Physio-Chemical and Microbiological Studies on Controlled Atmosphere Storage of Sugarbeets

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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 (12).[3] 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.

A storage system which could reduce sugarbeet respiration and thus inhibit sucrose degradation with a minimum of expense and inconvenience may find demand among sugar manufacturers.

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 also causes significant spoilage. Besides respiration, sprouting and microbiological spoilage losses are also significant. The losses vary considerably with temperature, but the average total loss amounts to about ½ pound of sugar per ton of beets per day (14). Attempts have been made since the beginning of the last century to minimize these losses by using forced ventilation, lime treatment, modified atmosphere (16), 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 a flow-through system. Kidd and West (8) in England were one of 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 et al. (3). The effect of different concentrations of these gases and the effect of various temperatures on germination and growth of certain fungi also were observed (4,10). The desirability of testing each commodity in

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various atmospheres was recognized, as in certain cases improper concentrations of gases caused poor texture, tissue breakdown, bicarbonate taste, and/or brown rot (13). These disorders made suitable substrates for microorganisms to grow profusely. The best concentrations for one product may be unsatisfactory for another product. Once appropriate concentration is established, however, CA storage has a number of advantages which include retardation of respiration, inhibition of microbial growth and of 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 the residual effect of CA in the commodity.

The investigations reported herein include our attempts to study 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° and 50° F.

Materials and Methods

Cultivar Utah-Idaho Number 7, an hybrid, F_1, was selected for these investigations. 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 makeup (6). Ten beets per sample were selected on the basis of uniformity of size, shape, and specific gravity for each treatment for four storage periods of 45, 90, 165, and 200 days. Beets were placed in mesh sacks, weighed, and then stored in 55-gallon barrels. A Tectrol generator, manufactured by Whirlpool Corporation, St. Joseph, Michigan, was set to produce basic atmospheres of 3% carbon dioxide and 5% oxygen. Zero, 3, 6, and 10% carbon dioxide and 5% oxygen concentrations were produced by using a series of pressure regulators, mixers, absorbers, and carbon dioxide.

The flow of atmospheres through the sealed barrels was regulated by employing flow meters and pressure regulators, as shown in Figure 1. The flow of gas was maintained at the rate of 105 cc of gas per minute per storage barrel. For certain experiments, Arcat-Arcosorb CA system of Atlantic Research Corporation, Mechanic Products Division, Alexandria, Virginia, was employed. Control beets were stored under identical storage conditions, and air was pumped in the barrels to flush out carbon dioxide produced by the beets during storage. The barrels used had a removable top which could be sealed gas tight to the rim. The top had a gas inlet, transparent window, and temperature-humidity meter. An outlet for flushing the gas was at the bottom of the barrels. The atmospheres in the storage

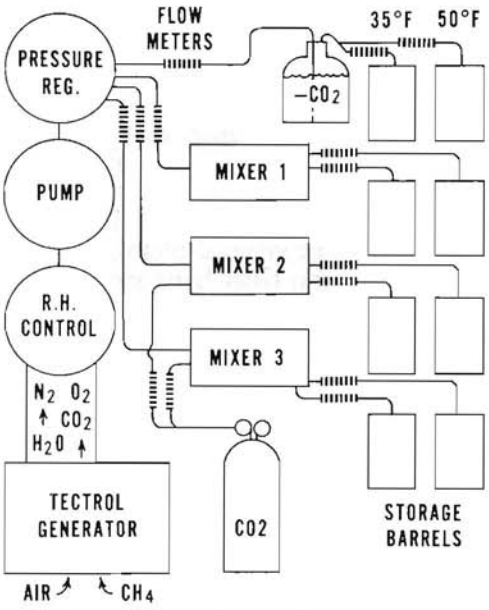

Figure 1.—Schematic diagram of controlled atmosphere storage system.

barrels were checked routinely by means of "Fyrite" gas analyzers. The samples were taken out periodically for respiration and chemical analyses to determine sucrose, reducing sugars, raffinose, and titratable acidity. The contents of the barrels opened during sampling were exposed to the atmosphere for no more than an hour.

Microbial growth and sprouting characteristics with different treatments were recorded. Organisms were then isolated, and incubated at 70° F under controlled atmosphere with same concentrations of gases as beets were stored to study the colony characteristics and growth patterns (9). Predominant organisms isolated from different treatments were treated with two antimicrobial agents, Difolatan and Tetracycline, to study the effective concentrations in inhibiting the growth. Respiration (0_2 uptake) was measured by using a Gilson respirator according to the procedure described by Stout and Spikes (15). The beets were then ground by Spreckels saw to fine particles for chemical analyses. Sucrose and reducing sugars were determined by the AOAC methods (1). Raffinose was determined enzymatically as described by Avigad et al. (2). Beet juice was filtered through the Whatman No. 1 filter paper and then titrated with 0.1 Na OH to pH 8.1 for estimating titratable acidity as described by Ruck (11). Statistical analyses were performed according to Cochran and Cox (5).

