

Respiration of Sugarbeets Following Harvest in Relation to Temperature, Mechanical Injury and Selected Chemical Treatment¹

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The loss of sugar from sugarbeets during storage is a significant economic factor. Some factors which contribute to this post-harvest loss include dehydration, decay, mechanical injury, sprouting, freezing, respiration and sugar conversions. Although quite variable, sugar losses alone have been estimated (6)³ at approximately 1 pound of sugar per ton of beets per day over the normal processing period, which may be as long as 4 months following harvest. Another estimate (2) places the average loss at more than 40 pounds of sugar per ton of beets piled, excluding processing loss. There is, therefore, great interest within the beet sugar industry to minimize this loss by improving the handling and storage techniques. Accurate assessment of the various factors contributing to the overall loss is of paramount importance in order to determine where improvement should be made, if proven economically feasible.

Knowledge of sugarbeet respiration and factors which influence its magnitude may be of value in developing improved handling and storage techniques. This study was initiated to determine the influence of temperature, mechanical injury and selected chemicals on the respiration rate of sugarbeets.

Materials and Methods

The sugarbeets (variety GW H-1) employed in these studies were grown near Fremont, Ohio. They were harvested on October 16 and November 6, 1967. Those from the first harvest were subjected to post-harvest chemical treatments, mechanical injury and ethylene treatment. Beets from the second harvest were employed for storage temperature and mechanical injury studies. The beets for both studies upon harvest were topped manually (cut just below the crown), and cleaned by a high pressure water spray to remove the adhering soil. The chemical treatment study consisted of 4 lots of approximately 30 beets each, fairly uniform in size, which received a 15-second immersion in one of the following treating solutions: 1) potassium azide - a res-

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³ Numbers in parentheses refer to literature cited.

piratory inhibitor (3 lbs/100 gal water), 2) Merck HZ 3456 - a morphactin⁴ (1 lb/100 gal water), 3) Botran - a fungicide⁵ (1.5 lbs/100 gal water), or 4) water only, which served as the control. Within 6 hours of harvest, six samples of three beets from each treatment lot (sample weight of approximately 3 kg) were weighed and placed in the APRIL⁶ system (4) to monitor the respiration rate in air at 20°C. Each sample was analyzed twice daily over a period of 10 days. In addition to the above treatments, two samples of four non-treated beets were cut in half lengthwise to simulate mechanical injury and opposite halves placed into two respirometer chambers. Two additional samples of three beets each were gassed with 1000 ppm of ethylene in a CO₂ free atmosphere for 12 hours at 20°C prior to commencing respiratory gas analysis.

For the temperature study, 5 replicates of three beets each were placed in respiration chambers at 0, 10, or 20°C, and respiratory analysis was commenced within 6 hours of harvest. In addition, five samples of three beets each were maintained at 0° C for 5 days prior to placement at 20° for respiratory measurements. This was to simulate a change of temperature effect on beet respiration. After 8½ days beets maintained continuously at 0, 10 or 20° C, were momentarily disconnected from the gas analyzing equipment and subjected to mechanical injury. Beets in four of the five chambers at each temperature were sliced into equal halves. All samples were returned to the respiratory chambers and reconnected for gas analysis for an additional 5-day period. Beets in the 5th chamber were not cut. Manipulation of the beets in this manner increased the surface area for gas diffusion and was designed to simulate severe mechanical injury that may occur during the harvesting and handling operation. The respiration data was processed by appropriate programs on the CDC 3600 computer. Best fit equations were obtained by the least squares procedure. Cumulative respiration data was obtained for various durations by integration of these equations. In some cases, the cumulative CO₂ evolution was obtained by approximate integration employing Simpson's modification of the prismoidal formula (8). Carbon dioxide production rates were converted to sugar loss and expressed as sucrose from the fact that 1 ml of CO₂ is derived from 1.274 mg of sucrose, assuming all the CO₂ evolved arose from complete oxidation of sucrose.

⁴ Methyl-2-chloro-9-hydroxy fluorene-9-carboxylate.

⁵ 2,6-Dichloro-4-nitroaniline.

