General Postharvest Physiology of the Sugarbeet Root

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The objective of this paper is to discuss some basic physiological processes that occur in the beet root after harvest, which affect sucrose losses.

It may be appropriate to determine first what is happening to sucrose during the time between harvest and processing. Table 1 is a summary of the losses in sucrose during 130 days of storage at 5 C. These data are the average for a 15-variety storage experiment. It is important to remember that these were near-ideal storage conditions: low temperature, no desiccation, and no mold.

Table 1. Source of sucrose losses in 130-day storage

	Changes: lb/ton/130 days		
Sucrose	-26		
Reducing sugars	+ 3		
Raffinose	+ 0.4		
Total sucrose to raffinose		0.54	
Reducing sugars from raffinose synthesis		0.13	
Reducing sugars from sucrose		2.87	
Sucrose lost to raffinose and reducing sugars			3.54
Sucrose lost by respiration			21.1
			24.6
Decrease in RWST			29.3

Storage temperature 5 C

The loss in total sucrose was 26 lb. Reducing-sugars increased 3 lb and raffinose 0.4 lb. Since two molecules of sucrose are required to make one raffinose molecule, 0.54 lb of sucrose went to raffinose, with 0.13 lb of reducing-sugar left over. Subtracting this 0.13 lb from the total reducing-sugar produced, leaves 2.9 lb of reducing-sugar derived directly from sucrose. Therefore, of the total 26 lb of sucrose lost during storage 3.5 lb went to raffinose and reducing-sugars.

In converting respiratory CO_2 to its equivalent of sucrose, we have 21 lb, or about 80% of the loss in total sucrose from respiration. The loss of recoverable white sugar per ton (RWST) was 29.3 lb, of which respiration accounted for 72%.

It is apparent that respiration is an important factor in determining sucrose losses in storage. However, in less-than-ideal conditions, conversion to raffinose and reducing-sugars may be of much greater importance.

Respiration Respiration in storage is the process whereby the root converts sucrose into energy to maintain its physiological integrity. The beet is still alive in storage and must remain so to prevent massive mold invasion.

Figure 1 depicts graphically the general conversions that take place in respiration. In the beet, the primary substrate for respiration is sucrose (Barbour and Wang, 1961). Sucrose is converted to glucose and fructose, which are then used as substrates for respiration. Amino acids can also act as substrates after deamination and conversion to organic acids. Since we are concerned here with aerobic respiration, oxygen is also required.

In the process of converting the substrates to carbon dioxide and water, energy is produced. However, the plant is not 100% efficient. Only part of this energy is trapped as ATP and is used to maintain biochemical functions within the cell. The energy that is not trapped is released as heat. This heat of respiration must be considered when pile-cooling requirements are calculated.

Figure 2 shows the respiration rate with time during storage. The rate is very high immediately after harvest, probably as a result of injury and the drastic metabolic changes that occur when a plant goes from a positive to a negative carbon balance. The respiration rate drops rapidly in the first week and then maintains a steady state for the remainder of the storage period.

Probably the single most important factor influencing the rate of respiration is temperature. As a rule of thumb, the rate will approximately double for each 10-C (18-F) rise in temperature.

Figure 3 is a composite of research results by several workers since the 1940's. The work of Barr et al (1940) covers the range of 35-95 F. Those of you concerned with short-term, high-temperature losses note that reducing the temperature from 95 to 85 F would reduce the respiration rate from 1.8 to 1.0 lb/T/day.

The vertical lines on the data of Stout, Nelson and Wood, and Wyse represent the range in respiration rates of the varieties used in their experiments. Note also that the respiration rate levels off at about 0.1 lb/T/day at 0 C or 32 F for all workers.

Another factor that has a tremendous effect on respiration rate is injury. The greater the degree of injury by topping and handling, the higher the respiration rate. Figure 4 shows data that I ran in cooperation with the GW Sugar Company on roots that ranged from minimal injury, hand dug and washed, to those machine-harvested and machine-washed. Note the systematic increase in respiration rate as injury is increased. Note also that the lines remained parallel after 12 days and did so for the entire 20-day duration of the experiment. Therefore, handling injury at harvest may affect respiration rates during the entire storage period.

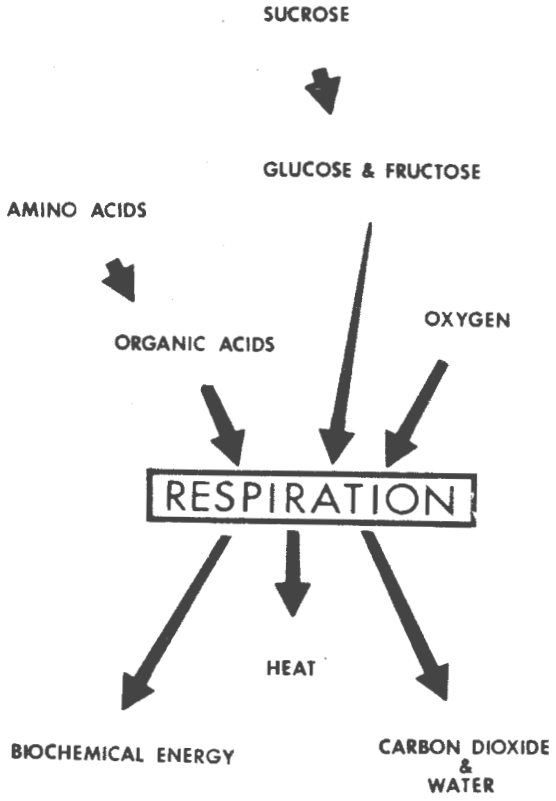

Figure 1. Diagram of important chemical conversions during respiration.

