

SOME ASPECTS OF THE PHYSIOLOGICAL BEHAVIOR OF MCINTOSH APPLE  
FRUITS FOLLOWING CONTROLLED ATMOSPHERE STORAGE

By

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AN ABSTRACT

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Harvested McIntosh apple fruits were stored for periods up to six months duration in controlled atmospheres of 5 percent carbon dioxide and 3 percent oxygen at 38° F and in regular air at 33° F. The apples, upon removal from storage at various intervals, were compared for the quality characteristics of appearance, texture, flavor, ripeness, flesh firmness, skin toughness, and quantity of cortical cell wall material. The respiratory behavior of the fruit was examined for influences of storage method, storage duration, high oxygen, ethylene gas, and apple volatiles. Other metabolic responses studied included polyphenoloxidase activity, aldehyde evolution, and ethylene evolution.

These studies confirmed the findings of others that apples stored under controlled atmosphere conditions were firmer and of better eating quality and market condition than comparable apples stored in regular air storage. These advantages of controlled atmosphere storage were retained when the fruit was held for seven days at 75° F after removal from storage. This was due to the initial difference at the end of the storage period, rather than differences in the rate of depreciation at the high temperature. Controlled atmosphere storage reduced the incidences of storage scald, mealy breakdown, and core browning associated with senescence over regular air storage.

The epidermal tissues of the controlled atmosphere apples were tougher, as measured by resistance to puncture, than those of apples stored in regular air when taken from storage and after holding for eight weeks in air at 33°F. Storage treatment had no significant effect on the total amount of cortical cell wall material of the apples.

A marked depressing effect of controlled atmosphere storage on the post-storage fruit respiration rate at 75°F became apparent after three months of storage in the first season and after six months in the second year. Controlled atmosphere storage caused a marked decrease in the respiration rate of the apples in air at 33° after removal from storage in the first season, but this effect was negligible in the second season. Ethylene treatments had no marked effect on the respiratory activity of apples stored for six months; whereas the volatiles given off by regular air-stored apples appeared to stimulate the post-storage respiratory activity of controlled atmosphere apples at 75°F. Post-storage treatment with 37 percent oxygen for a period of twelve days at 75°F had a slight depressing effect on the respiratory activity, measured by carbon dioxide evolution, of the apples stored under both storage treatments until March. Storage treatment had no marked effect on the respiratory quotient.

Apples from controlled atmosphere storage evolved aldehydes at

slower rates than apples which had been stored in regular air, but the patterns of increase in aldehyde production of apples from both storage treatments were similar during the twelve days of measurement at 75° F. These measurements were made in February, 1959, when the respiratory activity of apples from both storage treatments was approximately equal.

The post-storage rates of ethylene production at 33° F in air by the controlled atmosphere apples followed approximately the same curve throughout the season, but the rates of ethylene production by the regular air-stored apples decreased throughout the storage season. Controlled atmosphere apples removed from storage in March or later evolved ethylene at rates twice those of regular air storage apples after three to five weeks in air at 33° F and this difference in rates of ethylene evolution was maintained throughout the balances of the six to eight weeks of testing.

Carbon dioxide and ethylene evolution indicated a respiratory climacteric usually occurred in controlled atmosphere apples after removal from storage. Comparative rates of ethylene evolution, aldehyde evolution, catechol and citric acid oxidation following storage indicated the principal terminal oxidase systems of controlled atmosphere apples were more active than those of apples stored in regular air storage.

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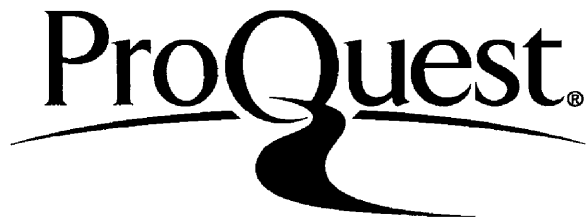
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## INTRODUCTION

The controlled atmosphere storage of apples has been extensively studied during the last thirty years. Much of this research has dealt with the chemical and physical changes occurring in the fruit during storage and with the optimum temperatures and concentrations of carbon dioxide and oxygen required by varieties in various areas of production. Few studies have emphasized the physiological behavior of apples after removal from controlled atmosphere storage. The need for such information has been accentuated in recent years by regulatory legislation for distinguishing and labelling controlled atmosphere-stored apples. Identifiable differences in properties and characteristics of controlled atmosphere-stored and regular air-stored apples seem essential for enforcement and success of such laws.