⁶ APRIL - Automated Photosynthesis and Respiration Integrating Laboratory.

Results and Discussion

The influence of various chemicals upon the respiratory rate of harvested sugar beets at 20° C is shown in Figure 1. Little difference was noted in the respiration rate of beets treated with the Botran fungicide or the morphactin compound in comparison to the non-treated beets. Similarity of respiration of control and fungicide treated beets indicates that microbial activity did not

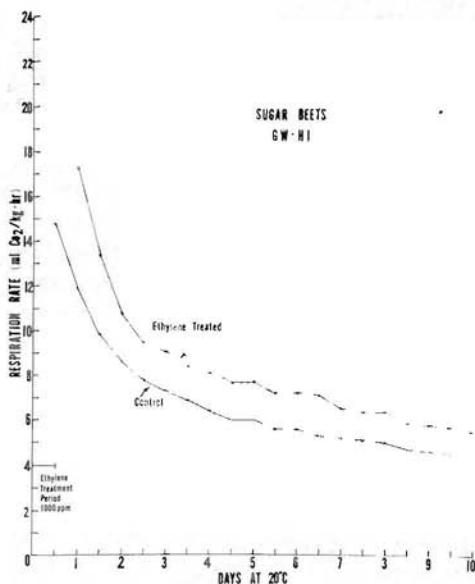


Figure 1.—Influence of post-harvest application of several chemicals on respiration rate of sugarbeets.

contribute significantly to the respiration measurements during the test period. The time-course change in respiration of non-treated beets is typical of results obtained by other investigators (7,10). The respiration rate declined sharply during the first 2 or 3 days following harvest after which a more or less steady respiration rate was observed through the test period of 10 days. The high rate observed for beets treated with potassium azide, a potent respiratory inhibitor, is difficult to interpret. It was presumed that the respiratory rate of potassium azide treated beets would be much lower than the control. However, potassium azide stimulated the respiration rate with the peak rate at approximately 11½ days following treatment after which the rate declined, reaching a steady state value after 5 or 6 days. By the 10th day following treatment the potassium azide treated beets were respiring approximately 60% higher than control beets. Similar data (not shown) were obtained for oxygen consumption

for all samples, thereby showing that the various chemical treatments were of no effect upon respiratory quotient. Had potassium azide poisoned mitochondrial oxygen uptake, the respiratory quotients would have been greater than 1. The pronounced effect of potassium azide on the respiration rate is particularly interesting since the beets had only received a 15-second immersion in the treating solution. Further, it is unlikely that the chemical penetrated much beyond the surface in contact with treating solution. Microbial activity would most certainly be destroyed by potassium azide at the concentration employed and, therefore, cannot be considered as contributing to the measured respiration rates.

Fitted lines of the time-course change of respiration rate measured as O_2 consumption are shown in Figure 2 for the control and the potassium azide treated beets. A 4th degree polynomial describes the data for the potassium azide induced respiratory behavior (see footnote to Table 2). A 3rd degree polynomial adequately described respiration of control beets and those receiving Botran or the morphactin.

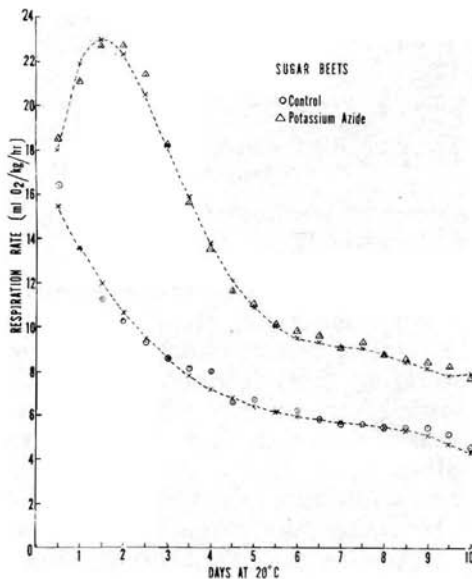


Figure 2.—Influence of potassium azide on respiration of sugarbeets.

