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Selected Physio-Chemical, Microbiological, and Agronomical Studies on the Controlled Atmosphere Storage of Sugarbeet (Beta Vulgaris) Roots

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SELECTED PHYSIO-CHEMICAL, MICROBIOLOGICAL, AND AGRONOMICAL STUDIES ON THE CONTROLLED ATMOSPHERE STORAGE OF SUGARBEET (BETA VULGARIS) ROOTS

by

Vinod V. Karnik

A dissertation submitted in partial fulfillment of
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of

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Vinod V. Karnik

Vinod V. Karnik

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

ABSTRACT

Selected Physio-Chemical, Microbiological, and Agronomical Studies on the Controlled Atmosphere Storage of Sugarbeet (Beta vulgaris) Roots

by

Vinod V. Karnik, Doctor of Philosophy

Utah State University, 1970

Major Professor: Dr. D. K. Salunkhe
Department: Food Science and Technology

The post-harvest physiology of sugarbeet (Beta vulgaris) roots was studied during controlled atmosphere (CA) storage at 35° and 50°F. Zero, 3, 6 and 10% carbon dioxide and 5% oxygen concentrations were employed to investigate the most beneficial concentrations of gases. Under the experimental conditions beets were stored successfully for 200 days. The maximum beneficial effects of CA were observed under 6% carbon dioxide and 5% oxygen at 35°F. Regardless of storage temperatures, sucrose retention was highest in the beets stored under CA, compared to conventional refrigeration (CR). Other beneficial effects include less hydrolysis of sucrose to reducing sugars and a decrease in raffinose accumulation. Fungal growth and sprouting were also inhibited significantly, under CA.

In the second phase of the studies, investigations were conducted on sugarbeets to study the effects of different levels of nitrogen fertilizer on the

optimum CA storage at 40°F. Regardless of the level of nitrogen fertilization, the beets stored under CA demonstrated beneficial effects as described earlier. In addition, respiration, measured on the whole beets, and amino nitrogen content of the beets were lower in the CA-stored beets than those stored under CR. Accumulation of citric acid and succinic acid was significant in the CA-stored beets.

(74 pages)

PART 1

PHYSIO-CHEMICAL AND MICROBIOLOGICAL STUDIES

INTRODUCTION

More than $30,000,000 are lost annually in the United States and Canada because of sucrose losses and spoilage during the storage of sugarbeets after harvest (Rush, 1968). In most beet growing areas, short harvest periods and limited capacities of processing plants necessitate long storage of sugarbeets. Therefore, the improvement of storage conditions is important to the sugarbeet industry.

The harvested beet is composed of living tissues that must remain alive to resist spoilage. During storage, respiration continues and sucrose, the reserved substrate, is metabolized to carbon dioxide and water with the liberation of heat. The heat of respiration given off by stored beets stimulates undesirable biochemical transformations of sucrose. Besides respiration, sprouting and microbiological losses are also significant. The losses vary considerably with temperature, but the average total loss amounts to about 1/2 pound of sugar per ton of beets per day (Stout, 1957). Attempts have been made since the beginning of the last century to minimize these losses by using forced ventilation, lime treatment, modified atmosphere (Wu et al., 1970) and several other methods, with varying degrees of success.

In the past decade, controlled atmosphere (CA) storage in conjunction with reduced temperatures and controlled humidity has gained a wide acceptance with many horticultural commodities. The CA storage involves maintaining

desired concentrations of carbon dioxide and oxygen in a gas-tight or flow-through system. Kidd and West (1930) in England were among the first to realize the potential of storage under certain gases. Subsequent to their work, CA storage was popularized in the United States through the work of Brooks, Bratley, and McColloch (1936). The effect of different concentrations of these gases and the effect of various temperatures on germination and growth of certain fungi also was observed (Brown, 1922, Littlefield et al., 1966). The desirability of testing each commodity in various atmospheres was recognized, as in certain cases improper concentrations of gases caused poor texture, tissue breakdown, bicarbonate taste, and/or brown rot (Smock, 1958). These disorders made suitable substrates for microorganisms to grow profusely. The best concentrations for one product may be unsatisfactory for another product. Once an appropriate concentration is established, however, CA storage has a number of advantages which include retardation of respiration, inhibition of microbial growth and sprouting, and retardation of general metabolism. The CA-stored fruit stays in better condition than fruit stored in air at the same temperature, even after it is taken out of controlled atmosphere because of residual effect of CA in the commodity.

Considerable literature is published on the effects of controlled atmosphere storage on fruits and vegetables (Kidd and West, 1930; Hulme, 1951; Young, Romani, and Biale, 1962) however, meager information is available on CA-storage studies with sugarbeets. The present study is concerned with the effects of different concentrations of carbon dioxide and oxygen on sucrose,

reducing sugars, raffinose, titratable acidity, respiration, microbial growth, sprouting characteristics, and other physio-chemical changes occurring in sugarbeet roots during storage at 35^{o} and 50^{o}F.

EXPERIMENTAL

Fall 1967 Experiments

Initial experiments with CA were conducted from October 26, 1967, until the end of April, 1968, mainly to standardize the methodology of CA system and to get acquainted with sugarbeets and their storage performance. The beets were stored at 35° and 50°F with the intention of maintaining atmospheres of 0, 3, 6 and 10 per cent carbon dioxide and 5 per cent oxygen. However, concentrations of CO_2 and O_2 did not remain constant as desired mainly because of unfamiliarity of operating generators to produce and maintain desired levels of gases. During this period analytical techniques were also standardized. No conclusive data were obtained with these experiments due to several modifications made during the course of investigations. It was noted, however, that beets varied considerably within the population and stratification of the population for uniform sampling was needed.

Summer 1968 Experiments

Pilot experiments were conducted in summer 1968 with beets secured from Bakersfield, California, to confirm the efficiency of the generators and the system to produce the desired levels of gases, to improve the sampling technique, and to minimize the problems which would otherwise be encountered in the experiments planned for fall, 1968. A small number of beets were stored

under CA for 60 days and no attempts were made to perform extensive analyses.