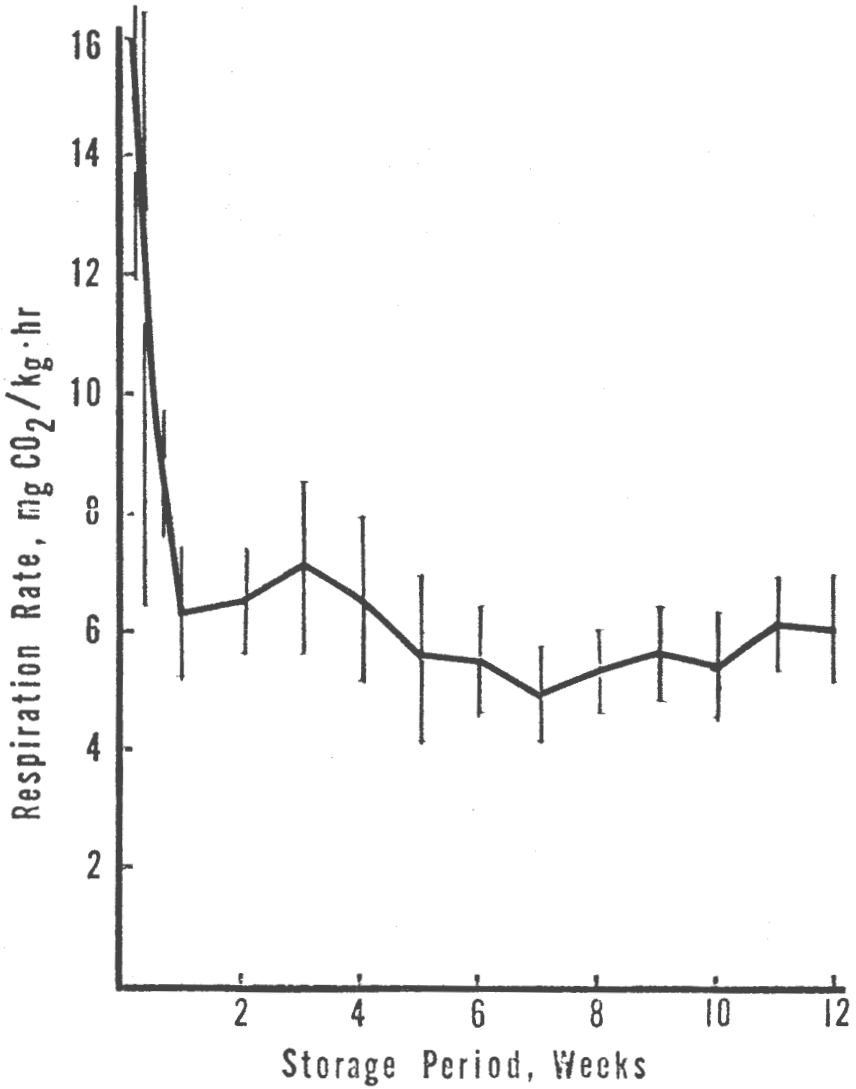

Figure 2. Average weekly respiration rate of fifteen varieties stored at 5 C. Vertical line depicts range between highest and lowest variety.

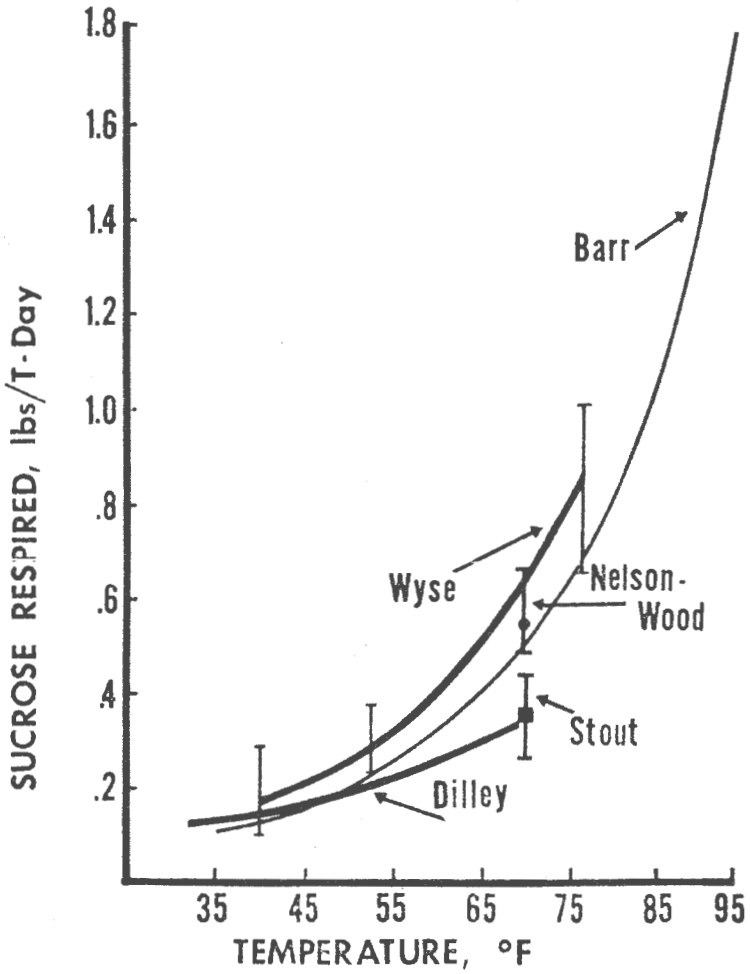

Figure 3. Effect of temperature on the respiration rate of sugarbeet roots.