The influence of these chemicals on the respiration rate expressed as cumulative respiration is shown in Tables 1 and 2. In Table 1, the respiration rates have been calculated for the 8th

Table 1.—Influence of selected post-harvest treatments on respiratory activity of sugarbeets.

Treatment	Respiration rate (static)*20°C		Respiratory quotient
	CO ₂ produced	O ₂ consumed	
	(ml/Kg/day)		
Control	111	114	0.97
Potassium azide 3 lbs/100 gal	175	193	0.91
Merck HZ 3456 1 lb /100 gal	149	156	0.96
Botran 1.5 lbs/100 gal	127	133	0.95
Sawed (lengthwise)	441	495	0.89
Ethylene (1000 ppm-12 hr)	140	163	0.86

* Based on average respiration rate for the 8th through 10th day following harvest. Beets harvested 10/16/67 at Fremont, Ohio. Variety GW H-1.

Table 2.—Influence of selected post-harvest treatments on respiration of sugarbeets^a.

Treatment	Cumulative respiration		Respiratory quotient
	CO ₂ produced	O ₂ consumed	
	(ml per Kg per 7 days at 20°C)		
Control	1227	1408	0.87
Potassium azide 3 lbs/100 gal	2150	2526	0.85
Merck HZ 3456 1 lb /100 gal	1362	1554	0.88
Botran 1.5 lbs/100 gal	1390	1594	0.87
Sawed (lengthwise)	6942	7638	0.91
Ethylene (1000 ppm-12 hr)	1578	1815	0.87

^a Beets harvested 10/16/67 at Fremont, Ohio. Variety GW H-1.

The following equations describe the respiration of sugarbeets in response to the various treatments as a function of time following harvest and treatment. The time variable (χ) is expressed as the number of 12 hour cycles. Respiration rate (y) is ml of CO₂ or O₂ per hour. These equations apply to Figures 1 through 4.

Control:

$$\begin{aligned} \text{CO}_2 &= 0.00434\chi^3 + 0.1751\chi^2 - 2.374\chi + 16.15 \\ \text{O}_2 &= -0.00361\chi^3 + 0.1544\chi^2 - 2.312\chi + 17.72 \end{aligned}$$

Potassium azide:

$$\begin{aligned} \text{CO}_2 &= 0.000144\chi^5 - 0.00892\chi^4 + 0.2044\chi^3 - 2.0435\chi^2 + 7.2813\chi + 11.28 \\ \text{O}_2 &= 0.000184\chi^5 - 0.01154\chi^4 + 0.26818\chi^3 - 2.7505\chi^2 + 10.468\chi + 10.03 \end{aligned}$$

Botran:

$$\begin{aligned} \text{CO}_2 &= -0.003822\chi^3 + 0.1605\chi^2 - 2.306\chi + 17.26 \\ \text{O}_2 &= -0.003693\chi^3 + 0.1634\chi^2 - 2.523\chi + 19.87 \end{aligned}$$

Morphactin HZ-3456:

$$\begin{aligned} \text{CO}_2 &= 0.003653\chi^3 + 0.1481\chi^2 - 1.982\chi + 15.36 \\ \text{O}_2 &= -0.003244\chi^3 + 0.1385\chi^2 - 2.02\chi + 17.20 \end{aligned}$$

Control + Ethylene:

$$\begin{aligned} \text{CO}_2 &= 0.000815\chi^4 - 0.0423\chi^3 + 0.7861\chi^2 - 6.372\chi + 26.78 \\ \text{O}_2 &= 0.001180\chi^4 - 0.06143\chi^3 + 1.1408\chi^2 - 9.1549\chi + 35.30 \end{aligned}$$

Mechanical injury:

$$\begin{aligned} \text{CO}_2 &= 0.00121\chi^5 - 0.06823\chi^4 + 1.3938\chi^3 - 12.098\chi^2 + 35.40\chi + 46.03 \\ \text{O}_2 &= 0.00129\chi^5 - 0.07263\chi^4 + 1.4783\chi^3 - 12.675\chi^2 + 35.16\chi + 58.99 \end{aligned}$$

through the 10th day following harvest, a period of relative stability of the respiratory activity. In Table 2, the data is cumulative respiration during the first week following harvest.

