amino acids excessive nitrogen fertilization is highly detrimental in terms of beet quality for processing (Rounds et al., 1958).

Because of the increased proportion of stored beets to the total beets processed, the effect of nitrogen fertilization on storage losses was studied by Dexter et al. (1966). In general their results indicated that the higher quality, low-nitrogen beets had superior storing characteristics since the loss of extractable sugar per ton was 30 percent less than for high nitrogen beets.

Phosphorus and Potassium Fertilization

Studies to determine the effect of potassium and phosphorus have indicated little or no effect on beet quality when rates are increased above adequate levels (Finkner et al., 1964; Finkner et al., 1958; Henry et al., 1961). Larmer (1937) noted that increased levels of phosphorus improved storage characteristics.

Harvest Date

Due to the rapid accumulation of sucrose in the root during the fall, delaying harvest will normally increase the RSPA* yield. However the cool temperatures during this period tend to promote the accumulation of raffinose. The amount of raffinose accumulated is related to the average daily temperature, incidence of frost and the genetic make-up of the variety (Finkner et al., 1959; Wood et al., 1956).

Beets harvested early lose less sucrose in storage than late harvested beets (Vajna, 1960). However, the soluble pectin content is higher (Silin, 1964).

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*See Appendix A for explanation of abbreviations.

MATERIALS AND METHODS

1966 Preliminary Experiments

In 1966, samples containing 10 beets each and weighing 16-20 pounds were stored either for 70 or 140 days at either 3 C or 11 C. Various degrees of desiccation were allowed to develop by storing the beets in single and double canvas bags and in polyethylene bags containing small perforated bags of wet wood shavings. These treatments were then designated as severe, moderate, and slight wilt, respectively. The clear juice from the beets in this experiment was used to develop rapid methods of raffinose and invert determinations to be used in subsequent studies.

1967 Experiments

The beets for the storage experiments in 1967 were grown near Sebewaing, Michigan. 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 in plots 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 sucrose, 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	(SL (129 x 133) ms x SP6322-0)	Hybrid Monogerm	Excellent

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

1968 Experiments

Beets for the 1968 experiments were grown near St. Charles, Michigan and received 500 pounds of 6-24-12 at planting. The beets were thinned to a 120 beets per 100 feet of row four weeks after planting. The variety used was the current commercial (SL (129 x 133) ms x SP6322-0). At harvest, all field replications were pooled for analysis.

Specific Gravity Sorting and Sample Preparation

After washing with high pressure cold water, the pooled samples were sorted by specific gravity (Dexter, Frakes, Wyse, 1969). The average specific gravity of beets from each field treatment (i.e., variety 1--high nitrogen) was determined by weighing a representative portion (40 pounds) in air and in water. Two salt solutions were then prepared for each field treatment one of which was 0.003 specific gravity units above the average and another 0.003 units below. This range corresponded to approximately 1 percent sucrose on beets. Thirty beets were then added to the tank containing the highest specific gravity. Those beets which floated were transferred to the second tank containing the lower specific gravity. The concentration of the two tanks was then adjusted so that twenty beets floated in the first tank and ten in the second. The beets which sank in the second tank were used for storage experiments. This method eliminated the very high and very low quality beets and increased sample uniformity 400 percent.

After sorting, the beets were rinsed to remove any adhering sodium chloride and after surface drying were made into samples of uniform weight for storage or immediate analysis.

All storage samples except those in the desiccation experiments were stored in 10" x 14" x 28" polyethylene bags. Each sample bag contained a one pint perforated polyethylene

bag containing wet wood chips to saturate the atmosphere and prevent wilting. In 1967 the wood chips were too wet and as a result free water formed on the beet surfaces causing some mold with prolonged storage. This problem was alleviated in 1968 by draining the chips before adding to the sample bags. Using this storage technique the loss in weight was less than 2 percent in 140-day storage (Figure 1). The concentration of oxygen and carbon dioxide inside the bags was measured by extracting a sample with a syringe and analyzing on a Perkin-Elmer Vapor-fractionator. The average oxygen content was 19.6 percent and the carbon dioxide ranged between 0.8 and 1 percent.

Outline of Major Storage Experiments

1967 Date of Harvest

Objectives:

1. To determine the effect of harvest date on storing ability with primary emphasis on marc breakdown during storage.
2. To determine the changes in the non-sucrose components during the harvest period and their subsequent effect later in storage.
3. To determine the differences between varieties in both their physical and chemical response to storage.

Procedure:

Three varieties of beets were harvested on October 6, October 26, and November 6. Ten beet samples, weighing 20 to 28 pounds, of three varieties (No's. 2, 3 and 5) grown at

Figure 1. Internal and external views of a beet stored for 140 days at 3 C.

two nitrogen levels were washed, sorted and stored for 65 and 130 days at 3 C. All storage treatments were replicated three times.

1967 Topping and Row Width

Objectives:

1. To determine the effect of harvest quality on losses in recoverable sugar in storage.
2. To determine if the crown affected the accumulation of non-sucrose constituents in storage.

Beets grown in blocks of 14 and 28 inch rows were topped either at the lowest leaf scar or by simply trimming off the terminal crown bud. Samples were stored for 100 days at 3 and 7 C.

Desiccation Experiment

Objectives:

1. To separate the effects of desiccation and temperature on losses of RSPT.
2. To determine the temperature at which specific sugar transformations occur.

Three levels of shrink were maintained by storing beets in a single canvas bag, a polyethylene bag and a polyethylene bag with wet chips added. All treatments were replicated three times and stored at 2, 4.5, 7.2, and 12.8 C for 35, 70, 105 and 140 days.

1967 Variety

Objective: To study the storage response of a wide spectrum of varieties.

Five varieties (see page 17) grown with 24 and 150 pounds of applied nitrogen were harvested on October 26. All samples were treated as in the 1967 date of harvest experiment. Storage was for either 65 or 130 days at 3 C.

1968 Date of Harvest

Objective: To confirm the results of the 1967 date of harvest experiment.

The commercial variety (SL (129 x 133) ms x SP6322-0) was harvested on September 1, October 1, and November 1 from a uniform stand in a field near Saginaw, Michigan. Four replications were stored for either 50 or 100 days at 3 C for each harvest date.

1968 Variety and Temperature

Objective: To determine the variety x temperature interaction with particular emphasis on sugar transformation.

Three varieties were harvested on November 10 from the sugarbeet breeding nursery at the Michigan State University Experiment Station.

Number	Variety
5	(SL (129 x 133) ms x SP6322-0)
6	SP6721-01 ms
7	129 x 4661 x 4661

Ten beet samples of each variety were stored for 100 days at either 3 C or 10 C. All treatments except temperature were replicated three times.

1968 Field Sprays

Objective: To determine the effect of several pre-harvest foliar sprays on the loss of RSPT in storage.

Preharvest foliar applications of vanadium sulfate (3#/acre), pyrocatechol (3#/acre) and CCC (2-chloroethyl-trimethylammonium-chloride at 3000 ppm) were applied to run off with a hand sprayer ten days prior to harvest. The rates of application of vanadium sulfate and pyrocatechol were those recommended by Dr. D. J. Wort (1968). No rain occurred between the time of application and harvest but the weather was cool (40-50 F) for most of the ten day period. Some leaf burn was noted on the plants treated with vanadium sulfate. Other treatments produced no visible effects. Samples were stored for either 50 or 100 days at 3 C with three replications.

1968 Vernalization

Objective: To determine if cold induction has an effect on invert accumulation and on the rate of respiration in storage.

MH-30 and GA_3 were applied ten days prior to harvest at concentrations of 3000 ppm. Chemicals were applied with a hand sprayer to "run-off." The crowns were trimmed at harvest to remove all petioles and the beets were stored at 5 C. Respiration rates were determined at weekly intervals

on three replications of each treatment. The chemical composition of a second lot of beets from each treatment was analyzed after five, eight and fifteen weeks of storage. These beets were cut on a slight diagonal to prevent crown bud injury and were then planted in sterilized soil in a greenhouse maintained at 18 to 21 C. A sixteen hour day was maintained with florescent lights. The incidence of bolting was recorded after six weeks.

1968 Controlled Atmosphere

Objective: To determine the effect of modified atmospheres on the accumulation of non-sucrose constituents in storage.

Gas mixtures of 5 percent oxygen, 5 percent carbon dioxide, and 90 percent nitrogen plus or minus 1000 ppm ethylene were purchased from Matheson. Each storage treatment consisted of three ten beet samples in separate containers. The gas mixtures were allowed to flow in series through three chambers (the replicate samples of each treatment) at a rate of approximately 1 ft^3/hr. The storage period was 8 weeks at 5 C.

Laboratory Analyses

The percent sucrose was determined by the Dexter, Frakes, and Snyder Method (1967) and the clear juice purity by a modification of the method of Carruthers (1962). The resulting clear juice samples were analyzed for potassium and sodium using a Coleman flame photometer. Total amino

acids were determined with ninhydrin by the method of Moore and Stein (1954). Reducing sugars were determined with 3, 5,-dinitrosalicylic acid (Bernfeld, 1951). A coupled enzyme system was developed for the determination of raffinose (Appendix B).

Dry matter determinations were made by weighing 25 grams of well mixed brei directly into tared weighing bottles and drying at 105 C for 48 hours. 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 appropriate temperature for 20 minutes. After incubation the samples were quantitatively transferred to tared 9 cm plastic Buchner funnels containing a powdered cellulose pad. The residue was then extracted with 25 C distilled water by repeated washing with ten 200 ml aliquots. The filters were dried at 105 C for 48 hours and the percent marc calculated as percent fresh weight.

The weight loss during storage was determined on all samples. All analyses expressed on beet weight were corrected to the original weight at harvest. All sucrose analyses were corrected for raffinose and invert sugar (see page 139 for correction equation).

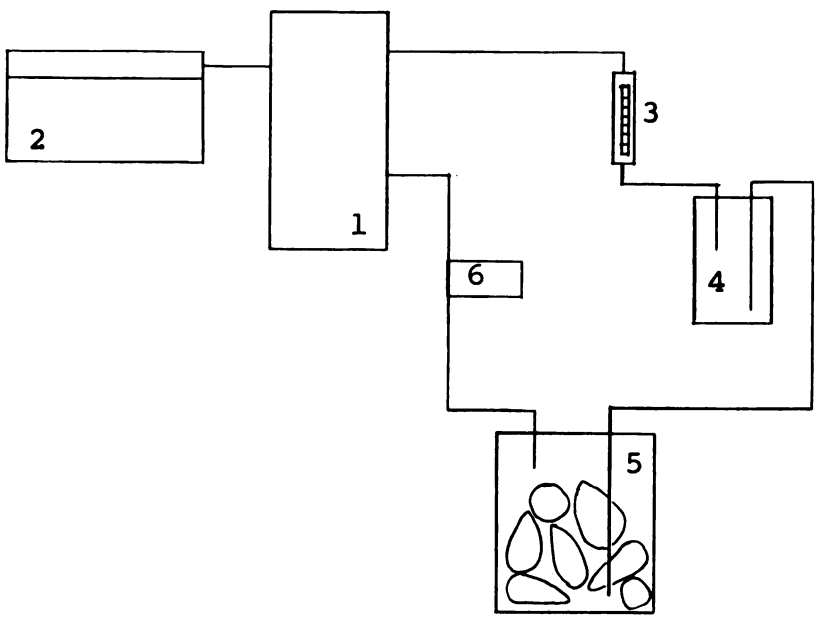
Respiration Analyses

The carbon dioxide evolved was monitored with a Beckman Model IR-2 Infrared Analyzer and a Sargent SR recorder. Respiration rates were determined by measuring the time required for the evolution of 20 mg of carbon dioxide.

The samples consisted of 10 beets in a five gallon pail with a tight fitting lid. The lids were sealed with plastic tape only during the actual period of analysis.

The air stream was pumped into the analyzer under a positive pressure of 60 cm of water with a diaphram pump (Figure 2). Pressure and flow rate were regulated with an adjustable flow meter. The air stream was passed through an ice bath to condense excess moisture before passing into the analyzer.

At the beginning of an analysis the sample container was sealed and coupled into the system to produce a closed circuit. Carbon dioxide was allowed to accumulate until a uniform rate of increase was obtained (1 to 2 minutes). At this point 10 cc or 20 mg of carbon dioxide was injected into the system and the recorder deflection noted. Since the samples were releasing carbon dioxide during the time required to reach a new equilibrium the deflection was measured by extrapolation back to the time of injection (Figure 3). The system was opened to the air to flush out the accumulated carbon dioxide. After flushing to atmospheric carbon dioxide concentration the system was again



1. Analyzer
2. Recorder
3. Flowmeter
4. Moisture trap
5. Sample
6. Diaphram pump

Figure 2. Diagram of carbon dioxide analyzing system.

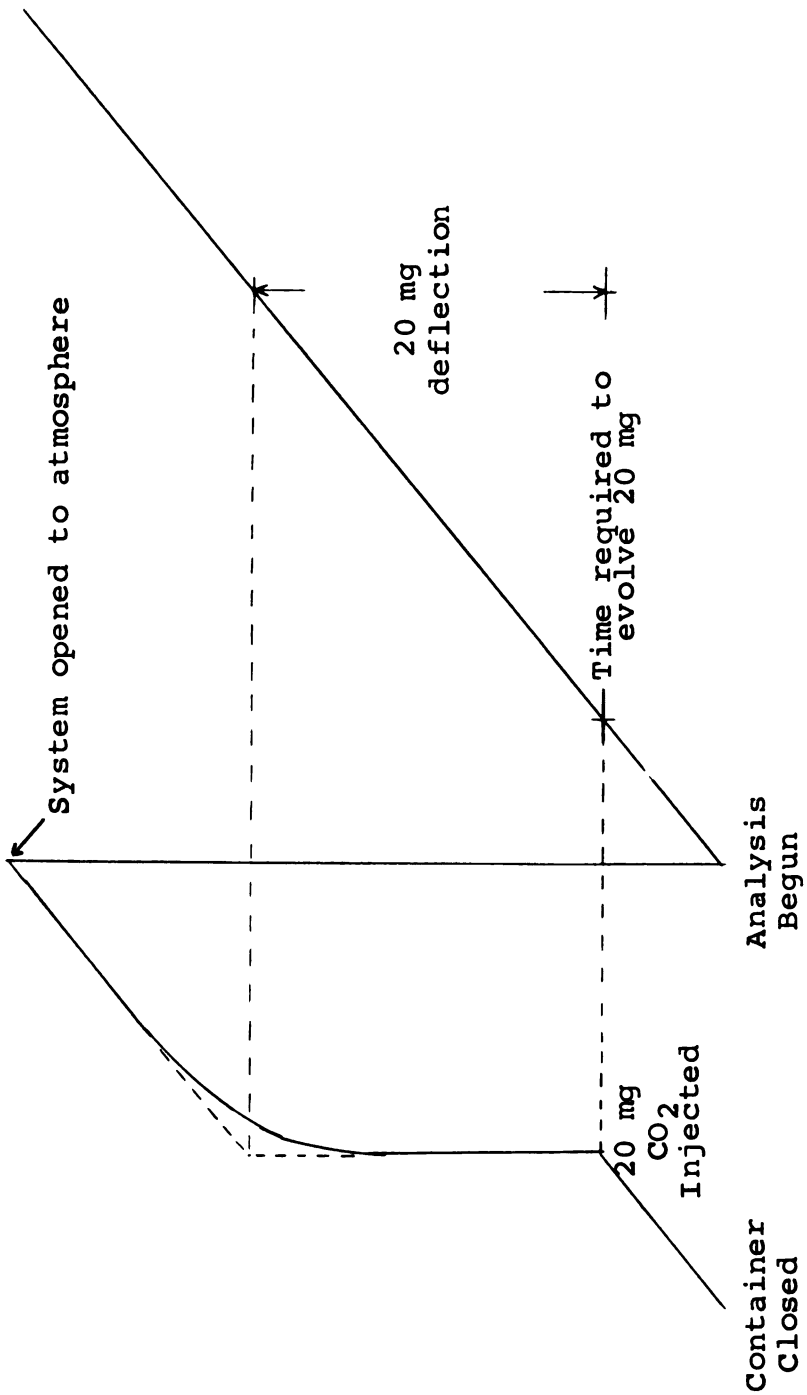


Figure 3. Method used to determine time required for sample to evolve 20 mg of CO_2.

closed and the time required for the sample to produce 20 mg of carbon dioxide was measured.

This method gave very reproducible results and eliminated errors due to changes in atmospheric pressure and instrument sensitivity over the extended period of the experiment.

RESULTS AND DISCUSSION

Preliminary Results

1966 Desiccation Experiment

The effect of desiccation on the loss of RSPT was studied at two temperatures. The results were used as a basis for planning subsequent studies. The clear juice was later used to develop rapid methods of analysis for raffinose and reducing sugar.

Raffinose

The raffinose content at harvest was very low (300 mg/100 RDS) but the average of all treatments increased almost three fold after 70 days of storage at 3 C (Figure 4). Between 70 and 140 days the average raffinose content decreased sharply. At 11 C the raffinose level decreased in a linear fashion to less than 100 mg/100 RDS after 140 days of storage.

The accumulation of raffinose was closely associated with the degree of desiccation during storage. Since the trends were the same, the wilt treatments for the two temperatures were pooled and the results given in Figure 5.

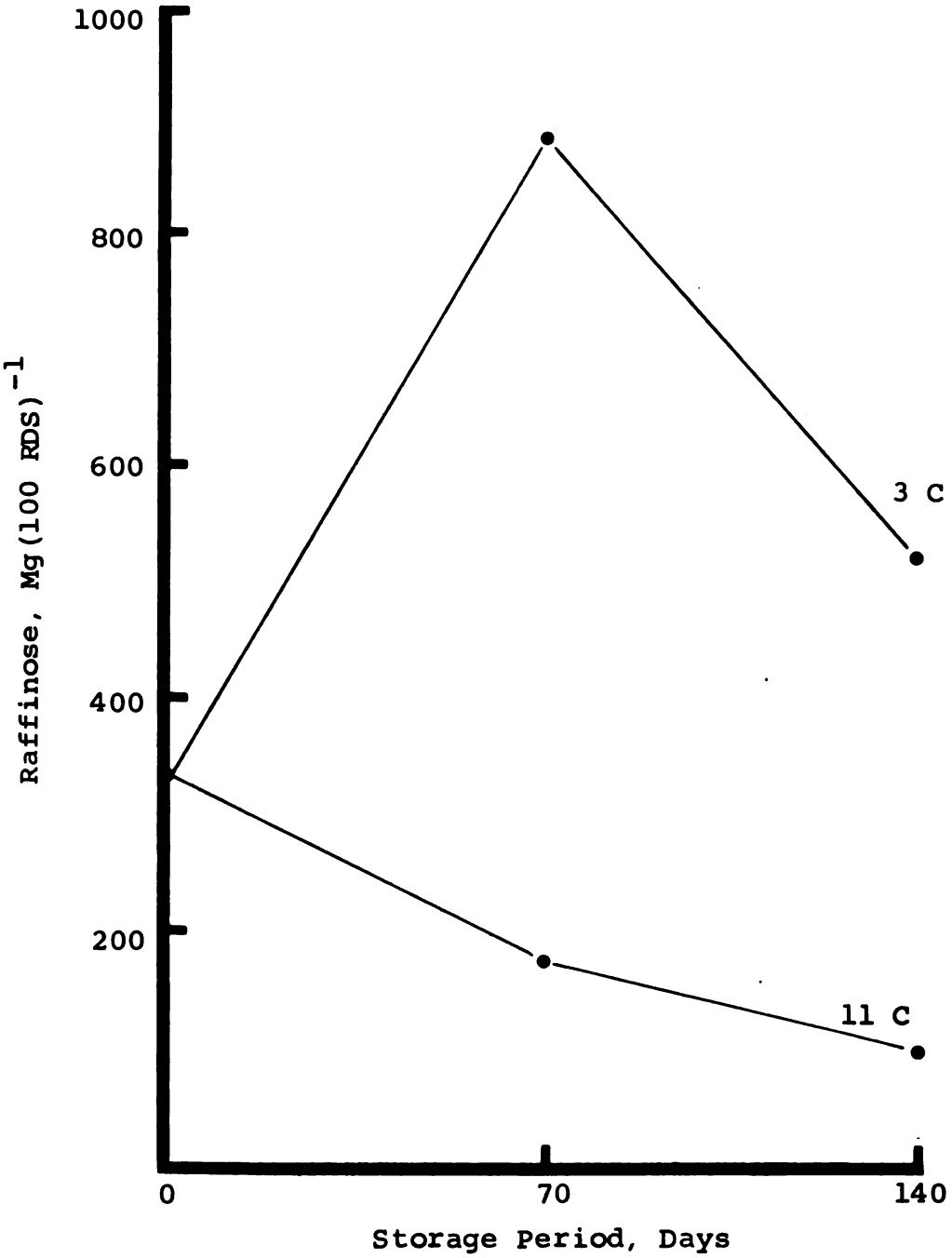


Figure 4. Effect of temperature on raffinose formation.

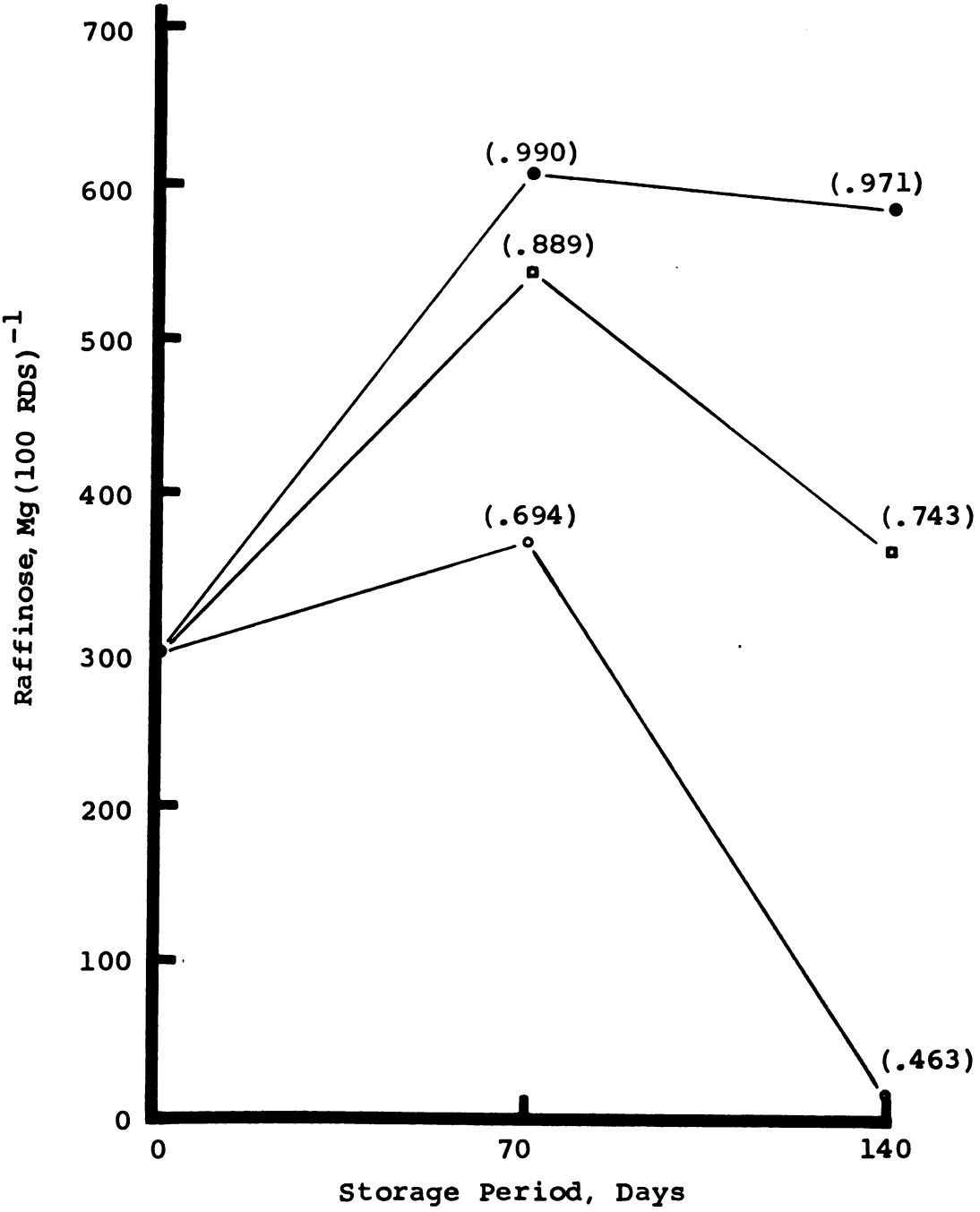


Figure 5. Effect of wilting on raffinose formation. No wilt ● ; slight wilt ▫ ; heavy wilt ○ ; wilt factor ( ).

Raffinose in samples which did not wilt in storage increased approximately 200 mg while in those allowed to wilt substantially it decreased to zero. After prolonged storage, the raffinose concentration in the slightly wilted samples was equal to that at harvest.

Reducing Sugars

Increasing the storage temperature 8 C, from 3 to 11 C, increased the reducing sugar accumulation by 100 percent (Figure 6). At 3 C the level decreased over the first 70 days but then increased at approximately the same rate as the 11 C storage. The rapid rise in the last 70 days was caused in part by small amounts of mold, particularly in the root tip area.

Beets which were not allowed to wilt accumulated very little reducing sugar at either 3 or 11 C, however, those wilted to 74 and 46 percent of their original weight more than tripled in reducing sugars (Figure 7). The highly wilted beets were in very poor physical condition and showed evidence of slight surface mold.

Chromatographic analysis* indicated the presence of kestose in amounts inversely proportional to the raffinose content. Treatments which increased raffinose decreased kestose.

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*Paper--Whatman #1; solvent--Butanol:Acetic Acid: Water, 4:1:5.

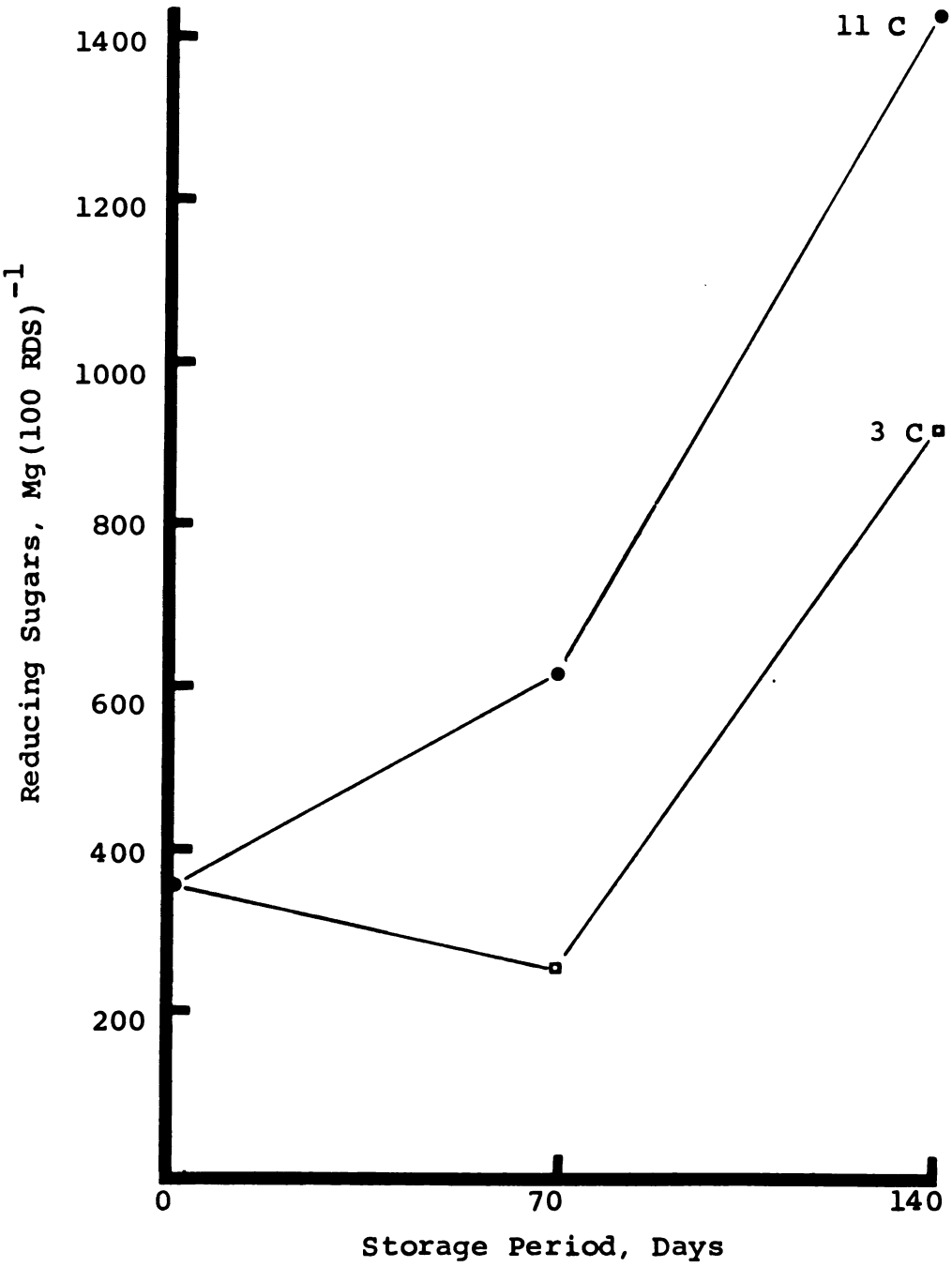

Figure 6. Effect of temperature on invert formation.