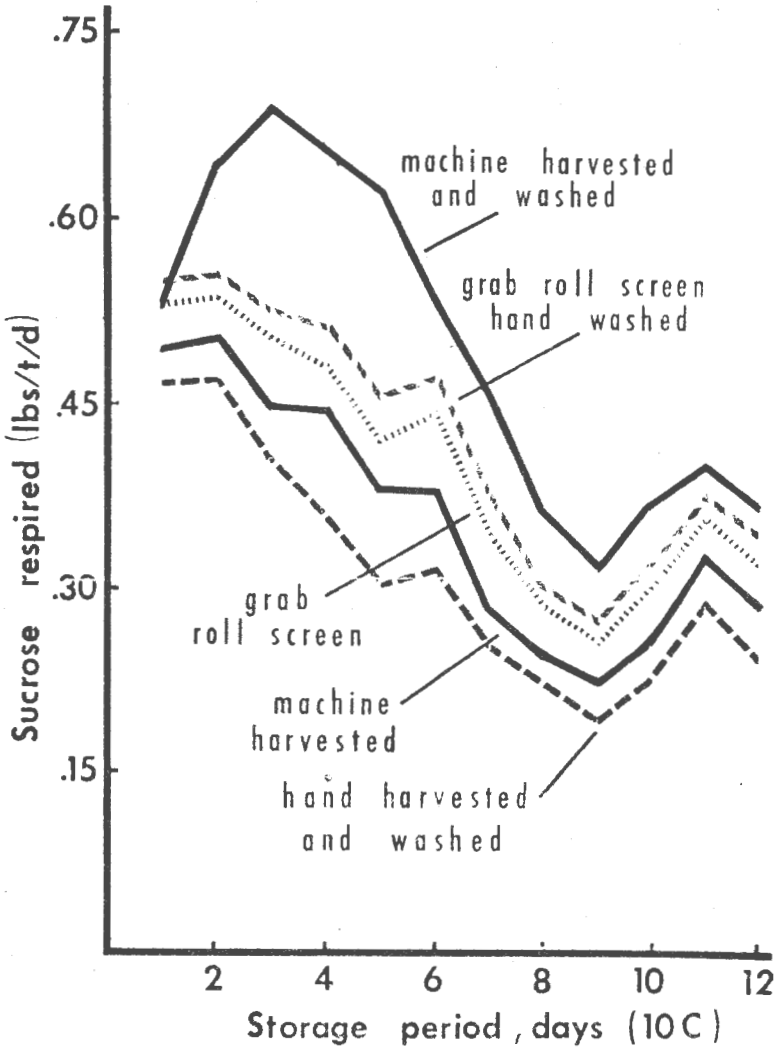

Figure 4. Effect of handling method on the respiration rate of sugarbeet root at 10 C.

Another factor affecting the rate of respiration is the oxygen content of the ambient atmosphere of the beet root. The theoretical effect of oxygen on respiration is depicted in Figure 5. As the oxygen concentration is decreased, respiration also decreases until the critical point is reached. This point is the concentration at which anaerobic respiration begins. At an oxygen concentration below this point, anaerobic respiration becomes an increasingly greater proportion of the total, and the respiration rate increases tremendously. Beets stored below the critical-point concentration would eventually die.

Figure 6 shows the results of a test on the effect of carbon dioxide and oxygen concentrations on beet-root respiration at 5 and 25 C. At 25 C (73 F), or about room temperature, after the oxygen content has been reduced from 20 to 2%, the same trend shown in Figure 5 is found. In 5% CO_2, the rate at high-O_2 is not affected, but the roots become anaerobic at between 5 and 10% O_2. Increasing the CO_2 content to 10% increases the respiration rate at all O_2 concentrations, and the beets become extremely anaerobic. Note that there is no apparent beneficial effect on respiration rates of increasing CO_2 levels at warm temperatures.

In the experiment at 5 C (40 F), the roots do not become anaerobic even at 2% O_2, but again 5% CO_2 has no effect. The rates at 5 C were so low that the 10% CO_2 treatment could not be studied. However, note that the respiration rates were reduced by about 50% by reducing the O_2 content to 5%. Therefore, it may be possible to reduce respiration losses by lowering the O_2 content of a beet pile. This could be done by covering the pile and recycling the air, thus allowing respiration to lower the O_2 content. However, the effect of temperature on respiration is much greater than that of oxygen.

Respiration is also affected by ethylene. Ethylene is a gaseous plant hormone, which causes apples, pears, bananas, and other fruits to ripen. Figure 7 is from a publication by Dilley, Wood, and Brimhall (1970) showing the effect of ethylene gas on the respiration rate of sugarbeet roots. The roots were exposed to 1,000 ppm of ethylene for 12 hours, and the subsequent respiration rate was monitored. The rate for ethylene-treated roots was consistently about 25% higher than that for the untreated control. The significant point is that many molds and injured tissues give off ethylene; therefore, one moldy beet could substantially increase the respiration rate of surrounding roots, even though they were not infected directly.

There have been reports in the literature that small roots respire faster than large roots (Table 2). However, this appears to be true only at high temperature. At 5 C (40 F) there is no difference in the respiration rates of large and small roots. Small roots obviously have more surface area per unit weight, which would allow a faster rate of gas exchange between the interior of the root and the ambient atmosphere. Small roots do have internal atmospheres closer to ambient that do large roots; but correlations between surface areas and respiration rates do not show good correlation with respiration rates within the larger size class (Table 3). This would indicate that surface area, and thus gas diffusion, is not limiting respiration rates in large roots.

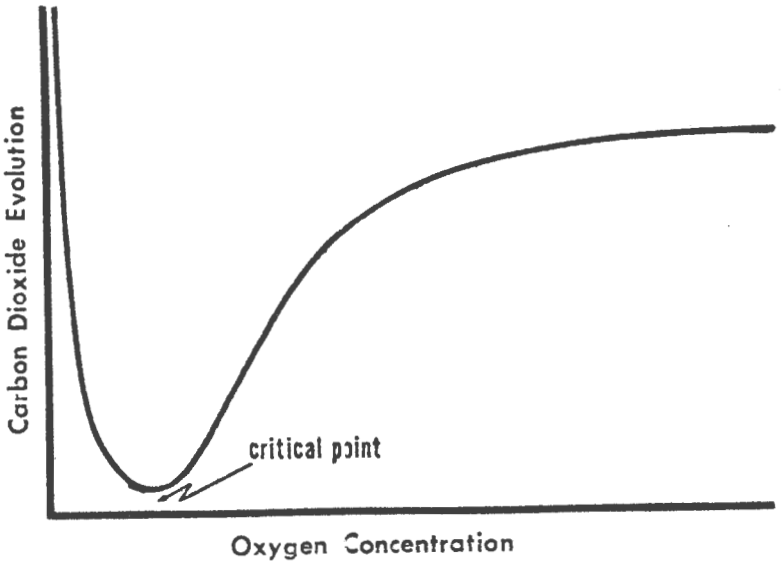

Figure 5. Theoretical response of respiration to changes in oxygen concentration.