The beets receiving a 12-hour exposure to ethylene at a concentration of 1000 ppm respired at a consistently higher rate throughout the 10-day period following harvest (Figure 3). The time-course change of the ethylene treated beets exactly paralleled that of the control, indicating that no marked qualitative change

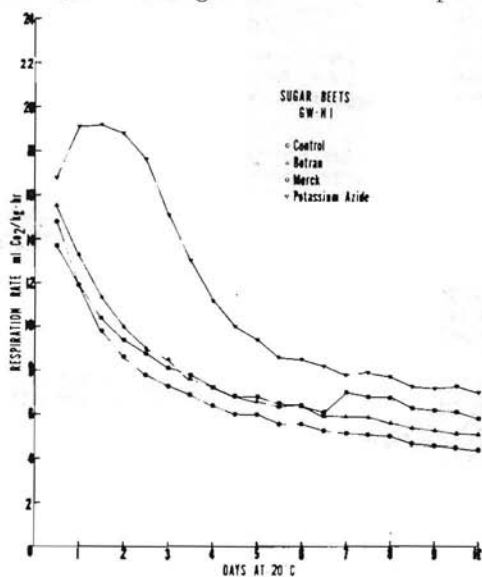


Figure 3.—Effect of ethylene treatment on respiration of sugarbeets as measured by CO_2 evolution.

in metabolism had occurred, but rather the general level of metabolic activity was increased. The effect of ethylene was maintained throughout the course of the experiment although the beets had only received a 12-hour exposure to ethylene. In fruit tissues, ethylene has been observed to induce an increased rate of respiration which is sustained if ripening processes are induced (1). This ethylene treatment was included since ethylene is naturally produced by plant tissues. Furthermore, ethylene may be a product of decay organisms developing on beets during storage. In addition, it has generally been observed that ethylene evolution is stimulated following wounding of a tissue (3). Ethylene did not alter the type of respiration, as the respiratory quotients are the same for ethylene treated as compared to control beets. Cumulative respiration data for ethylene treatment is also given for steady-state respiration, and respiration during the first week following harvest in Tables 1 and 2, respectively.

Mechanical injury caused by cutting the beets lengthwise induced a drastic increase in the respiratory rate of sugarbeets compared to uncut beets as measured by CO_2 production and O_2 consumption (Figure 4). There was no effect or injury on respiratory quotient throughout the time course of the experiment, indicating that respiratory metabolism was not quali-

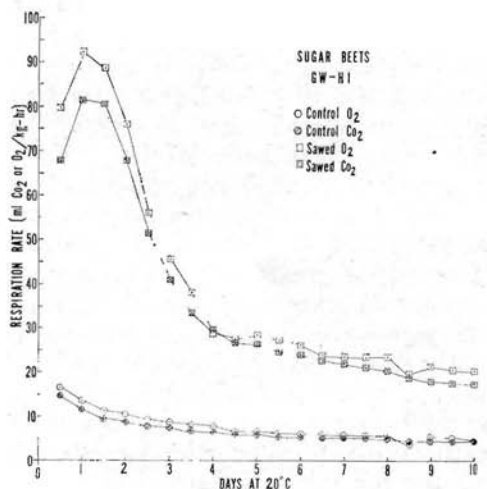


Figure 4.—Influence of mechanical injury on respiration rate of sugarbeets. Beets were cut longitudinally.

tatively altered from that of the control beets. Some of the increase in metabolic activity may be the result of a wound response at the cellular level, but only a small fraction of the total respiring cells were actually injured. The equation that best fits data for the mechanically injured beets is a 4th degree polynomial for both O_2 and CO_2 (see footnote to Table 2). A peak in metabolic activity was reached at approximately 1 day following mechanical injury, after which the respiration declined sharply reaching a steady rate of respiration at approximately 4 days. The steady-state respiration of mechanically injured beets remained from the 4th through the 10th day at a rate approximately 4 times that of the control beets. This respiratory behavior in response to mechanical injury may be explained in terms of a gas diffusion barrier. Cutting the beets exposes more surface area for gas diffusion and, in addition, the newly cut surface allows O_2 to gain entrance into the tissue very readily. Microbial development cannot be ruled out as a factor contributing to the high respiration rate of cut beets since no provision was taken to disinfect the beets. However, as noted earlier, beets dipped in Botran after topping and washing respired at the same