Other researchers have reported that apples which had been stored under controlled atmosphere conditions had a longer shelf life and a lower rate of respiration than comparable apples stored in regular air storage. The purpose of this research was to more intensively study the physiological behavior of McIntosh apples following storage under controlled atmosphere and regular air storage conditions, and to determine the time of inception of physiological differences during the storage period.

## LITERATURE REVIEW

Much of the research on the post-harvest physiology of apples has been summarized in excellent review articles by Smock (55), Biale (7), Pentzer and Heinze (48), Ulrich (64), and Hulme (31). Consequently, this review will deal with the specific effects of abnormal atmospheric concentrations of carbon dioxide and oxygen on harvested apple fruits, rather than with the general physiological changes which occur in apples after harvest.

### The Effects of Controlled Atmosphere Storage

Storage of apples in modified atmosphere has generally resulted in decreased rates of flesh softening and of changes in the ground color of the skin from green to yellow as compared to apples stored in regular air (36, 18, 54, 34, 66, 67). Van Doren (66) reported that controlled atmosphere storage also retarded the reduction of soluble solids in McIntosh apples.

Kidd and West (38), working with Bramley's Seedling apples stored at four levels of carbon dioxide (0.5, 5.0, 10.0 and 15.0 percent) in combination with four levels of oxygen (2.5, 5.0, 10.0 and 21.0 percent), found that both gases affected flesh softening. Although the level of carbon dioxide had a more pronounced effect on retention of flesh firmness than the level of oxygen, apples stored in 2.5 percent oxygen with only traces of carbon dioxide remained definitely more firm than apples stored in regular air. They reported similar results with King Edward VII apples.

Factors other than storage atmosphere may have considerable effect of flesh softening. Smock (56) found there were yearly variations for the McIntosh variety; for example, apples from controlled atmosphere storage were 5.1 pounds more firm than apples from regular air storage in 1945, but only 0.9 pounds firmer in 1940. Also, he has shown (61) that delays in placing the fruit into storage will markedly reduce the effect of controlled atmospheres on softening, and that storage temperatures below 38°F reduced the amount of softening (59). These lower temperatures, however, resulted in the development of off-flavors and functional disorders of the fruit in some years.

As a result of nine years of study with McIntosh apples stored in regular air at 32°F, and in 5 percent carbon dioxide with 2 to 3 percent oxygen at 40°F, Smock (56) concluded that controlled atmosphere storage lengthened the normal storage life of McIntosh apples by 48 to 150 days. Delays in establishing the controlled atmospheres, such as initial holding in regular air storage for one and two months at 32°F, decreased the 150 days of extra storage life of controlled atmosphere apples by 105 to 125 days, respectively. Kidd and West (34) working with Bramley's Seedling apples at various temperatures and combinations of oxygen and carbon dioxide, concluded that atmospheric control was of greater effect upon retardation of the change in ground color from green to yellow than upon extension of the storage life of the apples.

The retarding effect of modified atmospheres on the hydrolysis of proto-

pectin to pectin in McIntosh, Northwestern Greening, and Cortland apples was demonstrated by Van Doren (66). Fisher (18) obtained similar results with Delicious apples. Smock and Allen (53) found a retardation of protopectin hydrolysis in Elberta peaches, Bartlett and Hardy pears, and Wickson plums as a result of storage in modified atmospheres.

Fisher (17) stored Delicious apples in 2.5 percent oxygen and 5.0 percent carbon dioxide; 2.5 percent oxygen and 0.0 percent carbon dioxide; and 25.0 percent oxygen and 5.0 percent carbon dioxide at 40°F and in regular air at 32°F. He found the modified atmospheres retained flavor, but hastened the development of flesh mealiness. Apples stored in the presence of carbon dioxide were more mealy than comparable apples stored in an atmosphere free of carbon dioxide. Schomer and Sainsbury (52), working in the Pacific Northwest with Delicious apples, reported storage in 3 to 4 percent oxygen and 0.3 and 2.4 percent carbon dioxide at 30°F provided fruit of better appearance and texture than 30°F regular air storage, but the modified atmospheres tended to destroy the characteristic varietal flavor. They found small differences in texture and flavor between fruit in "low oxygen and 2.4 percent carbon dioxide" and fruit stored "with low oxygen and only a trace of carbon dioxide". Since the average oxygen level and temperature were slightly higher in the storage with 2.4 percent carbon dioxide than in the one containing a trace of carbon dioxide, these factors may have offset the effect of the higher level of carbon

dioxide. Further comparison of these conditions would seem to be desirable.