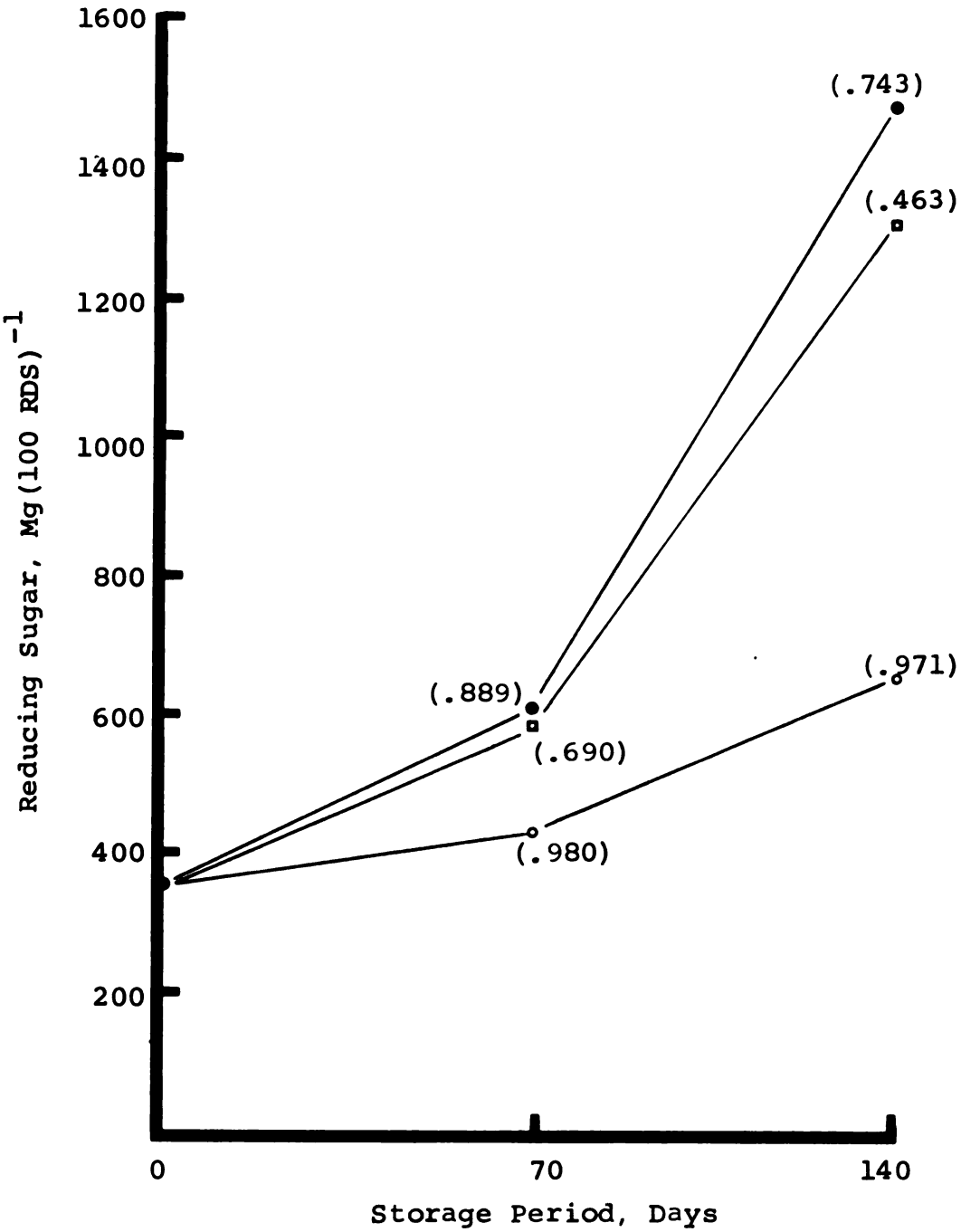

Figure 7. Effect of wilting on reducing sugar formation. No wilt ● ; slight wilt ▫ ; heavy wilt ° ; wilt factor ( ).

The results confirm the work of Atterson et al. (1963) who found the same relationship between wilting and trisaccharide formation. The formation of kestose indicates high invertase activity. Accumulation of reducing sugars in desiccated beets apparently was caused by this enzyme (Allen and Bacon, 1956).

These preliminary studies indicated the importance of controlling all storage variables particularly desiccation and surface molds when studying raffinose and invert formation. All subsequent samples were stored in polyethylene bags with a small amount of wet wood shavings added to prevent wilting.

FACTORS CONTROLLING THE MAJOR NON-SUCROSE COMPONENTS OF THE BEET AT HARVEST AND DURING STORAGE

Factors Controlling the Raffinose Content

Storage Temperature

The preliminary results indicated that the equilibrium temperature for raffinose production was between 3 and 7 C. In 1967 beets were stored at four temperatures, 2, 4.5, 7.2 and 12.8 C to better define the threshold temperature for raffinose production. However due to refrigeration failure the samples at 2 C were lost. Based on results of other experiments, raffinose content may double or triple in 65 days of storage (same variety) at 3 C, therefore 2 C was well below the threshold temperature. At 4.5 C (Figure 8) the raffinose content remained essentially constant and declined at all higher temperatures. In terms of raffinose accumulation, the ideal storage temperature would be slightly above 4 C.

Variety-Temperature Interaction

Three varieties were stored in 1968 at temperatures well above and below the threshold level for raffinose production. This was an attempt to develop a method for more

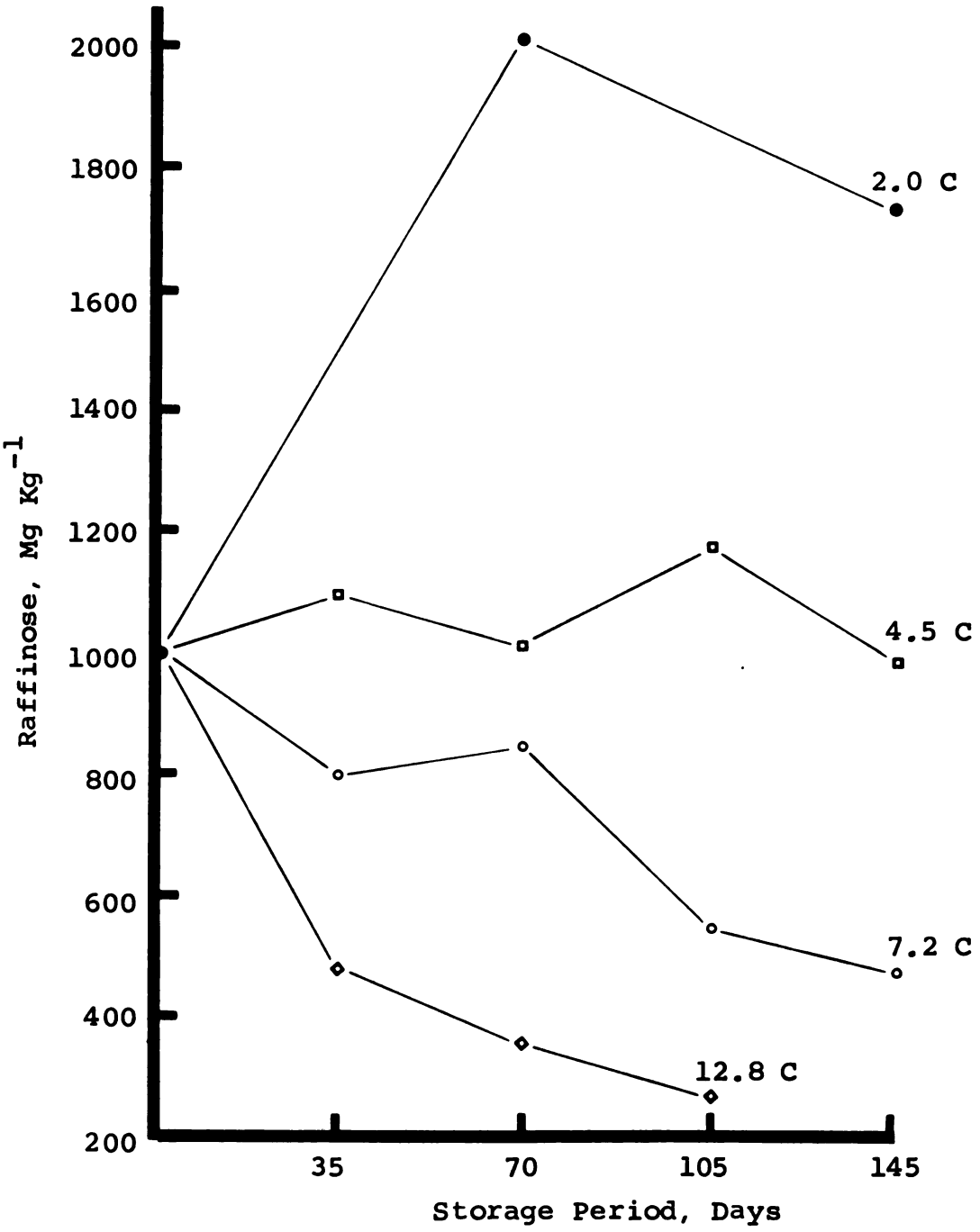

Figure 8. Effect of storage temperature on the raffinose content of beets stored for 35, 70, 105 and 145 days (1967).

critically detecting varieties prone to raffinose production. Raffinose increased substantially in all varieties tested previously at 3 C, while storage temperatures above 6 C resulted in a general decline in raffinose content.

Variety 5 had an intermediate raffinose content at harvest and variety 7 was very low considering that a week of freezing temperatures preceded harvest (Table 1). Variety 6 was extremely high at harvest, indicating sensitivity to temperature. In storage, variety 5 increased slightly (400 mg) in raffinose over the harvest level when stored warm and increased approximately 1000 mg when stored cold. Variety 7 reacted in the same manner but the raffinose doubled at 10 C and tripled at 3 C. Variety 6 nearly doubled at both storage temperatures. Variety 6 would be

Table 1. Influence of temperature in the production of raffinose by several varieties during storage for 100 days at 3 and 10 C (1968)

Variety	At Harvest	After Storage At 3 C	After Storage At 10 C
		Mg Kg^{-1}	
5	1288	2212	1693
6	2966	5015	4864
7	890	2602	1790
Average	1715	3310	2782

undesirable for processing after storage at either temperature because of its extremely high raffinose content.

The metabolic processes involved in raffinose production and degradation are very temperature sensitive and genetically controlled. Apparently two separate systems exist for raffinose production and degradation. At temperatures above 5 C the degradative system operates at a rate equal to or greater than the synthesizing system in most varieties. The opposite is true below 5 C. All the varieties tested had the mechanism for synthesizing considerable quantities of raffinose as evidenced by its general occurrence.

The turnover rate of the raffinose pool has not been studied and therefore no estimate of the amount of sucrose lost via this system can be made. The rate of accumulation and degradation (McCready and Goodwin, 1966) (see page 88) is rather slow but this merely indicates the differential rates of degradation and synthesis and not turnover rate. Ideally a superior variety would be one which did not accumulate raffinose.

Variety

Five varieties were stored in 1967 to study further the variety effect on raffinose content, reported to be substantial by Wood et al. (1956). At harvest there was a small but significant difference between varieties (Figure 9).

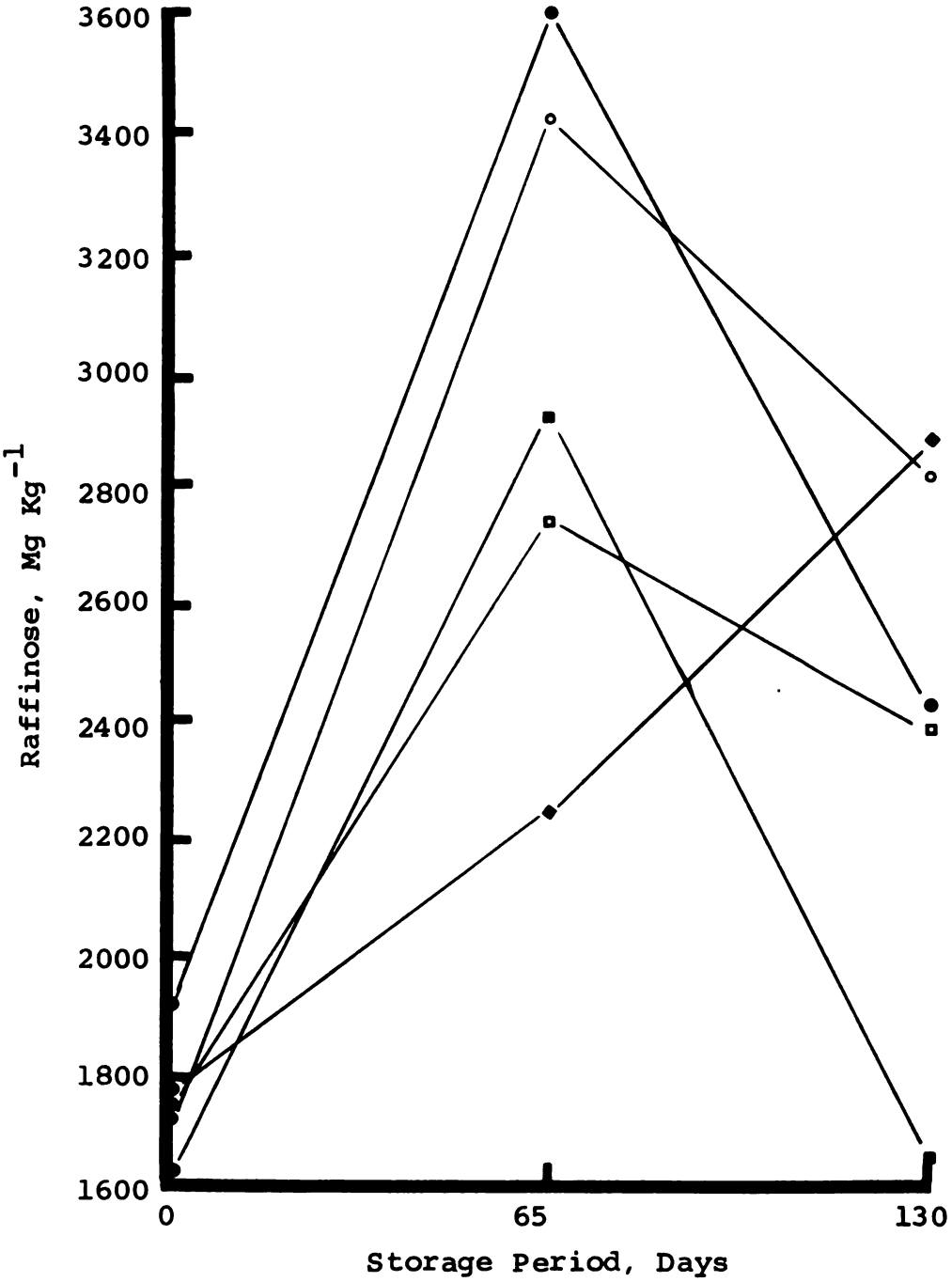

Figure 9. Variety x storage interaction for raffinose accumulation at 3 C. Variety: 1, ▫ ; 2, ○ ; 3, ◆ ; 4, ● ; 5, ■ .

Varieties 2 and 4 had higher levels in the first 65 days of storage causing their overall means to be significantly higher. Variety 3 reacted very differently than the other varieties by increasing in an almost linear fashion over the entire storage period.

Effect of Harvest Date

The raffinose content was four to six times higher at harvest in 1967 than in 1966. This was related to the very cool, damp weather during October in 1967. Prior to the November 6 harvest a severe frost caused visible freezing injury to some of the beet crowns. (No frost damaged beets were stored.)

The raffinose content increased approximately 35 percent from 1300 to 1850 mg/kg between the early and late harvests. There was little difference between harvest dates in the amount of raffinose accumulated after 65 days of storage at 3 C (Figure 10). The harvest date x removal interaction was significant due to the greater increase in raffinose for the early harvested beets. The very high raffinose levels at harvest and during storage caused a considerable error in the percent sucrose and clear juice purity determinations resulting in an overestimation of the RSPT. These results indicated the need for correcting the percent sucrose and CJP determination in stored beets and in some years, at harvest.

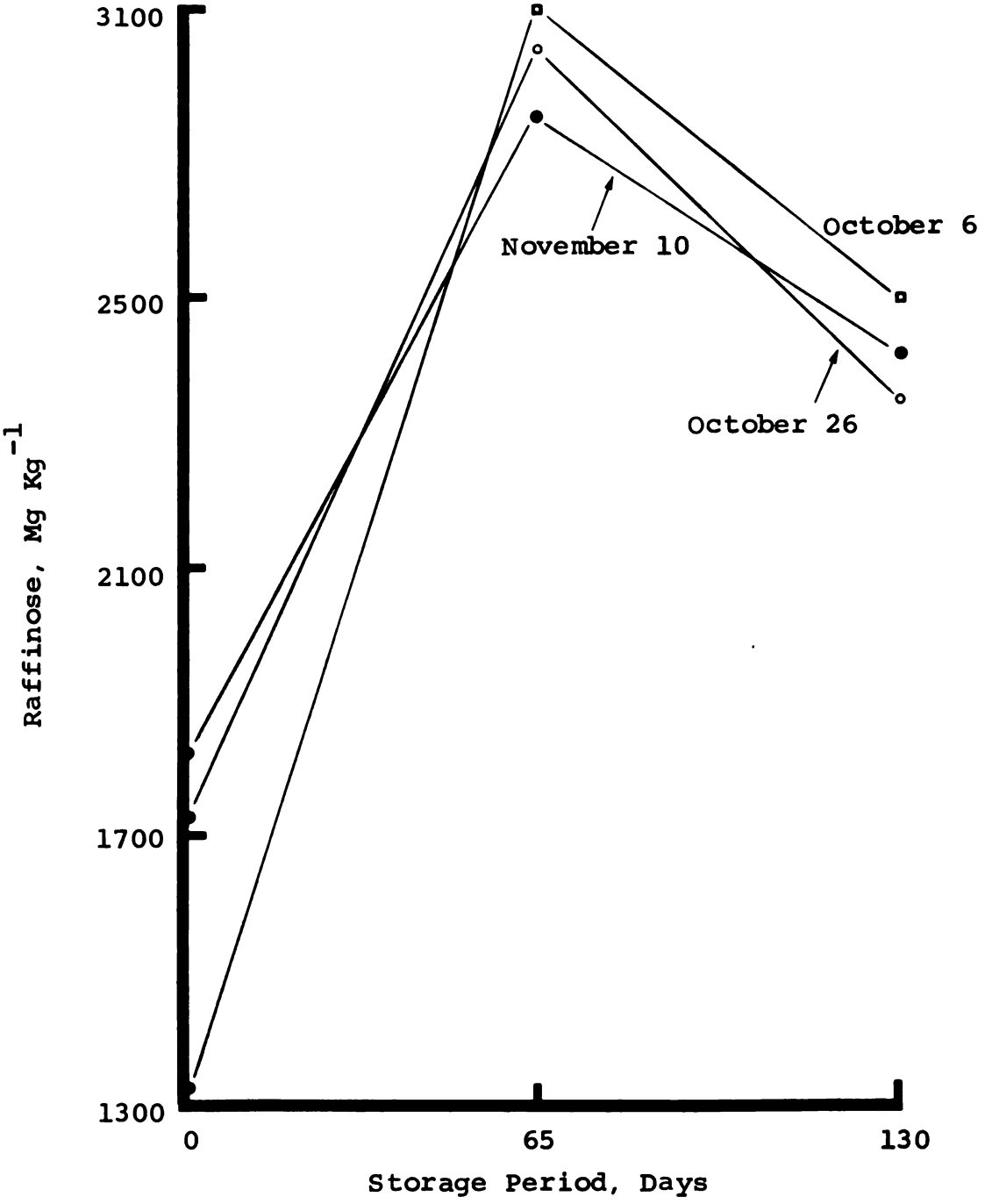

Figure 10. The effect of harvest date on the accumulation of raffinose during storage at 3 C in 1967.

In 1968 the effect of harvest date was again studied. The weather during the fall of 1968 was very warm and dry and the raffinose content at harvest was much lower than in 1967. However as in the 1967 the less mature beets showed the greatest increase in raffinose content in storage (Figure 11). The general decline with prolonged storage observed in 1967 occurred only in the case of the late harvested beets in 1968.

In the 1967 experiment there was a significant three-way interaction between harvest date, variety and length of storage (Table 2). Although the difference between varieties was statistically highly significant from a practical standpoint there was little difference. All three varieties increased in raffinose content during harvest at a very uniform rate. However there was a considerable difference between varieties in the amount of raffinose accumulated in the first 65 days of storage. Variety 5 was considerably lower for all harvests. Raffinose decreased in all varieties during the final 65 days of storage. Taking an average of each variety over the entire storage period variety 5 was significantly lower than varieties 2 and 3.

Effect of Nitrogen Fertilization

The raffinose content of varieties 2 and 3 showed little response to nitrogen fertilization (Table 3). However the 150 pounds of applied nitrogen per acre resulted in an increase in the raffinose content of 400 mg in variety 5.

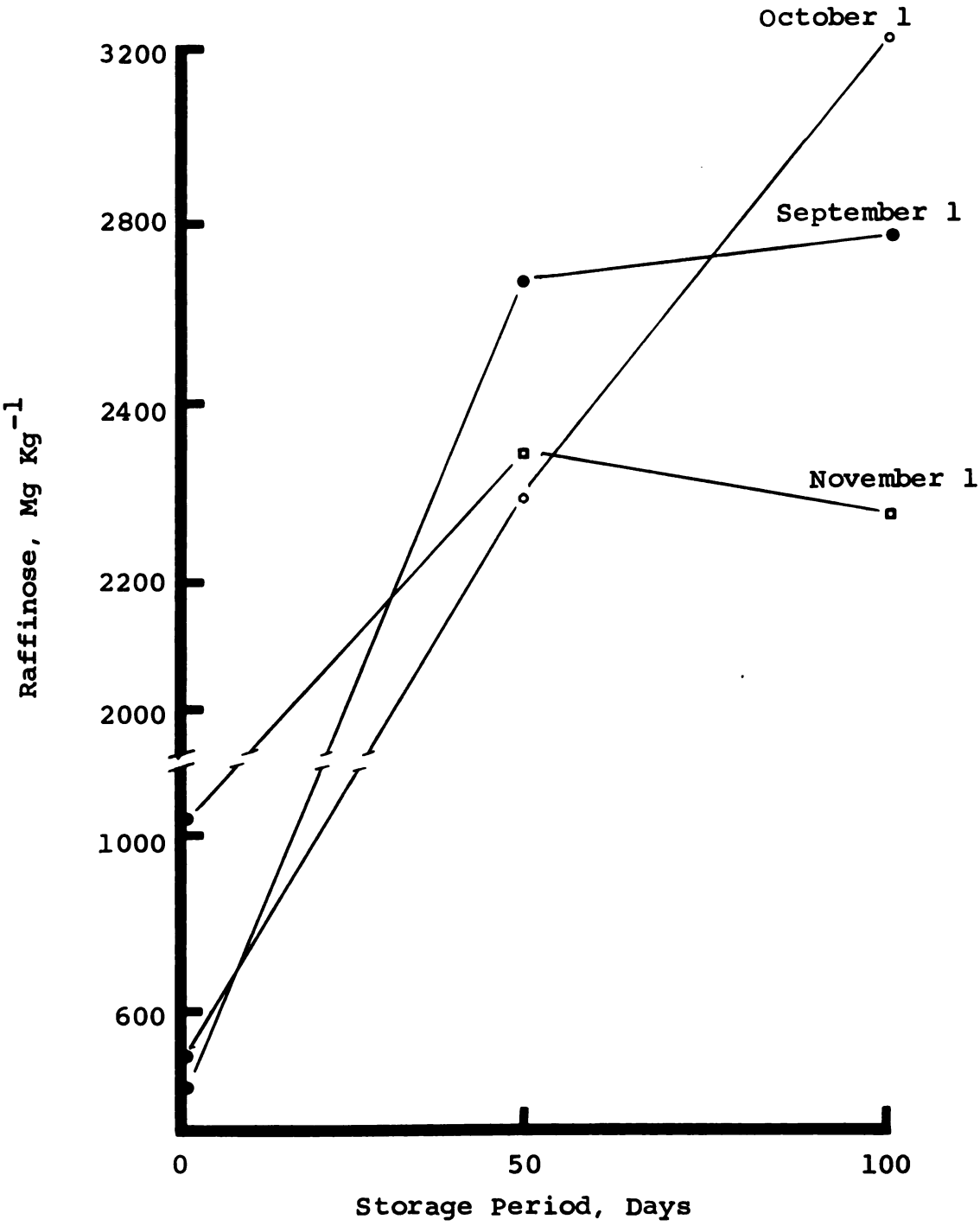

Figure 11. Effect of harvest date on raffinose accumulation in storage at 3 C.

Table 2. Interaction between harvest date, variety and length of storage on the raffinose content at harvest and after storage at 3 C in 1967

	October 6				October 26				November 6			
		Days in Storage				Days in Storage				Days in Storage		
Variety	At Harvest	65	130	Avg	At Harvest	65	130	Avg	At Harvest	65	130	Avg
		Mg Kg^{-1}				Mg Kg^{-1}				Mg Kg^{-1}		
2	1361	3240	2452	2351	1757	3427	2785	2656	1744	2886	2554	2395
3	1266	3415	2941	2540	1789	2216	2812	2272	2006	3140	2584	2577
5	1294	2618	2152	2021	1622	2927	1595	2048	1813	2358	2191	2121
Average	1306	3091	2515		1722	2860	2397		1854	2795	2443	

Table 3. Effect of nitrogen fertilization on the average raffinose content of three varieties stored at 3 C in 1967

	Nitrogen, #/A	
Variety	24#/A	150#/A
	Mg Kg^{-1}	
2	2428	2507
3	2524	2402
5	1872	2255
Average	2274	2388

There was no variety x nitrogen x removal interaction. The nitrogen x removal interaction (Table 4) combines three varieties and three harvest dates and was highly significant. Again high nitrogen fertilization increased the

Table 4. Nitrogen x removal interaction for raffinose accumulation in storage (1967)

		Days in Storage		
Nitrogen	At Harvest	65	130	Average
#/A		Mg Kg^{-1}		
24	1749	3017	2127	2298
150	1778	2954	2675	2469

raffinose content, but only 170 mg. Nitrogen had no effect on raffinose build-up during storage but did retard its decline between 65 and 130 days of storage.

Effect of Topping

Beets topped at the lowest leaf scar were higher in raffinose than beets with only the terminal crown bud removed (Table 5), suggesting that the crown area was apparently lower in raffinose than the tap root. Leaving the crown on caused a slightly greater accumulation of raffinose in storage at 3 C. However the topping x temperature interaction was not significant.

Table 5. Effect of topping on raffinose content of beets stored at 3 and 7 C

	At Harvest	Stored 100 Days At	
		3 C	7 C
		Mg Kg^{-1}	
Topped	1519	1876	596
Untopped	1330	2161	599

Preharvest Sprays

GA and MH-30 applied prior to harvest significantly increased the level of raffinose at harvest (Table 6). Beets from these treatments tended to accumulate more raffinose in storage than the control. However the spray x removal interaction was not significant.

The raffinose content doubled during the first 5 weeks of storage (Table 6). Although the content continued to increase, the treatment differences remained constant. Therefore in relatively short-term storage experiments it may be possible to screen varieties and treatments for their effect on raffinose accumulation.

Several other sprays (vanadium sulfate, pyrocatechol and CCC) did not affect raffinose content at harvest or during storage.

Table 6. Effect of giberellic acid and maleic hydrazide on the raffinose content of fresh and stored beets (1968)

Spray	At Harvest	Weeks at 5 C			Average
		5	8	12	
		Mg Kg^{-1}			
MH-30	1144	2673	3174	3341	2583
GA	1185	2943	3719	3430	2819
Control	904	2202	2714	3076	2224
Average	1078	2606	3202	3282	

Storage in Modified Atmospheres

Beets stored in modified atmospheres containing 5 percent carbon dioxide and 5 percent oxygen accumulated 2.5 times more raffinose than those stored at normal atmospheric concentrations (Table 7). The experimental design did not permit determining if the effect was caused by high carbon dioxide or by low oxygen. However the control containers accumulated approximately 1-2 percent carbon dioxide (due to insufficient air movement) and since these treatments did not result in accumulated raffinose the cause was apparently related to the low oxygen concentration. Ethylene, applied continuously throughout the storage period, had no effect.

Table 7. Effect of modified atmospheres on the accumulation of raffinose after 40 days of storage at 5 C

	Raffinose Content		
	Ethylene		
Atmosphere	0	1000 ppm	Average
		Mg Kg^{-1}	
Control	691	872	782
5% O_2, 5% CO_2, 90% N_2	1958	1899	1929
Average	1325	1386	
At harvest	890		

At normal levels of oxygen the raffinose content decreased slightly at 5 C. Decreasing the level of oxygen allowed raffinose to accumulate. Therefore raffinose accumulation was not only temperature dependent but also depended on oxygen concentration. Since oxygen directly affects the rate of respiration, the metabolism of raffinose may also be closely associated with respiration. Assuming oxygen affects the general metabolic level of the root, raffinose and invert would both be expected to change in relatively the same manner. However, oxygen had no effect on invert sugars. Therefore the metabolism of raffinose must be more directly associated to respiration than that due merely to the general metabolic level of the beet root. Whether the effect is due to the rate of synthesis or degradation could not be determined in this experiment.

The brei from beets stored under low oxygen did not turn black upon prolonged exposure to air at room temperature while the brei from beets stored under normal oxygen turned black immediately. Apparently under low oxygen either the phenol oxidase activity decreased or the accumulation of phenolic substrates did not occur.

Controlled atmosphere storage of beets may not be evaluated merely from the aspect of reduced respirational losses, but the changes in non-sucrose compounds must also be studied before it can be recommended for use.

Factors Controlling the Reducing Sugar Content

Preliminary results indicated that storage temperatures above 5 C accelerated accumulation of reducing sugars. In 1967 beets were stored at temperatures ranging from 2 to 12.8 C to determine if a threshold temperature similar to that found for raffinose existed for the accumulation of reducing sugars.

Storage Temperature

There was no significant difference between temperatures in the accumulation of reducing sugars in the first 70 days of storage (Figure 12). 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 and this could account for the rapid rise in invert after 105 days of storage. At 7.2 C the beets remained in perfect condition and the increase in reducing sugars was small. If molds and sprouting are prevented, beet roots can apparently tolerate prolonged storage in temperatures up to 7 C without appreciable accumulation of reducing sugars.

Variety-Temperature Interaction

A highly significant variety-temperature interaction for reducing sugar accumulation occurred in 1968 when samples of three varieties were stored at 3 and 10 C (Table 8).