Fall 1968 Experiments

Material and storage treatments

Cultivar Utah-Idaho Number 7, an F_1 hybrid, was selected for these investigations. Sugarbeets were grown on experimental farms of Utah State University. The roots were harvested by hand to avoid injuries, washed, and sorted according to their specific gravity to minimize variation in their sucrose content and their physical and chemical make up (Dexter and Frakes, 1966). Ten beets per sample were selected on the basis of uniformity of size, shape, and specific gravity for each treatment and for three storage periods of 45, 90, and 165 days. Beets were placed in mesh sacks, weighed and then stored in 55-gallon barrels under conventional refrigeration (CR) and controlled atmosphere (CA) at 35° and 50°F. A Tectrol generator, manufactured by Whirlpool Corporation, St. Joseph, Michigan, was set to produce basic atmospheres of 3 per cent carbon dioxide and 5 per cent oxygen (Figures 1 and 2). Zero per cent CO_2 treatment was produced by passing the gas mixture through a bottle containing saturated solution of potassium hydroxide. Six and 10% carbon dioxide concentrations were produced by adding excess CO_2 to the system from cylinders. The flow of atmospheres through the sealed barrels was regulated by employing flow meters and pressure regulators, as shown in Figure 3. The flow of gas was maintained at the rate of 105 cc of gas

Figure 1. Tectrol generator.

Figure 2. Fyrite gas analyzer for measuring the output concentration of gases from Tectrol generator.

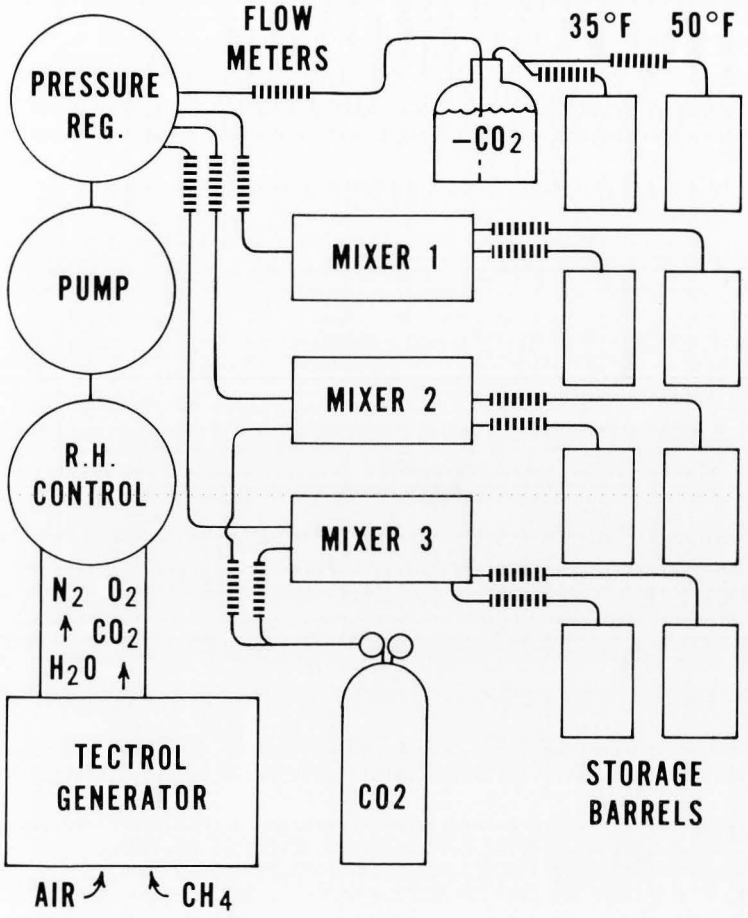

Figure 3. Schematic diagram of controlled atmosphere storage facilities.

Figure 4. Arcat-Arcosorb generator.

Figure 5. Routine sampling of gases in the storage barrels by Fyrite gas analyzers.

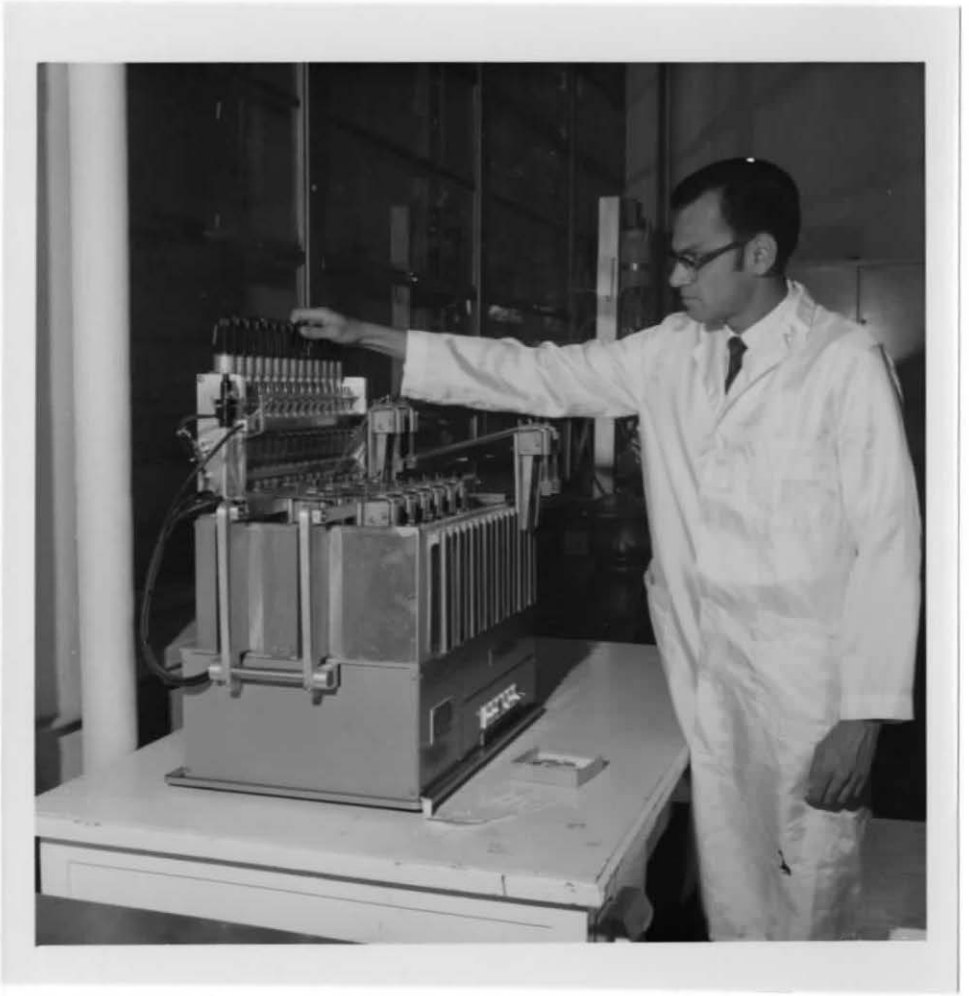

Figure 6. Gilson respirometer.