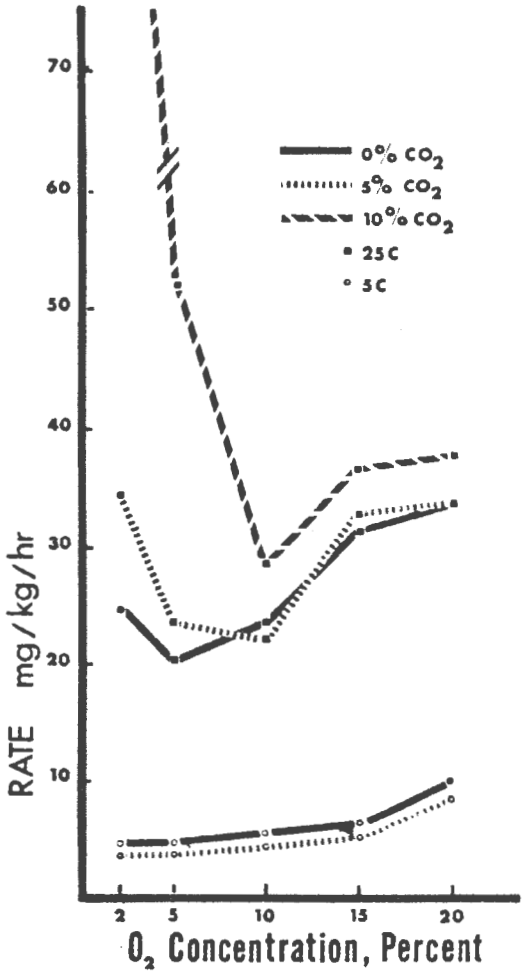

Figure 6. Effect of oxygen and carbon dioxide concentrations in the surrounding atmosphere on the respiration rate of sugarbeet root at 5 and 25 C.

Table 2. Respiration rates of large, medium, and small beets

Size - Wgt.	Respiration Rate 5 C	25 C
	lb/T/day	
Sm - 508 g	0.172a[1/]	0.913a
Med - 890 g	0.194a	0.905a
Lar - 1,600 g	0.166a	0.7868

[1/] Numbers followed by the same letter are not significantly different at the 5% level.

Table 3. Correlations of surface area and respiration rate

	Respiration Rate 5 C	25 C
	lb/T/day	
Ave. of 4 varieties all sizes	0.09	0.52
Small - 508 g	0.16	0.70
Med - 890 g	0.02	0.56
Lar - 1,600 g	0.11	0.18

Correlations between root characteristics and the respiration rate of roots at 5 C (40 F) are given in Table 4. The only consistently high correlation is with the DNA content per gm fresh weight, which is an index of cell number per unit weight. Therefore, the greater the number of cells, the higher the rate. This may also explain the differences between large and small roots. The goal of these studies was to find root characteristics that readily identify low-respiring lines for breeding studies. DNA is very difficult to measure and therefore would not be useful as a breeding tool.

Table 4. Correlations with respiration rate at 5 C.

	1970	1971
Sucrose	-0.06	-0.39
Weight	0.42	-0.53
Surface area	-0.64	0.10
Specific gravity	-0.77	-0.51
DNA	0.98	0.83
Ring structure number		0.23
mm/ring		-0.44
rings < 3 mm		0.19
" < 1.5 mm		0.29

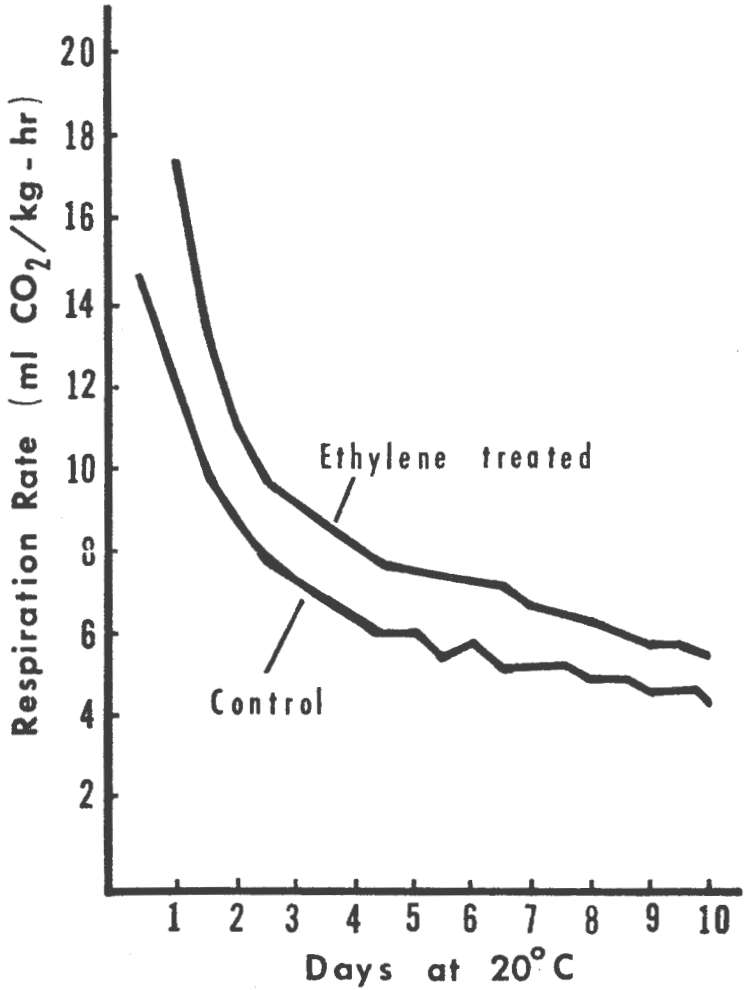

Figure 7. Influence of 1000 ppm ethylene pretreatment on the subsequent respiration rate of sugarbeet roots.

