Evaluation of Effects of Controlled Atmosphere Storage on Roots of Sugarbeets Grown at Various Levels of Nitrogen Fertilizer[1]

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Introduction

Nitrogen in combined form is one of the most important elements for the growth and development of sugarbeet plants. There has been a substantial increase in the rate of application of nitrogen fertilizers on sugarbeets during the last few decades. Associated with increased yields, which follow the excess use of nitrogen fertilizers, there has been decline in sugarbeet quality (7,19,21,23,24). Nitrate is the principal source of nitrogen utilized by sugarbeets. Nitrate uptake and metabolism require energy derived at the expense of sugar accumulation (13,21). The main source of reducing power in intact plants are the respiratory breakdown of carbohydrates and the photolysis of water in the chloroplasts. The ultimate H-donors in the initial step and the subsequent steps of nitrate reduction are the reduced pyridine nucleotides. This would be true whether nitrate reduction was coupled to respiration or photolysis of water. The ammonia formed is utilized in the synthesis of amino acids, proteins, betaine, and other nitrogenous compounds at the cost of sucrose synthesis (13). These impurities adversely affect the thin juice purity (17).

The studies by Dexter *et al.* (7) revealed the fact that high rate of nitrogen fertilizers not only affect the quality of the roots of sugarbeets at the time of harvest but also during the storage. These workers pointed out that high-level fertilizer beets deteriorated more quickly at warmer temperatures than those fertilized with medium or low-level nitrogen. The high nitrogen beets also accumulated more soluble impurities of an undetermined nature (7).

Since our earlier work indicated more retention of sucrose and improvement in the quality of sugarbeets stored under controlled atmosphere (CA), the present study was conducted to

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evaluate the effects of CA on the beets grown at different levels of nitrogen fertilizers. Sugarbeets were grown with three levels of nitrogen fertilizer. Control plots were left free of added fertilizer as initial total nitrogen content to soil was comprised of 2400 lbs. of total N per acre in the top one foot of soil. The other two plots were treated with 150, and 300 pounds per acre ammonium nitrate fertilizer. The beets grown under these conditions were stored under conventional refrigeration (CR) and under optimum controlled atmosphere (6% CO_2 and 5% O_2) at 40°F. In addition to the physio-chemical studies conducted earlier (11), changes in the total nitrogen, amino nitrogen, and organic acids were followed during storage.

Experimental

Sugarbeet (Utah-Idaho Number 7, an F_1 hybrid) roots used in these studies were grown on the experimental farms of Utah State University. Plots were treated with O, 150, and 300 pounds of added nitrogen fertilizer per acre in a randomized block design. The beets were harvested and sorted as described by Dexter and Frakes (6). The three groups were kept separate and 10 uniform beets from each group were sacked into two replications for storage. The conventional refrigeration (CR) beets were stored in air at 40°F, and those under controlled atmosphere (CA) were stored under 6% carbon dioxide and 5% oxygen at the same temperature.

Each sample consisting of 10 beets was analyzed immediately after storage intervals of 65, 130, and 200 days to observe the physical changes and changes occurring in respiration, sucrose, reducing sugars, raffinose total nitrogen, amino nitrogen, and organic acids.

Respiration. Respiration was measured on the whole beets as described by Claypool and Keefer (3). Beets were brought to an equilibrium with normal air at 40°F which required a period of 12 hours. The respiration was measured for one hour after equilibrium period.

Chemical analyses. Sucrose, reducing sugars, and total nitrogen were determined by A.O.A.C. (1) methods. Raffinose was determined enzymatically (2). Amino nitrogen was determined by using a modified Stanek-Pavlas reagent (20). Organic acids were determined as described by Humle and Wooltorton (10) with the following modifications.

An alcohol extract of sugarbeets was passed through cation exchange column, Dowex 50 W-X8, form, 200-400 mesh, to remove amino acids, proteins and other alcohol soluble materials. The eluent was then passed through an anion exchange column, Dowex 1-X8, Cl form, 400 mesh, which had previously been converted to the acetate form. The organic acids were fractionated using eluting solvents by gradient elution method. The fractions were titrated against 0.01 N NaOH using phenolphthalein as an indicator. Part of the acid fractions were identified by passing known organic acids and observing the fraction number of a peak against those of unknowns. The acid fractions were further identified by thin layer chromatography. Plates were sprayed for identification of spots with bromocresol green, a pH indicator. The spots were identified by their R_f values. R_f values of citric acid and malic acid were close, therefore, citric acid was confirmed by the method of Saffran and Densted (18).

The number of beets showing sprouting in each sample was recorded and microbial growth characteristics were studied as described by Kubica and Dye (12). Analysis of variance was performed and means were compared (4).