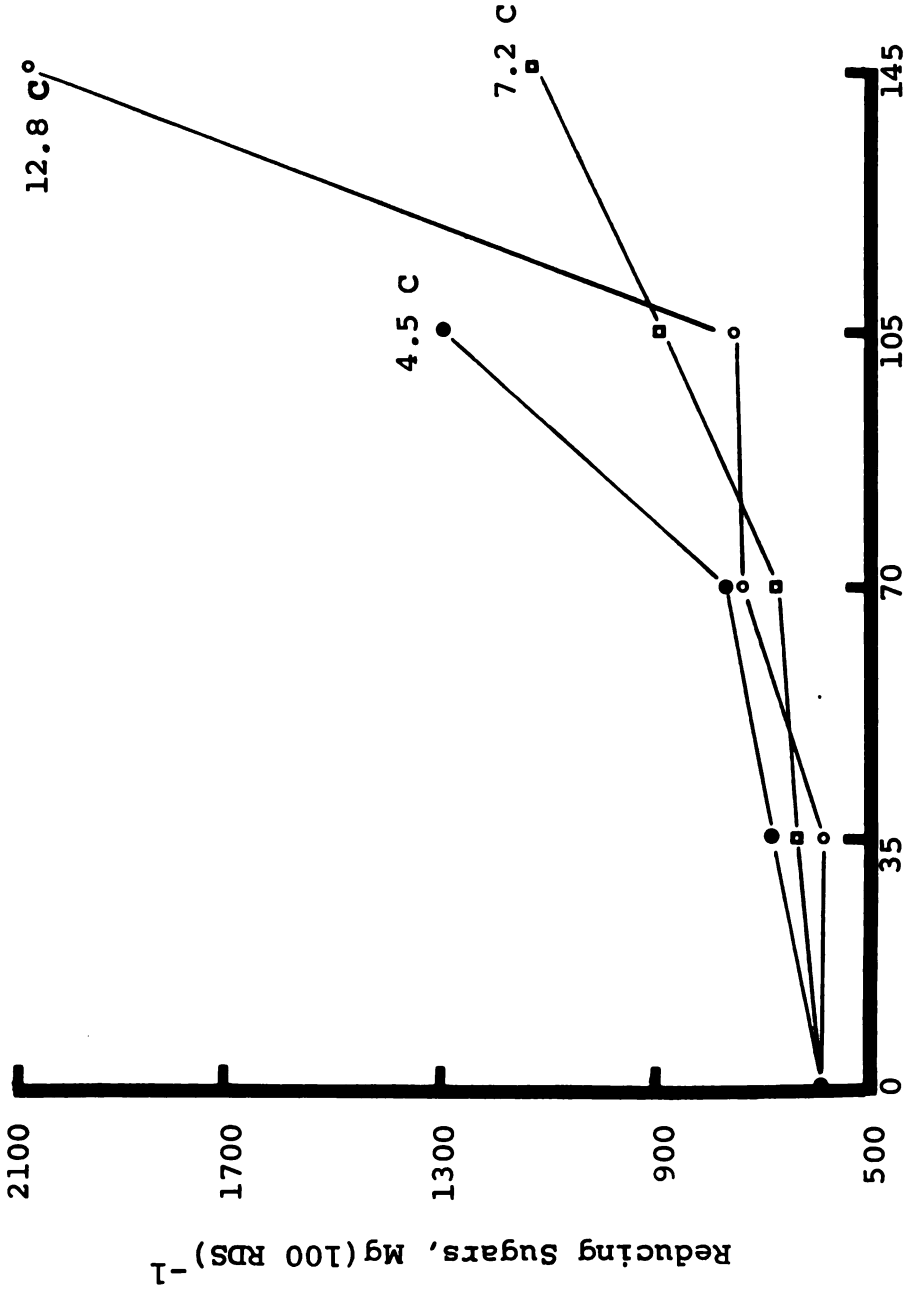

Figure 12. Effect of temperature on the accumulation of reducing sugars in beets stored for up to 145 days.

Table 8. Interaction of variety with temperature in the accumulation of reducing sugars after 100 days of storage at 3 and 10 C

Variety	At Harvest	Storage Temperature, C	
		3 C	10 C
		Mg Kg^{-1}	
5	656	658	1297
6	916	1095	1530
7	831	965	1115
Average	801	906	1314

The accumulation of reducing sugars was very small for all varieties stored at 3 C but all increased substantially at 10 C. The sensitivity of the varieties to 10 C storage was very different. Variety 5 doubled in concentration while variety 7 increased only 35 percent. Since no molds occurred in the 1968 storage it appears that the plant breeder may be able to select varieties which can be stored above 5 C without the production of reducing sugars.

Variety

The significant variety x removal interaction (Table 9) indicated that for the first 65 days of storage very little reducing sugar accumulated but in the last 65 days the reducing sugar content approximately doubled.

Table 9. Variety x removal interaction in reducing sugar accumulation (1967) for beets stored at 3 C

Variety	At Harvest	Stored (days) 65	130	Average
		Mg Kg^{-1}		
2	848	1241	1953	1347
3	638	856	2833	1442
5	745	1135	1931	1270
Average	743	1077	2239	

In this experiment the initial increase was a varietal characteristic typical of normal healthy beets in storage and the later rise was due to mold invasion. Although no varietal differences in susceptibility to mold invasion were noted, varietal differences have been shown by other workers (Gaskill, 1950).

Effect of Harvest Date

The reducing sugar content of the beet decreased slightly during the 1967 harvest period. The harvest x removal interaction was highly significant (Table 10). The early harvested beets remained lower throughout the 130 day storage period. The occurrence of very high levels of reducing sugar after 130 days of storage was due to small amounts of rot occurring in the crown region of the beets.

Table 10. Harvest x removal interaction for reducing sugar accumulation (1967) for beets stored at 3 C

Harvest Date	At Harvest	Stored (days)	
		65	130
		Mg Kg^{-1}	
October 6	771	944	1175
October 26	765	1203	2549
November 6	695	1084	2994

These regions were high in reducing sugars and since no attempt was made to remove them, the result was a high average reducing sugar content for the entire beet. Treatments did not differ in the amount of rotting which was due to moisture condensation between the polyethylene bag and the beet surface. Mold also occurred where free water condensed at the points of beet to beet contact. Mold formation is an important consideration if the practice of washing beets prior to storage is used commercially.

The harvest date x variety x removal date was highly significant (Table 11). Variety 3 remained low in reducing sugars for all harvest and removal dates, except for the 130-day storage of the late harvest. However this large increase was due to molding. In general, the early harvested beets did not increase as much in reducing sugars as the later harvested beets.

Table 11. Harvest x variety x removal interaction for reducing sugar accumulation of beets stored at 3 C (1967)

Variety	At Harvest	Stored (days) 65	Stored (days) 130	Average
		Mg Kg^{-1}		
October 6:				
2	864	997	1172	1011
3	663	798	987	816
5	787	1036	1366	1063
Average	772	944	1175	
October 26:				
2	869	1514	2650	1677
3	642	799	2636	1392
5	783	1295	2260	1446
Average	764	1202	2549	
November 6:				
2	810	1212	2038	1353
3	609	968	4775	2117
5	666	1072	2167	1301
Average	694	1084	2993	

In the 1968 date of harvest experiment the harvest x removal interaction was again significant (Figure 13). The problem of mold in the previous year was eliminated and the increase in reducing sugars with storage was not as great. The early harvested beets remained almost constant while the amount of reducing sugars in the late harvested beets doubled after 100 days of storage. Therefore two years data indicated that late harvested beets tended to accumulate more reducing sugars under prolonged ideal low temperature storage conditions than beets harvested prior to November 1. The September 1 harvest was extremely high in reducing sugars for fresh beets, the reducing sugars decreased substantially at the later harvests.

Variety-Nitrogen Interaction

There was a significant variety x nitrogen x removal interaction when five varieties were stored in 1967 (Table 12). The significant interaction occurred in the 65-130 day period of storage. Again this variation was apparently due to the incidence of molds. During the first 65 days of storage all of the varieties except variety 3 approximately doubled in reducing sugars. Variety 3 increased by only 25 percent. Nitrogen fertilization had no effect on the amount of reducing sugars accumulated in the first 65 days of storage.

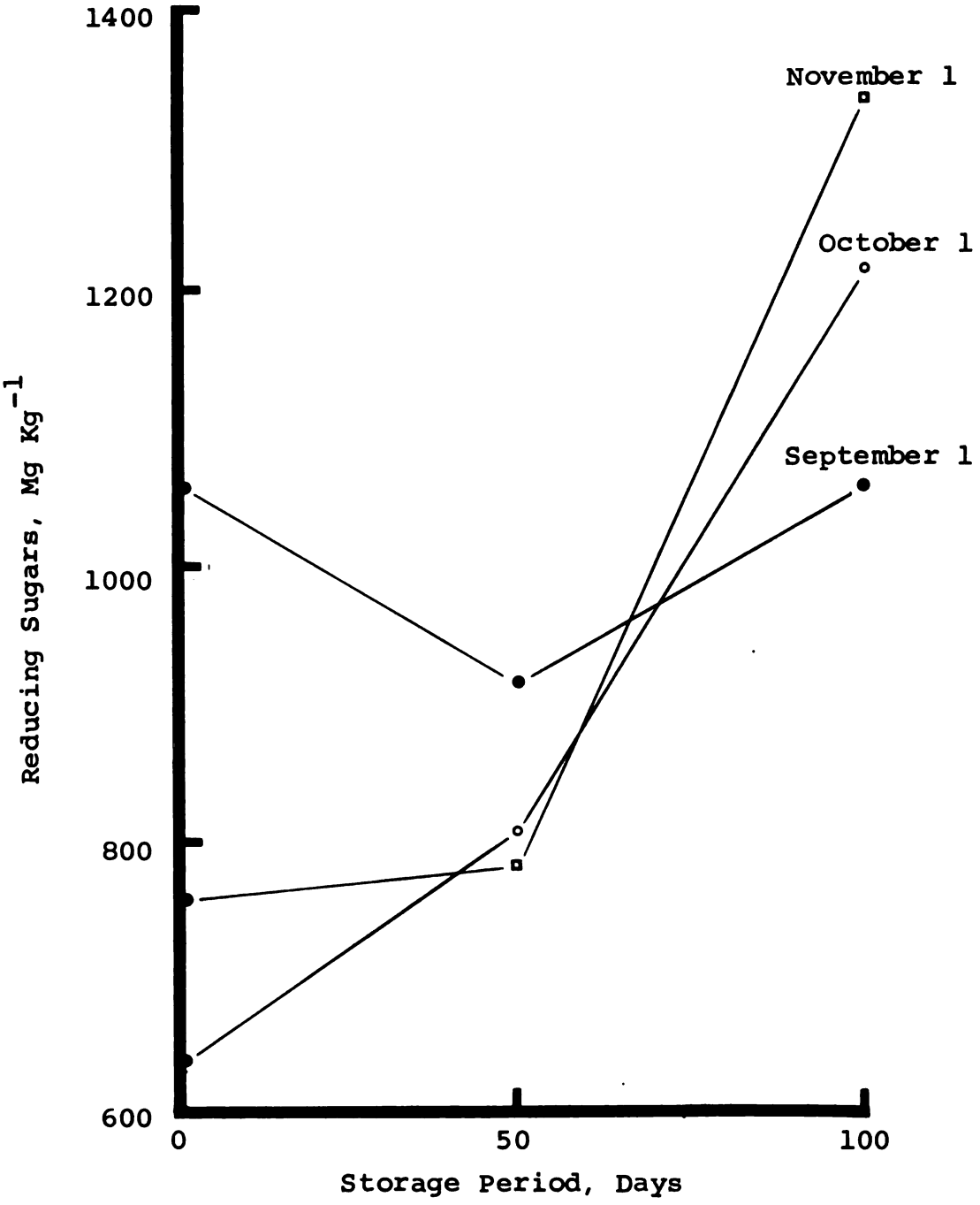

Figure 13. Effect of harvest date on the accumulation of reducing sugars in storage at 3 C.

Table 12. Variety x nitrogen x removal interaction for reducing sugar accumulation in five varieties grown on 24 and 150# nitrogen per acre and stored at 3 C for 65 and 130 days

	At Harvest			Stored 65 Days			Stored 130 Days		
Variety	24#	150#	Avg.	24#	150#	Avg.	24#	150#	Avg.
					Mg Kg^{-1}				
1	640	679	660	1021	1059	1040	4724	2697	3711
2	961	777	869	1626	1401	1513	1906	3393	2649
3	613	670	641	781	818	799	2314	3159	2736
4	628	685	656	1190	1099	1144	2653	2561	2607
5	794	772	783	1246	1342	2394	2579	1941	2260
Average	727	716		1173	1144		2835	2751	

The variety x nitrogen x removal interaction was also highly significant (Figure 14). All treatments increased essentially parallel in the first 65 days of storage. However in the final 65 days varieties 2 and 3 grown on high nitrogen increased by approximately 100 mg over the beets with los nitrogen. For variety 5 the reverse trend was true. The three-way interactions were apparently due to the incidence of molds and are therefore not indicative of normal beet responses. However these results indicate the drastic effect of only small amounts of mold on the composition and subsequent processing characteristics of stored beets (see page 147).

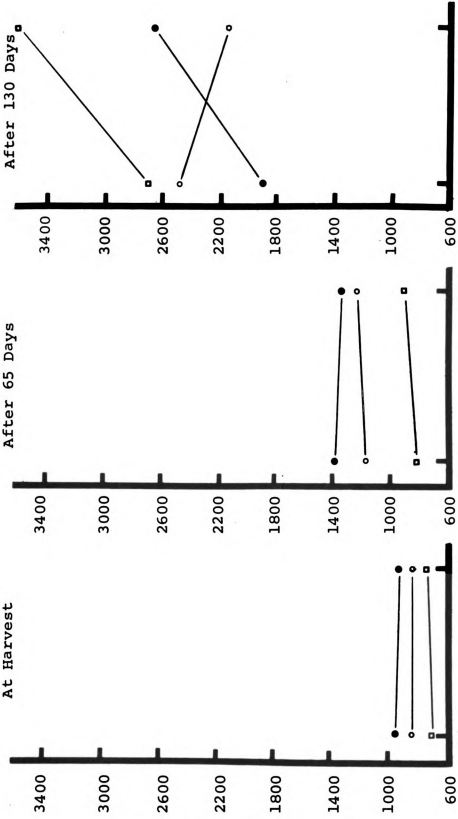

Figure 14. Effect of nitrogen fertilization on the accumulation of reducing sugars in three varieties during storage at 3 C. Variety: 2, ●; 3, ▪; 5, ○.

Effect of Topping

Although the increase in reducing sugars during storage was great, topping of beets prior to storage did not significantly affect the accumulation (Table 13).

Table 13. Effect of topping on the accumulation of reducing sugars after 50 and 100 days of storage (average of 3 and 7 C) (1967)

	At Harvest	Stored (days)	
		50	100
		Mg Kg^{-1}	
Topped	863	1891	3089
Untopped	905	1484	3305

Preharvest Sprays

The application of GA and MH-30 prior to harvest significantly increased the accumulation of reducing sugars after eight weeks of storage. Prior to eight weeks no significant difference existed (Figure 15).

Vanadium sulfate, pyrocatechaol and CCC were applied preharvest in an experiment separate from the GA and MH-30 application. Although no statistically significant spray x removal interaction was found all treatments were lower in

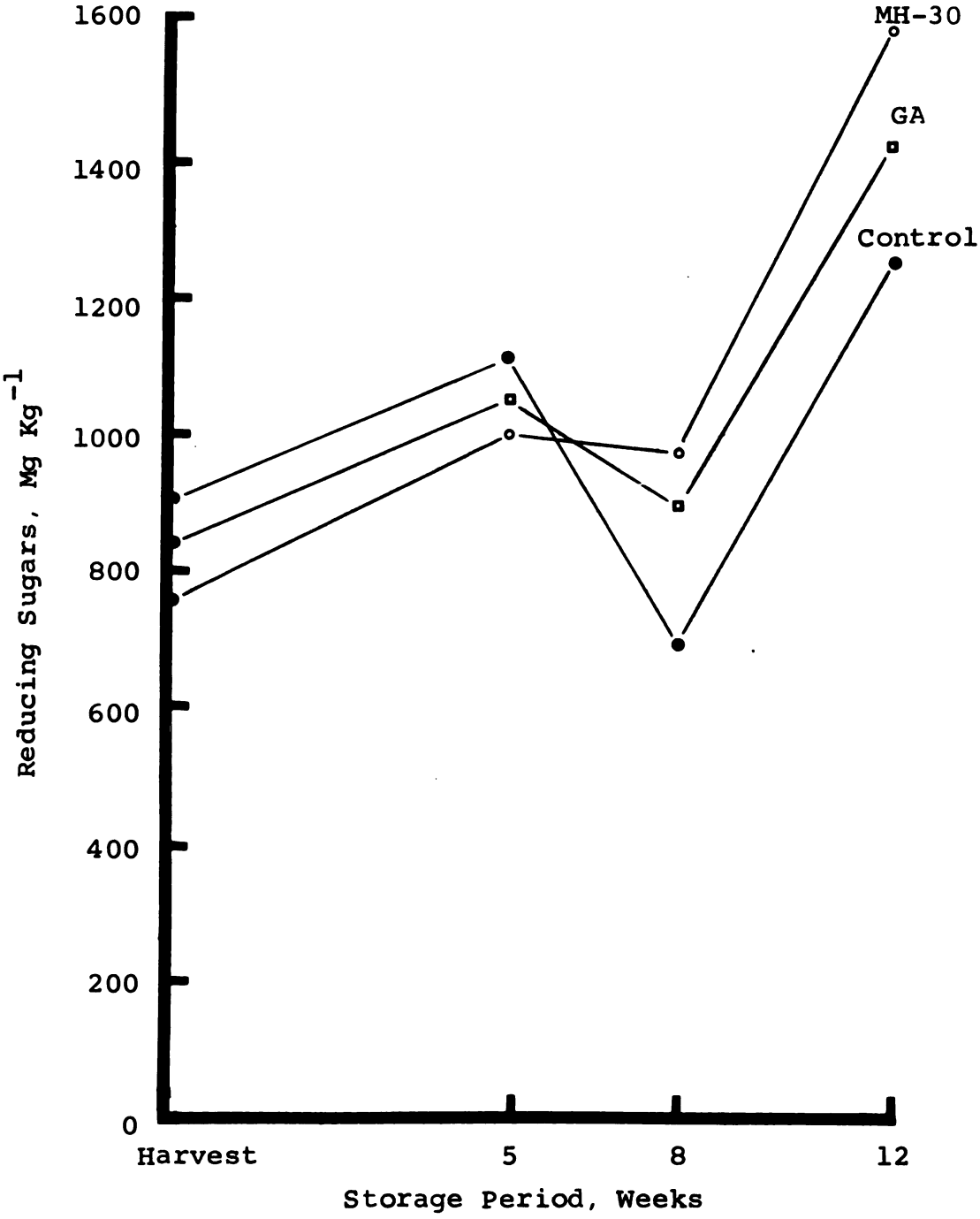

Figure 15. Effect of preharvest applications of GA and MH-30 on the reducing sugar content in storage at 5 C.

reducing sugars than the control at harvest and during storage (Table 14). The much reduced level with CCC treatment warrants further study.

Table 14. Effect of various preharvest foliar sprays on the accumulation of reducing sugars during storage at 4 C (1968)

Spray	At Harvest	Stored (days) 50	100	Average
		Mg Kg^{-1}		
Control	762	742	1274	926
Vanadium Sulfate	712	710	1116	846
Catechol	716	715	1133	855
CCC	698	647	930	759

Storage in Modified Atmospheres

Altering the carbon dioxide and oxygen concentration in the storage atmosphere had no effect on the accumulation of reducing sugars at 5 C (Table 15). Ethylene also had no effect. This is in contrast to the work reported previously where oxygen levels below 5-7 percent were reported to cause invert accumulation due to the onset of anaerobic respiration (Vajna, 1960).

Table 15. Effect of modified storage atmospheres on the accumulation of reducing sugars after 40 days of storage at 5 C

Atmosphere	Ethylene		Average
	0	1000 ppm	
		Mg Kg^{-1}	
Control	768	862	815
5% O_2, 5% CO_2, 90% N_2	906	909	910
Average	837	886	
At Harvest	831		

The ideal storage conditions under which these experiments were conducted produced atypical results compared to reducing sugar accumulation in commercial piles. Factories in Michigan have observed that the reducing sugar levels in the early stages of the campaign are approximately 1000 mg/kg and may increase to 9,500 to 12,500 mg/kg after prolonged storage. Therefore, the losses due to reducing sugar production in commercial operations are much greater than those observed in these experiments.

Factors Controlling the Amino Acid Content

Effect of Nitrogen Fertilizer

Beets grown with low nitrogen (24#/A) became nitrogen deficient several weeks prior to harvest, in 1967, as evidenced by leaf yellowing. The high nitrogen (150#/A) plants were still very succulent and green at the first harvest date (October 6). The free amino acid content of the root decreased almost linearly during the harvest period at the low nitrogen level (Figure 16, dotted lines). The beets were apparently nitrogen deficient and the free amino acid pool in the root was being depleted. The beets with high nitrogen remained high in free amino acids until October 25 after which time the amino acid content* declined rapidly. On November 6 the beets grown on high nitrogen were equal in amino acid content to the low nitrogen beets harvested on October 6.

The amino acid content declined during storage at 3 C. This was found previously by Dexter et al. (1966) and is apparently due to synthesis of new enzymes and other proteins. The beets grown on low nitrogen declined rapidly in amino acid content during the first 65 days of storage and then remained essentially constant at 1200 to 1500 mg/kg.

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*α-amino nitrogen times 10.

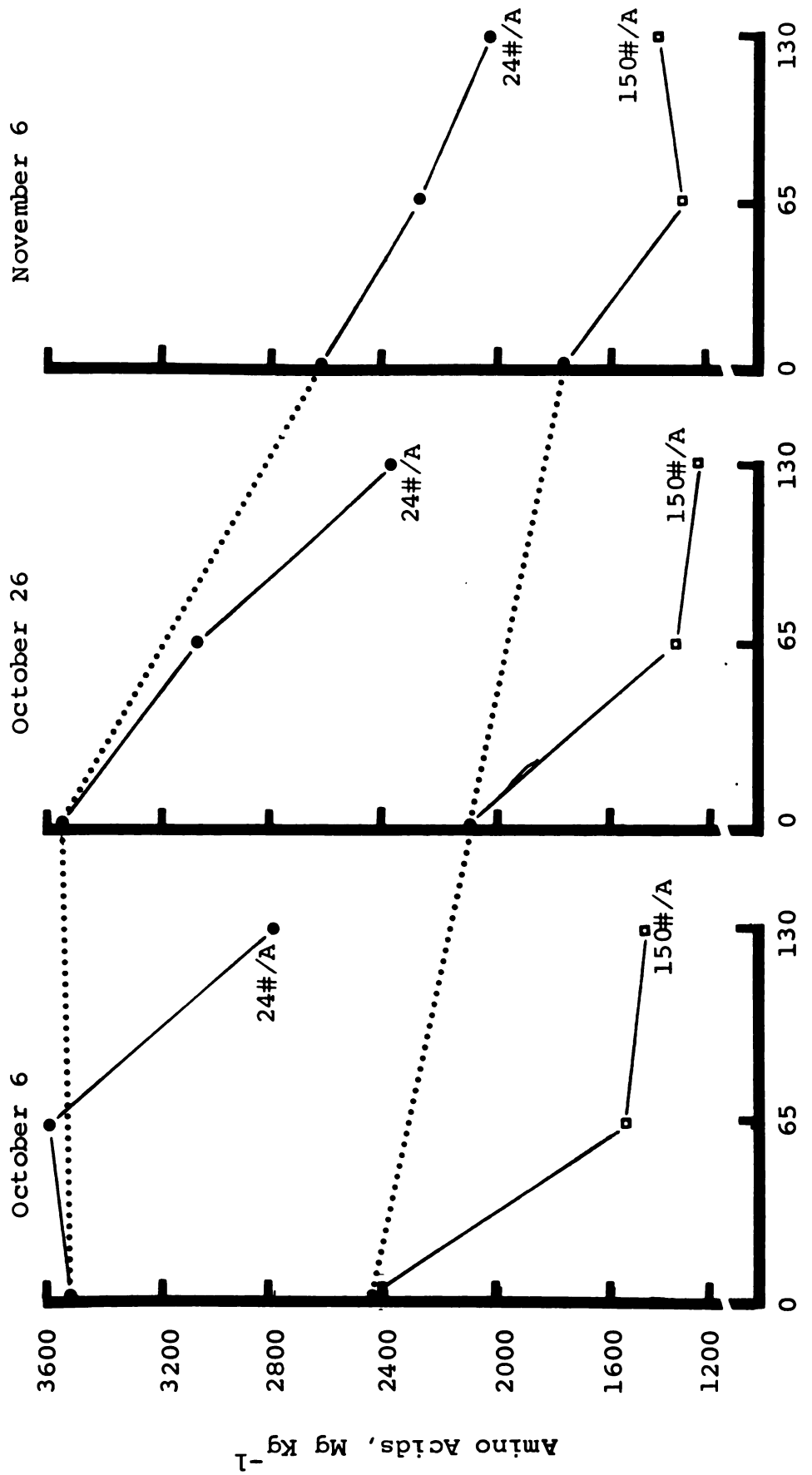

Figure 16. Harvest x nitrogen x removal interaction for amino acid content in beets stored at 3 C for 65 and 130 days.

The high nitrogen beets declined almost linearly (Figure 16). The rate of decline in amino acids of both the high and low nitrogen beets appeared to be related to the amino acid content at harvest (Figure 17). The higher the harvest level the greater the decline during storage.

Variety-Nitrogen Interaction

The variety x nitrogen interaction at harvest (Table 16) was significant indicating a substantial difference between varieties in their sensitivity to excessive nitrogen fertilization. Variety 5 was extremely sensitive having about twice the amino acid content at the 150 pound compared to the 24 pound per acre level of fertilization.

Table 16. Effect of nitrogen fertilization on the free amino acid content of three varieties at harvest (1967)

	Nitrogen Applied, Lbs/A	
Variety	24	150
	Mg Kg^{-1}	
2	1540	2462
3	1663	2832
5	1636	3360
Average	1613	2884

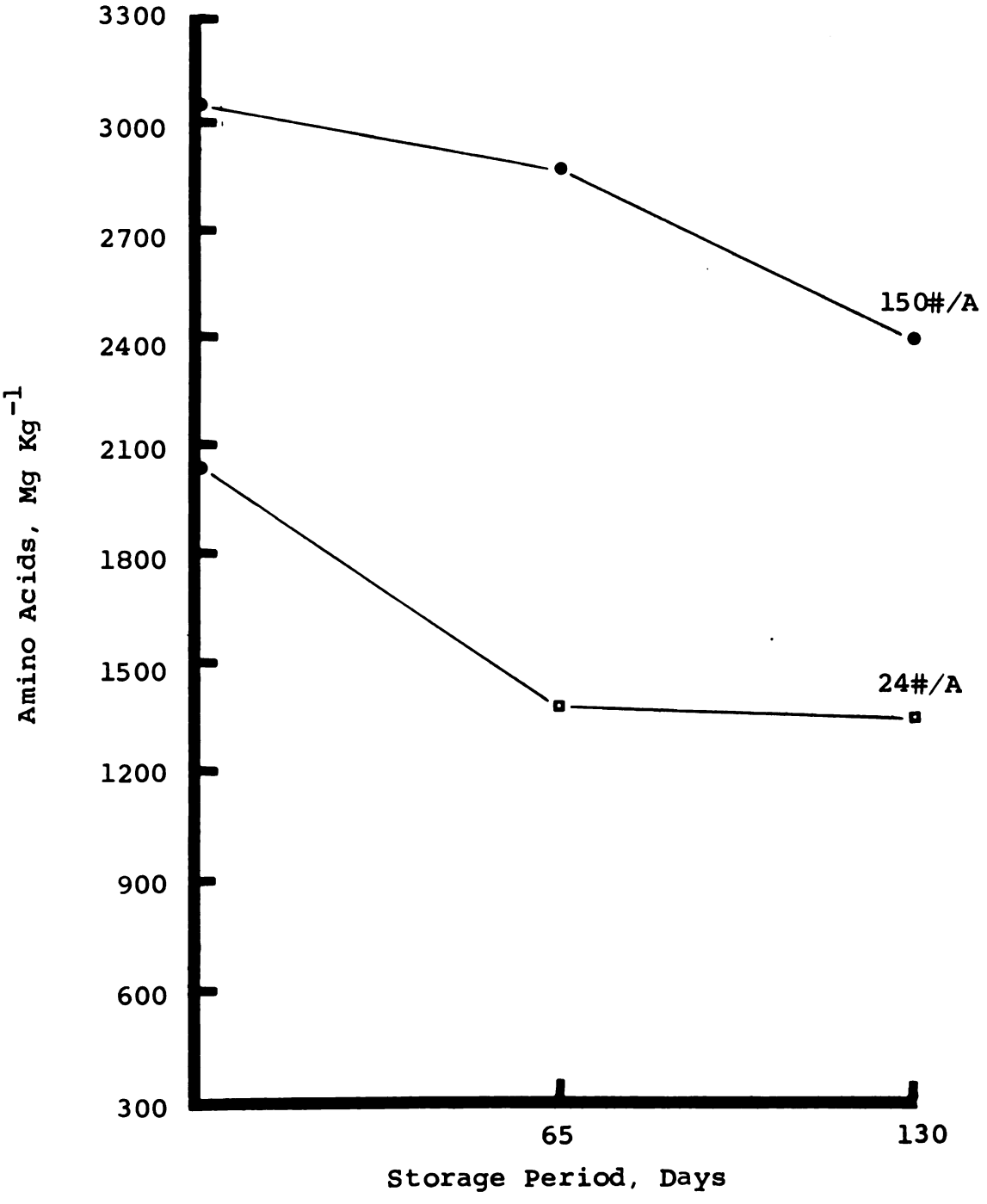

Figure 17. Amino acid content in storage at 3 C.

This sensitivity was also evident in the RSPA yield of variety 5 (p. 155). At the low level of nitrogen fertilization all varieties had essentially identical concentrations of amino acids.

There was no significant variety x nitrogen x removal interaction but the harvest x removal x nitrogen interaction was significant (Figure 16).

When five varieties were stored for 65 and 130 days at 3 C no variety x removal or nitrogen x removal interactions were found. However the variety x nitrogen interaction was highly significant (Table 17). Varieties 3 and 5 appeared to be highly sensitive to nitrogen fertilization since their amino acid content was almost doubled under high nitrogen fertilization.

Table 17. Variety x nitrogen interaction in amino acid content of five varieties

	Nitrogen Applied, Lbs/A	
Variety	24	150
	Mg Kg^{-1}	
1	1643	2153
2	1454	2493
3	1609	3036
4	1322	2443
5	1541	3564
Average	1514	2738

Variety-Temperature Interaction

Cold storage resulted in no accumulation of amino acids in three varieties stored at 3 C for 100 days in 1968 (Table 18). However at 11 C the amino acid content increased 50 percent. Variety 7 was relatively insensitive in its response to storage temperature. The extremely high amino acid content of variety 6 at harvest increased substantially at both temperatures. Variety 5 was sensitive to warm storage temperatures, accumulating 464 mg/kg.

Table 18. The effect of storage temperature on the amino acid content of beets stored for 100 days

Variety	Harvest	Storage Temperature	
		3 C	11 C
		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.

In storing beets at various temperatures, varieties differed considerably with respect to the level of raffinose, invert, and amino acids. These compounds make up the majority of the non-sucrose components in the clear juice. Through breeding and proper agronomic and storage practices the level of these important impurities can be controlled. The selection of a beet which would tolerate storage at 11 C without the accumulation of reducing sugars or amino acids appears to be possible and would greatly reduce the cost of refrigerated pile cooling.