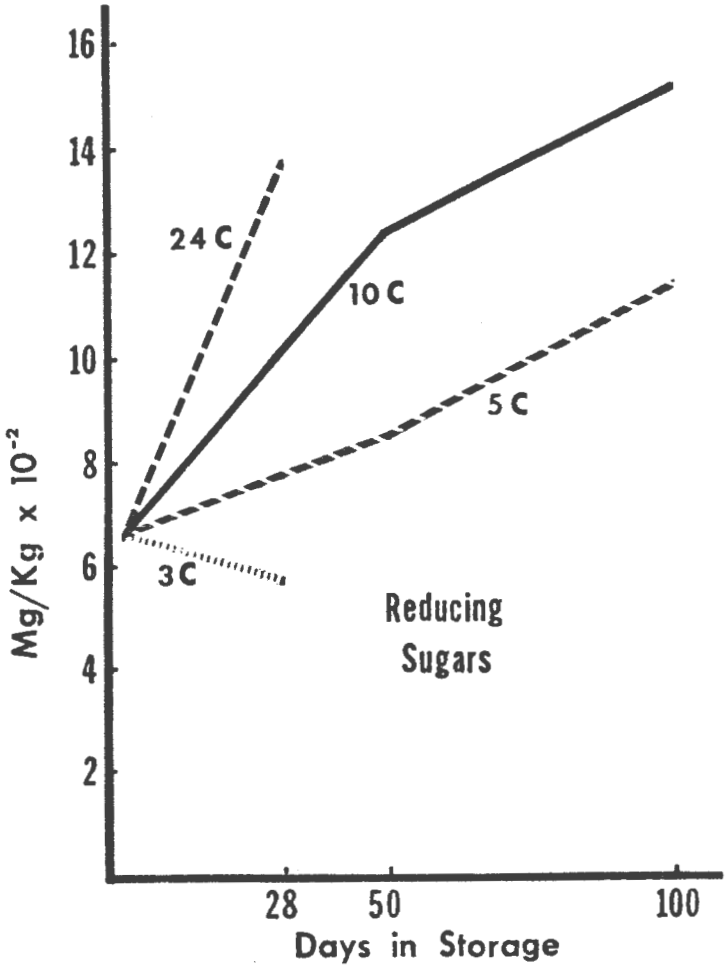

Figure 8. Reducing-sugar content of sugarbeet roots stored for 28 days at 3 and 24 C and 100 days at 5 and 10 C.

Biochemical conversions The second major source of sucrose loss is that of biochemical conversion to other metabolites. Invert sugars and raffinose are the primary metabolites formed from sucrose. Commercially, invert sugars develop primarily as a result of mold activity on the beet root. Moldy areas commonly contain high levels of invert sugars and essentially no sucrose. Therefore, very small amounts of mold increase the average invert levels substantially. However, invert sugars will accumulate even in beets stored under ideal conditions free of mold and desiccation. This accumulation is accentuated by increased temperature both in short- and long-term storage (Figure 8).

Another important impurity is raffinose. As you all know, raffinose accumulation is related to temperature (Figure 9). At temperatures below 40 to 45 F, raffinose accumulates. The threshold temperature where raffinose begins to accumulate is variety-dependent. In other words, it appears that all varieties accumulate raffinose at some temperature, but for some this temperature is much lower than for others.

Knowledge of the enzyme reactions that degrade sucrose and produce unwanted non-sucrose compounds may be beneficial in developing methods of chemical control.

Sucrose is readily converted to glucose and fructose by the enzyme invertase (Figure 10). The sugarbeet root contains two types of invertase, one with an optimum of pH 5, the other of pH 7.0. Acid invertase occurs at very low levels in the healthy, disease-free root. This enzyme also catalyzes the formation of kestose. However, very little is known about kestose metabolism.

Neutral invertase is a new enzyme that I recently found in the beet root. This enzyme is about 40-50 times more active than acid invertase, but it does not appear to make kestose.

Another very active enzyme in the beet root, which can degrade sucrose, is sucrose synthetase, which catalyzes the breakdown of sucrose to UDP glucose and fructose. UDP glucose can then be converted to UDP galactose, which combines with sucrose to produce raffinose. UDP glucose also acts as a precursor of cell-wall polysaccharides.

Figure 11 shows the change in activity of sucrose synthetase and acid invertase under several storage conditions. The activity of sucrose synthetase decreases during storage. More recent data show this decrease to be even more pronounced than shown in this figure. Invertase also decreases in most, but not all, instances. In other experiments, acid invertase activity may increase, but its activity is always very low.

The neutral invertase (pH optimum of 7.0 to 7.2) has an activity about 1/10 that of sucrose synthetase at harvest, but unlike that of sucrose synthetase, its activity remains high during storage. In preliminary experiments, this enzyme showed a good correlation with the reducing-sugar content of roots stored 175 days at 5 C (40 F) (Table 5). However, this correlation is not so clear in short-term storage.