Reducing sugars

Reducing sugars were determined by the Shaffer and Somogyi method (A.O.A.C., 1965).

Raffinose

Raffinose was determined enzymatically on pressed juice as described by Avigad et al. (1962).

Titratable acidity

Pressed beet juice was filtered through the Whatman No. 1 filter paper and then titrated with 0.1 N NaOH to pH 8.1 for measuring titratable acidity as described by Ruck (1963).

Physical Changes

Microbial growth

The microbial growth pattern on beets subjected to different treatments was recorded and organisms were isolated and identified on potato dextrose agar and trypticase soy agar. Isolated organisms were then incubated at 70°F under controlled atmosphere with the same concentrations of gases as beets were stored in to study the colony characteristics and growth pattern (Kubica and Dye, 1967). Predominant organisms isolated from different treatments were treated with two antimicrobial agents, Difolatan, and Tetracycline to study the effective concentrations in inhibiting the growth.

Sprouting

Sprouting characteristics were noted with different treatments and number of beets showing sprouting within treatments was recorded.

Statistical Analyses

Statistical analyses were performed for a split plot design according to Cochran and Cox (1962).

RESULTS AND DISCUSSION

Since the storage period of the average commercial sugarbeet pile is about 90 days, the initial experiments were planned to prolong this period to over 150 days. At the end of 165 days, certain samples of the beets appeared to have stored well under the experimental conditions. The experiments were further continued with remaining beets in storage for 200 days, although samples for all of the treatments were not available for this storage period. The information and statistical analyses presented are based on 165 days of storage for all the treatments and for 200 days of storage for the remaining available beets receiving certain treatments.

Respiration

The difference in the respiratory patterns at different temperatures is presented in Figure 7. At 35°F, CR beets demonstrated a lower rate of respiration than those stored under optimum (6% CO_2 and 5% O_2) CA. On the other hand, at 50°F, the beets stored under optimum CA showed lower rate of respiration than the CR. Since the activity of certain respiratory enzymes is considerably inhibited at 35°F compared to those at 50°F, it may be hypothesized that once the beets are exposed to air at room temperature (75°F) the activity of these enzymes increases, especially in the injured tissue.

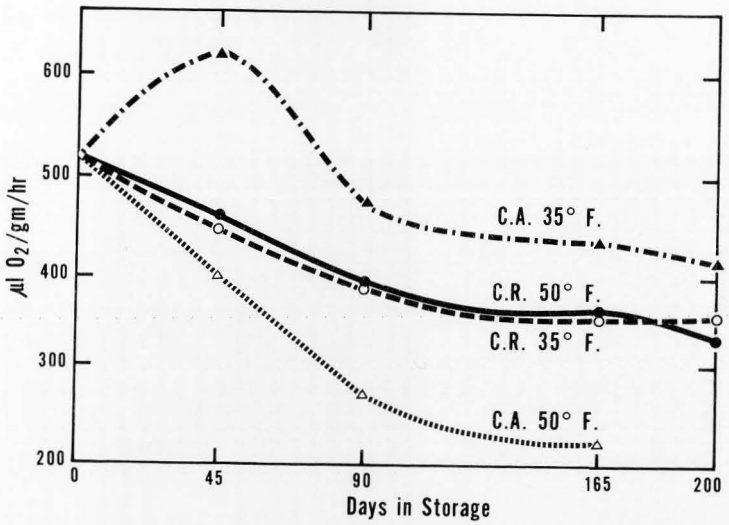

Figure 7. Effect of temperature and duration on controlled atmosphere (6% CO_2 and 5% O_2) storage on the respiration rate of sugarbeet discs.

Sucrose

On the basis of a stratified sampling technique, the beets contained 15.0% sucrose at the time of harvest. At the end of the storage period of 200 days at 35°F, 13% sucrose was retained (87.0% of initial value) in the optimum CA concentrations compared to 11.3% (75.5%) in the CR beets (Table 1). The loss of sucrose under optimum CA during the storage period averaged 54% of the loss occurring in the CR beets. Similarly, at the end of 165 days at 50°F storage, 12.3% (82.0%) sucrose was retained in the beets at 6% dioxide and 5% oxygen as compared to 11.0% (73.0%) in the CR beets indicating 67.5% as much loss of sucrose.

A toxic phenomenon was observed with high concentrations of carbon dioxide at the end of 90 days storage. Beets turned brown when stored at 10% carbon dioxide and 5% oxygen at both temperatures. They also were more susceptible to microbial attack and at the end of 90 days storage were dead as no respiration could be measured (Figure 8). Only 5.2% (34.9%) and 3.5% (23.1%) sucrose were retained at 35° and 50°F, respectively, at the end of 165 days, suggesting that concentrations of gases in the pile should be checked routinely. Anaerobic conditions, once developed, may lead to microbial spoilage of the entire pile.

Reducing Sugars

The initial content of reducing sugars in a 10-beet sample averaged 60 mg/100 gm. Reducing sugars had increased less in CA-stored beets than

Table 1. Effect of storage treatment and duration on per cent sucrose retention in sugarbeets at 35° and 50°F (per cent of original sucrose retained with initial concentration 15.0%)

Days in storage	Storage temp. (°F)	CR	CA treatments			
		0.03% CO_2 21.0% O_2	0% CO_2 5% O_2	3% CO_2 5% O_2	6% CO_2 5% O_2	10% CO_2 5% O_2
At harvest	35	100.0	100.0	100.0	100.0	100.0
45		97.3	96.3	99.2	97.9	91.5
90		93.7	93.2	95.6	96.6	85.0
165		86.9**	87.3	86.9	91.6**	34.9**
200		75.5	...	...	87.0	...
At harvest	50	100.0	100.0	100.0	100.0	100.0
45		94.7	94.6	96.7	98.4	84.7
90		89.8	90.3	91.7	94.4	75.8
165		73.0**	76.7**	80.5**	82.0**	23.1**
200		62.0	67.2	...	...	...

...Shortage of samples.