Effect of Topping

Topping of beets prior to harvest had no effect on the changes in the amino acid content at either 3 or 7 C storage. Topped beets were slightly lower in amino acids than untopped beets (1776 vs 2070) indicating that the crown was somewhat higher in amino acids than the tap root.

Preharvest Sprays

MH-30 and GA applied prior to harvest decreased the free amino acid content of the root at harvest. The amino acids of beets pretreated with GA also remained lower throughout storage, but with MH-30 they increased for the first 8 weeks of storage and then fell rapidly to the same level as the plants treated with GA (see Figure 18). MH-30 has been shown to inhibit pyrimidine synthesis which may have reduced new protein synthesis (Pavlinova, 1967).

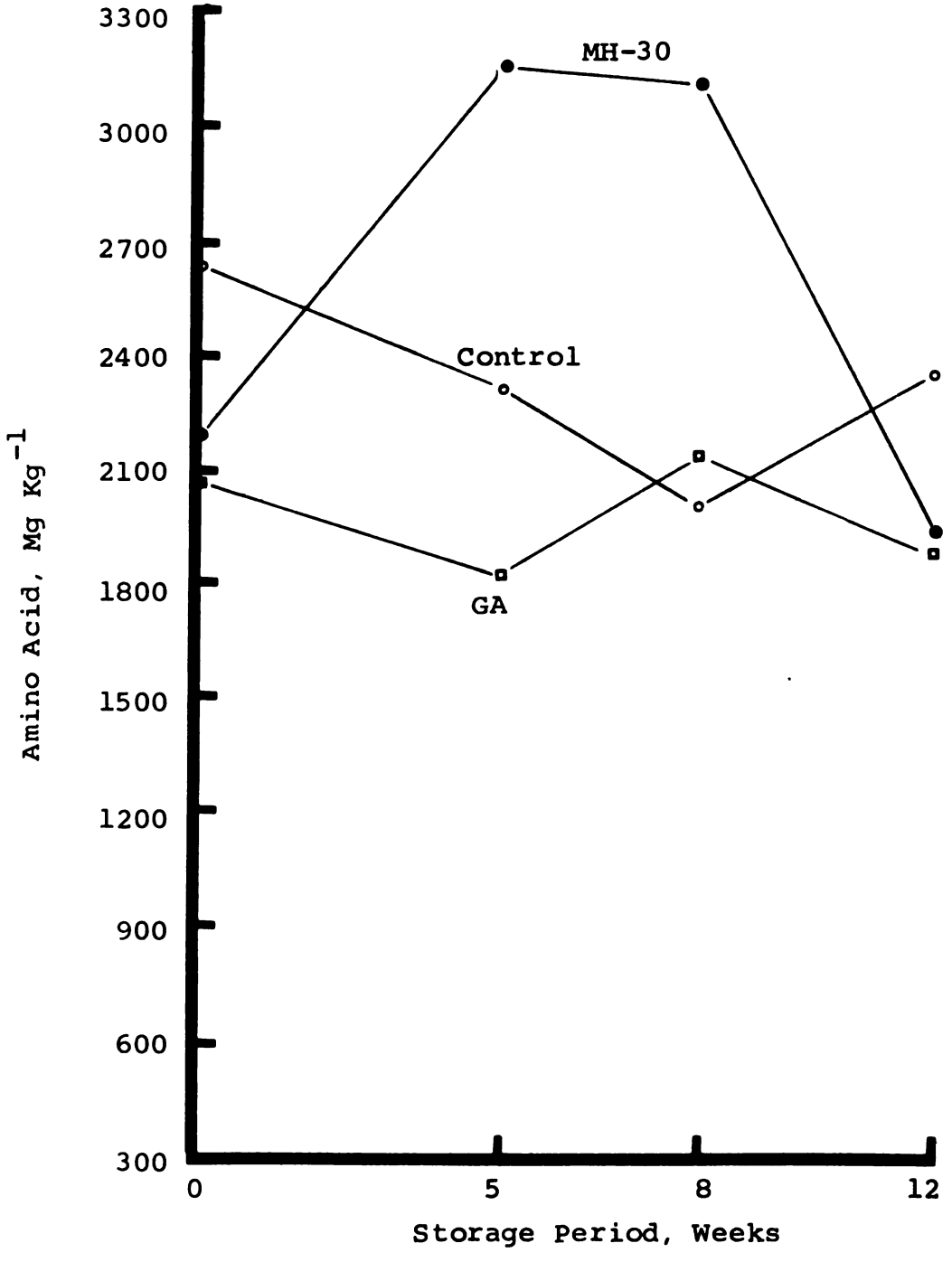

Figure 18. Effect of preharvest applications of MH-30 and gibberellic acid on the amino acid content of stored beets.

This could result in the accumulation of amino acids due to normal protein degradation.

Vanadium sulfate, pyrocatechol and CCC applied prior to harvest had no effect on the amino acid content either at harvest or after storage (Table 19).

Table 19. Effect of several preharvest sprays on the amino acid content of fresh and stored beets

Spray	Harvest	Days in Storage 50	100	Average
		Mg Kg^{-1}		
Control	2471	2072	2243	2262
$VaSO_4$	2251	2051	1668	1990
Pyrocatechol	2557	2204	1873	2211
CCC	2285	1753	1878	1972
Average	2391	2019	1916	

Storage in Modified Atmospheres

The storage of beets at 5 C in modified atmospheres for 40 days resulted in a 56 percent increase in amino acids over the control (Table 20). Ethylene (1000 ppm) increased the amino acid content 500 mg in normal atmosphere but caused a decline in the modified atmosphere. However the

increase in amino acids over the harvest level was much greater than in other experiments at the same storage temperature.

Table 20. Effect of modified atmospheres on the amino acid content of stored beets

	Ethylene		
Atmosphere	0 ppm	1000 ppm	Average
		Mg Kg^{-1}	
Control	1660	2114	1887
Modified Atmosphere	3126	2767	2946
Average	2393	2441	
At Harvest	808		

Factors Controlling the Sodium and Potassium Content of the Root

Sodium and potassium are two very important melassigenic constituents of the beet root (Stark, 1968; Khualkouskii, 1964; Silina, 1964). They have been proposed for use as a quality index in selecting breeding lines (Wood, 1958). Carruthers proposed their use in estimating the purity of the clear juice (1963).

Variety-Nitrogen Interaction

In 1967 the potassium and sodium content varied between varieties although the differences were not great (Table 21). Nitrogen fertilization had a major effect on both minerals and sodium in particular (Table 22). The nitrogen effect on sodium content was greater than the variety effect. This increase in sodium and potassium caused by high nitrogen fertilization was apparently due in part to the need for more base to neutralize the increased amino acid content of the beets grown on high nitrogen.

Nitrogen	Meq Amino Acids	Meq K and Na
150#/A	13	24 + 3 = 27
24#/A	7	21 + 2 = 23
Difference	6	4

Table 21. Effect of nitrogen fertilization on the sodium and potassium content of five varieties harvested on October 26, 1967

	Sodium			Potassium		
	Nitrogen			Nitrogen		
Variety	24#/A	150#/A	Average	24#/A	150#/A	Average
	Mg Kg^{-1}			Mg Kg^{-1}		
1	81	87	84	1531	1465	1498
2	78	96	87	1376	1571	1474
3	74	112	93	1159	1335	1247
4	67	107	87	1456	1566	1511
5	72	108	90	1325	1412	1369
Average	74	102		1369	1470	

Table 22. Effect of nitrogen fertilization on the average sodium and potassium content of three varieties harvested on three dates in 1967

	Sodium			Potassium		
	Nitrogen			Nitrogen		
Variety	24#/A	150#/A	Average	24#/A	150#/A	Average
	Mg Kg^{-1}			Mg Kg^{-1}		
2	79	97	88	1436	1681	1506
3	81	114	97	1287	1440	1310
5	77	110	93	1301	1321	1417
Average	79	107		1341	1481	

However the increase in free amino acids was greater than the combined increase in sodium and potassium.

Harvest Date

The harvest x removal interaction for potassium in 1968 was significant indicating that more potassium was available in stored than in fresh beets (Table 23). This same trend was found by Dexter (1966). The total potassium content in the root cannot change during storage, therefore the potassium in the beet apparently became associated with more soluble anions and was more readily squeezed out of the brei in the process of taking the sugar sample. In many of the other experiments the increased potassium in the clear juice after storage was nearly significant. In fact the increase was large enough to prevent using potassium as a basis for expressing changes in the chemical composition of the beets during storage. This method has been proposed by Vajna (1960), but its effectiveness would require an analysis of total potassium in the ash fraction.

Preharvest Sprays

Foliar sprays applied 10 days prior to harvest significantly affected the sodium content of the root at harvest and caused a significant increase in both the sodium and potassium content during storage (Table 24). Vanadium sulfate and CCC significantly reduced the level of sodium at harvest. Although the spray x removal interaction

Table 23. Effect of harvest date on the sodium and potassium content of fresh and stored beets in 1968

Harvest	Sodium				Potassium			
	At Harvest	Stored (days) 50	100	Average	At Harvest	Stored (days) 50	100	Average
	Mg Kg^{-1}				Mg Kg^{-1}			
September 1	107	75	100	94	1502	1373	1433	1436
October 1	57	91	53	67	1107	1421	1174	1234
November 1	123	148	141	137	1319	1464	1617	1467
Average	96	104	98		1309	1419	1408	

Table 24. Effect of several preharvest foliar sprays on the sodium and potassium content of fresh and stored beets

Spray	Sodium				Potassium			
	At Harvest	Stored (days) 50	100	Average	At Harvest	Stored (days) 50	100	Average
	Mg Kg^{-1}				Mg Kg^{-1}			
Control	123	157	142	140	1337	1474	1637	1483
Vanadium Sulfate	93	115	125	111	1452	1490	1568	1503
Pyrocatechol	143	130	153	142	1359	1373	1432	1388
CCC	93	115	109	105	1354	1389	1555	1432
Average	113	129	132		1375	1431	1548	
Sodium plus Potassium	1498	1560	1680					

was non-significant, the increase in sodium and potassium during 100 days of storage was highly significant. GA and MH-30 had no effect on the sodium and potassium content either at harvest or in storage.

Factors Controlling the Chloride Content

The chloride content of the clear juice is thought to have a high positive correlation to molasses purity (Stark et al., 1968). Increasing the chloride content increases both the quantity and purity of molasses, thus reducing RSPT.

Variety-Nitrogen-Harvest Date Interaction

The variety x nitrogen x harvest date interaction for chlorides was significant (Table 25). The average chloride content declined slightly with delayed harvest. The beets grown on low nitrogen contained a higher chloride content than the high nitrogen beets in all varieties except variety 3 harvested late. This is the only non-sucrose component which showed this response to nitrogen, all others increased with increased nitrogen applications. Variety 5 was significantly higher in chlorides on the average for all harvest dates.

The variety x nitrogen interaction has highly significant for the five varieties harvested on October 26 (Table 26). The beets grown on low nitrogen were again higher on

Table 25. Interaction between variety, nitrogen, and harvest date on the chloride content in 1967

Variety	October 6 Nitrogen 24#	October 6 Nitrogen 150#	October 26 Nitrogen 24#	October 26 Nitrogen 150#	November 6 Nitrogen 24#	November 6 Nitrogen 150#	Variety Average
				Mg Kg^{-1}			
2	217	137	196	133	210	157	175
3	206	164	133	171	168	171	167
5	255	220	252	182	189	166	212
Average	226	173	194	162	189	171	
Harvest Average	200		178		178		

Table 26. Variety x nitrogen interaction for chloride content of five varieties grown on high (150#) and low (24#) nitrogen

Variety	Nitrogen 24#/A	Nitrogen 150#/A
	Mg Kg^{-1}	
1	252	189
2	196	133
3	133	172
4	203	224
5	252	182
Average	208	180

the average but varieties 3 and 4 were slightly higher in chloride content at high nitrogen fertilization. There was no significant treatment x storage interaction in chloride content.

If the millequivalent decrease in chloride content with high levels of nitrogen is considered in the hypothesis that the increased base content is due to the higher concentration of amino acids, an interesting relationship develops. The chloride content was 1 millequivalent lower ($\frac{169}{35}$ vs $\frac{203}{35}$) under low nitrogen fertilization. Therefore the balance is as follows:

Nitrogen	Amino Acids	Chlorides	Total	Meq K and Na
		Mg Kg^{-1}		
24#/A	7	5.8	12.8	21 + 2 = 23
150#/A	13	4.8	17.8	24 + 3 = 27
Difference			5.0	4

In the field the acid-base balance of the beet is essentially balanced and any adjustments needed in processing arise either from reducing sugar and breakdown products or differential ion elimination in clarification.

Effect of Thermal Induction on Chemical Composition of Beets Stored at 5 C

Beets from the same origin as those used in the respiration study (p. 117) and stored at the same temperature (5 C) were analyzed after 5, 8 and 12 weeks of storage. Besides the usual chemical analyses beets from each treatment were planted in the greenhouse to determine the degree of thermal induction.

After 5 weeks of storage at 5 C, 87 percent of the GA treated and 56 percent of the control plants bolted. The plants treated with MH-30 produced only very stunted and deformed leaves. After 8 weeks of storage all plants from the GA and control treatments bolted. MH-30 inhibited normal sprout formation for the entire 15 week storage period.

Figure 19 is an average of all preharvest treatments and shows the effect of thermal induction on the raffinose, invert and amino acid content in storage. The non-induced beets were 35 percent higher than the induced beets in raffinose after 5 weeks in storage. However only the rate of accumulation was reduced because the induced samples increased to a high level after continued storage.

The results from previous years showed that after 60 days of storage at 5 C, the reducing sugars began to increase. Since approximately 60-70 days at 5 C fulfills the thermal requirement for bolting, induced samples might

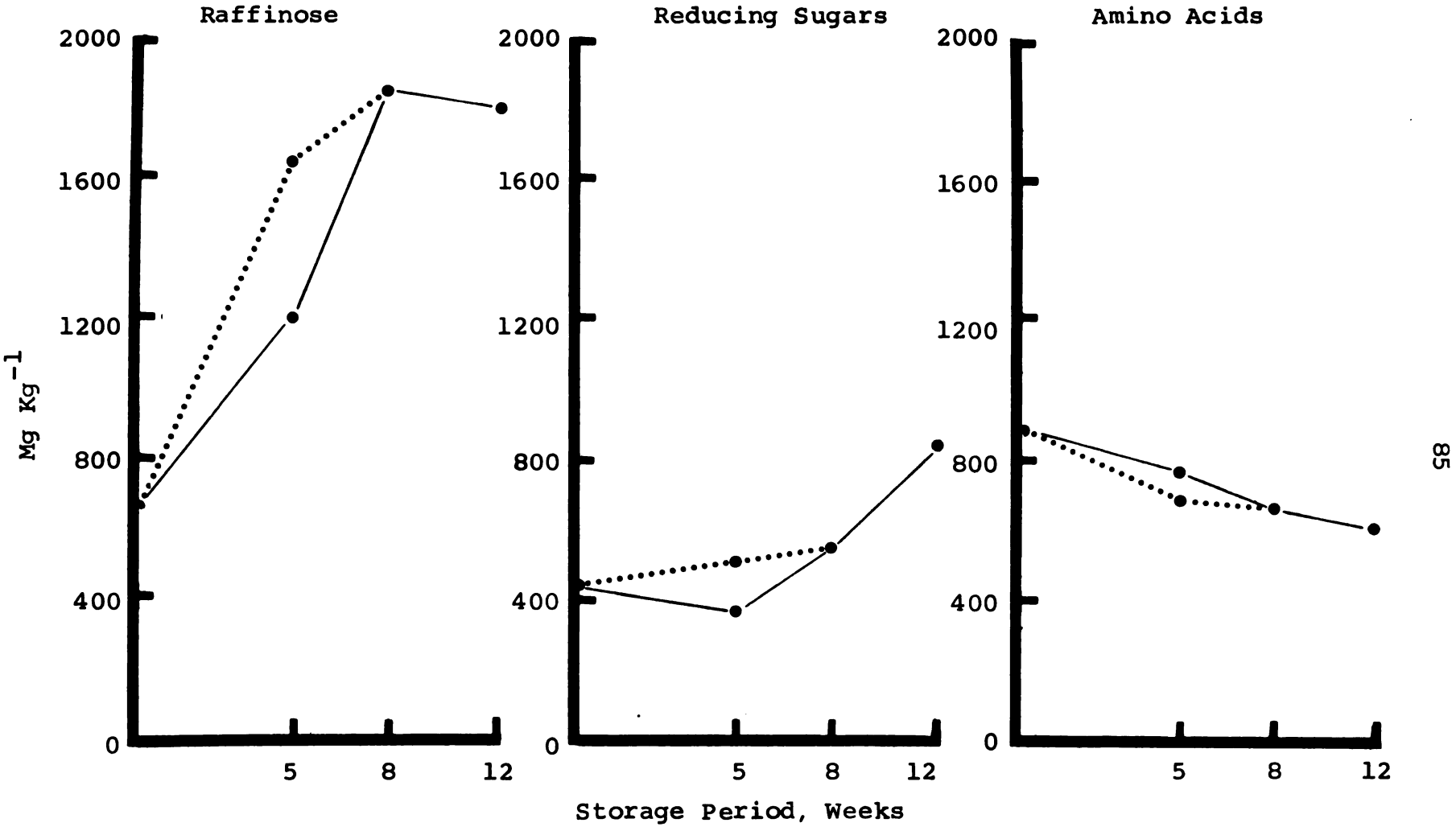

Figure 19. Effect of thermal induction on the raffinose, reducing sugar and amino acid content of beets stored at 5 C. Non-Induced, ···; Induced, —.

accumulate reducing sugars at a more rapid rate than the non-induced samples. However, the average of the three treatments shows that the non-induced plants were not significantly higher in reducing sugars. The reducing sugar content did increase rapidly later in the storage period but this could not be attributed to the induction process since all treatments were fully induced.

The non-induced plants were also lower in amino acid content than the induced plants, but again the differences were very small.

Table 27 gives the amino acid, raffinose, and reducing sugar content of the induced vs non-induced for all preharvest treatments. The difference between the induced and non-induced roots was greatest for the control. Induction decreased raffinose and reducing sugars 600 and 125 mg/100 RDS respectively. The GA treatments showed the same trend but the differences were much smaller. The plants treated with MH did not sprout normally so no analysis of induction could be made. However the trend for the MH treatment was the same as for the non-bolters in the control and GA treatments. From this tenuous information MH may have inhibited induction completely as opposed to the stimulation by GA (Stout, 1959; Stout, 1959). MH is considered a general growth retardant and has been shown to inhibit pyrimidine synthesis in the sugarbeet (Pavlinova, 1967; Kursanov, 1967).

Table 27. Effect of thermal induction on the raffinose, amino acid and reducing sugar content of beets stored at 5 C (1968)

	Raffinose				Reducing Sugar				Amino Acids			
Weeks Stored	0	5	8	12	0	5	8	12	0	5	8	12
						Mg/100 RDS						
Control												
Bolters		995	1602	1885		325	411	717		856	704	605
Non-Bolters	599	1609			442	458			976	640		
GA Treated												
Bolters		1449	2122	1983		439	518	846		707	708	597
Non-Bolters	697	1655			396	454			782	765		
MH-30 Treated												
Non-Bolters	601	1391	1834	1840	393	405	567	929	773	608	636	614
Averages												
Bolters		1222	1853	1836		382	499	831		781	683	605
Non-Bolters	648	1632			419	456			879	698		

Effect of Prestorage Heating on Raffinose, Reducing Sugar, and Amino Acid Content After Storage

Due to warm fall temperatures, interior pile temperatures may increase to 20-32 C for several days. Since this is a very common occurrence in commercial piles it seemed pertinent to study the effect of excessive heating early in the storage period on subsequent storage.

Samples were held at 27 C for 5 days in polyethylene bags. After the heating period the samples were placed in storage at 3 C.

The effect on the raffinose, reducing sugar and amino acid content after storage is given in Table 28. The raffinose content declined 30 percent during the five days at 27 C while the control at 3 C remained constant. However the heating treatment had no effect on the subsequent accumulation of raffinose in storage. The amino acid and reducing sugar levels were not affected.

Apparently beets can tolerate warm temperatures for short periods. The only loss would be due to the increased respiration. In the commercial pile these warm temperatures also facilitate mold growth and sprouting which are major factors contributing to sugar loss in commercial piles.

Table 28. Effect of postharvest heating on raffinose, invert, and amino acid production in subsequent cold storage (1968)

	At Harvest	Days Stored		
		5	50	100
		Mg Kg^{-1}		
		Raffinose		
27 C--5 days then at 3 C	1071	734	2152	1985
Control (3 C)	1071	1076	2352	2218
		Invert		
27 C--5 days then at 3 C	762	897	981	1290
Control (3 C)	762	939	744	1272
		Amino Acids		
27 C--5 days then at 3 C	2471	2635	2637	2303
Control (3 C)	2471	2700	2155	2243

Comparison of Analyzed vs Total Non-Sucrose Components of the Clear Juice

The preceding sections have discussed the content of several major impurities found in the clear juice at harvest and during storage. These non-sucrose components of the clear juice were composited as total analyzed impurities (TAI) and compared to the total impurities* in the clear juice as calculated from the clear juice purity (Table 29).

The TAI comprised approximately 65 percent of the total impurities in fresh and stored beets. However the changes in the TAI and non-TAI during storage varied widely between varieties.

The total impurities increased in the first 65 days of storage in all varieties. However varieties 2, 3 and 4 decreased slightly in total impurities during the second 65-day period. This indicates an apparent shift in the metabolic pattern of these varieties after prolonged storage. The change in the unknown impurities gives a good illustration of this shift (Table 29, Figure 20). In variety 1 the unknown impurities increased steadily during the entire storage period. In varieties 2, 3, 4 and 5 a decline in unknown impurities occurred in the last 65 days. This decline indicates a metabolic shift towards the catabolism of these unknown compounds possibly via respiration (see p. 122).

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*Impurities include all soluble non-sucrose constituents found in the thin juice.

Table 29. Comparison of the sum of analyzed impurities to the total calculated impurities in five varieties stored for 65 and 130 days at 3 C (1967)

Variety	Raffinose	Reducing Sugars	Amino Acids	Potassium + Sodium	Impurities TAI	Impurities Total	Impurities Unknown	Change in TAI	Change in Total	Change in Unknowns
					Mg Kg^{-1}					
At Harvest										
1	1766	659	2283	1553	6261	10407	4146			
2	1757	869	2348	1457	6431	10298	3867			
3	1789	641	2680	1272	6382	10159	3777			
4	1883	656	2256	1653	6448	10249	3801			
5	1622	783	3320	1479	7204	9853	2649			
Average	1763 (17)[a]	722 (7)[a]	2577 (25)[a]	1483 (14.8)[a]	6545 (64)[a]	10193	3648			
Stored 65 Days									0 to 65	
1	2773	1040	1594	1513	7280	11758	4478	1019	1351	+332
2	3427	1513	1987	1633	8568	12762	4194	2137	2464	+327
3	2216	799	2417	1355	6787	11449	4662	405	1290	+885
4	3585	1144	1730	1585	8044	12588	4544	1596	2339	+743
5	2227	1294	2183	1414	7118	11355	4237	-86	1502	+1588
Average	2846 (24)[a]	1158 (10)[a]	2054 (17)[a]	1500 (12.8)[a]	7559 (63)[a]	11982				
Stored 130 Days									65 to 130	
1	2411	3711	1456	1680	9258	13992	4734	1978	2234	256
2	2785	2649	1583	1593	8610	12007	3397	42	-755	-797
3	2812	2736	1870	1393	8811	13278	4467	2024	1829	-195
4	2403	2606	1661	1556	8226	12314	4088	182	-274	-456
5	1594	2260	2152	1482	7488	11495	4007	370	-140	-98
Average	2361 (19)	2792 (22)	1744 (14)	1541 (11.7)	8479 (67)	12617	4139	919	579	-258

[a]Number in parentheses = percent of total impurities.

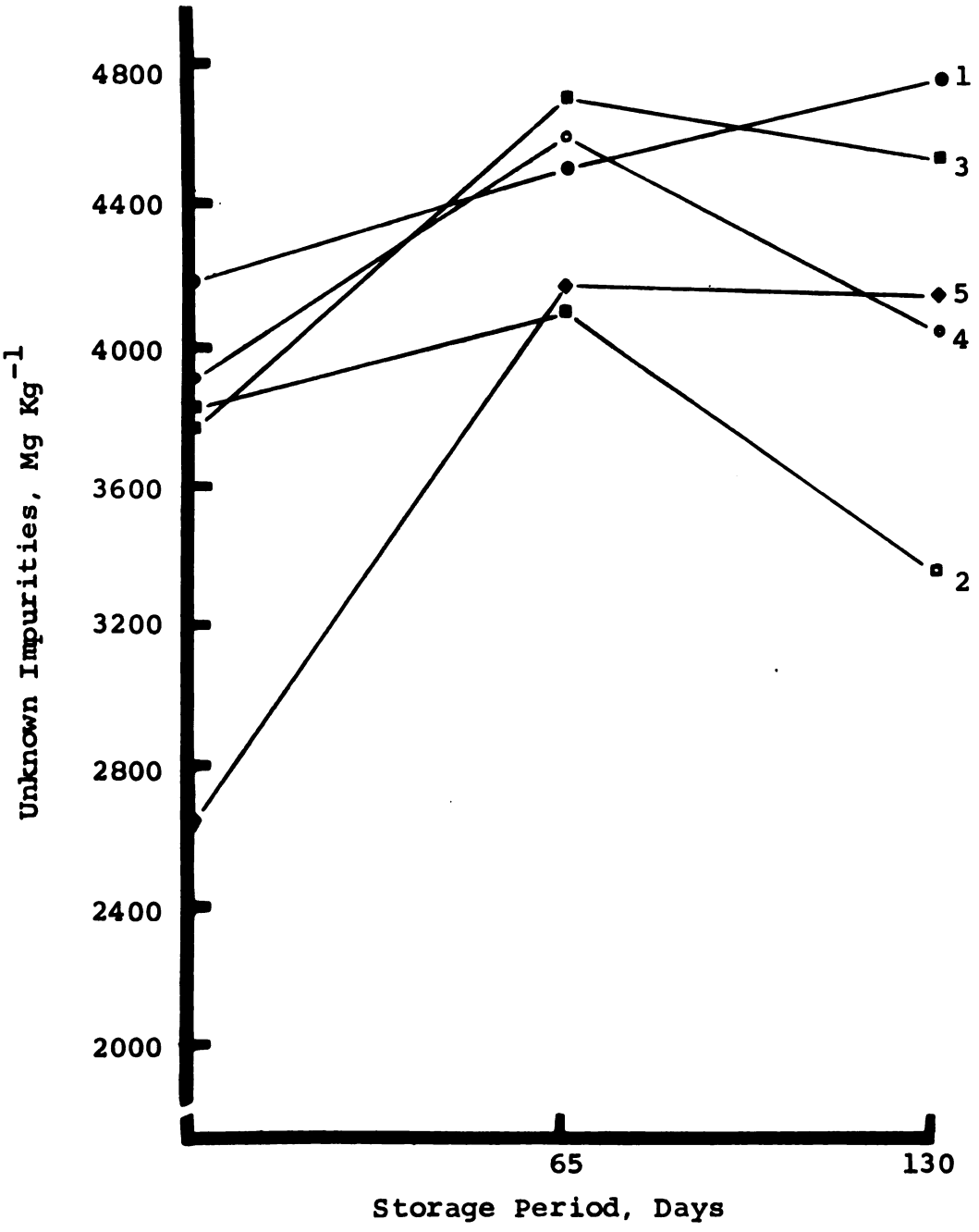

Figure 20. Change in non-TAI content of five varieties in storage 65 and 130 days.

In Table 30 the changes in the metabolicly active components of the TAI (raffinose, reducing sugars, and amino acids) are compared to the changes in total impurities. The increase in total impurities in the first 65 days of storage was due to the accumulation of raffinose and invert in varieties 1, 2 and 4. Varieties 3 and 5 did not accumulate large amounts of raffinose and invert but the increase in the total impurities was still considerable. After prolonged storage the total impurities decreased presumably because raffinose, amino acids, and some unknown impurities were catabolized.

The beet accumulates impurities, primarily raffinose and invert, during the early part of the storage period. With prolonged cold storage amino acids and some unknown impurities are catabolized presumably via respiration. This catabolism may occur at a rate greater than the accumulation of raffinose and invert and actually cause a decline in unknown as well as total impurities.

Comparing the very different chemical composition of the three varieties stored at 3 and 10 C illustrates this metabolic shift further (Table 31). The TAI were only 53 percent of the total non-sucrose compounds at harvest but increased to 64 percent in storage at 3 C. At 10 C the percent TAI remained constant. In varieties 6 and 7 the unknown impurities decreased approximately 400 mg/kg at 3 C.

Table 30. Increase in total impurities accounted for by raffinose, reducing sugars and amino acids in five varieties stored 65 and 130 days at 3 C (1967)

Variety	Increase in Total Impurities	Change in:			Sum of Changes in Raffinose, Reducing Sugars, Amino Acids
		Raffinose	Reducing Sugars	Amino Acids	
		Mg Kg^{-1}			
Stored 65 Days					
1	1351	1307	381	-689	999
2	2464	1670	644	-361	1953
3	1290	427	158	-263	322
4	2339	1702	488	-526	1664
5	1502	605	511	-1137	-21
Stored 130 Days					
1	2234	-662	2671	-138	1871
2	-755	-642	1136	-404	90
3	1829	596	1937	-547	1986
4	-274	-1182	1462	-69	211
5	-140	-633	966	-31	302

Table 31. Comparison of the total analyzed impurities (TAI) to the total impurities in three varieties stored for 100 days at 3 and 10 C (1968)

Variety	Raffinose	Reducing Sugars	Amino Acids	Sodium	Potassium	Impurities			Change in		
						TAI	Total	Unknown	TAI	Total	Unknowns
						Mg Kg^{-1}					
<u>At Harvest</u>											
5	1288	657	1241	120	1190	4496	9044	4548			
6	2212	916	2267	160	1443	6998	12753	5755			
7	1693	832	808	59	1040	4432	8409	3977			
Average	1731 (17)[a]	802 (8)[a]	1439 (14)[a]	113 (1)[a]	1224 (12)[a]	5309 (53)[a]	10069	4760			
<u>At 3 C</u>											
5	2966	659	1128	119	1142	6014	10706	4692	1518	1662	+144
6	5015	1096	2567	234	1586	10498	15859	5361	3500	3102	-394
7	4864	966	864	59	959	7712	11233	3521	3280	2824	-456
Average	4281 (34)[a]	907 (7)[a]	1520 (12)[a]	137 (1)[a]	1229 (10)[a]	8075 (64)[a]	12599	4524	2766	2530	-235
<u>At 10 C</u>											
5	890	1297	1705	135	1219	5246	10530	5284	750	1486	+736
6	2702	1530	3411	246	1553	9442	15216	5774	2444	2463	+19
7	1790	1115	1235	69	1057	5266	10002	4736	834	1591	+757
Average	1794 (15)[a]	1314 (11)[a]	2117 (18)[a]	150 (1)[a]	1276 (11)[a]	6651 (56)[a]	11916	4526	1343	1569	+227

[a]Number in parentheses = percent of total impurities.