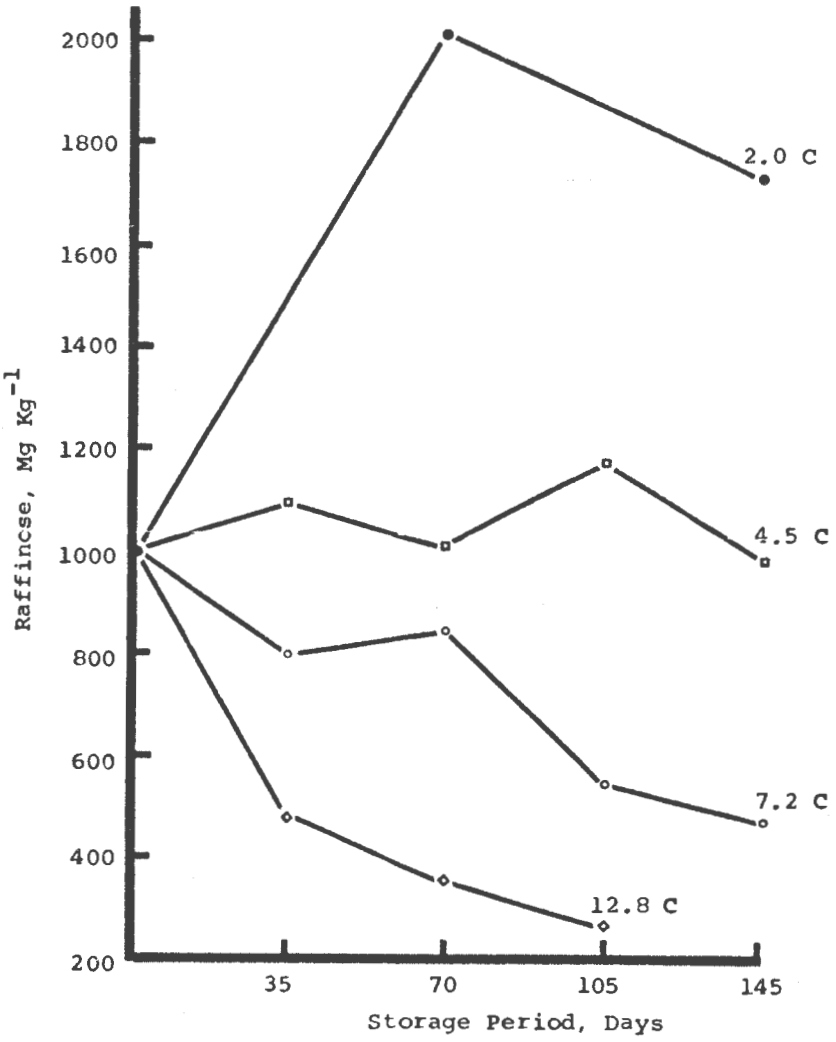

Figure 9. Effect of storage temperature on the raffinose content of sugarbeet roots stored for up to 140 days.

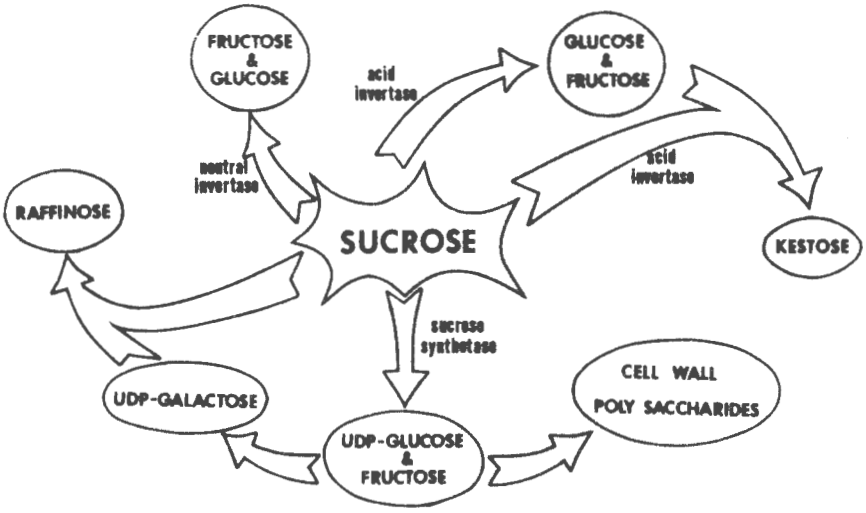

Figure 10. Postharvest sucrose metabolism in sugarbeet root tissue.

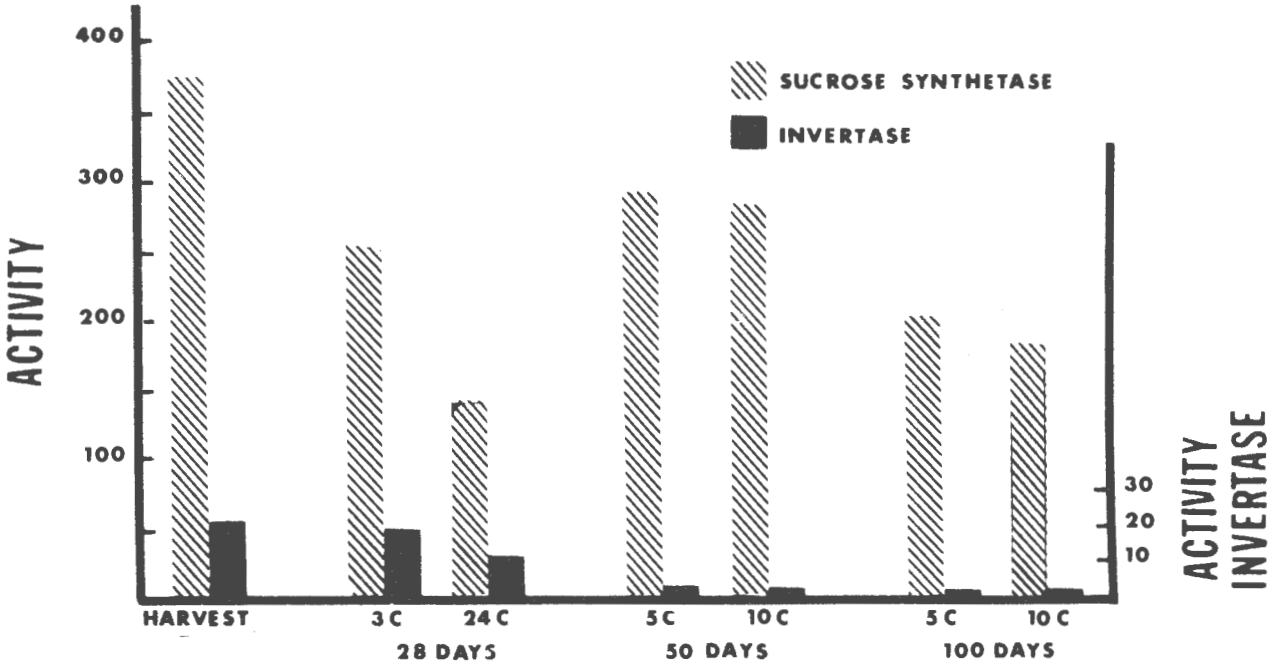

Figure 11. Sucrose synthetase and invertase activities in sugarbeet roots under various storage conditions.

Table 5. Relationship of acid invertase, neutral invertase, and sucrose synthetase activities to the reducing-sugar content of roots stored 175 days at 5 C.

Sample	Reducing sugars	Enzyme activity: Invertase pH 5	Invertase pH 7	Sucrose synthetase
	Relative/sucrose		mum/min/mg protein	
1	9	8	11.3	2.5
2	89	113	122	1.6
3	15	73	11.1	2.2
4	100	125	209	0.4
5	99	85	223	16
6	26	15	23	0.9
Correlation coefficients		0.83	0.96	0.39

We have now purified this enzyme to a pure protein and are currently working out its biochemical characteristics.