**At 165 days significant at 0.01 level compared with control.

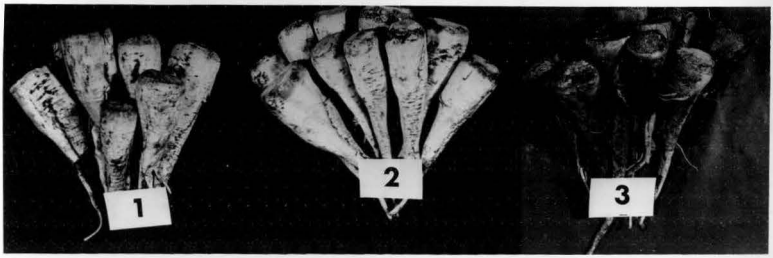

Figure 8. Effect of controlled atmosphere on the sugarbeets at 35°F (top) and 50°F (bottom) at the end of 165 days of storage: (1) control, (2) 6% CO_2 and 5% O_2, (3) 10% CO_2 and 5% O_2. (Note the adverse effect of excess CO_2.)

in the CR beets at both 35° and 50°F, as shown in Table 2, except for those stored at 10% carbon dioxide and 5% oxygen. At the end of 200 days storage, at 35°F in 6% carbon dioxide and 5% oxygen, 229 mg/100 gm of reducing sugars were observed compared to 334 mg/100 gm of beets in the CR group. This amounted to a 31% decrease in total reducing sugars in CA-stored beets. After 165 days at 50°F, CA-stored beets averaged 361 mg/100 gm of reducing sugars compared to 466 mg/100 gm in CR beets. Beets stored at 10% carbon dioxide had 384 mg/100 gm and 466 mg/100 gm reducing sugars at 35° and 50°F, respectively.

Raffinose

Raffinose content of the sugarbeets increased appreciably at 35°F as the storage period extended. Although CA storage appears to have some merit in controlling the raffinose accumulation (Table 3), this is primarily a temperature dependent phenomenon. Raffinose had increased from 24 mg/100 gm to 194 mg/100 gm under optimum CA compared to 222 mg/100 gm in CR beets at 35°F after 200 days storage.

Raffinose content of the beets was appreciably lower under storage at 10% carbon dioxide and 5% oxygen. However, beets stored under this treatment were significantly lower in sucrose and higher in other non-sugar constituents at the end of 90 days. At 50°F the net raffinose increase was less than at the lower temperature. At the end of 165 days storage at 50°F, 42 mg/100 gm raffinose was observed in beets stored at 6% carbon dioxide and 5% oxygen as compared to 50 mg/100 gm in the CR beets.

Table 2. Effect of storage treatment and duration on reducing sugar content in sugarbeets at 35° and 50°F expressed as mg/100 gm

Days in storage	Storage temp. (°F)	CR	CA treatments			
		0.03% CO_2 21.0% O_2	0% CO_2 5% O_2	3% CO_2 5% O_2	6% CO_2 5% O_2	10% CO_2 5% O_2
At harvest	35	60	60	60	60	60
45		198	124	102	87	135
90		217	175	139	128	391
165		293**	228**	158**	187**	384**
200		334	...	...	229	...
At harvest	50	60	60	60	60	60
45		313	287	253	217	268
90		352	331	317	305	428
165		466**	405**	426**	361**	466**
200		729	414	...	...	...

... Shortage of samples.

**At 165 days significant at 0.01 level compared to control.

Table 3. Effect of storage treatments and duration on raffinose content in sugarbeets at 35° and 50°F expressed as mg/100 gm

Days in storage	Storage temp. (°F)	CR	CA treatments			
		0.03% CO_2 21.0% O_2	0% CO_2 5% O_2	3% CO_2 5% O_2	6% CO_2 5% O_2	10% CO_2 5% O_2
At harvest	35	24	24	24	24	24
45		96	99	87	89	60
90		138	120	121	126	19
165		214**	200¯	188**	170**	14**
200		222	...	...	194	...
At harvest	50	24	24	24	24	24
45		39	34	37	37	30
90		48	41	45	39	32
165		50	37**	45¯	42**	20**
200		55	39	...	...	...

...Shortage of samples
**At 165 days significant at 0.01 level compared to control.
¯Non significant

Titratable Acidity

Titratable acidity, expressed as citric acid, increased from an initial 238 mg/100 gm to 328 mg/100 gm at the end of 45 days under optimum CA-stored beets at 35°F (Table 4). In contrast, considerable depletion of acids occurred (202 mg/100 gm) in the beets stored in CR at the same temperature. This trend continued, and at the end of 165 and 200 days of storage more acidity was observed in the beets stored under 6% CO_2 and 5% O_2 compared to the CR indicating an accumulation of acids of the Krebs cycle in the beet tissues. Beets stored under 10% carbon dioxide and 5% oxygen at both 35° and 50°F were highly acidic. This may be attributed to the profuse microbial growth and the aerobic and anaerobic fermentation products of microorganisms.

Sprouting and Microbial Growth

Sprouting and microbial growth were both temperature and CA dependent as indicated in Tables 5 and 6. At 35°F, in general, no sprouting was observed in CR or CA-stored beets. At 50°F, very distinct sprouting occurred in CR beets and sprouting was inhibited appreciably with beets under CA. Similar phenomenon, likewise, was observed with fungal growth. Generally, beets stored under CA, with the exception of those stored under high concentration of carbon dioxide, had much less fungal growth than with those stored under CR. Predominant fungi isolated included species of Penicillium, Fusarium, Botrytis, Rhizopus, and Aspergillus. Erwinia chrysanthemi, a soft rot-causing bacterium, was profuse

Table 4. Effect of storage treatment and duration on titratable acidity in sugarbeets at 35° and 50°F expressed as mg citric acid/100 gm

Days in storage	Storage temp. (°F)	CR	CA treatments			
		0.03% CO_2 21% O_2	0% CO_2 5% O_2	3% CO_2 5% O_2	6% CO_2 5% O_2	10% CO_2 5% O_2
At harvest	35	238	238	238	238	238
45		202	252	233	328	195
90		171	167	161	231	366
165		154	171⁻	160⁻	196**	956**
200		154	...	...	195	...
At harvest	50	238	238	238	238	238
45		226	274	202	242	101
90		178	259	173	169	1687
165		138	190**	165⁻	180**	2130**
200		119	161	...	...	...

...Shortage of sample.

**At 165 days significant at 0.01 level compared to control.

⁻Non significant.

Table 5. Number of sugarbeets per 10-beet sample showing significant sprouting at 50°F

Days in storage	CR	CA treatments			
	0.03% CO_2 21.0% O_2	0% CO_2 5% O_2	3% CO_2 5% O_2	6% CO_2 5% O_2	10% CO_2 5% O_2
At harvest	-	-	-	-	-
45	4	2	1	1	-
90	10	8	3	1	-
165	10	7	3	3	-
200	10	10	...	...	...

...Shortage of sample.
-No sprouting.