Again these varieties were apparently metabolizing the non-TAI components. At 10 C the unknown fraction increased greatly in variety 5 and 7 but in variety 6 the increase in TAI accounted for the total increase in impurities. These results further illustrate the variation between varieties in the interaction of storage temperature and the basic metabolic systems of the beet root.

Table 32 compares the change in total impurities to the change in raffinose, reducing sugars and amino acids in the variety-temperature experiment. The increase in total impurities was lower at 10 C. This was due partially to the lack of raffinose development. The increased recoverable sugar losses at warmer storage temperatures were primarily caused by increased respiration and not to increased impurities (assuming no rot, mold, or sprouting). However the impurities which increase under warm storage (amino acids, invert) are more melassigenic than raffinose and may result in a greater loss of sucrose to molasses (Carruthers, 1959). The increase in raffinose and invert compared to the increase in total impurities varies greatly between varieties. However these two components account for the majority of the impurities which accumulate in storage below 5 C. Above 5 C other impurities not analyzed in these experiments were accumulating as evidenced by the low proportion (63%) of the increase in total impurities accounted for by raffinose, amino acids and reducing sugars.

Table 32. Increase in total impurities accounted for by raffinose, reducing sugars and amino acids in three varieties stored 100 days at 3 and 10 C (1968)

Variety	Increase in Total Impurities	Change in			Sum of Changes in Raffinose, Reducing Sugars, Amino Acids	Change in Total Accounted for by TAI
		Raffinose	Reducing Sugars	Amino Acids		
		Mg Kg^{-1}				Percent
At 3 C						
5	1662	1678	2	-113	1567	94
6	3102	2803	180	300	3283	106
7	2824	3171	57	56	3284	116
Average	2529	2550	80	84	2711	105
At 10 C						
5	1486	-398	640	464	706	48
6	2463	490	614	1144	2248	91
7	1591	97	283	427	807	51
Average	1847	63	512	678	1254	63

Summary of the Factors Controlling Several of the Non-Sucrose Components in the Clear Juice

The variation in the chemical composition of the beet root was predominantly a varietal characteristic. In fact variety was the dominant factor controlling the non-sucrose content of the beet at harvest. Delayed harvest increased raffinose due to cool temperatures but the magnitude of this increase was strongly variety dependent.

Nitrogen fertilization had little effect on the analyzed non-sucrose components except the amino acids. However susceptibility to excessive nitrogen fertilization was strongly influenced by variety.

Under storage conditions which prevented excessive wilting, storage temperature played a dominant role in determining the impurity content of stored beets. Temperatures below 5 C caused an accumulation of raffinose while temperatures above 5 C caused a reduction in the raffinose content. However the threshold temperature and the amount of raffinose which accumulated were variety dependent.

Storage temperature also affected the reducing sugar content of stored beets but this was primarily a result of secondary effects on mold and sprout formation. These factors caused a rapid increase in reducing sugars. The interaction between temperature and variety for reducing sugar accumulation was not as great as that for raffinose but was significant.

Storage temperature also affected the change in amino acid content during storage. The amino acid content declined in storage below 8 C at a rate inversely proportional to the amino acid content at harvest. Again the response to temperature was variety dependent.

Topping beets prior to storage had little effect on the non-sucrose components in storage.

Delaying harvest reduced the accumulation of raffinose but increased the reducing sugar content in storage at 3 C.

Preharvest applications of maleic hydrazide and gibberellic acid increased the raffinose and reducing sugar content at harvest and after storage. GA decreased the amino acid content in storage while MH-30 caused an increase.

The low oxygen content of controlled atmosphere storage caused a 200 percent accumulation of raffinose and amino acids over the control. Reducing sugars were not affected.

Excessive nitrogen fertilization increased the sodium and potassium content of the beets but decreased the chloride content. The variety-nitrogen interaction was highly significant in all cases.

Fulfilling the thermal requirement for bolting caused an increase in the raffinose content in storage. The amino acid and reducing sugar contents were not significantly affected.

Short term high temperature treatments prior to storage reduced the raffinose content 35 percent in five days but had no effect on its accumulation later in storage. Reducing sugars and amino acids were not affected.

The TAI accounted for approximately 65 percent of the total impurities in beets stored at 3 C and a slightly lower percentage of the total in beets stored warm. With prolonged storage a metabolic shift occurred from the accumulation of raffinose and unknown impurities to the catabolism of these compounds. A decline in total impurities resulted in some instances. High sucrose losses in storage above 4 C are probably due to higher rates of respiration and not to the accumulation of impurities (if rot, mold and sprouting are prevented).

CHANGE IN MARC CONTENT DURING HARVEST AND IN STORAGE

In 1967 the percent marc was determined on all samples in the date of harvest and variety experiments. Fifty-gram brei samples were mixed with 200 ml of 70 C distilled water and incubated for 20 minutes at 70 C in a controlled temperature water bath. The mixture was then filtered through a powdered cellulose filter and washed with ten 200 ml aliquots of 25 C tap water. The residue was dried at 105 C for 48 hours and the marc content calculated as percent fresh weight.

At Harvest

Variety-Harvest Interaction

In 1967 varieties 2 and 3 were higher than variety 5 in percent marc for all harvest dates (Figure 21). The decline in percent marc for the October 6 harvest was caused by a heavy rain three to four days before harvest. As a result the beets took up water and all determinations based on percent fresh weight declined. Dry weather has been previously shown to increase the percent marc (Dubourg, 1960). However the total yield of marc per acre increased in a

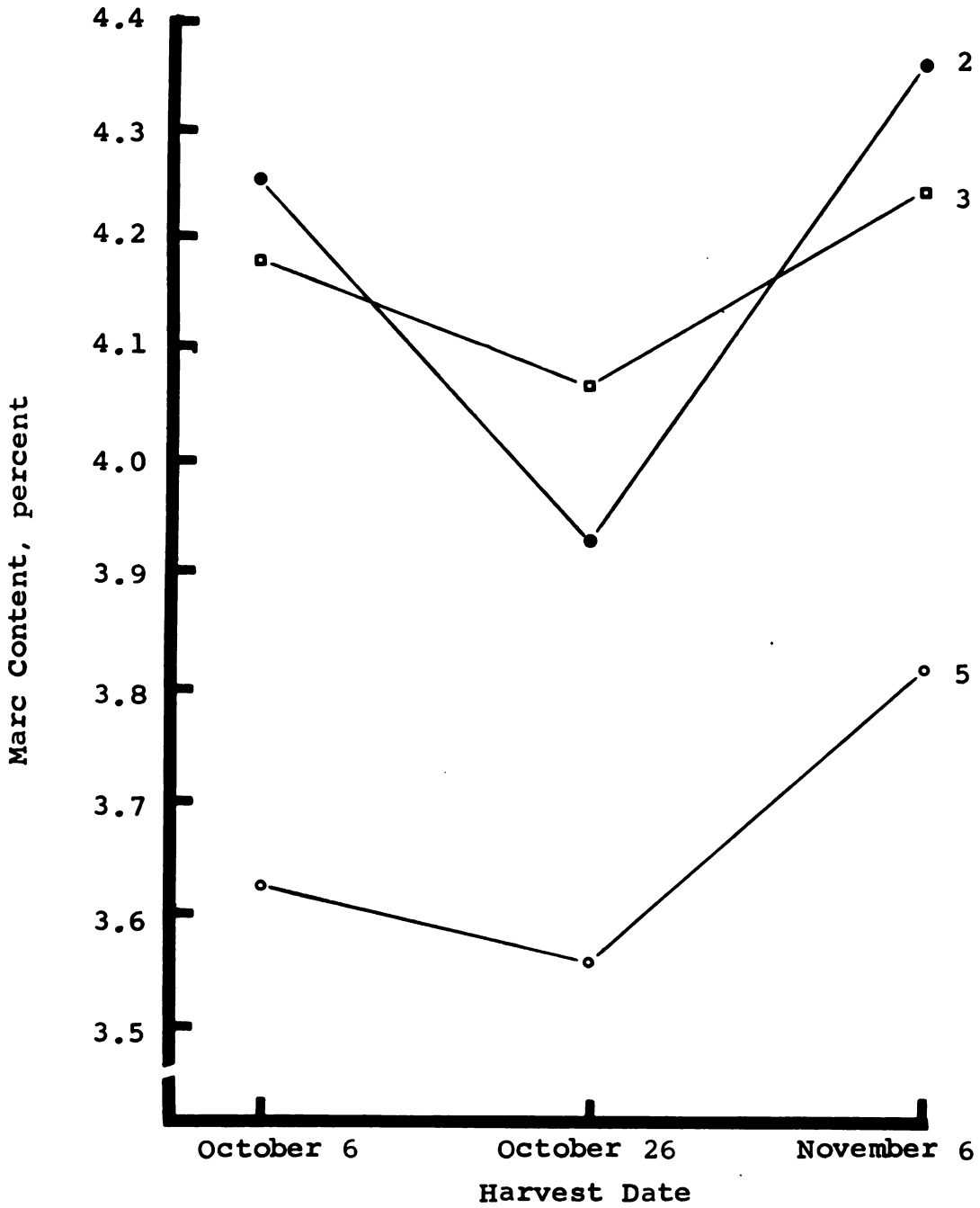

Figure 21. Percent marc of three varieties on three harvest dates in 1967.

linear fashion during the harvest period except for variety 3 (Figure 22).

Nitrogen

The percent marc was higher in the beets grown on low nitrogen at all harvest dates than in the beets grown on high nitrogen (average 4.2 vs 4.0). The beets grown on low nitrogen showed severe nitrogen deficiency and had stopped growing while the high nitrogen beets were still dark green and succulent. The higher percent marc in the low nitrogen beets may have been due to a maturation effect in the non-growing plants resulting in a shift in the pectin content to a more insoluble form.

Although the percentage marc was lower for the high nitrogen beets the total marc yield per acre was greater (Figure 23) since there were more tons of high N than of low N beets. These differences were small but the increased pulp yield per factory would be considerable.

During Storage

Changes in Marc During Storage

In 1967 after 65 and 130 days of storage at 3 C the average percent marc for three varieties increased 0.2 percent (Table 33). These results were very different from those found in the preliminary experiments of the previous year. The previous studies had shown a considerable decline in the percent marc during storage. The determinations were

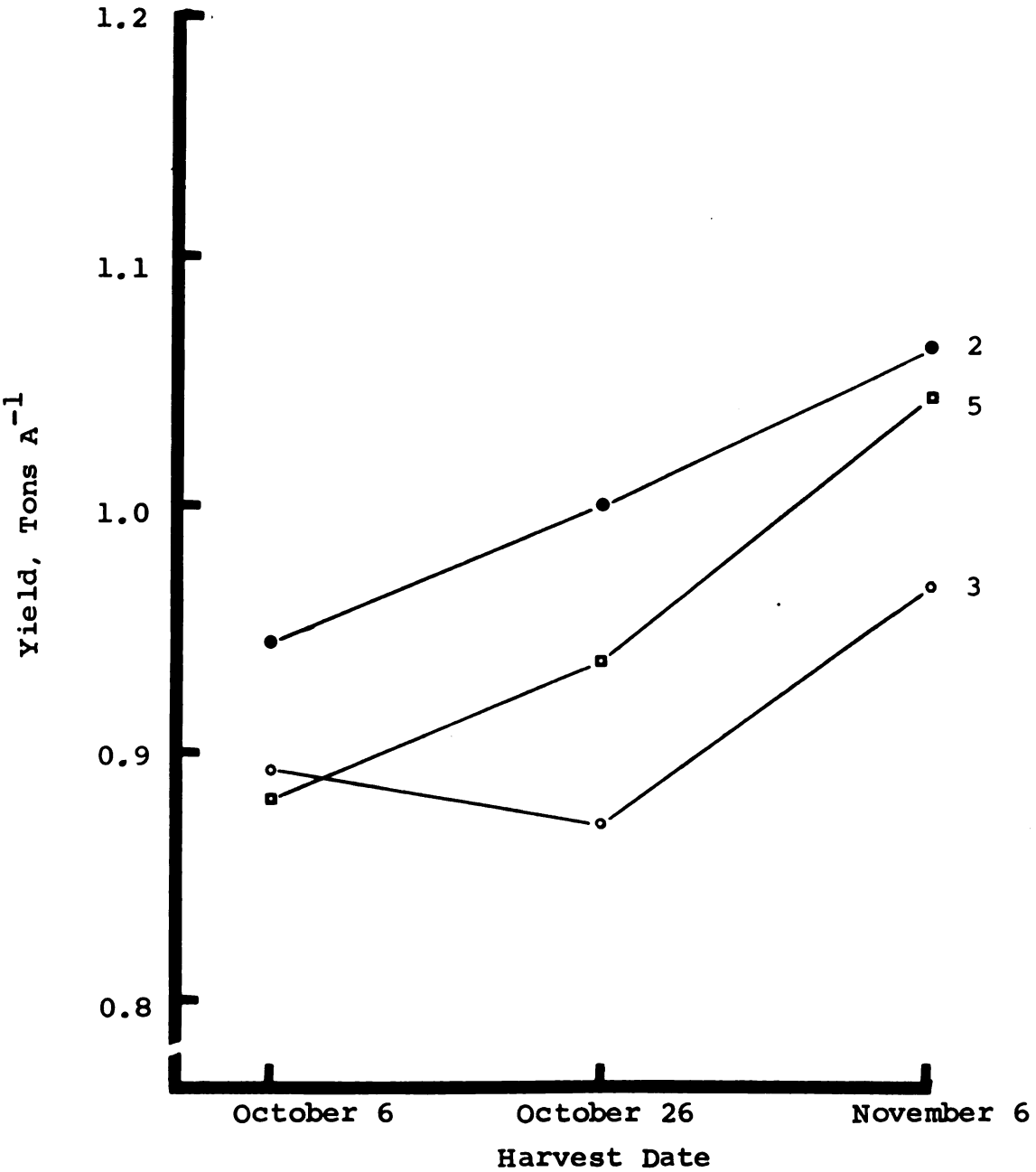

Figure 22. Yield of marc in tons per acre at harvest for three varieties in 1967.

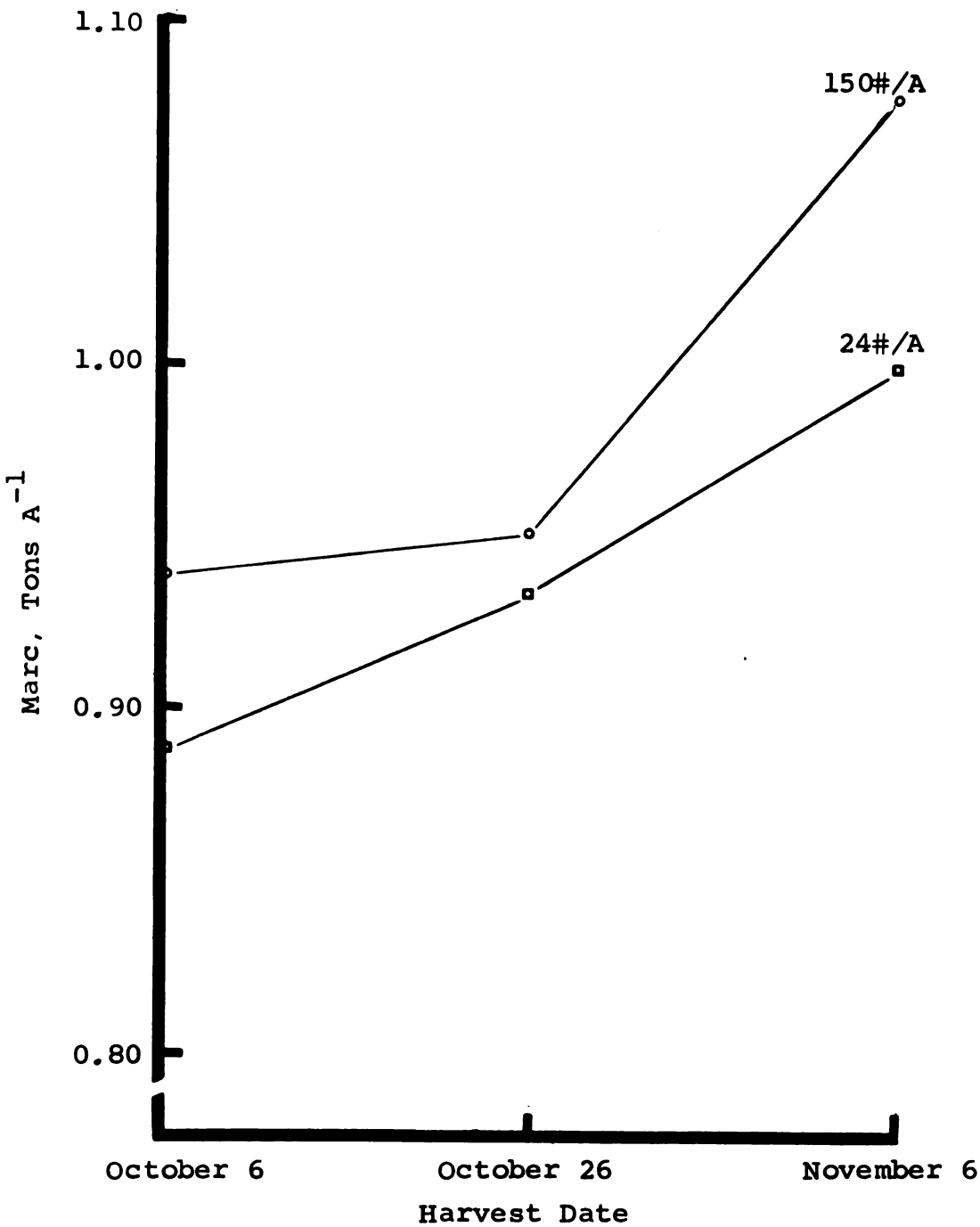

Figure 23. Marc yield of beets grown on high (150#/A) and low (24#/A) nitrogen on three harvest dates in 1967.

Table 33. Effect of variety on the percent marc at harvest and after storage at 3 C (70 C extraction) (1967)

Variety	At Harvest	Days Stored	
		65	130
		Mg Kg^{-1}	
2	4.19	4.40	4.32
3	4.16	4.20	4.38
5	3.67	4.02	3.96
Average	4.01	4.21	4.22

made at an extraction temperature of 80 C which should solubilize a greater proportion of the protopectins. Also in 1966 the beets were stored in canvas bags and considerable desiccation and molding occurred. This alone would have increased the solubility of pectic substances in hot water extractions (Silin, 1964).

In general, studies on the degradation of pectic substances in stored fruits and vegetables have shown a very pronounced conversion of insoluble pectins to more soluble forms (Joslyn, 1962).

Recently Wang (1961) and Barbour (1962) added sucrose ^{14}C to harvested beets. After several days of storage 20 percent of the counts added to the roots were found in the alcohol insoluble cell wall material. Apparently the sugarbeet continues to synthesize new cell wall materials after

harvest. Therefore it is physiologically possible for the percent marc to increase in storage.

The increase in the marc (70 C extraction) during storage was greatest for the October 26 harvest (Table 34). The heavy rainfall may have resulted in more soluble pectins at harvest which tended to "harden" under ideal storage conditions.

Table 34. Effect of harvest date on the percent marc at harvest and after storage at 3 C

Harvest Date	At Harvest	Days Stored	
		65	130
		Mg Kg^{-1}	
October 6	4.01	4.09	4.16
October 26	3.86	4.33	4.25
November 6	4.15	4.21	4.25

The five varieties harvested on October 26 showed no significant variety x removal interaction indicating that all varieties reacted the same, increasing approximately 0.3 percent on the average in marc.

The percent marc in 1968 was determined for the date of harvest and variety-temperature experiments. To study the marc stability problem further, the percent marc was determined by the same procedure as in 1967 but at three

extraction temperatures, 20, 70 and 80 C. All determinations were made in duplicate on four replications.

At harvest the percent marc varied some 20 percent between varieties when extracted at 20 C (Table 35). In other words variety 6 would yield 22 percent more pulp than variety 5 (Table 35). However the remarkable difference was that the marc of varieties 5 and 7 was 2-3 times more heat stable than variety 6 (70-80 C). Variety 6 had an extremely coarse, fibrous texture. The vascular bundles appeared to be highly lignified and convoluted throughout the entire root.

Table 35. Effect of extraction temperature on the percent marc of three varieties at harvest (1968)

	Extraction Temp., C			Difference		
Variety	20	70	80	20-70	70-80	20-80
			Percent			
5	4.06	3.78	3.65	-0.28	-0.13	-0.41
6	4.97	4.72	4.45	-0.25	-0.27	-0.42
7	4.10	3.90	3.81	-0.20	-0.09	-0.29

Variety-Temperature Interaction

The three varieties were stored for 100 days at 3 and 10 C and the marc analyses repeated (Table 36). The greatest decline occurred in the marc extracted at 20 C in 10 C storage. The decrease in the marc extracted at 70 C

Table 36. The average percent marc of three varieties at harvest and after 100 days of storage at 3 and 10 C

	Extraction Temperature		
	20 C	70 C	80 C
		Percent	
At Harvest	4.38	4.13	3.97
At 3 C	4.20	4.08	4.88
Loss	-0.18	-0.05	-0.09
At 10 C	4.11	4.01	3.98
Loss	-0.27	-0.12	....

and 80 C was negligible at either storage temperatures. These results partially explain the 1967 results where the marc at 70 C actually increased. This fraction was apparently very stable even at warm storage temperatures. This stability might be linked with resynthesis under ideal storage conditions.

The problem of reduced filtration rates when processing commercially stored beets seemed to be a solubilization of the pectic substances caused by rot and mold and not to a general and massive metabolicly controlled cell wall breakdown.

The three varieties reacted very differently when stored at 3 and 10 C. Variety 5 had the most unstable marc and actually lost more marc at 3 C than the other varieties

stored warm (Table 37). Variety 7 was the most stable of the varieties having negligible losses at either storage temperature. The 0.23 percent increase in the marc extracted at 80 C was significant and may confirm the stability theory proposed for the 1967 data. Variety 6 was more stable at 10 than at 3 C. This might indicate a variety-temperature interaction similar to that found for raffinose and amino acid production. However all marc losses were extremely small, except for variety 5 at 10 C, and would be considered negligible in normal factory operations.

Table 37. The loss in marc of three varieties stored 100 days at 3 and 10 C

Variety	Storage Temperature, C	Extraction Temperature, C		
		20	70	80
		Percent		
5	10	-0.56	-0.24	-0.15
	3	-0.31	-0.07	....
6	10	-0.07	....	....
	3	-0.21	-0.17	-0.08
7	10	-0.10	-0.12	....
	3	....	....	+0.23

Effect of Harvest Date

The 20 C marc remained constant between the September 1 and November 1 harvests (Table 38). However the 70 C marc increased slightly from 4.14 to 4.23. The same trend was found in the 70 marc in 1967. The 80 C marc remained constant during harvest. The new substances which caused the increase in the 70 C marc were apparently not heat stable and dissolved at the 80 C extraction temperature. The 70 C marc declined in storage until after 100 days it was essentially the same as the 80 C marc. The decline in the 20 marc during storage was essentially the same as the difference between the 20 and 80 C marcs at harvest (Figure 24). If this relationship held true it would be a method of

Table 38. Percent marc at three harvest dates and the loss in marc during 120 days of storage at 3 C

	Extraction Temperature, C			
	20	70	80	20-80
			Percent	
September 1				
At Harvest	4.32	4.14	3.95	-0.37
Stored	3.88	3.79	3.79	
Loss	-0.44	-0.35	-0.16	
October 1				
At Harvest	4.39	4.16	4.01	-0.38
Stored	3.92	3.77	3.72	
Loss	-0.47	-0.39	-0.29	
November 1				
At Harvest	4.34	4.23	3.95	-0.39
Stored	3.97	3.81	3.82	
Loss	-0.37	-0.42	-0.13	

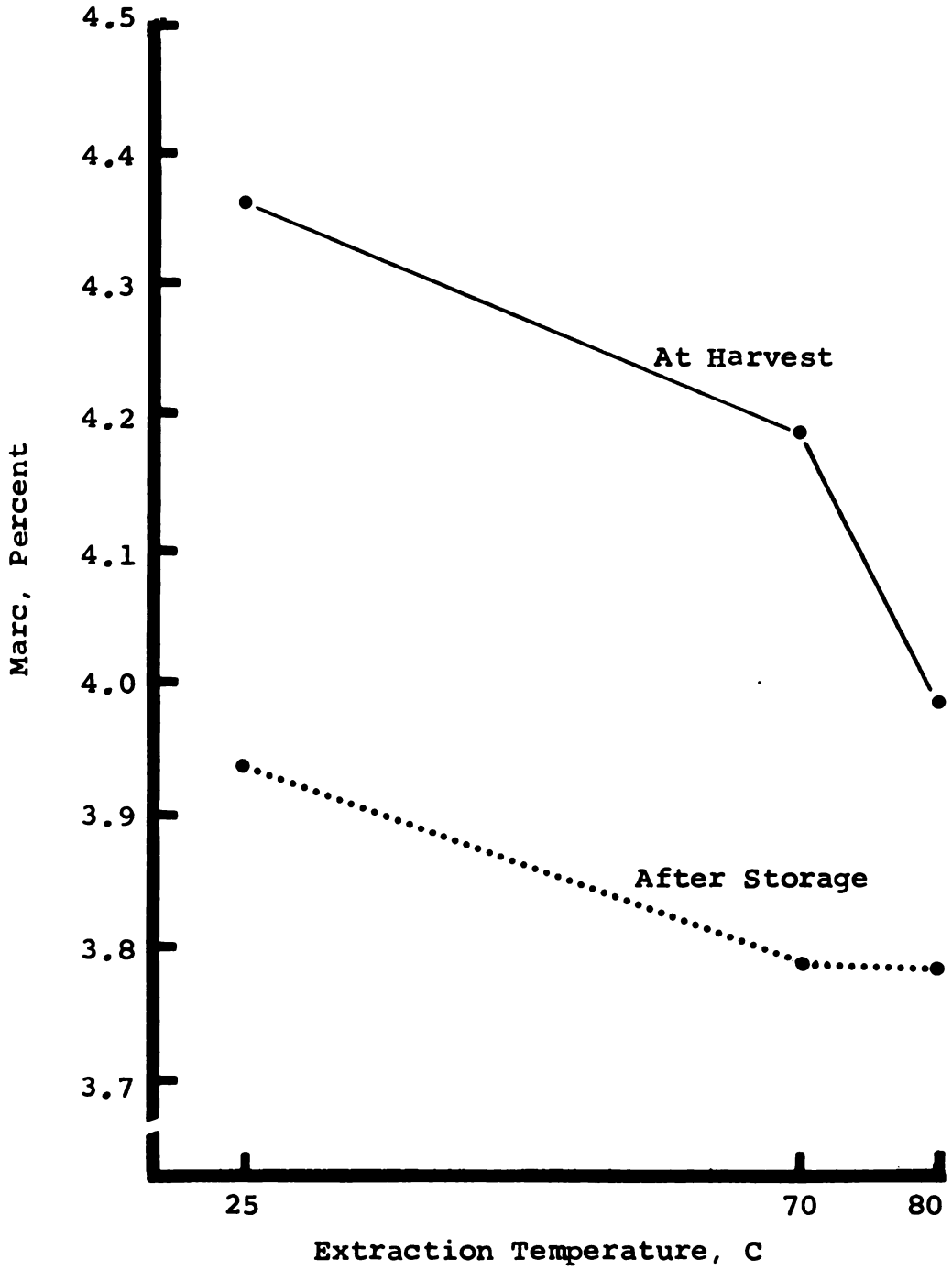

Figure 24. The effect of incubation temperatures on the percent marc before and after storage.

quickly screening varieties at harvest for their marc stability in storage. However the three varieties tested in 1968 did not follow this pattern (Table 35). Variety 3 was the most stable in storage and also had the lowest 20-80 difference at harvest. However variety 6 was also very stable in storage but had a high 20-80 value at harvest.

Summary of Marc Content

The percent marc at harvest is primarily a varietal characteristic, but is strongly influenced by environment, particularly rainfall. Cool wet weather decreased the percent marc, as did high rates of nitrogen fertilization.

The major loss of marc in storage occurred in the marc remaining after 20 C extraction. The portion remaining after 70 and 80 C extraction were relatively stable and actually increased in some cases under ideal storage conditions. Increasing the storage temperature from 3 to 10 C increased the loss of the 20 and 70 marc but had no effect on the 80 C fraction.

Harvest date had little effect on the percent marc at harvest or in storage.

Under ideal storage the marc appeared to be a very stable fraction of the beet root and showed evidence of being continuously resynthesized.

Considerable differences existed between varieties in marc stability in storage at both 3 and 10 C.

FACTORS INVOLVED IN THE DIRECT LOSS OF SUCROSE IN STORAGE

Respiration

Temperature, Injury and Wilting

The sucrose content of a beet decreases in storage by two pathways: respiration and sugar conversion. The research previously discussed has been directed primarily towards the study of sugar conversions during storage. In order to determine the proportion each pathway contributed to sucrose losses, several respiration experiments were conducted in 1968 (Table 39).

To prevent high rates of respiration in the first few days of storage, beets should not be injured nor allowed to wilt and should be cooled immediately.

Internal Atmosphere

An effective method for controlling respiration losses is refrigerated, controlled atmosphere storage. To test the possibility of using controlled atmospheres in beet storage a study of the internal atmosphere of the beet in relation to respiration rate was made.

Plugs 1 cm in diameter and 5 cm long were taken with a cork borer from the thickest portion of the beet root. The hole was then capped by inserting a rubber septum.

Table 39. Respiration rate of beets subjected to several postharvest treatments (1968)

Treatment[a]	Respiration Rate	
	Untopped	Topped
Cooled immediately to 5 C	4.0	....
Held at 24 C	12.0	16.5
Held at 24 C and allowed to wilt 10%		23.0*

[a]All treatments consisted of three replications of ten beets each.

*Based on original weight.

Gas samples were removed with a syringe after 24 hours. The beets were placed under water while withdrawing the samples to prevent air leaks. The carbon dioxide and oxygen composition of the sample was determined with a Perkin-Elmer Vapor Fractionator.