Some of the earliest studies in this country concerning the carbon dioxide storage of produce were made by Thornton (63). With apples, he found that injury caused by carbon dioxide was a function of variety and temperature as well as concentration of carbon dioxide. As temperatures were increased from 0.0° to 25.0°C, increased amounts of carbon dioxide injury occurred in Dutchess, Gravenstein, Delicious and McIntosh apples.

Smock (61) has conclusively shown that the development of storage scald, a sometimes serious functional disorder of McIntosh, was greatly inhibited and sometimes prevented by storage in controlled atmospheres. A direct relationship between the amount of scald in McIntosh apples in May and the hours of delay at 70° F before the fruit was placed in storage was demonstrated (61). The rapidity of oxygen reduction from 21 percent to 3 percent in the fall when the room was sealed also affected scald. An oxygen reduction period of two weeks gave 1 percent scald in March, whereas a four-week reduction period yielded 50 percent scald at the end of storage. Prior to Smock's findings, West (68) had believed that apples were more likely to scald in controlled atmosphere storage than in air storage because of the accumulation in the storage of scald-causing volatiles produced by the apples. Vickery et al. (67) reported modified atmosphere storage sometimes increased storage scald of certain varieties in Australia.

Kidd and West (32) employed temperatures of 1°, 5° and 10°C with various concentrations of carbon dioxide and oxygen and found with Bramley's Seedling apples that altering the atmosphere within the temperature range of susceptibility increased internal browning, but atmospheric alterations above the critical temperature decreased the occurrence of this physiological disorder. They pointed out that within the temperature range of susceptibility high levels of carbon dioxide tended to increase the amount of internal breakdown to a greater extent than decreased levels of oxygen (34). Allen and Smock (1) found internal browning of Yellow Newtown apples was controlled by storage in balanced atmospheres (percent carbon dioxide plus percent oxygen equalled 21 percent) of either 5 or 10 percent carbon dioxide at 45° F. Smock (61) has shown that brown core of McIntosh could be avoided by storage at 38° to 40° F in controlled atmospheres. The unfavorable effects of the high temperature on quality retention were more than offset by the modification of the storage atmosphere. Vickery et al. (67) reported reductions in Jonathan spot and soft scald by storage in modified atmospheres at 2.2°C for the first four weeks, followed by four weeks at 1.1°C, and subsequent storage at 0.0°C. Dewey et al. (13) found controlled atmospheres at 32° F controlled Jonathan spot and inhibited soft scald of Jonathan apples.

Changes in the chemical composition of fruit in controlled atmosphere storage have been studied. According to Smock (58), low oxygen in storage

reduced the normal loss of ascorbic acid in Yellow Newtown apples. Dalbro and Gumble (12) found the total vitamin C loss was less in Bramley's Seedling and Cox's Orange Pippin apples in air at 3° than in "gas" storage at 4.5° C.

Kidd and West (38) studied the effect of four levels of oxygen (2.5, 5.0, 10.0 and 21.0 percent) in combination with four levels of carbon dioxide (0.5, 5.0, 10.0 and 15.0 percent) on the sugar-starch, acid, and alcohol insoluble fractions of Bramley's Seedling apples stored at 1° and 5° C. Since all three fractions showed significant losses in storage, it was believed these fractions all served directly or indirectly as sources of carbon for the carbon dioxide produced by respiration. Oxygen levels of 2.5 and 5.0 percent minimized losses of all three fractions. Increases in the concentration of carbon dioxide up to 10 percent decreased losses in the sugar-starch fraction. At 5° C increases in carbon dioxide concentration decreased losses in the acid fraction; whereas, at 1° C increases in carbon dioxide increased losses in the acid fraction. At 5° C increases in the carbon dioxide level up to 10 percent decreased losses in the alcohol insoluble fraction; at 1° C, increases in carbon dioxide up to 15 percent decreased losses in the alcohol insoluble fraction. They further observed that increased carbon dioxide concentrations were much more effective than oxygen concentrations as low as 2.5 percent in reducing losses in these fractions.

The many studies of the post-harvest respirational activity of apples have

shown there is a normal pattern of respiratory behavior. As the apple matures its respiration rate decreases to a minimum level, which is followed by a period of increased rate of respiratory activity known as the climacteric rise, culminating in a peak rate of respiration or the climacteric. Following the climacteric, respiration declines slowly throughout the life of the apple (39, 35). The effects of modifying the atmosphere surrounding the fruit upon this respiratory pattern are of interest.