Results and Discussion

Since the storage period of the average commercial sugarbeet pile is about 90 days, our 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 3. At 35° F, control beets demonstrated a lower rate of respiration than those stored under CA. On the other hand, at 50° F, the beets stored under CA showed lower rate of respiration than the controls. 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 atmosphere at room temperature (75° F) the activity of these enzymes increases, especially in the injured tissue.

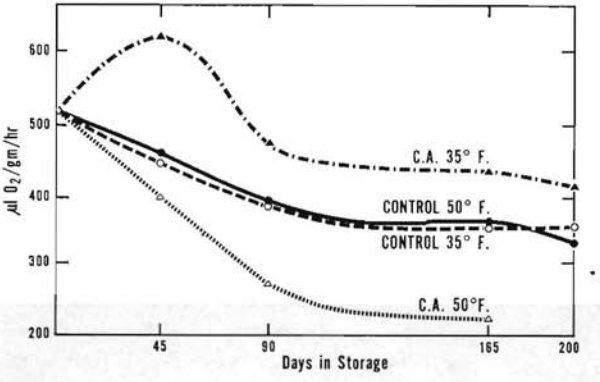

Figure 2.—Effects of temperature and controlled atmosphere (6% CO_2 and 5% O_2) storage on the respiration rate of sugarbeets.

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, 13.0% sucrose was retained (87.0% of initial value) in the best gas concentrations compared to 11.3% (75.5%) in the control beets (Table 1). The loss of

Table 1.—Effect of storage treatment and duration on percent sucrose retention. [Percent of original sucrose retained with initial concentration (15.0%)].

Days in storage	Storage temp. (°F)	Control	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
0	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	----
0	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.

sucrose under CA during the storage period averaged 54% of the loss occurring in the control beets. Similarly, at the end of 165 days at 50° F storage, 12.3% (82.0%) sucrose was retained in the beets at 6% carbon dioxide and 5% oxygen as compared to 11.0% (73.0%) in the control beets indicating 65% 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 susceptible to microbial attack and at the end of 90 days' storage were dead as no respiration could be measured (Figure 2). 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, indicatng that concentrations of gases in the pile should be checked routinely. Anaerobic condition, once developed, may lead to microbial spoilage of the entire pile.

Figure 3.—Effect of controlled atmosphere on the sugarbeets at 35° F 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.

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 in the control beets both at 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

Table 2.—Effect of storage treatment and duration on reducing sugar content, expressed as mg/100 gm.

Days in storage	Storage temp. (°F)	Control	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
0	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	.....
0	50	60	60	60	60	60
45		313	287	253	217	268
90		352	331	347	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.

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 control 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 control 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 a temperature-dependent phenomenon. Raffinose had increased from 24 mg/100 gm to 194 mg/100 gm in CA-stored beets compared to 222 mg/100 gm in control beets at 35° F after 200 days storage.

Raffinose content of the beets was appreciably low under storage at 10% carbon dioxide and 5% oxygen. However, beets stored under this treatment were significantly low in sucrose and high in other nonsugar 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% oxygen as compared to 50 mg/100 gm in the control beets.

Table 3.—Effect of storage treatments and duration on raffinose contents expressed as mg/100 gm.

Days in storage	Storage temp. (°F)	Control 0.03% CO_2 21.0% O_2	CA treatments 0% CO_2 5% O_2	3% CO_2 5% O_2	6% CO_2 5% O_2	10% CO_2 5% O_2
0	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	-----
0	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.
-N.S.

Titratable Acidity

Titratable acidity, expressed as citric acid, increased from initial 238 mg/100 gm to 328 mg/100 gm at the end of 45 days in the CA-stored beets at 35° F (Table 4). In contrast, considerable depletion of acids occurred (202 mg/100 gm) in the beets stored in air at the same temperature. This trend continued, and at the end of 165 and 200 days of storage more acidity was observed in the CA-stored beets compared to the controls indicating an accumulation of tricarboxilic cycle acids 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 their profuse microbial growth and the aerobic and anaerobic fermentation products of microorganisms.

Table 4.—Effect of storage treatment and duration on titratable acidity expressed as mg citric acid/100 gm.