rate as control beets. Furthermore, potassium azide treatment would essentially surface sterilize the cut surface of the beets ruling out microbial activity as contributing to the respiration rate. In fact, potassium azide stimulated respiratory gas exchange as noted earlier. The rapid decline between the 1st and the 4th day following cutting may result from dehydration of the cut surface and creation of a less permeable diffusion barrier. It is not known if wound healing due to periderm formation was responsible for the decrease in respiration rates during this period. From the 4th through the 10th day following cutting, respiration declined slowly, but paralleled that of control beets. The fact that the respiratory rate of cut beets leveled out at a much higher value than control beets is probably explained in that the diffusion barrier at the cut surface, continued to be more permeable than the uncut surface of the control. In addition, the surface area of the cut beets was greater. The magnitude of respiratory activity of cut vs control beets is clearly seen in the data of Tables 1 and 2. The cumulative respiration calculated for the 8th through the 10th day following harvest for the cut beets is nearly four times that of control beets. When calculated for the first 7 days following harvest the difference is even greater in view of the markedly stimulated respiration during the first 4 days following the mechanical injury.

The influence of temperature on respiratory rate of sugarbeets is shown in Figure 5. The time-course change of respiration at 20°C for beets in this experiment paralleled, but was slightly higher than that obtained in the earlier experiment. At 10°C, however, the respiration declined more quickly following harvest and by the 5th day had reached steady-state values. At 0°C the respiration remained low and quite stable for a period of approximately 5 days, after which the rate declined and reached a steady-state value by the 8th day. An interesting aspect of this temperature data is seen in comparing 0 with 10°C respiration. Note that between the 4th and the 6th day following harvest the 10°C respiration is only slightly higher than that at 0°C. At the 5th day the temperature coefficient (Q_{10}) is approximately 1.1 between 0 and 10°C and 2.35 between 10 and 20°C. At 1½ days following harvest the temperature coefficient between 0 and 10°C was 2.64 and between 10 and 20°C was 1.3. By the 8th day steady respiration rate was apparent for beets at 0, 10, or 20°C and the temperature coefficient between 0 and 10°C and between 10 and 20°C was 1.41 and 1.46, respectively. One would expect that the temperature coefficient would remain approximately constant through this 8-day period providing metabolic activity was qualitatively the same. Since it did not, some inter-

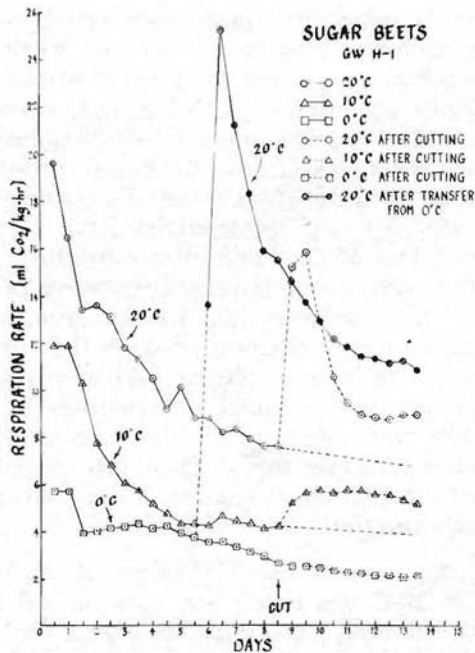


Figure 5.—Influence of temperature, change in temperature, and mechanical injury on respiration rate of sugarbeets as measured by CO₂ evolution.

mediary metabolite may have accumulated at 10°C that acted as a restraint on metabolism. Alternatively, conversion of oligosaccharides to readily metabolizable monosaccharides may account for the relatively high respiration rate at 0°C, since sugars accumulate in beets as in other storage organs at low temperatures (5). Further evidence is the respiratory response upon transfer from 0 to 20°C. Beets maintained for 5½ days at 0°C and then transferred to 20°C exhibit markedly stimulated respiratory activity in comparison to those beets not exposed to the low temperature. Accumulation of readily metabolizable carbohydrates such as reducing sugars at the low temperature may be responsible for this increased respiration rate at the higher temperature. The decline in respiration at 20°C parallels, but remains at a higher level for beets receiving a low temperature treatment in comparison to those not.