Marc stability Another important aspect of postharvest metabolism is that related to cell-wall or marc stability. During commercial storage, the marc or pulp yield per ton of beets declines. This is particularly true if mold or desiccation occur. However, under good storage conditions at low temperature, the percent marc may actually increase (Table 6). The fact that the sugarbeet has a very stable marc fraction is not too surprising, considering that sugarbeet is a biennial and thus normally overwinters to complete the life cycle.

Table 6. Evidence of marc stability under good storage conditions

Variety	At harvest	Stored 65 days	Stored 130 days
	%	%	%
SP63194-0	4.19	4.40	4.32
0 2 Clone	4.16	4.20	4.38
USH20	3.67	4.02	3.96
Mean	4.01	4.21	4.22

Extraction temperature 70 C
Storage temperature 3 C
$LSD_{.05}$ means - 0.09%

To substantiate that the increase in marc was caused by cell-wall synthesis, beet roots were fed sucrose -C^{14}, and the incorporation of radioactivity into cell-wall material was determined after 3 weeks (Table 7). Cell-wall material was isolated by extracting the root in hot 80% ethanol. This extraction removed 90% of the radioactivity fed. The residue (cell-wall fraction) was extracted with 80 C water, which removed an additional 5%. This would represent the pectin-type compounds. The hot-water-stable residue contains the remaining 5% of the applied radioactivity. This confirms the synthesis of cell-wall material during storage, using sucrose as the substrate.

Table 7. Evidence for synthesis of cell-wall polysaccharides during storage of sugarbeet roots.

Fraction	Percent on dry weight	% of total cpm fed
80% hot-ethanol-soluble or respired	74	90
Soluble in 80 C H_2O	5.2	4.1
Residue	20.8	4.9

^{14}C - sucrose was fed to beet roots by removing a plug from the thickest part of the root and adding 2 ml of sucrose containing 0.2 mcuries sucrose -U-^{14}C. After 3 weeks in storage at 5 C, the roots were extracted with 80% boiling ethanol and 80 C H_2O, and the radioactivity in each fraction was determined.

SUMMARY

About 70% of the sucrose losses under good storage conditions are caused by respiration, the remainder are a result of sucrose conversion to raffinose, reducing sugars, etc.

The major factors influencing the respiration rates of sugarbeet roots in storage are temperature and injury.

Temperature and desiccation are two major factors influencing the accumulation of impurities. In beets free of mold, warm temperature increases reducing-sugar accumulation. Desiccation increases mold susceptibility and reducing-sugar accumulation.

In beets free of mold and desiccation, sucrose synthetase and acid invertase appear to be of minor importance in controlling reducing-sugar accumulation.

Under storage at low temperature and in the absence of mold and desiccation, the beet root is capable of maintaining a stable marc by synthesizing new cell-wall polysaccharides.

REFERENCES

1. Barbour, Royal D., and C. H. Wang. 1961. Carbohydrate metabolism of sugar beets. I. Respiratory catabolism of mono and disaccharides. J. Amer. Soc. Sugar Beet Technol. 11:436-442.

2. Barr, C. Guinn, E. M. Mervine, and R. A. Bice. 1940. A preliminary report on the effect of temperature and beet conditions on respiration and loss of sugar from beets in storage. Proc. Amer. Soc. Sugar Beet Technol. Part 1:52-65.

3. Dilley, David R., Ralph R. Wood, and Phillip Brimhall. 1970. Respiration of sugarbeets following harvest in relation to temperature, mechanical injury and selected chemical treatments. J. Amer. Soc. Sugar Beet Technol. 15:671-683.

4. Nelson, R. T., and R. R. Wood. 1950. Respiration and spoilage studies employing a modification of method developed by Stout and Fort. Proc. Amer. Soc. Sugar Beet Technol. 6:660-663.

5. Stout, Myron. 1954. Some factors that affect the respiration rate of sugarbeets. Proc. Amer. Soc. Sugar Beet Technol. 8:404-409.

6. Wyse, R. E., and S. T. Dexter. 1971. A study of changes in the marc content of the sugarbeet during storage. J. Amer. Soc. Sugar Beet Technol. 16:289-298.

DISCUSSION

Tanner: At what temperature does respiration actually stop?

Wyse: In Figure 3 you will notice that all the lines tended to converge at a tenth of a pound per ton loss at 32 F. At 32 F (0 C) the root is not frozen. However, respiration would stop when the beet is frozen solid.

Fox: You showed a graph of respiration vs different atmospheres of CO_2 and O_2. At the higher temperature there was a very great effect of having different atmospheres but a much smaller effect at 5 C. What would be your conclusion then on the need for controlled atmosphere storage of sugarbeets if you were going to store them at 5 C.

Wyse: We can store beets at 5 C in controlled atmospheres and significantly reduce the losses over storage in air. Although that line looked flat at 5 C, we did reduce the respiration rate by 50% when O_2 was reduced to 5%, but by far the most important factor is reducing the temperature to 5 C. What we do after that is dictated by economics.

Bichsel: Do we know anything about enzymatic activity when a beet is frozen? Can we assume that essentially nothing is going on.

Wyse: I personally have not studied enzymatic activity at very low temperatures. This is difficult to do because the rates are very slow. At temperatures near 0 C when the beet is not frozen, enzymatic activity is obviously maintained because the beet remains alive and respiration is still occurring. When the beet is frozen however, enzymatic activity would be extremely slow if in fact non-existent.

Fox: We find a lot of temperatures in piles particularly in the rims between 10 F and 25 F and the interior of the piles are all around 28 to 32 degrees. These piles are still losing a lot of sugar somehow. At least we can't get it in the bag after they've been stored that way for a long time.

Wyse: But did those losses occur while they were frozen or did they occur at some other time? For example, does the loss occur in the cool-down and the warm-up period or when they went into the warm flume water after they had been frozen?

Barr: Referring to your curves on raffinose accumulation with temperature. Were they experimentally determined under your best storage conditions? How reproducible are those curves for a variety and between roots?