Results and Discussion

Respiration. The respiration rate at harvest averaged 20 mg CO_2/kg beet/hour (Table 1). Respiration decreased gradually

Table 1.—Effect of fertilizer, storage treatment, and duration on respiration rate of sugarbeets at 40°F expressed as mg CO_2/kg/hour.

Fertilizer[a] added (Pounds N/acre)	Storage treatment	Days in storage			
		At harvest	65	130	200
None	CR	19.2	12.3	10.8	12.2[a,b]
	CA	19.2	11.9	8.6	9.4
150	CR	20.4	11.4	12.2	13.4[a]
	CA	20.4	9.3	9.6	10.8
300	CR	19.8	10.5	9.7	14.5[a]
	CA	19.8	9.9	8.6	10.4

[a] Main effects of fertilizer nonsignificant.
[b] Storage treatments significant at 0.05 level.

and remained at a steady level until 130 days of storage and increased again at the end of 200 days. Regardless of the fertilizer level, beets stored in CA had a lower rate of respiration than the CR beets. At the end of 200 days of storage, 23, 20, and 28% decrease in respiration rate was observed in CA with 0, 150, and 300 pounds of added fertilizer beets respectively. The rate of respiration seems to be in direct correlation with sucrose retention data (Table 2). The increased respiration rate at the end of 200 days of both CR and CA-stored beets may be attributed to the additional respiration of the heavy growth of fungi present on the sugarbeets at this time of storage.

Sucrose. Sucrose content at harvest differed with varying levels of nitrogen fertilizer. Beets grown without added fertilizer (hereafter referred to as 0 level) contained 13.2% sucrose. Those grown with 150 and 300 pounds of added fertilizer contained

Table 2.—Effect of nitrogen fertilizer, storage treatments, and duration on percent sucrose retention, reducing sugars, and raffinose content in sugarbeets at 40°F.

Fertilizer added (Pounds N/acre)	Storage treatment	Sucrose retention (%) Days in storage				Reducing sugars (mg/100gm) Days in storage				Raffinose (mg/100gm) Days in storage			
		0	65	130	200	0	65	130	200	0	65	130	200
None	CR	100[a]	97.7	94.7	80.3*[b]	95	281	347	419	31	92	117	124
	CA	100	98.5	97.9	86.2	95	223	282	374	31	86	94	114
150	CR	100	96.1	88.2*	78.7*	110	260	360	445	34	76	97	118
	CA	100	95.3	93.7	84.3	110	186	302	413	34	79	95	108
300	CR	100	96.6	91.5	78.6	123	259	410	511	31	88	104	123
	CA	100	99.1	95.7	82.1	123	288	330	427	31	78	101	117

[a] Initial sucrose content of 13.2, 12.7, and 11, respectively, with 0, 150, and 300 pounds N per acre taken as 100%.
[b] Storage treatment significant at 0.05 level.
CR = Conventional refrigeration.
CA = Controlled atmosphere.

12.7 and 11.7% sucrose respectively. At the end of 200 days of storage, beets grown with 0 level nitrogen retained 86.2% sucrose under CA compared to 80.3% under CR (Table 2). This amounted to 23% more sucrose retention under CA than under CR. Likewise, beets grown with 150 and 300 pounds added nitrogen retained 26% and 16% more sucrose respectively, under CA. These results suggest that CA storage inhibits the metabolic breakdown of sucrose regardless of nitrogen fertilizer levels.

Reducing sugars. The initial reducing sugar content of beets grown with 0 level nitrogen was 95 mg/100 gms. The reducing sugar content was increased to 110, and 123 mg/100 gms, for the 150 and 300 pound added nitrogen treatments respectively. This indicated that beyond certain levels of nitrogen fertilizer beets accumulate reducing sugars which are undesirable impurities. Reducing sugars increased during storage. However, the reducing sugars were lower in CA storage compared to CR regardless of nitrogen fertilizer level or length of storage (Table 2). The increase in reducing sugars, at the end of storage, was 10.7, 7.2, and 16% less respectively under CA than in CR with increasing levels of nitrogen fertilizer.

Raffinose. At harvest the raffinose content did not vary significantly with increasing levels of nitrogen. Initial concentration ranged from 31 to 34 mg/100 gms of beets as shown in Table 2. As the storage period lengthened the raffinose content increased. However, beets stored under CA accumulated less raffinose than those stored under CR. The decrease in raffinose accumulation under CA, compared to CR, at the end of 200 days of storage, average 8.0, 8.5, and 4.9% for 0, 150, and 300 pounds nitrogen treatments, respectively.

Total nitrogen. At harvest, the total nitrogen content of the beets varied from 0.177% to 0.219% with increasing levels of fertilizer (Table 3). Added fertilizer demonstrated an adverse effect in accumulating nitrogenous impurities, expressed

Table 3.—Effect of fertilizer, storage treatments, and duration on percent total nitrogen and ppm amino nitrogen content in sugarbeets at 40° F.