Respiration data for mechanically injured beets at 0, 10 and 20°C are also given in Figure 5. The respiratory response to mechanical injury at these three temperatures is further evidence that gas diffusion may be a limiting factor to the metabolic activity of sugarbeets. The metabolic demands for oxygen at

low temperature is sufficiently low, however, that gas diffusion is not a limiting factor in respiration. Furthermore, at the lower temperature, a greater quantity of oxygen would be dissolved in the tissue fluids. Therefore, as observed, beets cut at 0°C should have a respiration rate similar to intact beets. At 10°C, however, the cut beets exhibited a higher respiration rate than uncut beets, and at 20°C the effect was still greater. This response is what one would expect if the surface of the beet were acting as a barrier to entrance of O₂. The intercellular air space volume of beets and other root crops is very low, being of the order of 0.5 to 2% of the total volume (9). In tissues with a low intercellular air space volume, diffusion of gases into or out of these tissues is limited. The bulk of the gas diffusion must take place in solution and the rate is much slower than in a gas phase. Microbial activity may contribute to the respiration rates obtained in the temperature study since no special precautions were taken to retard their development. No visual signs of fungal or bacterial contamination were evident.

It is interesting to note that the effect of mechanical injury on respiration at 20°C was much less pronounced for beets harvested November 6 than for beets harvested October 16. For the early harvest of October 16, the respiration rate of mechanically injured beets reached a value of approximately 90 ml of CO₂/kg/hr on the 1st day; whereas control beets at the same time had a respiration rate of approximately 12 ml/kg/hr. Mechanically injured beets for the second harvest (November 6, reached peak values of approximately 16 ml/CO₂/kg/hr vs control values of approximately 8 ml within 1 day following mechanical injury, then declined rapidly. Steady-state respiration values were apparent in 3 days while those of beets harvested October 16 required 6 days. One marked difference between these two experiments was, however, that the beets of the second harvest were held for 8½ days prior to mechanical injury. The respiration rates of control beets at 20°C for both the first and second harvest were similar (compare 20°C respiration rate in Figures 2 and 5) during the first 8½ days. In fact, the effect of change in temperature from 0 to 20°C for beets of the second harvest induced a more marked respiratory response than cutting.

The marked difference in respiratory response to mechanical injury of beets at these two harvests may indicate that gas diffusion becomes less of a limiting factor on respiration rate as the harvest season progresses. This may be a result of an increase with growth in the amount of intercellular air space which markedly increases gas diffusion in bulky storage organs. Greater availability of readily metabolizable substrates in beets at a less

mature state in development may be responsible for the marked stimulation of respiration that is observed upon cutting. Further studies are needed to establish the factors responsible for the respiratory response to mechanical injury.

The similarity in the time-course change of respiration of mechanically injured beets to beets receiving a post-harvest treatment with potassium azide suggests an indirect effect of potassium azide, perhaps upon the permeability characteristics of the beets. The respiratory curves in both instances are described by similar 4th degree polynomials. Furthermore, the time required to reach the respiratory peak and to establish steady-state respiration are similar, differing in magnitude only. If microbial activity was responsible for the high respiration rate of mechanically injured beets, it is highly unlikely that the respiratory kinetics would so closely parallel that of the azide treated beets which should be quite free of microbial activity. As mentioned previously, it is doubtful that the potassium azide would affect the bulk of the cells beyond the surface.