Hulme (28) demonstrated a direct proportionality between respiration rate and the protein nitrogen content of Bramley's Seedling apples. He found a strong negative correlation between the change in protein content and the change in asparagine content during the period of decreased respiration preceding and during the climacteric rise. In other words, during the climacteric rise there was a steady decrease in asparagine content and a steady increase in protein content. When ethylene gas was used to stimulate the climacteric rise in later studies (29), he found a net rise in protein content. Hulme et al. (27) further demonstrated that delaying the climacteric rise of Bramley's Seedling apples by treatment with 10 percent carbon dioxide and 20 percent oxygen at 10°C also delayed the increase in protein nitrogen. The apples stored in the high carbon dioxide atmosphere respired more slowly and had a lower percentage protein nitrogen than comparable apples stored in air at the same temperature throughout the 150-day period of study. Van Doren (66) found

the ratio of protein nitrogen to total nitrogen was lower in controlled atmosphere-stored McIntosh at 40° than in McIntosh stored in air at 32° F throughout the storage season.

Kidd and West (36) reported the first extensive studies of the effect of carbon dioxide and oxygen on the respiration rate of apples. They found lowering the temperature of fruit decreased the height and steepness of the climacteric rise; increased levels of carbon dioxide and decreased levels of oxygen suppressed or postponed the climacteric and decreased the respiration rate; the minimum level of oxygen, below which alcohol formed, increased with increasing age of the fruit. They found (37) that preclimacteric apples showed an immediate but transitory increase in respiratory activity when submitted to higher than normal concentrations of carbon dioxide at storage temperatures. Kidd and West (33) showed that oxygen levels of 1 to 2 percent depressed the respiration rate of Conference pears at 1° C by 50 percent, whereas, 5 percent oxygen depressed respiration only slightly when compared to regular air, and 100 percent oxygen caused a respiration rate that was practically the same as in regular air. Smock (54) reported that McIntosh apples stored in regular air at 32° F respired two to three times faster than comparable fruit stored at 40° F in 2 percent oxygen and 5 percent carbon dioxide.

The effect of modified atmospheres on respiratory activity varies with

the kind of fruit. Higher than normal oxygen tensions had no effect on the position and magnitude of the climacteric of avocado (5), but caused a consistently higher climacteric peak of the respiration curve and increased carbon dioxide evolution in lemons (6).

Biale (5) found a close correlation between oxygen uptake and carbon dioxide evolution throughout the climacteric rise of avocado at 15°C. Since dinitrophenol had no effect on respiration rate during the climacteric, Miller, Bonner and Biale (43) concluded that the climacteric rise in avocado was due to a natural uncoupling of the oxidative phosphorylation system from the electron transfer system.

Another hypothesis, which may not conflict with the above, on the cause of the climacteric in apples was postulated by Pearson and Robertson (47). With Granny Smith apples, they attributed the climacteric rise in respiration to a shift in adenosine-triphosphate/adenosine-diphosphate (ATP/ADP) ratio. The addition of ATP, 2, 4-dinitrophenol (DNP), sodium succinate, sodium citrate, and sodium malate to apple slices brought about an increase in oxygen uptake, but the Krebs' cycle acids increased oxygen uptake only during the climacteric rise. They believed the concentration of adenosine-phosphate remained about constant, but a relatively low amount of ADP before the climacteric rise held respiration at a low level. It was conjectured that at the time of the climacteric rise, the maintenance of protein and other unstable

compounds already synthesized made high demands on the energy carrying system. These demands caused a drop in the ATP/ADP ratio, and the subsequent build-up of ADP allowed for an increase in respiration.

The theory of Hulme and Neal (30) and Neal and Hulme (45) on the cause of the climacteric rise differed from the two mentioned above. Their work with Bramley's Seedling apples revealed an increase in the respiration quotient during the climacteric rise. They found that addition of malate to tissue slices brought about the following reaction under aerobic conditions:  $\text{malate-2H} \rightarrow \text{pyruvate plus CO}_2 \rightarrow \text{acetaldehyde plus CO}_2$ . Oxygen uptake was not influenced by this malate decarboxylation. Addition of succinate, on the other hand, stimulated oxygen uptake as well as carbon dioxide production. They further found that malate injected into preclimacteric Bramley's Seedling apples caused a higher and earlier climacteric rise. They theorized that the increased carbon dioxide production during the climacteric rise at least in part was due to this malate decarboxylation reaction.