Normal beets had an internal atmosphere composition of approximately 2 to 2.5 percent CO_2 and 18 percent O_2 with a respiration rate of approximately 16 mg CO_2/Kg-hr at 10 C. When beets were coated with paraffin the CO_2 increased to 5 percent and the O_2 decreased to 13 to 14 percent with a respiration rate of 8 mg CO_2/Kg hr at 10 C.

These short term experiments indicate that respiration rates could be decreased some 50 percent by modifying the carbon dioxide and oxygen content inside the beet.

Since the oxygen concentration in the root was not reduced drastically in these experiments, the rate of respiration in the beet root seemed very susceptible to inhibition by high carbon dioxide concentrations. Stout (1954) found that increasing the oxygen concentration to 35 percent increased the respiration rate. Oxygen diffusion into the bulky root tissue apparently was limiting the rate of respiration. The surface area of the beet, a factor in controlling diffusion rates, is highly correlated to the rate of respiration. The increased rate of respiration of wilted beets might be related to the increased air space in the root allowing faster gas exchange.

Carbon Monoxide Inhibition of Respiration

Carbon monoxide is a potent inhibitor of the metal containing terminal oxidases. Carbon monoxide competes with O_2 for the reduced form of cytochrome oxidase. However the affinity of CO is only one-tenth that of O_2 (Hackett, 1960). Therefore a 10:1, $CO:O_2$ ratio should give a 50 percent inhibition of the oxidases. Wort *et al.* (1959) found a mixture of 95 percent carbon monoxide and 5 percent oxygen caused a 15 percent inhibition of oxygen uptake in thin disks of beet root tissue. From his results he concluded that the active terminal oxidases in the beet contained copper.

Due to the bulky nature of the beet root, the use of many respiration inhibitors has been largely unsuccessful (Vajna, 1960). Carbon monoxide should be of practical importance, if effective, due to its ease of application.

To test the effectiveness of carbon monoxide as a respiration inhibitor in intact beet roots the following experiment was performed. Six samples, consisting of ten uniform beets each, were placed in sample containers as described previously. Three samples were connected in series with a potassium hydroxide CO_2 trap and the air circulated with a diaphram pump. Carbon monoxide was then added to the system using a gas burette to the desired concentration. A six hour equilibrium was allowed after each carbon monoxide addition before the rate of respiration was determined. The three other samples were used as controls. The samples were held at 5 C.

Very low levels (1-2%) of carbon monoxide (Figure 25) inhibited the carbon dioxide output by 20 percent. Increasing the concentration to 10 percent did not increase the degree of inhibition. Between 10 and 20 percent carbon monoxide the rate of respiration declined steadily to 65 percent of the control.

Gassing beets in covered piles with carbon monoxide may reduce the sugar losses occurring in the first few days of storage. A 20 percent reduction in these losses would be of substantial economic interest.

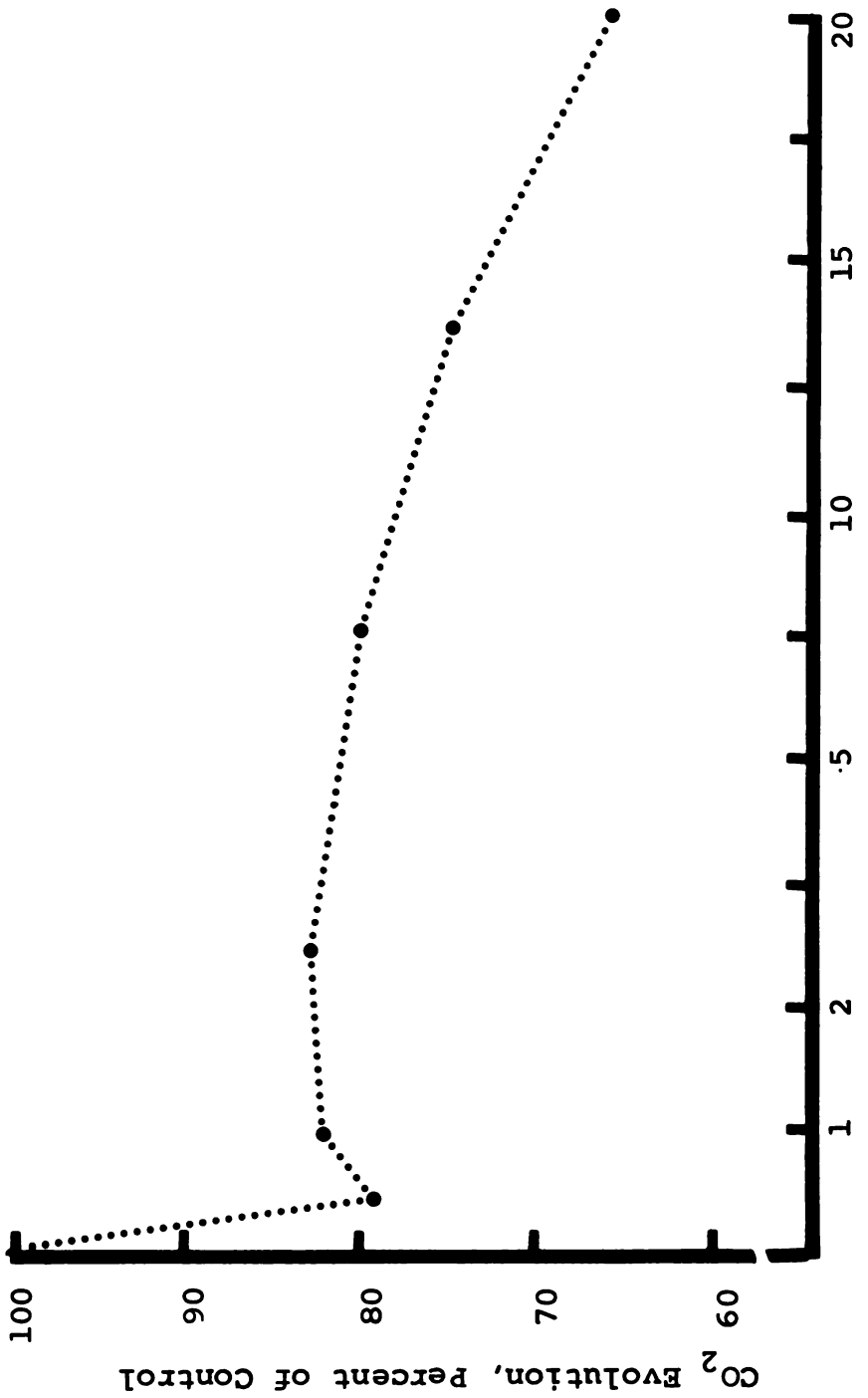

Figure 25. Carbon monoxide inhibition of carbon dioxide evolution.

Effect of Preharvest Sprays on Thermal Induction and Respiration

The respiration rate of samples treated with GA and MH-30 prior to harvest were monitored periodically for 102 days at 5 C.

Samples of GA and MH treated roots were removed after 5, 8 and 12 weeks of storage in the same cooler as the respiration experiment and the degree of thermal induction was determined. Beet halves were planted in sterilized soil in the greenhouse at 18 to 21 C in 16 hour days and the percent bolters recorded after six weeks.

Due to the rapid fluctuations in the respiration rate immediately after harvest (Dilley, 1969; Stout, 1957) the first respiration analyses were delayed until seven days after the beets were placed in storage.

After 30 days in storage the rate of respiration for the plants treated with GA was 15 percent higher than the control (Figure 26). At this time 87 percent of the plants treated with GA were induced to flower as compared to only 57 percent of the control plants. Sprouting was inhibited to such an extent in the plants treated with MH that no induction observations could be made. Although no definite conclusion can be drawn, the degree of induction appeared to affect the respiration rate. The increase in respiration between 70 and 110 days was probably due to surface desiccation.

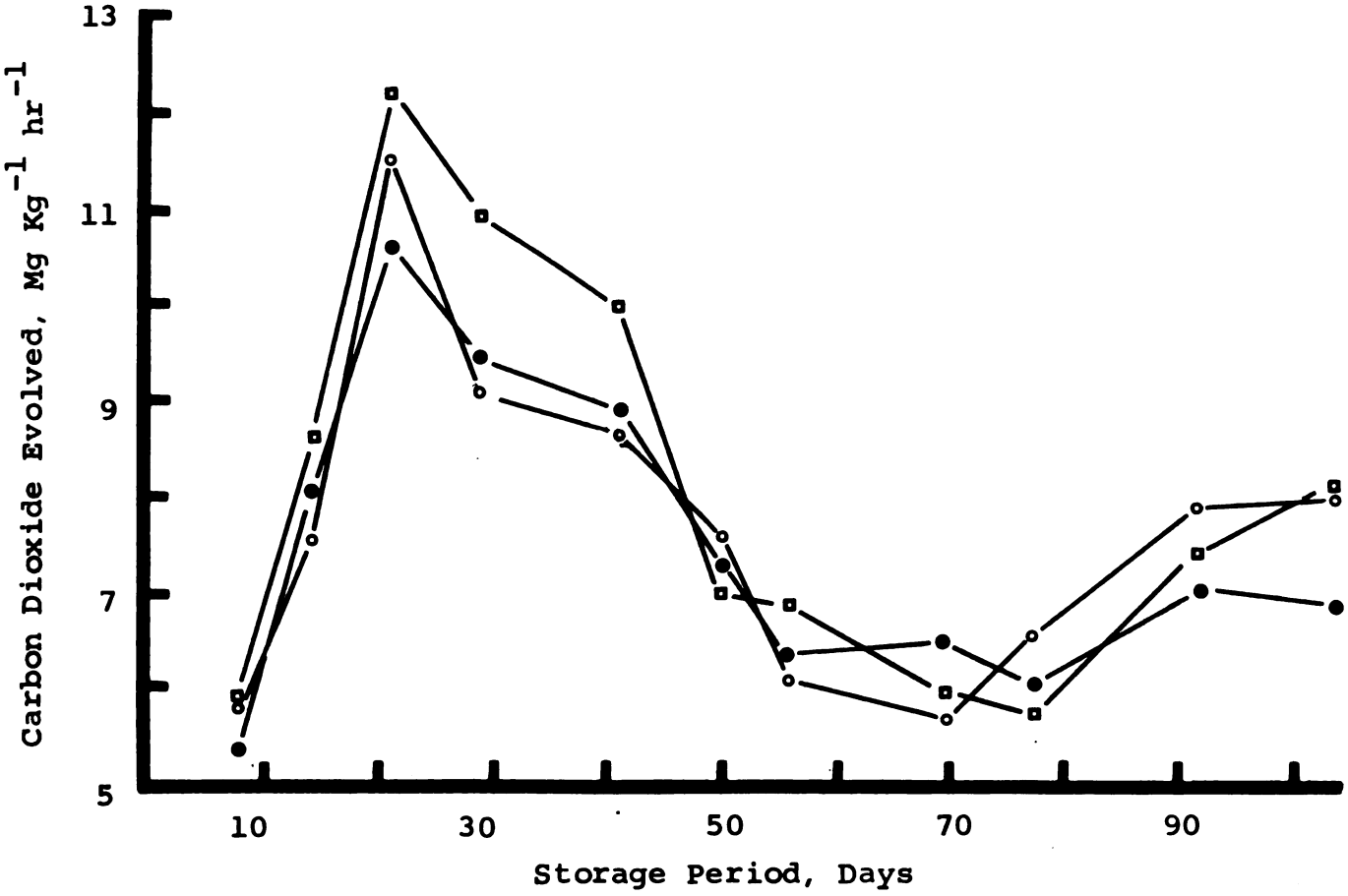

Figure 26. Carbon dioxide evolution of samples treated with MH-30, gibberellic acid and stored for 102 days at 5 C. MH-30, ∘ ; GA, ▫ ; Control, ● .

Stout (1949) found a high correlation between respiration rate and the degree of flower induction. The respiration rate increased with prolonged cold temperatures. When the beets were subjected to temperatures of 23 C to cause a reversal of induction the rate of respiration declined. However he did not report the temperature at which the respiration analyses were made which would be a prime factor in explaining his results. Moving beets from the cold to a warmer temperature caused a burst in the rate of respiration lasting for 2-3 days (Dilley, 1969). This increase in respiration was apparently due to substrate availability and not to a change in carbon dioxide solubility (Stout, 1954).

Lang (1956) reported that GA would fulfill the thermal induction requirements of some biennials. Gibberellic acid has been shown to partially fulfill the thermal requirements for induction in sugarbeets (Stout, 1959; Snyder and Wittwer, 1959; Stout and Owen, 1959).

The very prominent increase in respiration for the first 20 days of storage has not been shown previously. Previous workers found the respiration rate declined to a low level after a high rate in the first few hours (Dilley, 1969; Stout, 1957). However the present results correlate very well with observations of commercially stored beets. In the first few weeks of storage the beets go through a "sweat" period when pile temperatures are hard to control.

Again in late January the pile temperatures increase causing substantial sugar losses. These increased pile temperatures may be due to the heat of respiration (Figure 27).

Proportion of Sucrose Losses Accounted for by Respiration

Carbon Dioxide Evolution

Loss of sucrose through respiration is generally considered to account for most of the total sucrose loss in storage. Previous workers have shown that the decrease in sucrose and the total carbon dioxide evolved were not significantly different (Vajna, 1960). The proportion of the sucrose lost by carbon dioxide evolution varied between varieties (Stout, 1950). However the incidence of mold in his experiments caused an unusually high accumulation of invert sugars which might have caused considerable overestimation of sucrose losses by polarimetry. Normally the sucrose losses might be expected to be greater than the loss accounted for by carbon dioxide evolution since reducing sugars and trisaccharides are formed from sucrose.

The total carbon dioxide evolved during the 102 day storage period described in the previous section was 18.8 gm/kg. Table 40 gives a balance sheet for the losses due to respiration and sugar conversions. The 4.7 gm/kg excess carbon dioxide evolved apparently represented the respiration of non-sucrose compounds derived from carbohydrates other than sucrose. This excess was approximately equal to

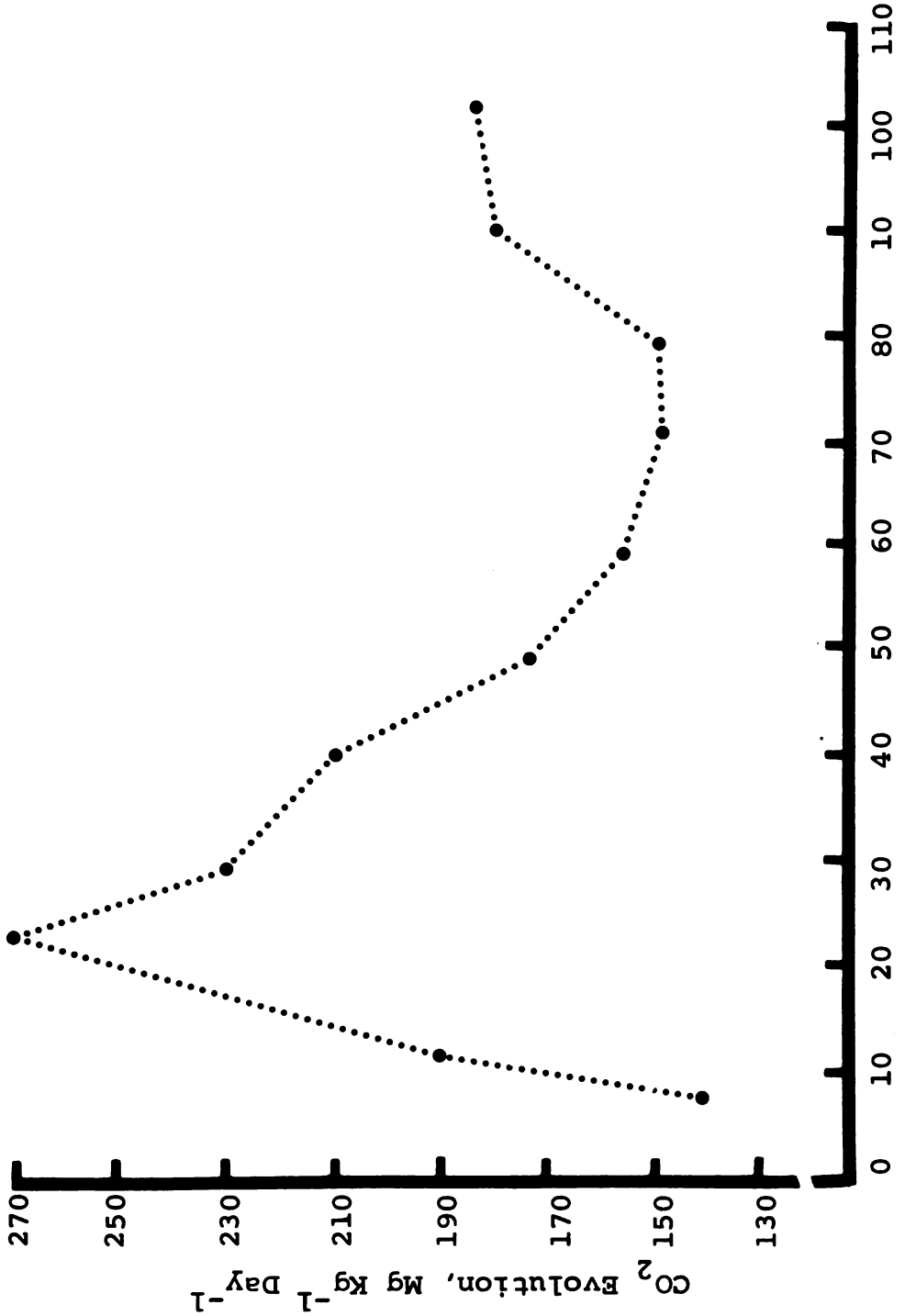

Figure 27. Carbon dioxide evolution during 102 days of storage at 5 C.

Table 40. Proportion of sucrose losses accounted for by respiration and interconversions in 112 day storage at 5 C

		gm/kg
CO_2 lost		18.85
Sucrose lost		8.00
Raffinose lost		0.90
Invert gained		1.40
Total sugar loss	8 + 0.9 =	8.90
Total sugar gained		1.40
Net loss		7.50

Since 1 gm of CO_2 is derived from 0.648 gms of sucrose, 18.85 gms CO_2 is equivalent to 12.21 gms of sucrose respired.

$$\begin{array}{r} 12.2 \\ \underline{-7.5} \\ 4.7 \text{ gms/kg excess } CO_2 \text{ evolved} \end{array}$$

the amount of insoluble cell wall material which became soluble in this type and length of storage (see marc section). It was therefore possible that part of the cell wall polysaccharides were hydrolyzed and subsequently respired.

Sucrose vs Dry Matter Losses

In 1967 the amount of respiration in storage was estimated by measuring the loss of dry matter. Although this method was not as precise as measuring actual carbon dioxide evolved it was a good estimate.

No significant differences were found between agronomic practices in the amount of dry matter lost.

The proportion of sucrose lost by respiration and transformation decreased in the last 65 days of the 130 day storage period (Table 41).

Table 41. Average loss of dry matter and sucrose in 1967 date of harvest study after 65 and 130 days of storage at 3 C

Days Stored	Sucrose Loss	Dry Matter Loss
	Pounds/Ton	
65	-8	-12
130	-12	-22

Dry matter losses, which can occur only by gaseous evolution, were considerably greater than the sucrose losses in all experiments. These results substantiate the loss of total impurities in the clear juice which may occur in the final 65 days (page 90).

The difference between dry matter and sucrose losses varied among varieties. In variety 1 and 4 the losses were equivalent, while in the other three varieties the dry matter loss was much greater (Table 42).

Table 42. Loss of dry matter and sucrose in five varieties during storage at 3 C for 130 days

Variety	Sucrose Lost	Dry Matter Lost	Non-Sucrose Compounds Respired
		Pounds/Ton	
1	21.6	24.0	2.4
2	4.6	28.6	24.0
3	14.8	16.4	1.6
4	13.0	15.6	2.6
5	13.4	23.6	10.2
Average	13.5	21.6	

The non-sucrose constituents in the beet root normally increased during storage resulting in a decreased clear juice purity. If the difference between sucrose loss and dry matter losses came from only the soluble fraction of the beet the purity would increase considerably. The slight decline in total impurities in the clear juice was several magnitudes less than the dry matter minus sucrose losses. Therefore the cell wall or insoluble fraction must contribute to any increased impurities in the clear juice and also to the carbon dioxide evolved. However it was possible that the source of non-sucrose compounds respired, is partly precipitated by lime and therefore their loss would not be reflected in the C.J.P. determination.

Enzymatic Degradation of Sucrose in Stored Beets

Enzyme Analysis

The reducing sugars produced in stored beets occur either as a result of mold activity or enzymatic degradation of sucrose by endogenous enzymes. Prolonged storage at temperatures above 5 to 8 C caused reducing sugars to accumulate. This response to temperature was diametrically opposite that commonly found in other plants; i.e., potatoes (Pressey, 1968).

Acid invertase activity in fresh beet tissue was very low and is often assumed to be zero (Bacon, 1961; Vaughan and MacDonald, 1967). However invertase activity

could be induced by aging beet disks in sterile aerated water. Avigad (1968) found that, due to its easily reversible nature, the sucrose synthetase in beet root was capable of producing reducing sugars.

pH Profile of Sucrase Activity in Root Homogenates

In a preliminary study* the amount of sucrose inversion in a brei preparation over the pH range of 4.0 to 8.5 was determined. Two hundred grams of fresh beet tissue was homogenized in a Virtis Blender at 10,000 RPM for one minute. The grinding medium contained EDTA, 10^{-4}M; NaCN, 10^{-3}M in phosphate buffer, 10^{-3}M at pH 7, and was cooled to 0 C prior to use. Ten ml of the homogenate was added to test tubes containing 10 ml of a 2 x 10^{-2}M buffer (pH 4 to 5 acetate; pH 6 to 7 citrate-PO_4; pH 8 to 9 Tris) and 0.05 molar sucrose. The samples were mixed and allowed to equilibrate at 30 C for 10 minutes. Ten ml of the reaction mixture were removed and two ml of 1 percent neutral lead acetate were added to stop the enzymatic reaction and clarify the solution. The increase in reducing power one hour later was measured using the tetrazolium method (Carruthers, 1955).

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*The preliminary investigation reported in this section was conducted in the Research Laboratory of the British Sugar Corporation in Norwich, England. The work was a continuation of a study of enzymatic inversion in factory diffusers previously undertaken by J. V. Dutton and D. Grierson of the British Laboratory.

Results were expressed as relative activity with the peak activity at pH 5 taken as 10. All samples were run in quadruplicate with boiled controls at each pH.

The acid invertase activity at pH 5.0 was very low as reported previously (Figure 28). However the striking increase in hydrolytic activity at pH 7 was totally unexpected. As yet no characterization of this peak has been made, but it might be due to the reversal of sucrose synthetase (Avigad, 1968).

Distribution of Hydrolytic Activity in the Beet Root

To determine the localization of the two enzymes in the beet root the homogenate was filtered through nylon bolting and the filtrate centrifuged at 30,000 g for 20 minutes. The resulting supernatant was a pale yellow. The inversion activity was determined on the homogenate and supernatant at pH 5 and 7.2.

The activity at pH 7.2 occurred primarily in the supernatant while the activity at pH 5 was presumably attached to the cell wall (Table 43). Previous workers have reported that invertase was associated with the cell wall in beet disks (Vaughan and MacDonald, 1967). The possible importance in sugar transport across cell membranes has been implied, but never proved (Steward, 1965). Vaughan and MacDonald (1967) found no alkaline invertase activity in fresh beet discs. The invertase activity which developed with

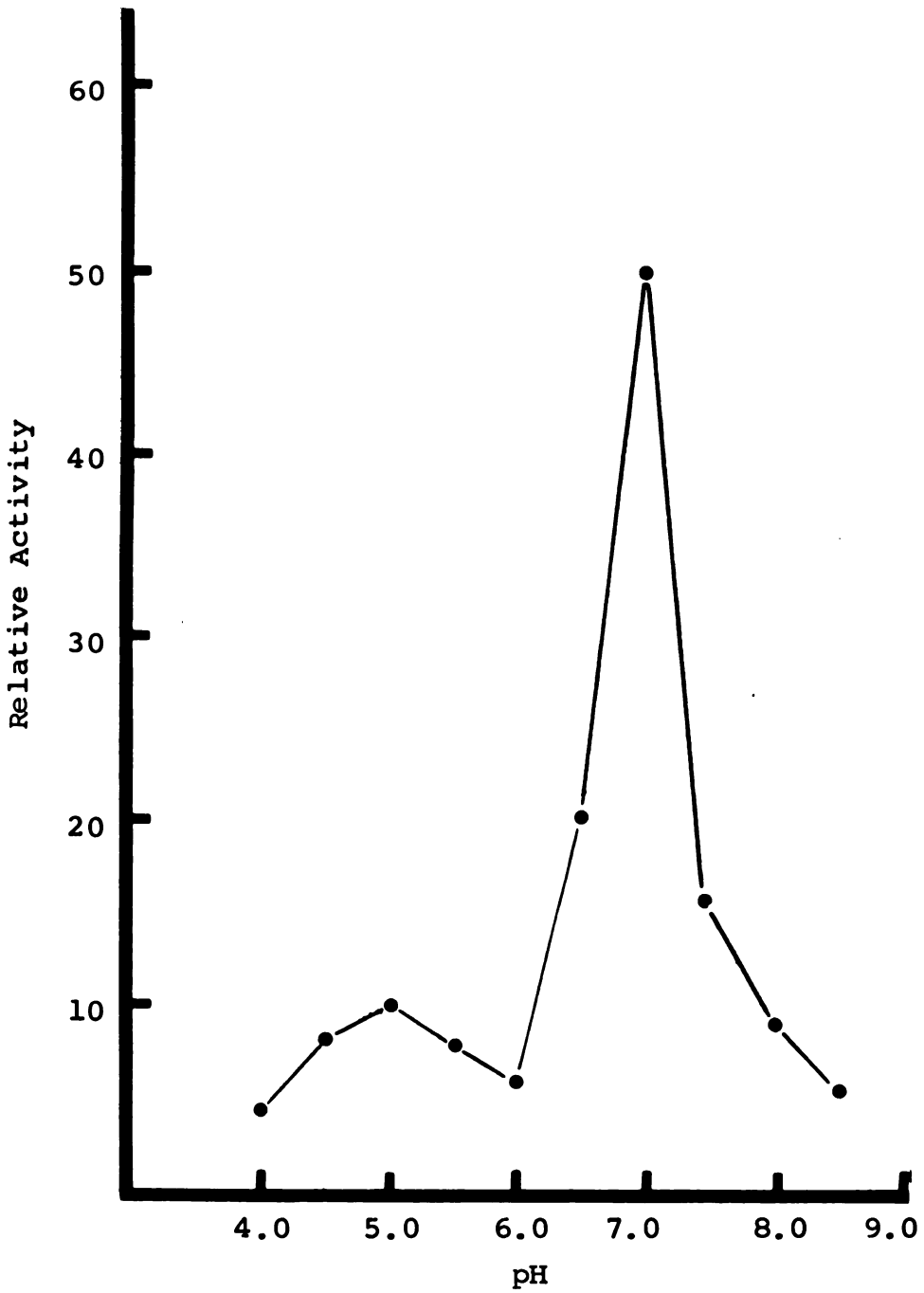

Figure 28. Effect of pH on the hydrolysis of sucrose by a beet root homogenate.

Table 43. Distribution of inversion activity at pH 5 and 7.2 between supernatant and cell wall material

pH 5	Percent
Supernatant	32
Cell Wall	68
pH 7	
Supernatant	69
Cell Wall	31

aseptic washing appeared in the cell wall and then moved into the cytoplasm and had a pH optimum at 5 (MacDonald, 1968).

Possible Importance of Hydrolytic Activity at pH 5 and pH 7

In an attempt to determine the possible relative importance of these two enzymes in beet metabolism their activity was measured in beets stored at 3 and 11 C. Assuming that the enzyme was operating at maximum velocity *in vitro* if the milligrams of reducing sugar produced by the activity at pH 5 was less than or equal to the rate of carbon dioxide evolution it could be assumed that this enzyme was probably of secondary importance in sucrose breakdown in storage.

Sections of beet root were passed through a grater to produce thin slices less than 1 millimeter thick and approximately two millimeters wide. Ten grams of these slices were placed in 150 milliliter beakers containing

50 ml of phosphate buffer 2 x 10^{-2}M at pH 7.2 or acetate buffer 2 x 10^{-2}M pH 5.0, EDTA 10^{-4}M sodium cyanide 10^{-3}M and sucrose 0.05 M. The assays were run at 30 C. All determinations were run in duplicate with boiled controls. The entire experiment was repeated twice and the results given are an average of the two runs.

The hydrolytic activity at pH 5 was approximately twice as great in beets stored at 11 C as in beets stored at 3 C (Table 44). The opposite was true for the hydrolytic activity of pH 7.2. This may indicate that the accumulation of reducing sugars at warm temperatures was due to increased invertase activity and not to the enzyme active at pH 7.2. The activity at pH 7.2 doubled at low storage temperatures but reducing sugars did not (see Figure 12). The lower activity at pH 5 in the 3 C storage may be due to the

Table 44. Effect of storage temperature on the enzymatic inversion of sucrose at pH 5 and 7

Storage Temperature	pH 5	pH 7
	Mg Kg^{-1}/Hr	
11 C*	66	76
3 C*	39	140
3 C**	33	129

*Assayed at 30 C.

**Assayed at 3 C (different lot of beets).

presence of a temperature dependent inhibitor. Such an inhibitor having a molecular weight of 18,000 to 23,000 was found by Pressey (1968) in cold stored beets.

When the assay was carried out at 3 C, the same as the low storage temperature, the activity was 33 and 129 mg/kg/hr (Table 44). Carbon dioxide evolved due to respiration at 5 C was approximately 10-15 mg/kg/hr. Since 1 mg carbon dioxide released in respiration equals 0.648 mg sucrose, the carbon dioxide evolved at 5 C (10-15 mg/kg/hr) was derived from 6.5 to 9.8 mg of sucrose. If it is assumed that the acid invertase activity attached to the cell wall was not involved in supplying the hexose sugars as substrates for respiration, the soluble invertase could supply 33 x 0.32 or 10.6 mg/kg·hr of hexose sugars. Therefore the relatively low invertase activity was theoretically great enough to supply the substrate for respiration in the beet root.

Effect of Several Preharvest Sprays on Hydrolytic Activity at pH 5 and 7.0

Using the same thin slice assay, the activity of the two enzymes was determined in beets treated prior to harvest with pyrocatechol, vanadium sulfate and CCC. Pyrocatechol and vanadium sulfate reduced the activity of both enzymes. CCC reduced invertase activity slightly at pH 5 but had no effect on the activity at pH 7 (Table 45).