Fertilizer[a] added (Pounds N/acre)	Storage treatment	Total N (%) Days in storage				Amino N (ppm) Days in storage			
		0	65	130	200	0	65	130	200
None	CR	0.177	0.191	0.194	0.195	500	550	495*[b]	410
	CA	0.177	0.197	0.183	0.182	500	490	390	365
150	CR	0.206	0.210	0.210	0.206	650	595	605	535
	CA	0.206	0.194	0.212	0.207	650	560	580	510
300	CR	0.219	0.221	0.227	0.220	745	755	700	675
	CA	0.219	0.218	0.219	0.219	745	720	645	640

[a] Main effects of fertilizer significant at 0.01 level.
[b] Storage treatments significant at 0.05 level.

as total nitrogen. During storage under CR and CA, no significant change in total nitrogen content was observed.

Amino nitrogen. Amino nitrogen, at harvest, increased in concentration with increasing levels of nitrogen fertilizer (Table 3). The amino nitrogen concentration decreased gradually with time both under CR and CA storage. At the end of 200 days, 9.0, 3.8, and 4.7% less amino nitrogen under CA was observed. It may be hypothesized that either degradation of proteins or amino acid transformation is inhibited under CA.

Organic acids. Four major acid fractions indentified include citric acid, malic acid, oxalic acid, and succinic acid. Other fractions observed in trace quantities were not identified. Table 4 shows that the citric acid concentration was reduced after

Table 4.—Effect of fertilizer, storage treatment, and duration on organic acid content in sugarbeets at 40° F expressed as mg/100 gms.

Fertilizer[a] added (Pounds N/acre)	Identified fractions	Days in storage		
		At harvest	200 CR	200 CA
None	Citric acid	20.3	19.0	24.0[a,b]
150		23.2	18.2	25.1
300		22.6	22.0	28.0
None	Malic acid	4.6	2.7	2.8
150		4.3	2.5	3.5
300		5.0	4.3	4.4
None	Oxalic acid	6.8	5.8	6.3
150		7.4	4.5	5.8
300		6.3	7.2	5.6
None	Succinic acid	2.9	2.5	5.9[a]
150		2.9	2.4	4.2
300		3.4	3.4	4.2

[a] Main effects of fertilizer nonsignificant.
[b] Storage treatment significant at 0.05 level.

200 days in storage under CR while it increased in the beets stored under CA. A similar trend was observed with succinic acid. There was no significant difference in the concentration of malic acid and oxalic acid under either CR or CA storage. Accumulation of succinic acid in storage has been reported due to inhibitory effect of CO_2 on dehydrogenases (14,15). Ranson *et al.*, (16) demonstrated that with increasing levels of carbon dioxide, besides succinic acid, accumulation of pyruvic acid occurs. Their data suggested that in high concentration of CO_2 some enzymes involved in the production of citric acid from pyruvic acid were markedly affected, in addition to succinic dehydrogenase. The data with sugarbeets under CA indicate that besides succinic acid, citric acid also accumulates. Accumulation of succinic acid may be due to blocking of succinic dehydrogenase. Accumulation of citric acid may be due to an inhibition of aconitase. In-

creased concentration of succinic and citric acid may also be attributed to an increased rate of formation through the dark fixation of CO_2 as follows:

Pyruvic acid + $CO_2 \rightleftharpoons$ Oxalacetic acid.

Oxalacetic acid in turn forms citric acid (5).

The above data can be correlated with sucrose and respiration data presented in Figures 1, 2 and 3. Sucrose retention

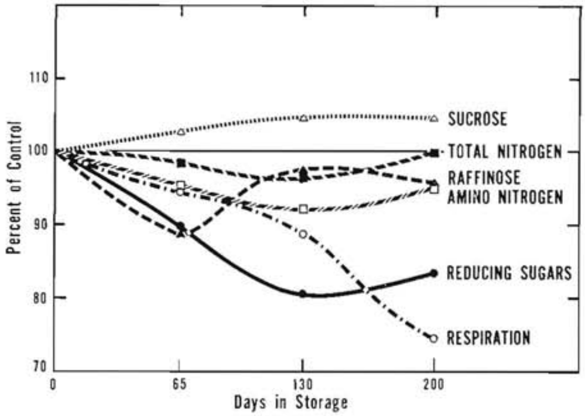

Figure 1.—Effects of controlled atmosphere storage on sucrose, reducing sugars, raffinose, amino nitrogen, total nitrogen, and respiration of sugarbeets grown at zero level of nitrogen fertilizer.