Table 6. Effect of storage treatments and duration on mold growth on sugarbeets at 35° and 50°F[a]

Days in storage	Storage temp. (°F)	CR	CA treatments			
		0.03% CO_2 21.0% O_2	0% CO_2 5% O_2	3% CO_2 5% O_2	6% CO_2 5% O_2	10% CO_2 5% O_2
At harvest	35	-	-	-	-	-
45		-	-	-	-	-
90		+	-	-	-	-
165		++	+	-	-	++++
200		+++	...	...	+	...
At harvest	50	-	-	-	-	-
45		+	+	-	-	-
90		+++	++	++	+	++++
165		++++	+++	++	++	++++
200		++++	++++	...	...	...

[a] Growth characteristics as defined by Kubica and Dye (1967).

... Shortage of sample

++++ Confluent growth (more than 500 colonies).

+++ Almost confluent (200-500 colonies)

++ 100-200 colonies.

+ 50-100 colonies.

- No growth (below 50 actual count).

in control beets. Fungi isolated from CA-stored beets had inhibited mycelial growth.

In the early period of storage, species of Penicillium, Aspergillus, and Rhizopus were predominant. Subsequent to 90 days of storage, however, profuse growth of Fusarium and Botrytis superseded other organisms. This growth pattern was observed both in the CR and CA-stored beets.

The growth pattern of fungi isolated on potato dextrose agar and incubated under CA is presented in Table 7. Inhibition of colony diameter was observed with increasing concentrations of carbon dioxide indicating that fungal metabolism was also controlled with CA as that of the roots. Table 8 shows the effects of "Difolatan" and "Tetracycline" on the growth of organisms. At 10 ppm concentrations of "Difolatan," fungal growth was inhibited with predominant organisms. Although favorable and objectional reports on use of fungicides are reported in literature, no studies with the above mentioned antimicrobial agents are known with sugarbeets. Tetracycline, likewise, demonstrated inhibition of growth of Erwinia chrysanthemi at 5 ppm concentrations. Since much of the microbial problem is associated with fungi, the use of "Difolatan" as a dip may be considered more favorably before beets go in pile for storage. Other reports on use of Difolatan with horticultural commodities have shown promising results (Do et al., 1966).

Data presented above indicate the effects of controlled atmosphere on the metabolic processes and storage physiology of sugarbeets. Although lower temperature is an important factor in storage, the optimum concentration of

Table 7. Effect of controlled atmosphere on the colony diameter in centimeters of microorganisms grown on potato dextrose agar at 70°F

Organism	CR	CA treatments			
	0.03% CO_2 21.0% O_2	0% CO_2 5% O_2	3% CO_2 5% O_2	6% CO_2 5% O_2	10% CO_2 5% O_2
Aspergillus sp.	2.5	2.2	2.2	1.8	1.0
Botrytis sp.	2.0	1.8	1.0	1.0	0.7
Fusarium sp.	3.5	2.5	2.5	1.5	1.0
Penicillium sp.	3.0	3.0	3.0	4.0	2.5

Table 8. Effect of Difolatan and Tetracycline on the growth of microorganisms at 70°F on potato dextrose agar and trypticase soy agar[a]

Organism	Chemical	CR	ppm concentrations 0.1	1.0	3.0	5.0	10.0
Aspergillus sp.	Difolatan	++++	++++	+++	+	-	-
		+++	+++	++	34	-	-
Botrytis sp.	Difolatan	++++	+++	1	-	-	-
		+++	++	-	-	-	-
Fusarium sp.	Difolatan	++++	+++	9	-	-	-
		++++	+++	24	-	-	-
		++++	+++	11	-	-	-
Penicillium sp.	Difolatan	++++	++++	+	12	-	-
		+++	++	+	4	-	-
Rhizopus sp.	Difolatan	+++	++	-	-	-	-
		+++	++	-	-	-	-
Erwinia sp.	Tetracycline	++++	+++	++	±	-	-
		+++	++	+	±	-	-

[a]Growth characteristics as defined by Kubica and Dye (1967).
. . . Shortage of sample.
++++ Confluent growth (more than 500 colonies).
+++ Almost confluent (200-500 colonies).
++ 100-200 colonies.
+ 50-100 colonies.
- No growth (below 50 actual count).

carbon dioxide and oxygen does slow the degradation of sucrose. It is believed that CA inhibits the respiratory enzymes (Biale, 1960). The increase in reducing sugars is dependent upon the invertase activity and is proportional to the increase (rise) in temperature. The above results indicate that less sucrose was hydrolyzed under CA storage, regardless of the temperature. Raffinose accumulation may be considered as a temperature-dependent phenomenon as CA had a slight effect in controlling the raffinose buildup. Figures 9 and 10 show the correlation between sucrose degradation and synthesis of raffinose. An inverse relationship is observed between reducing sugars and titratable acidity. It is evident from data that certain acids accumulate in the storage tissue of the beet roots. The accumulation of acids may be attributed to the controlled respiratory rate during storage.

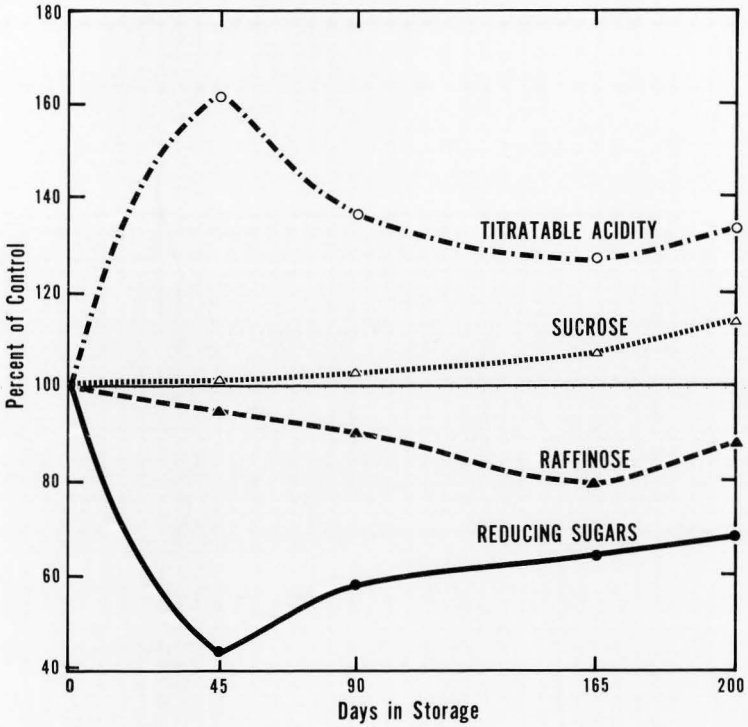

Figure 9. Effect of controlled atmosphere (6% CO_2 and 5% O_2) storage and duration on sucrose, reducing sugars, raffinose, and titratable acidity in the sugarbeets at 35°F, in relation to control.