Wyse: They were determined under conditions which prevented weight loss and mold growth. I do not know how consistent this response is from beet to beet, but they reproduce very closely for a

given variety. The threshold temperature at which raffinose begins to accumulate appears to be quite consistent for a given variety. It is this threshold temperature which appears to be affected by genetics. All varieties accumulate raffinose at some temperature, but this temperature is much lower in some than others.

Bichsel: You suggested the possibility of using enzyme inhibitors applied as sprays or dips to reduce enzymatic activity. How far away are we from that kind of control?

Wyse: A lot of work is required before this will become a reality. To inhibit a key enzyme, we must first identify that enzyme and then find an inexpensive means of control. I should also mention that control of one enzyme by this method is extremely difficult. Most inhibitors will inhibit all enzymes with the same type of active site, so many enzymes in the root would be affected by applying such compounds. At this point in time we need to determine the key enzymes and then screen compounds to control them. The problem is that for many chemicals, their effect on enzymes and plant metabolism is not known.

Dickenson: At what temperature would you have the lowest respiration rate, the least raffinose and reducing sugar accumulation?

Wyse: Obviously, the lower the temperature the lower the respiration rate. However, an experiment which needs to be run is the determination of respiration rates at near freezing temperatures. In some plant material, potatoes for example the rate actually increases at low temperature. I doubt whether this occurs in sugarbeet, however. At low temperature reducing sugars are not a problem, raffinose is. So here variety becomes important. In general the lower the temperature, the better off we are.

Watkins: Your desiccation data was almost identical to ours, but I was wondering how you keep your desiccation rates so low.

Wyse: In the desiccation experiments the no-wilt controls were roots stored in 3 mil plastic bags containing a 1 pint perforated poly bag of wet wood chips. The large sample bag had 5-10 holes made with a paper punch to allow gas exchange.

Watkins: We use somthing similar but have more holes. Under our conditions we find that raffinose does not build up.

Barr: How does temperature affect the biochemical pathways of raffinose and reducing sugar accumulation?

Wyse: We do not know the biochemical pathway of raffinose synthesis and degradation in the beet root. What I presented here today is what obviously has to occur based on what we know occurs in other plants. We do not know specifically what enzymes are involved in sugarbeet. Therefore, the temperature effect is not known. But I think we can assume that the

effect of low temperature is to decrease the rate of raffinose degradation more than the rate of synthesis. The opposite being true for warm temperature.

In the case of reducing sugars (in the absence of mold) which are probably being utilized primarily as substrates for respiration the same logic would hold. However, in potatoes the temperature effect on reducing sugar accumulation is actually due to an inhibitor of invertase which fluctuates with temperature.

Barr: Are you talking about reducing sugars per se or are you actually measuring glucose and fructose individually? In the conversion of sucrose to raffinose you end up with one 6 carbon sugar left over. Can you show that you have one of the six carbon sugars in excess?

Wyse: I measure reducing sugars in total using the dinitrosalicylic acid reagent. I do not measure glucose and fructose individually. At low temperature when raffinose is being accumulated, we should theoretically be able to show fructose to be in excess of glucose. I have not done this. The problem you have is that glucose and fructose are easily interconverted in plants so what you measure in an extract may not be indicative of the active pathway.

Barr: Is there any difference between glucose and fructose in the rate of their metabolism to carbon dioxide?

Wyse: Probably not from a practical standpoint, but I do not have any data from sugarbeets to answer your question definitely.

Hobbis: You indicated that rough handling increased respiration rates. Is this due to respiration by the beet or could it mean that rough handling has allowed invasion by bacteria and mold and it is their respiration we are seeing.

Wyse: That is a good point. I believe it is due to root respiration for the following reasons. It is a typical response of plant material to increase in respiration rate when injured. When a sugarbeet is injured the rate increases even when the root is held in ideal storage conditions. In most cases the wound will heal and no mold develops. The other aspect is that you are talking about a root which weighs a kilogram as compared to maybe a gram at most of micro-organisms. If you look up the respiration rate of fungi in the literature, you will find that they would contribute a very insignificant part of the total respiration measured. So I think we are talking about the physiological response of a plant to injury.

Dickenson: When you injure a root and rupture cells how does this increase respiration.

Wyse: There have been experiments done on plants other than sugarbeet showing that wound respiration is due to ethylene production.

For example, if wounded plant material is placed in a vacuum to eliminate ethylene, the wound respiration is greatly reduced.

Dickenson: Is this a response of the plant tissue to try to heal this wound?

Wyse: This phenomenon is not well understood. Obviously to heal the wound the plant must initiate production of suberin and other compounds requiring energy but whether ethylene triggers this useful energy production is not known.

Frakes: When you measure respiration by carbon dioxide evolution, what percentage of that carbon dioxide comes from sugars and what comes from nonsugars?

Wyse: I think we can safely assume that nearly 100% comes from sucrose. There was work done by Wang and Barbour at Oregon and reported in the JASSBT showing this to be true. Using radioactive sucrose he showed that a very high percentage of the carbon dioxide evolved came from sucrose. The primary second source would be amino acids. The protein content does decline during storage as do the amino acids indicating that possibly amino acids are deaminated and utilized as substrates for respiration. But this would be small compared to the total CO_2 evolved.

Hobbis: What effect do freezing and thawing have on ethylene production by the cell and resulting respiration rates? Do you see that in a similar light as the physical injury?

Wyse: If you freeze the root and ice crystals form, cells are broken the result being much the same as hitting it on the surface. So I would expect the response to be much the same.

Bichsel: If ethylene would accumulate down in the pile at sufficient concentrations to accelerate respiration would you not also have an excess of CO_2 which would retard respiration.

Wyse: First in sugarbeet roots carbon dioxide does not significantly affect respiration. Carbon dioxide is a competitive inhibitor of ethylene, so if you had high CO_2 you would automatically reduce the effect of ethylene. In ventilated piles I'm sure that neither would be a problem.

Bichsel: Is there a possibility this build up would occur if the pile were sealed and recycling of air used extensively?

Wyse: I think it's a possibility. The reason we ran the experiment on the effect of oxygen and carbon dioxide was to determine what effect these would have on respiration in covered piles. It appears that the only detrimental effect occurs at warm temperatures.