The effect of temperature on respiration rate of sugarbeets at the various temperatures employed is summarized as cumulative respiration in Tables 3 and 4 for steady-state respiration and respiration during the first 7 days, respectively. In Table 3 it is evident that temperatures much below 10°C are not greatly beneficial in reducing the respiration rate; whereas, a temperature change between 10 and 20°C is of considerable influence. This

Table 3.—Influence of temperature on respiration rate of sugarbeets following harvest.

Temperature (°C)	Respiration rate (static)*		Respiratory quotient
	CO ₂ produced	O ₂ consumed	
	(ml per Kgm per day)		
0	74	76	0.97
10	103	117	0.88
20	178	188	0.95

* Based on cumulative respiration for the 6th through the 8th day at the various temperatures following harvest. Harvest made on 11/6/67 at Fremont, Ohio. Variety GW H-1.

Table 4.—Influence of temperature on respiration rate of sugarbeets during first week following harvest*.

Temperature (°C)	Cumulative respiration		Respiratory quotient
	CO ₂ produced	O ₂ consumed	
	(ml per Kgm per 7 days)		
0	684	683	1.00
10	1054	1099	0.96
20	1896	1980	0.96

* Beets harvested 10/16/67 at Fremont, Ohio.

may have bearing on the practicality or design of low temperature storage facilities for sugarbeets since it would appear that provisions to maintain temperatures much below 10°C are not necessary. Maintenance of temperatures near 0°C, which is difficult since beets are stored out-of-doors and are, therefore, exposed to widely varying temperatures, does not appear to be warranted from this short-term storage temperature and respiration data.

The influence of various treatments calculated as sugar loss is given in Tables 5 and 6 for the steady-state respiration period and respiration during the first week following harvest, respectively. The magnitude of sugar loss, particularly with mechanically injured beets is rather striking with respect to the initial sugar content. An industry estimate of 1 lb of sugar lost per ton of beets per day from all sources during the storage season may be conservative. At 20°C (Table 5) beets may lose ¼ lb. of sucrose per ton per day from respiration alone. Beets, however, are not stored at this high a temperature during the bulk of the storage period. Rather, temperatures are generally below 10°C

Table 5.—Influence of selected post-harvest treatments on loss of sugar from sugarbeets.

Treatment	Daily sugar loss at 20°C*	
	gms of sucrose	pounds of sucrose
	kgm of beets per day	tons of beets per day
Control	0.141	0.282
Potassium azide 3.0 lbs/ 100 gal	0.223	0.446
Merck HZ 3456 1.0 lbs/ 100 gal	0.190	0.380
Botran 1.5 lbs/ 100 gal	0.162	0.324
Sawed (lengthwise)	0.562	1.124
Ethylene (1000 ppm-12 hr)	0.178	0.356

* Based on average daily CO₂ production from the 8th through the 10th day following harvest.

Table 6.—Influence of selected post-harvest treatments on loss of sugar from sugarbeets.

Treatment	Sugar loss (first week)*	
	gms of sucrose	pounds of sucrose
	kgm of beets	ton of beets
Control	1.563	3.126
Potassium azide 3 lbs/100 gal	2.739	5.478
Merck HZ 3456 1 lb/100 gal	1.735	3.470
Botran 1.5 lbs/100 gal	1.770	3.540
Sawed (lengthwise)	8.844	17.688
Ethylene (1000 ppm-12 hr)	2.010	4.020

* Based on cumulative CO₂ production during first week following harvest at 20°C.

through much of the storage season. However, warm harvest period temperatures of 20°C or higher are encountered. Furthermore, temperatures greater than 20°C are possible within the beet piles due to the heat of respiration.

Mechanical injury undoubtedly is a significant factor contributing to respiratory loss of sugar from sugarbeets. The damage to beets in the harvesting and handling operations may be extensive. The increase in surface area of beets as a result of mechanical injury (breakage, cracking, topping or chipping) markedly increases the respiration rate as seen in Figures 3 and 5, and in the sugar loss data of Tables 5 and 6. Possibly, suitable artificial diffusion barriers such as waxes or synthetic materials could be utilized to reduce gas diffusion, and thereby partial restriction of the respiration rate without causing fermentation, and, therefore, be of value in reducing respiratory losses.

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