Days in storage	Storage temp. (°F)	Control 0.03% CO_2 21.0% O_2	CA treatments 0% CO_2 5% O_2	3% CO_2 5% O_2	6% CO_2 5% O_2	10% CO_2 5% O_2
0	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	-----
0	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 samples.
*At 165 days, significant at 0.01 level compared to control.
-N.S.

Sprouting and Fungal Growth

Sprouting and fungal growth were both temperature and controlled atmosphere dependent as indicated in Tables 5 and 6. At 35° F, in general, no sprouting was observed both in control as well as CA-stored beets. At 50° F, very distinct sprouting occurred in control beets and sprouting was inhibited appreciably with beets under controlled atmosphere. Similiar phenomenon, likewise, was observed with fungal growth. Generally, beets stored under controlled atmosphere, with the exception of those stored under high level of carbon dioxide, had much less fungal growth than with those stored under conventional methods of refrigeration. Predominant fungi isolated included species of *Penicillium, Fusarium, Botrytis, Rhizopus,* and *Aspergillus. Erwinea chrysanthemi,* a soft rot-causing bacterium, was profuse in control beets. Fungi isolated from CA-stored beets had inhibited mycelial growth.

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

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

..... Shortage of samples.
-No sprouting.

Table 6.—Effect of storage treatments and duration on mold growth[a].

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

[a] Based on Kubica and Dye (9).
..... Shortage of sample
++++ Confluent growth (more than 500 colonies)
+++ Almost confluent (200-500 colonies)
++ 100-200 colonies
+ 50-100 colonies
- Now growth (Below 50 actual count)

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 control and CA-stored beets.

The growth pattern of fungi isolated on potato dextrose agar and incubated under controlled atmosphere is presented in Table 7. Inhibition in 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 *Erwinea 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 (7).

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

	Control	CA treatments			
Organism	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 organisms at 70° F on potato dextrose agar and trypticase soy agar.

		Control	ppm concentrations				
Organism	Chemical		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	+++	++	-	-	-	-
		+++	++	-	-	-	-
Erwinea sp.	Tetracycline	++++	+++	++	±	-	-
		+++	++	+	±	-	-

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 carbon dioxide and oxygen does slow the degradation of sucrose. It is believed that CA inhibits the respiratory enzymes thus controlling the glycolytic breakdown. The increase in reducing sugars is dependent upon the invertase activity and is proportional to the increase (rise) in temperaure. Our results indicate that less sucrose was hydrolyzed under controlled atmosphere 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 4 and 5 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.

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

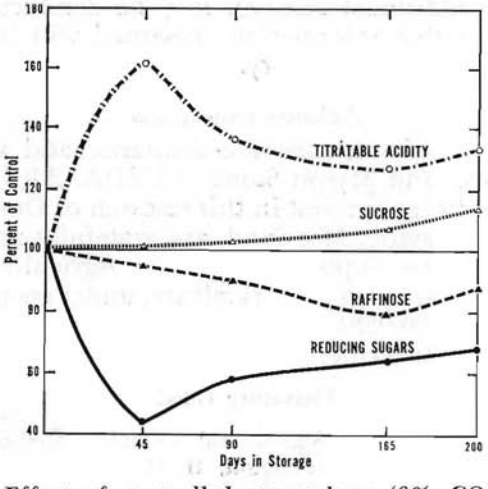

Figure 4.—Effect of controlled atmosphere (6% CO_2 and 5% O_2) storage on sucrose, reducing sugars, raffinose, and titratable acidity in the sugarbeets at 35° F.

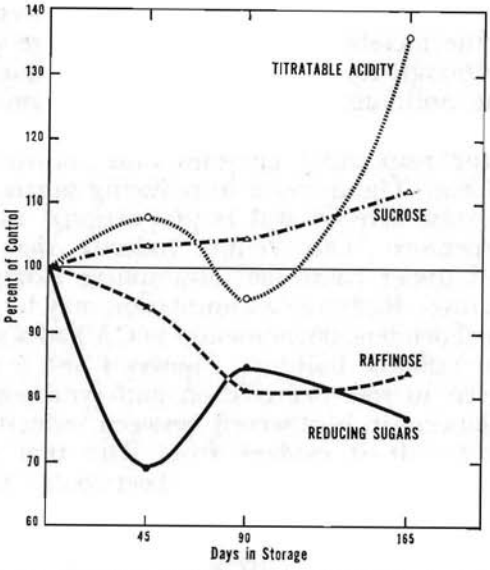

Figure 5.—Effect of controlled atmosphere (6% CO_2 and 5% O_2) storage on sucrose, reducing sugars, raffinose, and titratable acidity in the sugarbeets at 50° F.

control of raffinose accumulation—the index of quality. Fungal growth and sprouting were also inhibited significantly. Our 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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