Table 45. Effect of preharvest sprays on the degradation of sucrose by two enzymes assayed at pH 5 and pH 7

	pH 5	pH 7
	Mg Kg^{-1}	
Pyrocatechol	23	78
Vanadium sulfate	22	94
CCC	26	142
Control	39	140

The accumulation of reducing sugars was slight in relation to the potential hydrolytic activity contained in the beet root. The enzymatic hydrolysis of sucrose in stored beets therefore appeared to be highly controlled if the beets were not allowed to mold or wilt.

Summary of Factors Influencing the Direct Loss of Sucrose in Storage

The rate of respiration in the beet root could be maintained at a very low level by rapidly cooling the beets immediately after harvest and keeping injury at a minimum. Wilting increased respiration losses drastically. The respiration in the beet was apparently very susceptible to regulation by high levels of carbon dioxide in the root. Gassing covered piles with carbon dioxide may be a method of reducing respiration losses. Low levels of carbon

monoxide also had a very inhibition effect on respiration in intact roots.

Thermal induction of the beet root in storage increased the rate of respiration in the early part of the storage period.

Measurements of total respirational losses by carbon dioxide analysis and dry matter loss indicated that carbohydrates other than sucrose comprise a considerable, but variable portion, of the substrate for respiration.

The hydrolysis of sucrose in the beet root occurred via two pathways. Acid invertase with a pH optimum of 5 occurred in the beet at very low levels of activity. Hydrolytic activity at pH 7 is approximately 4-5 times the pH 5 activity and appeared to be due to the reversal of sucrose synthetase. The low activity of invertase was apparently large enough to supply hexose sugars for the carbon dioxide evolved in respiration.

Storage at 11 C doubled the hydrolytic activity at pH 5 but decreased the activity at pH 7 to 50 percent of that in 3 C storage. Pyrocatechol reduced the activity at pH 7 by 50 percent over the control. Vanadium sulfate reduced the pH 7 activity by 33 percent but CCC had no effect. All preharvest sprays decreased the activity at pH 5. The hydrolysis of sucrose in the beet root was apparently a highly controlled process since no substantial amount of reducing sugar accumulated if the beet root was maintained in a normal condition free from molds.

ASSESSMENT OF QUALITY IN STORED BEETS

The most common method for evaluating the quality of fresh beets is by determining the percent sucrose and clear juice purity. These determinations can be made rapidly and used to estimate the amount of bagged white sugar which can be recovered from a ton of beets.

Carruthers (1961a, 1961b, 1961c) proposed an alternative method whereby the clear juice purity could be estimated by correlation with the sodium, potassium, amino acid, and betaine content of the clear juice. The correlation equation was as follows:

$$\text{Purity} = 100.9 - (0.00143\ (2.5\ \text{sodium} + 3.5\ \text{potassium} + 10\ \text{amino nitrogen} + \text{betaine}))$$

This method appears to have merit both in evaluating fresh beets (Henry et al., 1961) and factory juices (Carruthers et al., 1963). Since the compounds used to calculate the impurity index do not change appreciably in storage, the index would be of little apparent value in assessing the quality of stored beets, in which changes in other substances assume major proportions.

Use of the Impurity Index in Evaluating Stored Beets

A comparison of apparent impurities, total analyzed impurities (TAI) and impurity index from the experiment in 1967 on date of harvest is given in Table 46. In general the total analyzed non-sucrose constituents tend to correlate very well with the apparent impurities. The difference between the two (unknown impurities) is approximately a constant 2500 mg/kg at harvest and during storage. However the impurity index remains essentially constant in storage and therefore the difference between the apparent impurities and the impurity index increases. Therefore the impurity index is of little value in assessing the technological value of stored beets.

Formula for Correcting Sucrose Determinations for Raffinose and Invert

The estimated RSPT often increased in short term storage (Dexter, 1966; Larmer, 1937; McCready, 1966). Therefore a formula was needed to correct the sucrose determination for optically active compounds in the clear juice to produce a more accurate estimate of the sucrose concentration in the beet and in the clear juice.

Raffinose has 1.6 times the optical activity of sucrose (sp. act. 104 vs 66.5). At a high concentration of raffinose, considerable error can be introduced into sucrose determinations. Even small corrections to the sucrose

Table 46. Comparison of total impurities with the total analyzed impurities and the impurity index on the evaluation of three varieties stored 65 and 130 days at 3 C (1967)

Variety	Total Impurities	TAI	Unknown[a] Impurities	Impurity Index	Unaccounted[b] for Impurities
			Mg Kg^{-1}		
At Harvest					
2	6671	4096	2575	3930	2741
3	6626	3860	2766	3763	2863
5	6419	4008	2411	4221	2198
Stored 65 Days					
2	7606	5177	2429	3837	3769
3	7574	4621	2953	3757	3817
5	7211	4777	2434	3900	3311
Stored 130 Days					
2	7570	5043	2527	3758	3812
3	8480	5757	2723	3534	4946
5	6914	4847	2067	3891	3023

[a]Unknown Impurities = Total - TAI.

[b]Unaccounted for Impurities = Total - Impurity Index.

determination may make considerable difference in the clear juice purity. Reducing sugars primarily glucose and fructose occur in the beet roughly equal concentrations and can therefore be called invert sugars. This combination of glucose and fructose has a specific activity of -40. In cases of high invert production in storage invert sugars may also contribute to errors in the determination of sucrose.

Since specific rotation is based on the weight of the optically active compound in solution the following relationships can be made.

$$1 \text{ mg raffinose} = \frac{104}{66.5} \text{ (mg sucrose)} = 1.59 \text{ (mg sucrose)}$$

$$1 \text{ mg invert} = \frac{40}{66.5} \times \frac{\text{mg sucrose}}{2} = 0.302 \text{ (mg sucrose)}$$

Therefore 1 mg of raffinose would appear as 1.59 mg sucrose using polarimetric methods. A similar relationship can be made for invert. As a result of the above relationships the following equation can be derived.

$$\text{Corr \% S} = \text{App C.J.\% S} - \frac{1.59 \text{ mg/ml Raff.} - 0.302 \text{ mg/ml Invert}}{10 \times \text{Density (mg/ml)}}$$

Assuming there is no loss of raffinose or invert in sample clarification* and that the raffinose and invert

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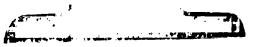
*This assumption is not entirely true (Appendix C). Some reducing sugars are lost by alkali decomposition. Since fructose is the most reactive the actual correction should no doubt be more positive than the -40 used here.



concentration per gram of sugar is a constant in the beet and in the clear juice the following relationship is also valid.

$$\text{Corr \% S on Beets} = \text{App \% S on beets} \times \frac{\text{Corr C.J. \% S}}{\text{App C.J. \% S}}$$

These equations were used to correct all sucrose determinations reported in the following section.

The magnitude of the corrections on fresh and stored beets from several experiments are given in Table 47.

The corrections are obviously substantial both at harvest and after storage. It is apparent that any storage experiments evaluated by RSPT measurements must be corrected for raffinose and invert.

Base-Acid Balance in the Clear Juice

During the processing campaign the lime salts normally increase significantly and require the addition of considerable amounts of sodium carbonate to reduce calcium salts and prevent acidic conditions in the evaporators. However, the addition of sodium carbonate results in a considerable loss of sucrose into the molasses (approximately 5 lbs of sucrose per pound of sodium carbonate added). The changes in the acid-base balance of the clear juice may be an important supplement to clear juice purity and RSPT calculations, particularly in evaluating stored beets.

Table 47. Magnitude of corrections required due to errors in polarimeter reading caused by raffinose and invert*

Five Varieties (1967)

Variety	At Harvest			Stored at 3 C for 65 Days			Stored at 3 C for 130 Days		
	S	CJP	RSPT	S	CJP	RSPT	S	CJP	RSPT
	%	%	lb	%	%	lb	%	%	lb
1	0.26	1.51	13.7	0.41	2.32	21.6	0.27	1.63	14.3
2	0.25	1.51	13.3	0.50	2.91	26.3	0.36	2.18	19.1
3	0.27	1.56	14.0	0.33	1.89	17.3	0.36	2.19	19.2
4	0.28	1.70	14.7	0.54	3.07	28.2	0.30	1.90	16.0
5	0.23	1.37	12.3	0.43	2.51	22.5	0.19	1.12	9.8

Variety-Temperature Experiment (1968)

Variety	At Harvest			Stored 100 Days at 3 C			Stored 100 Days at 11 C		
	S	CJP	RSPT	S	CJP	RSPT	S	CJP	RSPT
	%	%	lb	%	%	lb	%	%	lb
5	0.18	1.13	9.8	0.45	2.76	23.8	0.10	0.65	5.4
6	0.32	2.17	17.0	0.76	5.33	40.1	0.38	2.67	20.1
7	0.24	1.42	12.9	0.74	4.39	39.2	0.25	1.55	13.2

*All corrections in these experiments were negative.

During storage, the cations, sodium and potassium, and the anion chloride remain constant since they are not metabolized by the beet. The amino acids remain fairly constant but in general decline slightly during storage. A large part of the acids in thin juice which contribute to high lime salts in stored beets are derived from the alkaline decomposition of invert sugars during lime defecation. The literature reports that in general one invert sugar produces two acids each with a molecular weight of 90 (McGinnis, 1951). However, our studies of alkaline decomposition of glucose and fructose indicate that the molecular weight is closer to 120 (Appendix C). In other words, the invert sugars produced from one molecule of sucrose would produce three molecules of acid upon decomposition. However the alkaline decomposition of invert produces a yellow color due to the presence of browning reaction products. These must be reduced by the addition of sulfur dioxide. The SO_2 produces more acid in the juice which must also be neutralized with sodium carbonate. Therefore in the following calculations 90 will be used as the milliequivalent (meq) weight of the acids derived from invert to partially compensate for the required SO_2 addition.

The base-acid balance for five varieties grown on high (150#/A) and low (24#/A) nitrogen and stored for 130 days was calculated using the following equation.

$$\frac{\text{mg K}}{39.1} + \frac{\text{mg Na}}{22.9} - \frac{\text{mg Amino Acids}}{140} - \frac{\text{mg invert}}{90} - \frac{\text{mg Cl}}{35} = \text{Residual Alkalinity}$$

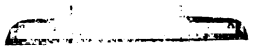
(all values are in mg/100S)

Since a slight excess of base is required to maintain alkalinity during evaporation this relationship will be designated as residual alkalinity.

Since K, Na, Cl and amino acids are essentially constant during storage, the fluctuations in invert sugar will determine the amount of acid which must be neutralized as Ca Salts or by the addition of Na_2CO_3. Table 48 indicates the magnitude of the changes in residual alkalinity during storage.

Table 48. The residual alkalinity of five varieties stored at 3 C for 130 days

Treatment	At Harvest	After Storage
	(meq/100S)	(meq/100S)
Variety 1	+7.4	-13.0
Variety 2	+4.4	+1.6
Variety 3	+1.4	-5.8
Variety 4	+7.6	-6.2
Variety 5	-2.2	-7.6
Variety 5 @ 11 C	-2.2	-29.0
Average all varieties grown on:		
150# N/A	+1.2	-9.2
24# N/A	+5.8	-6.4



Although the optimum residual alkalinity for maximum processing efficiency is not known, beet variety, agronomic and storage practices obviously all provide a practical means for manipulating this balance.

Utilizing the Residual Alkalinity in the Calculation of the RSPT

Many factory chemists assume a loss of 5 pounds of sucrose into the molasses for each pound of Na_2CO_3 added in order to produce the proper base-acid balance. Assuming this 5 pound loss to be accurate, the following relationship between sucrose lost by Na_2CO_3 addition and the base-acid balance of the clear juice can be made (Table 49).

Therefore 0.26 percent of the total sucrose in the beet is lost for each meq of Na_2CO_3 added to correct the residual alkalinity of the clear juice to a value of +11.* In two experiments the correction in the RSPT at harvest was only 2 to 3 pounds (Table 50 and Table 51). However in long term storage where reducing sugars accumulated the correction was considerable.

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*Previous workers have indicated that an effective alkalinity of 2.24 or a K + Na - Amino nitrogen balance of 16 should allow efficient factory operation. The beet contains approximately 2 meq of Cl and up to 3 meq of invert/100 S can normally be tolerated without causing difficulty in processing (Dexter et al., 1969; Frakes, 1969; Anderson-Smed, 1963). Therefore 16 - 2 - 3 = 11.

Table 49. Calculation of sucrose losses due to sodium carbonate addition

Assume 5 lbs sucrose lost/# Na_2CO_3 added

or 5#/454,000 mg Na_2CO_3

or 5#/8566 meq Na_2CO_3

or 5.8×10^{-4}#/meq Na_2CO_3

or 0.26 gm sucrose/meq.

Therefore 0.26 percent of the total sucrose will be lost for each meq Na_2CO_3 added/100 gm sucrose.

or 5.2 x meq/100 S base deficit x percent sucrose = lbs sucrose lost per ton of beets due to sodium carbonate addition.

Also, since 53 mg Na_2CO_3 = 1 meq and

90 mg invert = 1 meq,

$\frac{53}{90}$ x 5# = 2.9# loss to molasses/lb of invert sugar

2.9# + 1# = 3.9# of sucrose lost per pound of invert sugar.

Table 50. Effect of base-acid balance corrections on the RSPT of five varieties at harvest and after storage at 3 C (1967)

Variety	Base-Acid Balance	Na_2CO_3 Required	Sucrose Lost	Total Sucrose	Suc. Lost	RSPT	Corr RSPT
		Meq	%	lb/T	lb	lb	lb
At Harvest							
1	8.6	1.4	0.36	324.4	1.2	284.4	283.2
2	7.5	2.5	0.65	313.3	2.0	273.7	271.7
3	4.9	5.1	1.33	320.7	4.3	281.5	277.2
4	9.6	0.4	0.10	308.1	...	268.6	268.6
5	2.2	7.8	2.03	323.6	6.6	285.6	279.0
Average					2.8		
Stored 65 Days							
1	6.0	4.0	1.04	329.6	3.4	285.1	281.7
2	6.9	3.1	0.81	318.1	2.6	270.4	267.8
3	4.5	5.5	1.43	322.9	4.6	279.8	275.2
4	8.3	1.7	0.44	324.1	1.4	277.1	275.7
5	3.3	6.7	1.74	317.7	5.5	274.2	268.7
Average					3.5		
Stored 130 Days							
1	-4.4	14.4	3.74	302.8	11.3	251.1	239.8
2	2.3	7.7	2.00	308.7	6.2	263.3	257.1
3	2.0	8.0	2.08	305.8	6.4	256.6	250.2
4	0.8	9.2	2.39	294.9	7.0	248.8	241.8
5	-1.5	11.5	2.99	310.3	9.3	266.8	257.5
Average					8.0		

Table 51. Effect of base-acid balance corrections on the RSPT of three varieties at harvest and after storage at 3 and 10 C (1968)

Variety	Base-Acid Balance	Na_2CO_3 Required	Sucrose Lost	Total Sucrose	Suc. Lost	RSPT	Corr RSPT
		Meq	%	lb/T	lb	lb	lb
At Harvest							
5	8.7	1.3	0.34	308.3	1.1	272.7	271.6
6	8.9	1.1	0.29	272.8	0.8	225.2	224.4
7	6.0	4.0	1.04	327.8	3.4	294.2	290.8
Average					1.8	264.0	262.0
Stored 100 Days at 3 C							
5	9.1	0.9	0.23	306.1	0.7	265.1	264.4
6	14.1	...	....	254.8	...	197.3	197.3
7	4.3	5.7	1.48	316.4	4.7	273.3	269.0
Average					1.8	245.5	243.6
Stored 100 Days at 10 C							
5	3.8	6.2	1.61	294.1	4.7	253.8	249.1
6	4.3	5.7	1.48	257.1	3.8	201.6	197.8
7	2.9	7.1	1.85	304.2	5.6	265.5	259.9
Average					4.7	240.3	235.6

Quality Evaluation of Fresh and Stored Beets on the Basis of Recoverable Sugar*

In the following results, corrections for raffinose, reducing sugars, and the residual alkalinity were applied as discussed previously.

Recoverable Sugar Yields at Harvest

The RSPA (Recoverable Sugar Per Acre) increased substantially during the 1967 harvest season (Table 52). The weather was dry until just prior to the October 26 harvest when approximately 2 inches of rain fell. As a result the percent sucrose decreased for this harvest due to water uptake but the increased tonnage more than compensated to increase the RSPA with delayed harvest. Varieties 2 and 5 yielded more RSPA when grown on low nitrogen (24 pounds per acre) compared to high (150 pounds per acre) nitrogen (Table 52). Variety 5 which is extremely susceptible to luxury consumption of nitrogen (page 68) showed a substantial yield increase under low nitrogen fertilization.

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*$RSPT = 20\ [(\%\ S - 0.3)(1 - 1.667\ (\frac{100-CJP}{CJP}))]$

Table 52. Increase in RSPA yields of three varieties at harvest grown on high (150#/A) and low (24#/A) levels of nitrogen during 1967

	Harvest Date			
Variety	October 6	October 26	November 6	Average
		pounds/acre		
2H	5768	6426	6655	6283
2L	6082	6529	2792	6468
Average	5925	6478	6724	
3H	5977	5820	6766	5988
3L	5733	5700	6282	5905
Average	5855	5760	6224	
5H	6349	6375	7398	6707
5L	6763	7438	7670	7290
Average	6556	6907	7534	

Effect of Harvest Date on Storage Characteristics

In 1967 beets from three harvest dates were stored for 65 and 130 days at 3 C (Table 53). The RSPT data were rather erratic and the responses in storage are difficult to explain. The early harvested beets exhibited a sharp drop in RSPT after 65 days of storage but after 130 days averaged the same as the freshly harvested beets. This trend was true for all varieties and nitrogen treatments harvested on October 6. Although the loss of RSPT was greatest in the beets harvested on November 6 compared to October 26, the increased tonnage which occurred during this period resulted in a greater net RSPA after storage for the late harvested beets.

Table 53. Effect of harvest date on the percent sucrose, clear juice purity, and RSPT at harvest and after storage at 3 C (1967)

Harvest	Sucrose	CJP	RSPT	RSPT
	%	%	lb	lb
At Harvest				
October 6	16.2	93.5	273	6279
October 26	16.0	94.0	272	6582
November 6	16.6	94.0	285	7097
Stored 65 Days				
October 6	15.5	92.3	257	5911 (-368)[a]
October 26	16.0	93.1	266	6437 (-145)
November 6	16.4	93.8	279	6947 (-150)
Stored 130 Days				
October 6	16.2	93.7	275	6324 (+46)
October 26	15.4	92.6	247	5977 (-605)
November 6	15.5	92.4	245	6101 (-996)

[a]Parentheses indicate loss in storage.

The date of harvest experiment was repeated in 1968 with somewhat different results. The late harvested beets lost only 10 pounds of RSPT after 100 days of storage at 3 C as compared to 16 and 32 pounds for the earlier harvests (Table 54). Although the losses incurred during storage were very different in the two years the bagged sugar production for the campaign would have been substantially increased both years by delaying harvest. Delayed harvests would also facilitate the lowering of pile temperatures by ventilation due to the normally lower air temperatures. The primary disadvantage of delayed harvest is the possibility of inclement weather conditions which could prevent harvesting altogether. Vajna (1960) reported that although earlier harvested beets respired at a greater rate the sugar losses in storage were lower.

Comparison of Varieties in Storage on the Basis of RSPT

Three varieties were harvested on October 6, October 26, and November 6, 1967 and stored for 65 and 130 days at 3 C. The October 6 harvest gave unexplainable results as previously mentioned and will not be discussed further.

Losses in RSPT were negligible for the first 65 days of storage and no differences between varieties existed (Table 55). After 130 days of storage the inferior keeping quality of variety 3 was evident.

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Losses in RSPT were negligible for the first 65 days of storage and no differences between varieties existed (Table 55). After 130 days of storage the inferior keeping quality of variety 3 was evident.

Table 54. Effect of harvest date on the percent sucrose, CJP and RSPT in storage (1968) at 3 C

Harvest	Sucrose	CJP	RSPT
	%	%	lb
At Harvest			
September 1	16.4	94.7	292
October 1	15.3	97.3	284
November 1	16.2	94.5	288
Stored 50 Days			
September 1	15.8	93.7	275 (17)[a]
October 1	15.2	94.0	266 (18)
November 1	15.9	93.6	276 (12)
Stored 100 Days			
September 1	15.0	93.5	260 (32)
October 1	15.5	93.4	268 (16)
November 1	16.4	92.6	279 (9)

[a]Parentheses indicate loss in storage.

Table 55. The decline in RSPT of three varieties harvested October 26, November 6 and stored for 65 and 130 days at 3 C (1967)

Variety	Harvest	Days Stored		Loss in 130 Days
		65	130	
		pounds		
October 26 Harvest				
2	267	263	251	16
3	273	271	240	33
5	274	264	251	23
November 6 Harvest				
2	281	273	257	24
3	290	285	221	69
5	283	279	257	26

The keeping quality of variety 5 was not affected by harvest date, but the other varieties lost considerably more RSPT when harvested late. However the incidence of surface mold after 130 days of storage was much greater in varieties 2 and 3 and this may account for the greater losses (see invert discussion on page 52).

Variety-Nitrogen Interaction

Five varieties grown on 150 and 24 pounds of nitrogen per acre were harvested on October 26, 1967 and stored for 130 days. When beets were grown on low nitrogen, the RSPA at harvest was greater for varieties 2, 3, and 5 but lower for varieties 1 and 3 (Table 56). The much higher

Table 56. Recoverable sugar per acre yield at harvest and after 130 days of storage for five varieties grown on 24 and 150 pounds nitrogen per acre and stored at 3 C

	At Harvest		Stored 130 Days		Loss in Storage	
	Nitrogen		Nitrogen		Nitrogen	
Variety	24#/A	150#/A	24#/A	150#/A	24#/A	150#/A
	pounds					
1	6731	7178	5587	6214	1194	964
2	6856	6536	6444	6235	412	301
3	5889	6220	5308	5473	581	747
4	7200	6659	6421	6089	779	570
5	7949	6612	7148	6264	801	348
Average					753	586

RSPA for variety 5 under low nitrogen fertilization would indicate that this line is extremely sensitive to excessive nitrogen fertilization. Since this variety has been used in many of the nitrogen studies in Michigan the results of these studies may have exaggerated the detrimental effects of excessive nitrogen application.

The effect of nitrogen fertilization on the storage losses (RSPA) varied between varieties but the beets grown on low nitrogen lost 167 pounds more sugar on the average. Variety 2 was definitely the superior storing variety at both nitrogen levels. Although the RSPA losses in storage for variety 5 grown on low nitrogen were among the highest,

its overall RSPA yield after storage was 700 pounds per acre greater than any of the other varieties tested. The result was a greater sugar yield after 130 days of storage than any other variety at harvest. Therefore, though sugar losses were greater total production was increased by storing high quality beets.

Table 57 gives the average RSPT over three harvest dates for three varieties grown on high and low nitrogen. Although variety 5 stored relatively better when grown on high nitrogen, it had a greater RSPT after 130 days of storage when grown on low nitrogen (Table 57). In contrast, variety 2 had similar losses for both nitrogen levels, but again it had a greater RSPT after 130 day storage when grown on low nitrogen. Variety 3 appeared to have a relatively lower loss when grown on low rather than high nitrogen. It is apparent that there is a great difference in the response of varieties to nitrogen in terms of RSPT at harvest and also in the preservation of RSPT in storage. The average loss in RSPT was the same for both nitrogen levels, however, to evaluate a variety or agronomic practice the yield and the losses in storage must be evaluated to determine the best variety from a total economic standpoint.

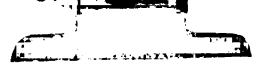
Preharvest Sprays

None of the preharvest foliar sprays (MH, GA, CCC, $VaSO_4$, Pyrocatechol) affected the loss of RSPT in storage.

Table 57. The effect of nitrogen fertilization on the loss of RSPT of three varieties in storage at 3 C (1967)

Variety	At Harvest			Stored 65 Days			Stored 130 Days		
	Nitrogen		Variety Average	Nitrogen		Variety Average	Nitrogen		Variety Average
	24#/A	150#/A		24#/A	150#/A		24#/A	150#/A	
	Pounds								
2	282	264	273	268	264	266	270 (12)[a]	252 (12)[a]	261
3	293	267	280	288	250	269	263 (30)	230 (37)	247
5	293	261	277	275	259	267	272 (21)	250 (11)	261
Average	289	264		277	258		268 (21)	244 (20)	

[a]Parentheses indicates loss in storage.



Previously Wittwer and Hansen (1951) reported maleic hydrazide reduced sucrose losses. D. J. Wort (1968) also reported that Va SO_4 and pyrocatechol reduced sucrose losses in the first 30 days of storage.

Modified Atmosphere

The loss of RSPT in modified atmosphere storage* was significantly decreased in comparison to the samples stored in air (Table 58). The amount of sucrose conserved was much higher but the accumulation of impurities caused a greater loss in purity in the modified atmosphere. However other combinations of carbon dioxide and oxygen may alleviate the decreased purity problem. The 1000 ppm ethylene increased sugar losses, presumably by increased respiration (Dilley, 1969).

Proportion of Recoverable Sugar per Ton Loss in Storage Accounted for by the Direct Loss of Sucrose

In Tables 59 and 60 the loss of total sucrose is compared to the loss in RSPT. In all varieties, except 2, the loss of sucrose by respiration and conversion accounts for approximately 50 percent of the total loss in RSPT. In variety 2 the loss due to increased impurity content was much greater than the loss by respiration and transformations.

---

*5% O_2, 5% CO_2, 90% N_2 $\pm$ 1000 ppm ethylene.

Table 58. Effect of modified atmosphere on clear juice purity, sucrose and RSPT after 40 days at 5 C (1968)

	Ethylene								
	0 ppm			1000 ppm			Average		
Atmosphere	CJP	S	RSPT	CJP	S	RSPT	CJP	S	RSPT
	%	%	lb	%	%	lb	%	%	lb
Control	95.3	15.2	271 (20)[a]	95.1	15.0	264 (27)	95.2	15.1	268 (24)
Modified Atmosphere*	94.4	15.9	279 (12)	94.0	15.5	264 (27)	94.2	15.7	271 (20)
At Harvest	95.1	16.4	291						

[a]Parentheses = RSPT loss in storage.

*5% O_2, 5% CO_2, 90% N_2.

Table 59. Proportion of RSPT losses due to loss of sucrose in five varieties stored 130 days at 3 C (1967)

Variety	At Harvest		Stored 130 Days		Loss in Storage		Sucrose Loss as Percent of Total RSPT Loss
	Total Sucrose	RSPT	Total Sucrose	RSPT	Total Sucrose	RSPT	
	lbs/ton	lbs	lbs/ton	lbs	lbs/ton	lbs	percent
1	324	283	303	240	-21	-43	49
2	313	272	309	257	-4	-15	27
3	321	277	306	250	-15	-27	56
4	308	269	295	242	-13	-27	48
5	324	279	310	258	-14	-21	67

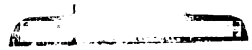


Table 60. Proportion of RSPT losses due to loss of sucrose in three varieties stored 100 days at 3 and 10 C (1968)

Variety	At Harvest		Stored 100 Days at:									
			3 C					10 C				
					Loss of:					Loss of:		
	Total Sucrose lbs/ton	RSPT lbs	Total Sucrose lbs/ton	RSPT lbs	Total Sucrose lbs/ton	RSPT lbs	Sucrose Loss as Percent of RSPT Loss	Total Sucrose lbs/ton	RSPT lbs	Total Sucrose lbs/ton	RSPT lbs	Sucrose Loss as Percent of RSPT Loss
5	308	272	306	264	2	8	25	294	249	13	23	57
6	273	224	255	197	18	27	67	257	198	16	26	62
7	327	291	316	269	11	22	50	304	260	23	31	74

Of the 8 pounds of RSPT lost by variety 5 at 3 C only 2 pounds or 25 percent was by the direct loss of sucrose. Varieties 6 and 7 corresponded very closely to the 1967 results. Increasing the storage temperature increased the direct loss of sucrose more than the indirect loss.

These results indicate the need for future research not only in the area of direct sucrose losses by respiration but also in the area of non-sucrose accumulation. Respiration losses can be reduced by controlled atmosphere storage or inhibitors, but the usefulness of these methods may be negated by the RSPT losses due to lower clear juice purity.

Summary of Recoverable Sugar Yields at Harvest and After Storage

Estimates of recoverable sugar are valuable measures of quality in fresh beets. After storage the estimate appears to be less accurate but is still an excellent guideline if the proper corrections for optically active compounds and for the decline in residual alkalinity are made.

Delayed harvest increased the RSPT and RSPA yields. The effect of harvest date on sucrose losses in storage varied considerably from year to year. Although high quality beets showed greater losses in storage, net yields per acre were increased by storing high quality beets.

Low nitrogen fertilization increased RSPA yields at harvest and after storage in three out of five varieties. The effect of nitrogen on storage losses depended strongly

on variety. Four out of five varieties lost more RSPA in storage when grown on 24 lbs of nitrogen per acre as compared to 150 lbs/acre. However the RSPA yields after storage were still higher in the low nitrogen beets. Variety appeared to be the most significant prestorage affecting recoverable sugar losses in storage.

Slightly over 50 percent of the RSPT lost in storage was by respiration. The remainder was due to a loss in purity. Storage at warmer temperatures increased respiratory losses as well as the losses resulting from the accumulation of impurities. Although the results differed between varieties the proportion of sucrose lost by respiration is approximately the same at 3 and 10 C.

Storage in modified atmospheres containing 5 percent oxygen, 5 percent carbon dioxide and 90 percent nitrogen significantly reduced losses in RSPT.

Preharvest applications of maleic hydrazide, gibberellic acid, CCC, vanadium sulfate, and pyrocatechol had no effect on the recoverable sugar losses in storage.

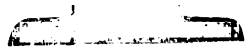
SUMMARY AND CONCLUSIONS

The effect of various agronomic and storage practices on the physical and biochemical factors affecting the loss of sucrose during storage or in subsequent processing were studied. The changes in the major impurities during storage were determined and their effect on processing evaluated. Loss of sucrose in storage occurred either directly via respiration and sugar conversions or indirectly by changes in the chemical composition of the beet which decreased recovery in the factory.

Slightly over 50 percent of the total RSPT losses were the result of the direct loss of sucrose in storage. Of these direct losses, 90 percent was by respiration. The sucrose lost by conversion to reducing sugars, raffinose, and other oligosaccharides accounted for only a small proportion of the total sucrose lost in storage. The remainder of the RSPT losses were the result of a decrease in clear juice purity. Therefore any future attempt to reduce storage losses must consider, not only respiration losses but also the losses due to the accumulation of melassigenic non-sucrose compounds.