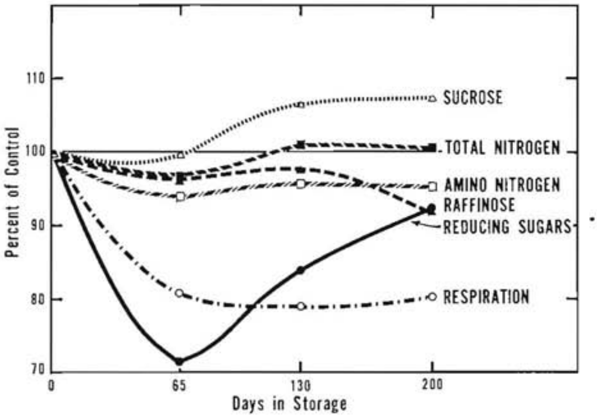

Figure 2.—Effects of controlled atmosphere storage on sucrose, reducing sugars, raffinose, amino nitrogen, total nitrogen, and respiration of sugarbeets grown with 150 lb/A of nitrogen fertilizer.

and inhibition of respiration appear to be directly associated. It may be stated that with the inhibition of respiration more

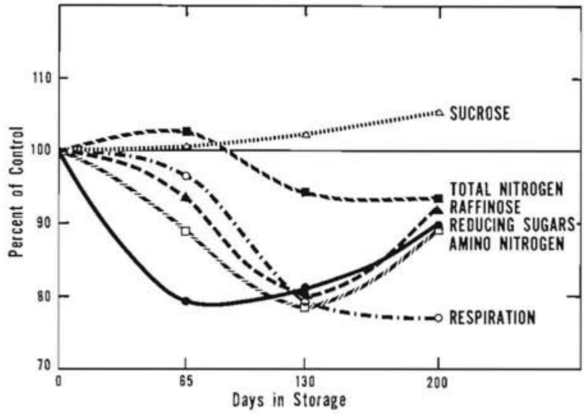

Figure 3.—Effects of controlled atmosphere storage on sucrose, reducing sugars, raffinose, amino nitrogen, total nitrogen and respiration of sugarbeets grown with 300 lb/A of nitrogen fertilizer.

sucrose is retained. Accumulation of acids may be due to an inhibition of sucrose decomposition. This is supported by the amino nitrogen data. Joy (13) indicated that the sugarbeet root is a primary organ of glutamic acid synthesis. The transaminase reactions play a role in roots to synthesize other amino acids needed for protein synthesis from glutamic acid. It mav be possible then that in CA this mechanism is inhibited.

Sprouting and microbial growth. Sprouting characteristics and fungal growth pattern are reported in Tables 5 and 6. Beets stored under CA, irrespective of the fertilizer level, demonstrated inhibited sprouting and fungal growth. Until the end of 130 days of storage, no significant difference in microbial flora was observed with different levels of fertilizer treated beets. At the end of storage, however, beets grown with 300 pounds nitrogen fertilizer demonstrated profuse microbial growth in CR and CA-stored beets. Isolated species included, *Penicillium, Aspergillus, Rhizopus,* and *Fusarium.* Soft rot causing organisms of the *Erwinia* species were observed in CR and CA stored beets.

Table 5.—Effect of fertilizer, storage treatment, and duration on number of sugarbeets per 20-beet sample showing sprouting at 40° F.

Fertilizer added (Pounds N/acre)	Storage treatment	Days in storage			
		At harvest	65	130	200
None	CR	0	4	7	14
	CA	0	1	3	5
150	CR	0	4	10	15
	CA	0	0	1	6
300	CR	0	4	10	15
	CA	0	0	4	7

Table 6.—Effect of fertilizer, storage treatment, and duration on mold growth of sugarbeets at 40° F.[a]

Fertilizer added (Pounds N/acre)	Storage treatment	Days in storage			
		At harvest	65	130	200
None	CR	—	+	+++	++++
	CA	—	—	++	+++
150	CR	—	+	+++	++++
	CA	—	—	+	+++
300	CR	—	+	+++	++++
	CA	—	—	+	++++

[a] Growth characteristics as defined by Kubica and Dye (12).
++++ Confluent growth (more than 500 colonies).
+++ Almost confluent (200-500 colonies).
++ 100-200 colonies.
\+ 50-100 colonies.
— No growth.

Summary and Conclusion

Beneficial effects of controlled atmosphere storage were observed in beets grown with different levels of nitrogen fertilizer. Respiration rate was significantly reduced with beets stored under CA. Sucrose retention was higher with beets stored under controlled atmosphere irrespective of the level of fertilizer added. Likewise, less accumulation of reducing sugars, raffinose, and amino nitrogen was observed with beets stored under CA. Total nitrogen content did not change in any treatment during storage. Accumulation of citric acid and succinic acid was observed with beets stored under CA. Fungal growth and sprouting were significantly inhibited under CA.

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