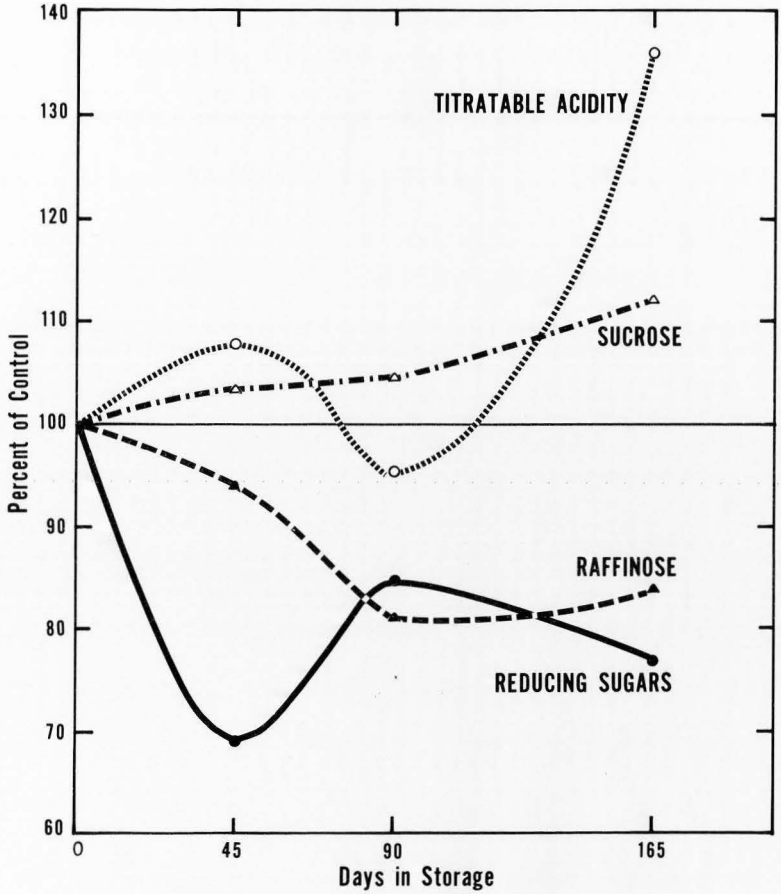

Figure 10. Effect of controlled atmosphere (6% CO_2 and 5% O_2) storage and duration on sucrose, reducing sugars, raffinose, and titratable acidity in the sugarbeets at 50°F, in relation to control.

SUMMARY AND CONCLUSIONS

The controlled atmosphere storage of sugarbeets showed promising results when beets were stored for 200 days under experimental conditions. The maximum beneficial effects of CA were observed under 6% carbon dioxide and 5% oxygen at 35°F. Regardless of storage temperatures, sucrose retention was highest in the beets stored under CA. Other beneficial effects of CA include less hydrolysis of sucrose to reducing sugars and control of raffinose accumulation--the index of quality. Fungal growth and sprouting were also inhibited significantly. These studies indicate the guidelines of beneficial concentrations of gases and chemicals under laboratory conditions. It is suggested, however, that additional research may be conducted with commercial piles so that information presented will be of practical value to the sugarbeet industry.

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PART II

NITROGEN FERTILIZER STUDIES

INTRODUCTION

Application of nitrogen fertilizers has substantially increased the yield of sugarbeets in the last few decades. Nitrogen in combined form is one of the most important elements for the growth and development of sugarbeet plants. Deficiency in nitrogen fertilization may be a limiting factor in plant growth. Although nitrogen fertilization in excess of the plant needs enhances the yield per acre, sucrose concentration is reduced and the quality of beets is substantially inferior. Several investigators have reported on the beneficial and harmful effects of nitrogen fertilization (Haddock et al., 1959; Snyder and Tolbert, 1966; Stout, 1961; Tolman and Johnson, 1958; Ulrich, 1942). Stout (1961) pointed out that when the sugarbeet plant absorbs nitrogen as nitrate ions from soil it expends energy which comes from the metabolic processes synthesizing sucrose. It requires eight electrons to reduce one nitrate ion to ammonia. Electrons which are available for reducing CO_2 in photosynthesis are utilized in reducing NO_3 to NH_3. The NH_3 formed stimulates the synthesis of amino acids, proteins, betaine, and other nitrogeneous compounds at the cost of sucrose synthesis. These impurities affect the thin juice purity in the processing (Rounds et al., 1958).

Dexter, Frakes and Nichol (1966) in their studies with three levels of nitrogen fertilization--30, 90, and 230 pounds per acre--concluded that low levels of fertilizer produced less sucrose while the sucrose content increased

with the medium level of nitrogen. At high levels of nitrogen, however, beets were low in sucrose content. These studies also indicated 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 soluble impurities of an undetermined nature. Likewise, extensive studies of Haddock, Linton, and Hurst (1956), Stout (1964), Ulrich (1942), and Snyder and Tolbert (1966) have demonstrated that sucrose accumulation in the sugarbeet roots is inhibited by the high level of nitrogen fertilization.

In view of the above mentioned studies of several investigators, beets were grown with three levels of nitrogen fertilizer. Control plots were left free of added fertilizer as the initial total nitrogen content was 2400 pounds of total N per acre in the top 1 foot of soil. High nitrogen content may be attributed to the residual effect of alfalfa crop from the preceeding year. 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 $40^{\circ}F$. In addition to the physio-chemical studies conducted in Part I, 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 0, 150, and 300 pounds of added nitrogen fertilizer per acre in a randomized block design. The beets were harvested and sorted as described in Part I. The three groups were kept separate and 10 uniform beets from each group were sacked in two replications for storage. The CR beets were stored in air at 40°F, and those under controlled atmosphere were stored under 6% carbon dioxide and 5% oxygen at the same temperature.

The samples were analyzed at harvest and 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 (1942) (Figure 11). Beets were brought to an equilibrium with normal air at 40°F which required a period of 12 hours. The respiration was measured for 1 hour after equilibrium period.

Figure 11. Claypool Keefer Apparatus for measuring respiration.

Chemical Analyses

Sucrose, reducing sugars, and raffinose content were determined as described in Part I.