The rate of respiration in storage was determined by carbon dioxide evolution or loss of dry matter. The results from both methods indicated that in addition to sucrose, ether carbohydrate components were acting as substrates for carbon dioxide evolution. Respiration losses immediately after harvest were substantially reduced by preventing injury, desiccation, reducing storage temperatures and controlled atmosphere storage. The respiration rate of the beet root was markedly reduced by high levels of carbon dioxide and carbon monoxide. The results of this study indicate that the direct loss of sucrose via respiration could be reduced on an economically feasible commercial scale by (1) reducing harvesting and handling injury and (2) covering the pile with a material capable of increasing the carbon dioxide content, and of preventing desiccation.

In processing, the amount of sucrose lost to molasses increases as the period of storage of commercial beets is extended. In this study the amount of sucrose lost to molasses was estimated using the percent sucrose and clear juice purity. The clear juice purity normally decreased during storage due to a decline in sucrose content and an increase in the non-sucrose components of the clear juice. Under the ideal storage conditions used in this study the TAI (raffinose, reducing sugars, amino acids, chlorides, sodium, and potassium) comprised approximately 65 percent of the total impurities in the clear juice at harvest and during storage below 4 C. Storage at warm temperatures



caused the accumulation of impurities not analyzed in this study. However the increase in total impurities was essentially identical at both cold (3 C) and warm (10 C) storage temperatures. Beets stored at higher temperatures contained more acids (reducing sugars, amino acids) and were therefore theoretically more melassigenic.

Variety played a major role in determining the chemical composition of the beet at harvest. The interaction of variety and nitrogen fertilization were significant for amino acid, sodium, potassium and chloride content. Substantial differences existed between varieties in relation to the RSPT losses in storage, the accumulation of raffinose and the stability of marc. Harvest date and topping had only minor effects on the chemical composition of the beets in storage. In general agronomic practices played a minor role compared to storage practices in controlling the chemical composition of the beet.

During low temperature storage (below 4 C) the major non-sucrose component accumulating was raffinose. High levels of raffinose caused a considerable overestimation of sucrose by polarimetric methods. Since raffinose cannot be degraded during processing it accumulates in the factory and is a primary nuisance to processing. Raffinose accumulation was a function of temperature but the amount accumulated was variety dependent. Although all varieties in this study accumulated raffinose, substantial differences in sensitivity to temperature existed between varieties.

Reducing sugars accumulated during storage primarily as a result of mold or desiccation. The accumulation of reducing sugars in storage as a result of these factors was many times greater than that due to variety or agonomic practices. Invertase apparently played a secondary role in the hydrolysis of sucrose in healthy beets. Reducing sugars have a threefold detrimental effect on processing. Not only is (1) sucrose lost directly by hydrolysis to reducing sugars, but (2) reducing sugars are degraded to acids and (3) contribute to the formation of highly colored clear juices due to non-enzymatic browning reactions. The excess acids produced in processing must be neutralized with sodium carbonate to prevent acid inversion. In commercial piles the accumulation of reducing sugars occurs to a much greater extent than that found in this study. However, on a commercial scale, the accumulation of reducing sugars could be reduced considerably by eliminating freezing and thawing on the pile surface, and preventing excessive pile heating.

In the absence of acids derived from invert, the amino acids are the major contributors of acidity to factory juices. The amino acid content at harvest was primarily a function of the rate of applied nitrogen. The degree of luxury consumption of nitrogen was strongly variety dependent. In storage at temperatures below 7 C the amino acid content normally declined, while above 7 C the content may increase. Storage in atmospheres of low oxygen (5%) and

high carbon dioxide (5%) caused a substantial accumulation of amino acids. Since the major factors controlling the amino acid content of the root are nitrogen fertilization and variety, control of the amino acid content through breeding and proper agronomic practices should be relatively easy.

The quantity of anions absorbed by the beet root during growth was equal to the cations. This equilibrium relationship of ions is also nearly ideal for efficient factory processing. However due to preferential elimination of ions during juice clarification, the formation of acids by alkaline decomposition of reducing sugars, and the deamination of amides produces excess acids in the processing juices from stored beets. Therefore any method used to evaluate the quality of stored beets must consider the melassigenic nature of the method used to amend the composition of process juices from stored beets. Since five pounds of sucrose is lost for each pound of sodium carbonate added, agronomic and storage practices which increase the acidity of process juices must be avoided (high nitrogen fertilization, warm storage temperatures, sprouting, molds).

Not only is the loss of sugar in the factory due to changes in the chemical composition of the clear juice, it is also related to the physical integrity of the root. However under ideal storage conditions, the marc remained reasonably stable to extraction temperatures in the 70-80 C

range and showed evidence of resynthesis. The percent marc was variety dependent and influenced greatly by environment (rainfall and nitrogen fertilization). Marc stability in storage was influenced most by variety and storage temperature.

APPENDICES

APPENDIX A

ABBREVIATIONS USED

CCC: 2-chloroethyl-trimethylamonium chloride

CJP: Percent clear juice purity

EDTA: Ethylenediamine tetraacetic acid

GA: Gibberellic acid

Invert sugars: equimolar quantities of glucose and fructose

MH-30: Maleic hydrazide

mg/100 s: Milligrams per 100 gms of sucrose

mg/kg: Milligrams per kilogram fresh weight

Reducing sugars: Sugar containing a free aldehyde group capable of being oxidized

RSPA: Recoverable sugar per acre (bagged sucrose)

RSPT: Recoverable sugar per ton (bagged sucrose)

RDS: Refractometric dry solids--total soluble dry matter

%S: Percent sucrose on beets

TAI: Total analyzed impurities, includes raffinose, reducing sugars, amino acids, sodium, potassium, chlorides.

APPENDIX B

DEVELOPMENT OF ENZYME METHOD FOR THE DETERMINATION OF RAFFINOSE

A method was developed to determine rapidly the amount of raffinose in the clear juice via a coupled enzyme system composed of galactose oxidase and peroxidase. Galactose oxidase is a metalloenzyme containing one gram atom of copper per mole (Amaral, 1963) which will oxidize the C_6 position of galactose as well as some of its derivatives and polymers. The enzyme has a pH optimum of 7.0. Galactose oxidase will react with free or bound (as in raffinose) galactose to produce D-galacto-hexodialdose and hydrogen peroxide (Avigad, 1961). The hydrogen peroxide can then be reduced to water by peroxidase concurrent with the oxidation of an appropriate chromagen to produce a detectable color.

Four mg of galactose oxidase (20 units, 2.5 mg peroxidase and 2.5 mg of O-tolidine were mixed and diluted to 50 ml (Avigad, 1961). Due to the high pH (9.2) of the clear juice samples to be analyzed 1 ml of a 0.1 M phosphate buffer pH 7 was added to the reaction mixture. Although more dilute buffers were tried this concentration gave the most linear results.

The reaction was initially stopped by addition of 6 ml of 0.1 M glycine buffer pH 9.7. However the sensitivity of the procedure for low concentrations of raffinose could be increased by stopping the reaction with 2 ml 0.05M EDTA by complexing with the copper ion.

In the initial studies 2 ml of the enzyme preparation, 1 ml of 0.1 M phosphate buffer gave a linear absorbance at 425 mμ for raffinose standards over the range of 0 to 0.6 mg. The reaction was linear for up to 90 minutes. A 60 minute incubation time was used for all routine analysis. This length of time was sufficient to develop readable color at the lower concentrations.

The volume of the clear juice samples was limited to 0.25 ml due to irregular color development which occurred when larger volumes were used. This problem could be eliminated by deionizing the sample with a mixed bed resin (Bio Rad AG 501 - x 8, 20-50 mesh).

The final procedure used for all routine analysis was as follows:

- 2 ml enzyme reagent mixture (Worthington Biochemical, Freehold, New Jersey). Reagent was made to volume with 0.05 M phosphate buffer pH 7.0
- 0.25 ml sample containing 0-0.3 mg raffinose
- incubate at 30 C for 60 minutes
- 3 ml 0.05 M EDTA added to stop reaction
- read at 425 mμ on Beckman DB spectometer.

Chromatographic analysis of clear juice samples from many treatments did not reveal the presence of free galactose or galactinol which could act as substrates for the assay.

APPENDIX C

INVERT DEGRADATION

During the clarification of sugarbeet diffusion juice the pH is raised to approximately 11.5 with calcium oxide and the mixture heated to 75 C to decompose the reducing sugars present. Glucose and fructose under these conditions break down to form acids. It was necessary to determine the average molecular weight of these acids in order to predict the amount of sodium carbonate to be added to neutralize the acids during the processing of stored beets.

Mixtures of glucose, fructose and sucrose were heated at various temperatures up to 80 C in a three-necked reaction flask containing 0.125 N sodium hydroxide pH 11.5. The reaction mixture was heated with an electric mantle controlled by a rheostat. An aliquot of the mixture was taken at the beginning and end of the alloted heating period and the net loss in reducing sugar and gain in acid were determined. The equivalent weight of the acid produced was calculated as the grams of reducing sugar decomposed divided by the equivalents of acid produced.

Although the results were quite variable ranging from 110 to 130 grams per equivalent the results were well above those of 90 reported previously in the literature (Silin, 1964; McGinnis, 1950). Carruthers (1968) indicated that in extensive work by his laboratory, the average value was shown to be approximately 120.

The chemically more reactive fructose began to decompose at 58 C in 15 percent sucrose or 10 C lower than glucose. It is therefore very important not to heat the limed-pressed juice sample above 60 C in the determination of clear juice purity if the assumption that no invert is decomposed in this process is to be valid. This assumption will be assumed valid in all calculations but due to the light yellow color of the clear juice samples it was apparent that some variable proportion of the reducing sugars was lost during clarification.

BIBLIOGRAPHY

BIBLIOGRAPHY

Allen, P. J., and J. S. D. Bacon. 1956. Oligosaccharides formed from sucrose by fructose-transferring enzymes of higher plants. Biochem. J. 63:200-206.

Amaral, D. L. Bernstein, D. Morse and B. Horecken. 1963. Galactose oxidase of Polyporus circinatus: a copper enzyme. J. Biol. Chem. 238:2281.

Andersen, E., and E. Smed. 1963. The chemical composition of sugarbeets and the effective alkalinity and the sugar loss in molasses. Paper presented to the 12th Cen. Ass. S.I.T.S.

Atterson, A., A. Carruthers, J. V. Dutton, D. Hibbert, J. F. T. Oldfield, M. Shore and H. J. Teaque. 1963. Chemical changes occurring in sugarbeets during storage, freezing, wilting. 16th Ann. Tech. Conf. B.S.C.

Avigad, B., D. Amaral, C. Asensio and B. Horecker. 1961. Galactdialdolase production with an enzyme from the mold Polyporus circinatus. Biochem. Biophys. Res. Comm. 4:474.

Avigad, G. 1968. Study of enzymatic degradation of sucrose in sugarbeet tissues, to provide information of improved procedures in the handling of sugarbeets for processing into sugar and other useful products. Final Report Project #UR-A(10)50-25. The Hebrew Univ. of Jerusalem Dept. of Biol. Chem. Jerusalem, Israel.

Bacon, J. S. D. 1961. The development of invertase activity in washed slices of beet tissue. Biochem. J. 79:200.

Bacon, J. S. D., I. R. MacDonald and A. H. Knight. 1965. The development of invertase activity in slices of the root of Beta vulgaris L. washed under aseptic conditions. Biochem. J. 94:175.

Barbour, R. D., and C. H. Wang. 1961. Carbohydrate metabolism of sugarbeets. Respiratory catabolism of mono and disaccharides. ASSBT 11:436-442.

Barr, C., E. M. Guinn and R. A. Rice. 1940. A preliminary report on the effect of temperature and beet conditions on respiration and loss of sugar from beets in storage. Proc. ASSBT Part 1:52-65.

Bernfeld, P. 1961. Enzymes of starch degradation and synthesis. Adv. Enzymology 12:379-395.

Brown, R. J. 1955. Non-sucrose constituents of beetsugar processing liquors of the Rocky Mountain Area. Agricultural and Food Chemistry 3:346-353.

Carruthers, A., and A. E. Wootton. 1955. Colorimetric method for the determination of invert sugar in the presence of sucrose. International Sugar J. 57:193.

_____, and J. F. T. Oldfield. 1956. Pectin and Polysaccharides in beet juices and molasses. 9th Ann. Tech. Conf. BSC.

_____, _____, A. E. Wootton, and M. Shore. 1957. The destruction of sucrose and invert sugar in beet juice processing. Presented to: Commission Internationale Tecknique De Sucrerie.

_____, J. V. Dutton, J. F. T. Oldfield, M. Shore, and H. J. Teaque. 1959. The composition of sugar beet and chemical changes occurring during processing. 11th Annual Tech. Conf. BSC.

_____, _____, _____, _____, and _____. 1960. Juice composition in relation to factory performance. 13th Ann. Tech. Conf. BSC.

_____, and J. F. T. Oldfield. 1960. Methods for the assessment of beet quality. In: The Technological Value of the Sugar Beet, Elsevier Publishing Company.

_____, _____, J. V. Dutton, L. C. Manning, and M. Shore. 1961. Juice components in relation to sugar quality. 14th Ann. Tech. Conf. BSC.

_____, and _____. 1961. Methods for the assessment of beet quality. Int. Sugar Journal 63:72-74, 103-105, 137-139.

_____, J. V. Dutton, J. F. T. Oldfield and M. Shore. 1962. The interaction of amino acids and glucose in factory juices. 15th Ann. Tech. Conf. BSC.

Carruthers, A., J. F. T. Oldfield and H. J. Teaque. 1962. Assessment of beet quality. 15th Ann. Tech. Conf. BSC.

_____, J. V. Dutton, J. F. T. Oldfield, C. W. Elliott, R. K. Heaney and J. J. Teaque. 1963. Estimation of sugars in beet molasses. 16th Ann. Tech. Conf. BSC.

_____. 1963. Quality for processing. Journal of the Nat. Inst. of Agri. Botany 9:427-431.

_____, J. F. T. Oldfield and H. J. Teaque. 1965. The influence of crown removal on beet quality. 18th Ann. Tech. Conf. Brit. Sugar Corp.

_____, and _____. 1966. The influence of crown removal on beet quality. Int. Sugar Journal 68:297-301.

_____, _____, and H. J. Teaque. 1967. The influences of crown removal on beet quality. 18th Ann. Tech. Conf. BSC.

_____. 1968. Personal communication.

Claassen, H. 1943. Pectin and its influence on juice purification and the filterability of carbonation juice. Centr. Zuckerind 50:221, 1942 From C.A. 37:6922.

Dedek, J. 1948. The chemistry of lime salts in sugar beet juices. ASSBT 5:595-610.

Dexter, S. T., M. G. Frakes and Grant Nichol. 1966. The effect of low, medium and high nitrogen fertilizer rates on the storage of sugar beet roots at high and low temperature. ASSBT 14:147-159.

_____, and _____. 1967. A rapid method of sorting sugar beets for storage. ASSBT 14:350-356.

_____, _____, and F. W. Snyder. 1967. A rapid and practical method of determining extractable white sugar as may be applied to the evaluation of agronomic practices and grower deliveries in the sugar industry. ASSBT 14:433-454.

_____, _____, and R. E. Wyse. 1969. Sorting sugar-beets by specific gravity to obtain more uniform samples for post-harvest studies. ASSBT. In Press.

_____, _____, and _____. 1969. Storage and clear juice characteristics of topped and untopped beets grown in 14 and 28 inch rows. A prescription for beet quality. ASSBT: In Press.

Devillers, P. L., M. R. Loilier and J. C. Chartier. 1966. Studies on the respiration of beet after harvesting. Ind. Alim. Agric. 83:901-911.

Dilley, D. R., F. F. Wood and Phillip Brimhall. 1969. Respiration of sugarbeets following harvest in relation to temperature, mechanical injury and selected chemical treatment. ASSBT. In Press.

Downie, A. R. 1948. Sugar beet storage experiment, 1947. ASSBT 5:660-664.

Drachovska, M. 1961. Results of an examination of the technological value of sugar beet. Zucker 14:182-187.

Dubourg, J. P. Cevillers et P. Naffa. 1960. Methods D'Etimation de la Valeur Technique de La Betteraue Sucriere. In: The Technological Value of the Sugar Beet. Proceedings of the XI Session of the Commission Internationale Technique De Sucreie, Frankfurt.

Dutton, J. V., A. Carruthers and J. F. T. Oldfield. 1961. The synthesis of sucrose by extracts of the root of the sugar beet. Biochem. J. 81:266-272.

Fan, Der-Fong and David Sidney Feingold. 1969. Nucleoside Diphosphate-sugar 4-Epimerase. I Uridine Diphosphate Glucose 4- Epimerase Of Wheat Germ. Plant Physiol. 44:599-604.

Finkner, R. E., D. B. Ogden, P. C. Hanzas and R. F. Olson. 1958. II. The effect of fertilizer treatment on the calcium, potassium, raffinose, galactinol, nine amino acids and total amino acid content of three varieties of sugar beets grown in the Red River Valley of Minnesota. ASSBT 10:272-280.

_____, M. D. Finkner and R. F. Olson. 1959. Effects of storage on raffinose content of sugar beets. I. Varietal changes occurring during storage. ASSBT 10:481-488.

_____, J. F. Swink, C. W. Doxtator, R. F. Olson, and P. C. Hanzas. 1959. Changes in raffinose content and other characteristics of sugar beet varieties during six different harvest dates. ASSBT 10:459-566.

_____, D. W. Grimes, and G. M. Herron. 1964. Effect of plant spacing and fertilizer on yield, purity, chemical constituents and evapotranspiration of sugar beets in Kansas. II. Chemical constituents. ASSBT 12:699-714.

Frakes, M. G. 1969. Personal communication.

Frydman, R. B., and E. F. Neufield. 1963. Synthesis of galactosylinositol from peas. Biochem. and Biophysical Res. Comm. 12:121-125.

Gaskill, J. O. 1950. Effects of wilting, drought and temperature upon rotting of sugarbeets during storage. ASSBT 6:653-659.

Goodban, A. E., J. Benjamin Stark and H. S. Owens. 1953. Content of sugarbeet processing juices. J. Agr. Food Chem. 1:261-264.

Hac, L. R., A. C. Walker and B. B. Dowling. 1950. The effect of fertilization on the glutamic acid content of sugarbeets in relation to sugar production. ASSBT 6: 401-411.

Haddock, J. L., D. C. Linton and R. L. Hurst. 1956. Nitrogen constituents associated with reduction of sucrose percentage and purity of sugarbeets. ASSBT 9:110-117.

_____, P. B. Smith, A. R. Downie, J. T. Alexander, B. E. Easton and V. Jensen. 1959. The influences of cultural practices on the quality of sugarbeets. ASSBT 10:290-301.

Hackett, D. P. 1960. Respiratory inhibitors. Encyl. Plant Physiol. 12(2):30.

Hangyal, K. 1966. Changes in the technical value of sugarbeet with various types of storage. Zucker, 19:279-285; 303-310.

Henry, J., R. Vandewijer and R. Pieck. 1960. The technical value of sugarbeets in 1960. Presented to the 14th Annual Technical Conf. of the B. S. Co.

Joslyn, M. A. 1962. The chemistry of protopectin: A critical review of historical data and recent developments. Advances in Food Research. 11:1-106.

Kertesz, Z. I. 1951. The Pectic Substances. Interscientific Pub. Inc., New York.

Khelemskii, M. Z., N. T. Poedinok and M. L. Pelts. 1963. Influences of the temperature factor on variation in the quality of beet during storage. Trudy CINS 11:31.

Khelemskii, M. Z., E. A. Vorobeva and M. L. Pelts. 1963. Influence of beet storage period on the activity of enzymes and the content of trisaccharides, organic acids and amino acids. Trudy CINS 11:18.

_____, N. T. Poedinok and M. L. Pelts. 1963. Effect of the temperature factor on the change in beet quality during storage. Trudy Tsentv. Nauch.-Issled. Inst. Sahar. Prom. 11:31-44.

Khualkovkii, T. P. 1964. Melassigenic coefficients of non-sugars in standard molasses as a function of their effect on molasses viscosity and sugar solubility. Sakar. Prom. 38:580-583.

Kursanov, A. L., and O. A. Pavlinova. 1967. Sugar accumulation as a function of growth processes in sugarbeet roots. Fiziologiya Rastenii 14:21-28.

Lang, A. 1956. Induction of flower formation in biennial hyoscyanmus by treatment with gibberellin. Naturwissenschaften 43:284-285.

Larmer, F. C. 1937. Keeping qualities of sugar beets as influenced by growth and nutritional factors. J. Agr. Res. 54:185-98.

MacDonald, I. R. 1968. Personal communication.

McCready, R. M. 1966. Polysaccharides of sugarbeet pulp a review of their chemistry. ASSBT 14:260-270.

_____, A. E. Goodban, R. Ratner, and A. Ulrich. 1966. Sugarbeet and purified juice quality in relation to non-sugar constituents. ASSBT 14:91-96.

_____, and J. C. Goodwin. 1966. Sugar transformations in stored sugarbeets. ASSBT 14:197-205.

McGinnis, R. A. 1951. Beet-Sugar Technology. Reinhold Publishing Corp.

_____. 1954. Round-table discussion of "lime-salts." ASSBT 8:310-315.

Milner, Y., and G. Avigad. 1964. The UDP-glucose: D-fructose glucosytransferase system from sugarbeet roots. Israel J. Chem. 2:316.

Moore, S., and W. M. Stein. 1954. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J. Biol. Chem. 211:907.

Moreno, A., and C. E. Cardini. 1966. A raffinose-sucrose transglactosidase from wheat germ. Plant Physiol. 41:909-910.

Nelson, R. T., and R. R. Wood. 1950. Respiration and spoilage studies employing a modification of method developed by Stout and Fort. ASSBT 6:660-663.

_____, and R. K. Oldemeyer. 1952. Preliminary studies applicable to selection for low respiration and resistance to storage rots of sugarbeets. ASSBT 7:400-406.

Neumann, N. P., and J. O. Lampen. 1967. Purification and properties of yeast invertase. Biochemistry 6:468-475.

Oldfield, J. F. T., J. V. Dutton, D. Grierson, R. K. Heaney and H. J. Teaque. 1968. Effect of draft and of inversion on the quality of juice in beet diffusers. 19th Ann. Tech. Conf. BSC.

Owens, H. S., E. A. McComb, and G. W. Deming. 1954. Composition and percentage of marc in some varieties of sugarbeets. ASSBT 9:267-271.

_____, J. B. Stark, A. E. Goodban and H. G. Walker, Jr. 1955. Application of composition knowledge to beet sugar technology. Agr. and Food Chem. 3:350-353.

Ogden, D. B., R. F. Finker, R. F. Olson and P. C. Hanzas. 1958. The effect of fertilizer treatment upon three different varieties in the Red River Valley of Minnesota for: stand, yield, sugar, purity and non-sugars. ASSBT 10:263-271.

Pack, D. A. 1924. The storage of sugarbeets. Facts about sugar XIX, No. 8, 174-180; No. 9, 208-209; No. 10, 232-235; No. 11, 251-253.

_____. 1926. The effect of moisture on the loss of sugar from sugarbeets in storage. Journal Agr. Res. 32:1143-1152.

Palmer, J. M. 1966. The influence of growth regulating substances on the development of enhanced metabolic rates in thin slices of beet-root storage tissue. Plant Physiol. 41:1173-1178.

Pavlinova, O. A., M. F. Prasolova, and E. A. Ivanova. 1967. Mechanism of maleic acid hydrazide on growth and sugar accumulation in sugar beets. Fiziologiya Rastenii 14: 992-996.

Pressey, R. 1968. Invertase inhibitors from red beet, sugarbeet, and sweet potato roots. Plant Physiol. 43:1430-1434.

Pridham, J. B., and W. Z. Hassid. 1965. Biosynthesis of raffinose. Plant Physiology 40:984-986.

Rorabaugh, G., and L. W. Norman. 1956. The effect of various impurities on the crystalization of sucrose. ASSBT 9:238-252.

Rounds, H. 1950. Second carbonation studies based on commercial application of the "effective alkalinity" concept. ASSBT 6:551-564.

Rounds, H. G., G. E. Ruch, D. L. Oldemeyer, C. P. Parrish and F. N. Rawlings. 1958. A study and economic appraisal of the effect of nitrogen fertilization and selected varieties on the production and processing of sugarbeets. ASSBT 10:97-116.

Schales, O., and S. S. Schales. 1941. A simple and accurate method for the determination of chloride in biological fluids. J. Biol. Chem. 140:879.

Schneider, F., and D. Schleplake. 1963. The behavior of non-sugars during sugar extraction. Zucker. 16:503-509.

Silina, N. P. 1964. Effect of potassium and sodium salts on molasses sugar content. Sakhar. Prom. 38:728-731.

Silin, P. M. 1964. Technology of beet-sugar production and refining (Translated from the Russian, Israel Program for Scientific Translations LTD.) U.S. Dept. of Commerce.

_____. 1965. Evaluation of the technological properties of sugarbeet for selection. Priklad. Biokhim. Mikrobiologo 1:90-94.

Silina, N. P. 1966. Melassigenic coefficients of individual non-sugars. Sakhar. Prom. 40:15-18.

Snyder, F. W., and S. H. Wittwer. 1959. Some effects of gibberellic acid on stem elongation and flowering in sugarbeet. ASSBT 10:553-561.

Snyder, F. W., and N. E. Tolbert. 1966. Influences of nitrogen nutrition and season on photosynthetic incorporation of $C^{14}O_2$ into sucrose and other soluble compounds of the sugarbeet. Bot. Gaz. 127:164-170.

Sorokina, T. I. 1963. The influence of sugar content on the level of sugar losses in beets during storage. Sakhar. Prom. 37:925.

Stark, J. B., and R. M. McCready. 1968. The relationship of beet molasses composition to true purity. Part 1 Composition. ASSBT 15:61-71.

_____, _____, and A. E. Goodban. 1968. The relationship of beet molasses composition to true purity. Part 2 Statistical evaluation. ASSBT 15:73-84.

Steward, F. C. 1965. Plant Physiology: A Treatise. Vol IB: Photosynthesis and Chemosynthesis. Academic Press, New York.

Stout, M. 1949. Relation of oxidation-reduction potential, respiration and catalase activity to induction of reproductive development in sugarbeets. Bot. Gaz., pp. 438-449.

_____, and C. H. Smith. 1950. Studies on the respiration of sugarbeets as affected by bruising, by mechanical harvesting, severing into top and bottom halves, chemical treatment, nutrition and variety. ASSBT 6:670-679.

_____. 1954. Some factors that affect the respiration rate of sugarbeets. ASSBT 8:404-409.

_____. 1957. Respiratory losses from sugarbeets soon after harvest. ASSBT 9:350-353.

_____, and J. D. Spikes. 1957. Respiratory metabolism of sugarbeets. ASSBT 9:470-475.

_____, and F. V. Owen. 1959. Effect of gibberellic acid on rate of bolting of annual beets. ASSBT 10:302-310.

_____. 1959. Some effects of gibberellic acid on the physiology of sugar beets. ASSBT 10:306-310.

Straus, J. 1962. Invertase in cellwall of plant tissue cultures. Plant Physiol. 37:342-348.

Sugarbeet Research, 1964 Report. 1964. Determination of revoverable sugar using formula proposed by Great Western Research Laboratory, Denver. USDA-ARS Bluebook, CR-4-64. Pg. 155.

Swink, J. F., and R. E. Finkner. 1956. Galactinol-weight relationships in breeding for resistance to the sugarbeet nematode. ASSBT 9:70-73.

Ulrich, A. 1954. Growth and development of sugarbeet plants at two nitrogen levels in a controlled temperature greenhouse. ASSBT 8:325-338.

_____. 1955. Influence of night temperature and nitrogen nutrition on the growth, sucrose accumulation and leaf minerals of sugarbeet plant. Plant Physiology 30: 250-257.

Vajna, S. 1960. Sugar Beet Storage, Translated by Michael Schalit, Storage of sugarbeets a literature survey, The Great Western Sugar Company Research Laboratory: Rpt No. RL 65-005 May, 1965.

_____, and T. Vajna. 1965. Determination of sugar losses in sugarbeets during storage. Z. Zuckerind 15:387.

Vajna-Papp, M. 1964. New measurements of the respiration rate and sugar loss in stored beet. Zeitsch. Zuckerind. 89:67-73.

Vandewijer, R. 1962. The treatment of clear first carbonation juice. 15th Ann. Conf. of the BSC.

Vukov, K., and L. Barany. 1964. Influences of storage on alterations of the technological quality of sugarbeets. Cukoripar 17:19.

Vaughan, D., and I. R. MacDonald. 1967. Development of soluble and insoluble invertase activity in washed storage tissue slices. Plant Physiol. 48:456-458.

_____, and _____. 1967. Invertase development in storage tissue disks of Beta vulgaris; its nature, extent and location. J. Expt. Botany 18:578-586.

Wang, C. H., and R. D. Barbour. 1961. Carbohydrate metabolism of sugarbeets. II. Catabolic pathways for acetate, glyoxylate, pyruvate, glucose and gluconate. ASSBT 11:44-454.

Walker, A. C., L. R. Hac, A. Ulrich and F. J. Hills. 1950. Nitrogen fertilization of sugarbeets in the woodland area of California. I. Effects upon glutamic acid content sucrose concentration and yield. ASSBT 6:362-371.

Walker, H. G., E. S. Rorem and R. M. McCready. 1960. Compositional changes in diffusion juices from stored sugar beets. ASSBT 11:206-214.

Wallenstein, H. D., K. Bohn, G. Kagelmann and G. Willer. 1962. Results of beet storage experiments in 1957-1961. Zuckererzeugung 6, suppl. No. 2,1.

_____, and G. Kagelmann. 1966. Control of mass change during sugarbeet storage by determining the content of invariable constituents particularly potassium. Zeitsch. Zuckerind. 91:189-194.

Wittwer, S. H., and C. M. Hansen. 1951. The reduction of storage losses in sugarbeets by preharvest foliage sprays of maleic hydrazide. Agron. J. 43:340-341.

Wohlert, W. 1962. Respiration tests on different varieties of beta-beets. Zuckererzeugung 6:303.

Wood, R. R., R. K. Oldemeyer and H. L. Bush. 1956. Inheritance of raffinose production in the sugarbeet. ASSBT 9:131-138.

_____, H. L. Bush, and R. K. Oldemeyer. 1958. The sucrose-sodium relationship in selecting sugarbeets. ASSBT 10:133-137.

Woolley, D. G., and W. H. Bennet. 1959. Glutamic acid content of sugarbeets as influenced by soil moisture, nitrogen fertilization, variety and harvest date. ASSBT 10:624-630.

Wort, D. J. 1968. Personal communication. University of British Columbia, Vancouver, Canada.

Zhadan, V. Z., and M. Z. Khelemski. 1962. Dependence of sugar losses in respiration of beet on storage temperature. Sakhar. Prom. 36(10):55.