Total nitrogen

Total nitrogen was estimated by the Kjeldahl method (A. O. A. C., 1965).

Amino nitrogen

Amino nitrogen was determined by using a modified Stanek Pavlas reagent (Stout, 1954).

Organic acids

An alcohol extract of sugarbeets was passed through cation exchange column, Dowex 50 W-X8, H 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 1X8, Cl form, 400 mesh, which had previously been converted to the acetate form. The organic acids were fractionated by the gradient elution method (Hulme and Wooltorton, 1958). 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 numbers of a peak against those of unknowns. The acid fractions were further identified by spotting on thin layer chromatography plates and developing by butanol:acetic acid:water (12:3:5, v/v/v). The plates were

sprayed for identification of spots by using 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 Safran and Densted (1948).

Physical Changes

Sprouting and microbiological growth characteristics were studied as described in Part I.

Statistical Analyses

Analysis of variance was performed and means were compared (Cochran and Cox, 1962).

RESULTS AND DISCUSSION

Respiration

The respiration rate at harvest averaged 20 mg CO_2/kg beets/hour (Table 9). Respiration decreased gradually 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 is in direct correlation with sucrose retention data (Table 10). 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 the end of 200 days 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 12.7 and 11.7% sucrose, respectively. The initial sucrose content in the beets indicated that the soil contained an adequate

Table 9. 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*
	CA	19.2	11.9	8.6	9.4
150	CR	20.4	11.5	12.2	13.4*
	CA	20.4	9.3	9.6	10.8
300	CR	19.8	10.5	9.7	14.5*
	CA	19.8	9.9	8.6	10.4

[a]Main effects of fertilizer non-significant.
*Storage treatments significant at 0.05 level.

Table 10. Effect of fertilizer, storage treatment and duration on per cent sucrose retention in sugarbeets at 40°F

Fertilizer[b] added (Pounds N/acre)	Storage treatment	Days in storage			
		At harvest	65	130	200
None	CR	100[a]	97.7	94.7	80.3*
	CA	100	98.5	96.9	86.2
150	CR	100	96.1	88.2*	78.7*
	CA	100	95.3	93.7	84.3
300	CR	100	96.6	91.5	78.6
	CA	100	99.1	95.7	82.1

[a]Initial sucrose concentration of 13.2, 12.7, and 11.7, respectively, taken as 100% with 0, 150, and 300 pounds N per acre.
[b]Main effects of fertilizer significant at 0.01 level.
*Storage treatments significant at 0.05 level.

available nitrogen for the sucrose synthesis because the added nitrogen did not prove beneficial. 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 10). 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 110 and 123 mg/100 gms for the 150 and 300 pounds added nitrogen treatments, respectively. This indicated that beyond certain levels of nitrogen fertilizer beets accumulate reducing sugars which are undesirable impurities. During storage reducing sugars increased. However, the reducing sugars were lower in CA storage compared to CR, regardless of nitrogen fertilizer level or length of storage (Table 11). The increase in reducing sugars, at the end of storage, was 10.7, 7.2, and 16% less under CA than in CR with increasing levels of added fertilizer, respectively.

Raffinose

At harvest the raffinose content did not vary significantly with increasing levels of nitrogen. Initial concentration averaged from 31 to

Table 11. Effect of fertilizer, storage treatment and duration on reducing sugar content in sugarbeets at 40°F, expressed as mg/100 gms

Fertilizer[a] added (Pounds N/acre)	Storage treatment	Days in storage			
		At harvest	65	130	200
None	CR*	95	281	347	419
	CA	95	223	282	374
150	CR	110	260	360	445
	CA	110	186	302	413
300	CR	123	259	410	511
	CA	123	288	330	427

[a]Main effects of fertilizer non-significant.
*Overall storage treatments significant at 0.05 level.

34 mg/100 gms of beets as shown in Table 12. 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, averaged 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 13). Added fertilizer demonstrated an adverse effect in accumulating nitrogeneous impurities, expressed 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 14). 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.

Table 12. Effect of fertilizer, storage treatment and duration on raffinose content in sugarbeets at 40°F, expressed as mg/100 gms

Fertilizer[a] added (Pounds N/acre)	Storage treatments	Days in storage			
		At harvest	65	130	200
None	CR	31	92	117*	124
	CA	31	86	94	114
150	CR	34	76	97	118
	CA	34	79	95	108
300	CR	31	88	104	123
	CA	31	78	101	117

[a]Main effects of fertilizer non-significant.
*Storage treatments significant at 0.05 level.

Table 13. Effect of fertilizer, storage treatment and duration on per cent total nitrogen in sugarbeets at 40°F

Fertilizer[a] added (Pounds N/acre)	Storage treatment	Days in storage			
		At harvest	65	130	200
None	CR	0.177	0.191	0.194	0.195
	CA	0.177	0.197	0.183	0.182
150	CR	0.206	0.201	0.210	0.206
	CA	0.206	0.194	0.212	0.207
300	CR	0.219	0.221	0.227	0.220
	CA	0.219	0.218	0.219	0.219

[a]Main effects of fertilizer significant at 0.01 level.

Table 14. Effect of fertilizer, storage treatment and duration on ppm amino nitrogen in sugarbeets at 40°F

Fertilizer[a] added (Pounds N/acre)	Storage treatments	Days in storage			
		At harvest	65	130	200
None	CR	500	550	495*	410
	CA	500	490	390	365
150	CR	650	595	605	535
	CA	650	560	580	510
300	CR	745	755	700	675
	CA	745	720	645	640

[a]Main effects of fertilizer significant at 0.01 level.
*Storage treatment significant at 0.05 level.

Organic Acids

Four major acid fractions identified include citric acid, malic acid, oxalic acid, and succinic acid. Other fractions observed in trace quantities were not identified. Table 15 shows that the citric acid concentration was depleted after 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 both CR and CA storage. Accumulation of succinic acid in storage has been reported due to an inhibitory effect on dehydrogenases (Neal and Hulme, 1958; Miller, 1960). Ranson, Walker, and Clark (1960) demonstrated that with increasing levels of carbon dioxide, besides succinic acid, accumulation of pyruvic acid occurs. Their data suggested that at higher concentrations of CO_2 some enzymes involved in the production of citric acid from pyruvic acid were markedly affected, in addition to succinic oxidase. The data with sugarbeets under CA indicate that besides succinic acid citric acid accumulates. Accumulation of succinic acid may be due to blocking of succinic dehydrogenase. Accumulation of citric acid may be due to inhibition of aconitase, which can be correlated to the decreased amount of amino nitrogen in the CA-stored beets. Increased 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:

$$\text{Pyruvic acid} + CO_2 \longrightarrow \text{Oxalacetic acid.}$$

Oxalacetic acid in turn forms citric acid (Davies, Giovanelli, and Rees, 1964).

Table 15. 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*
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*
150		2.9	2.4	4.2
300		3.4	3.4	4.2

[a]Main effects of fertilizer non-significant.
*Storage treatment significant at 0.05 level.

The above data can be correlated with sucrose and respiration data presented in Figures 12, 13 and 14. Sucrose retention and inhibition of respiration are in direct correlation. It may be stated that with the inhibition of respiration more sucrose is retained. Accumulation of acids may be due to inhibition of sucrose decomposition. This is also in direct correlation with amino nitrogen data. Joy (1967) indicated that the sugarbeet root is a primary organ for glutamic acid synthesis. Thus transaminase reactions play a role in roots from which protein synthesis is accomplished. It may be possible that in CA this mechanism is inhibited.

Sprouting and Microbial Growth

Sprouting characteristics and fungal growth patterns are reported in Tables 16 and 17. 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.

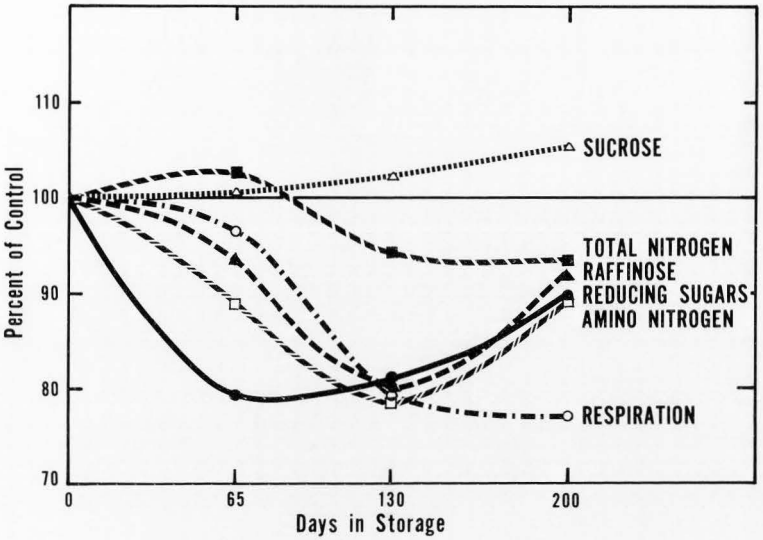

Figure 12. Effect of controlled atmosphere storage and duration on sucrose, reducing sugars, raffinose, amino nitrogen, total nitrogen and respiration at 40°F in sugarbeets grown with no added nitrogen fertilizer, in relation to control.

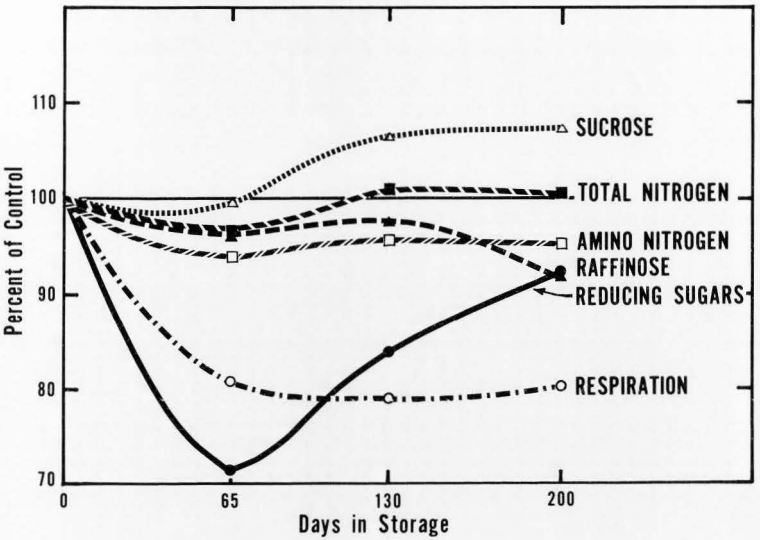

Figure 13. Effect of controlled atmosphere storage and duration on sucrose, reducing sugars, raffinose, amino nitrogen, total nitrogen and respiration at 40°F in sugarbeets grown with 150 pounds of added nitrogen fertilizer, in relation to control.

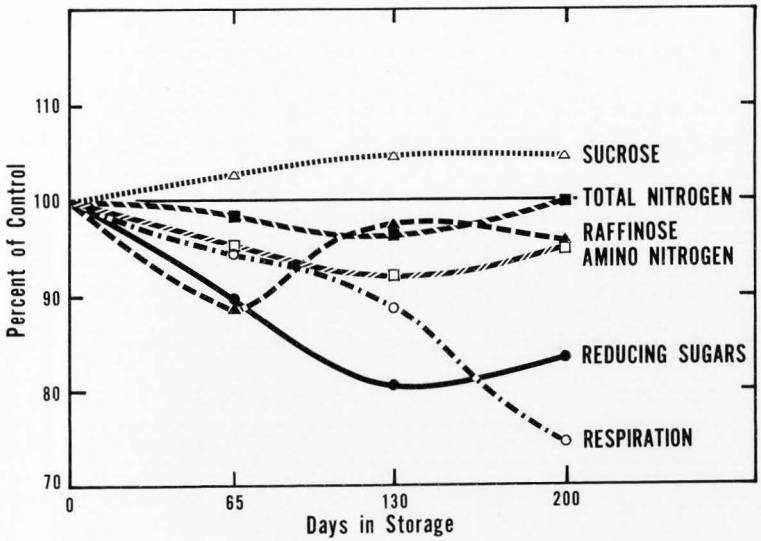

Figure 14. Effect of controlled atmosphere storage and duration on sucrose, reducing sugars, raffinose, amino nitrogen, total nitrogen and respiration at 40°F in sugarbeets grown with 300 pounds of added nitrogen fertilizer, in relation to control.

Table 16. Effect of fertilizer, storage treatment and duration on number of sugarbeets per 20-beet sample showing sprouting at 40^{o}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 17. 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 (1967).
++++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 with beets grown with different levels of nitrogen fertilizer. 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 with any treatment during storage. Accumulation of citric acid and succinic acid was significant with beets stored under CA. Fungal growth and sprouting were significantly inhibited under CA. Respiration rate was significantly reduced with beets stored under CA.

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VITA

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