The magnitude of the effect of apple volatiles upon the respiration rate of other apples according to Smock (58) depended upon: varieties, temperature, sample size, and the variety and degree of ripeness of the apples providing the emanations.

To substantiate their observation that ripening fruit caused considerable damage to green fruit in "gas" storage, Kidd and West (40) stored Bramley's

Seedling apples in atmospheres of 10 percent carbon dioxide and 15 to 20 percent oxygen at 10° C, and added ethylene (1,000 ppm) for two days at three different times. The respiration rate of the fruit was stimulated but recovered from the effects of the first two applications. The third treatment caused stimulation without recovery, which indicated that the successive dosages were additive. Griffiths and Potter (19), working with King Edward VII apples, found respiration activity was highest in air, intermediate in 8 percent carbon dioxide and 13 percent oxygen when the fruit volatiles were allowed to accumulate, and lowest under the same levels of carbon dioxide and oxygen when the volatiles were flushed away continuously. Fidler (15) reduced respiration by 20 percent for apples in "gas" storage when the ethylene was constantly removed from the storage room atmosphere.

Artsikhovskaya and Sokolova (4) found that lowering the oxygen concentration had no effect on the concentration of ethyl alcohol in the flesh of apple fruit, but increased the acetaldehyde concentration. The temperature coefficient for ethyl alcohol formation was approximately one, and 1.2 to 1.8 for acetaldehyde formation. Thompson and Heulin (62) found that storage of Granny Smith apples in 6 percent oxygen at 20° C decreased the amount of alcohol in the flesh when compared with check fruit stored in regular air at the same temperature. Hulme (26) has shown temperature had a greater effect on alcohol and acetaldehyde accumulation of Worcester Pearman apples at 1° and 4° C in regular air than in an atmosphere of 5 percent carbon dioxide

and 2.5 percent oxygen. After 225 days at 1°C, the apples stored in air had seven times as much alcohol and three times as much acetaldehyde as the apples stored under the modified atmosphere. At the end of the same storage period at 4°C, apples stored in regular air had 16 times as much alcohol and eight times as much acetaldehyde as comparable fruit stored in the modified atmosphere. These workers (62, 26) felt the accumulation of alcohol and acetaldehyde in the regular air stored fruit was caused by a gradual failure in the oxidative systems which accompanied normal senescence.

Potter and Griffiths (50) found the rate of evolution of ethylene and odorous fraction gases by King Edward VII apples was slower in an atmosphere of 5 percent oxygen and 10 percent carbon dioxide at 15°C than in regular air at the same temperature. Fidler (15) found the rate of volatile evolution by apples was slower under modified atmosphere conditions than in air at 38°F. However, the concentration of volatiles was higher in "gas" storage rooms than in regular air storage rooms (15). He found the level of ethylene was higher, often greatly, than the odorous fraction of volatiles in these storages (14). He estimated the loss of carbon in the form of volatile compounds was about 0.3 percent of that lost as carbon dioxide. Griffiths and Potter (19) reported the accumulation of volatiles in "gas" storage rooms decreased the rate of volatile production by the apples. They found the ethylene to odorous fraction ratio was greater when the volatiles were allowed to accumulate than when all the volatiles were constantly flushed from the 8 percent carbon

dioxide and 13 percent oxygen chambers. Fidler (16) practically eliminated ethylene gas from a sealed storage by ozonization, but the apples developed severe lenticel spotting. When ozonization was discontinued after 170 days of storage, the concentration of ethylene increased rapidly.

### The Residual Effect

An extended shelf life of apples removed from storage under modified atmospheres has been reported by Kidd and West (36), Allen and Smock (1), Van Doren (66), Smock (54), Rasmussen (51), and Dewey et al. (13). Van Doren (65) used the term residual effect "to describe the partially inactivated condition of fruit which is induced by the modified atmosphere treatment, i. e., the normal rate of softening of the apple tissue as well as the green-to-yellow color changes of the skin of the fruit are markedly retarded both during and after the period of storage".

Smock (54) reported controlled atmosphere-stored McIntosh apples went through a normal respiratory curve in air at room temperature when removed from storage in January, March, and May. At no time did the controlled atmosphere fruit respire as rapidly as the air-stored fruit. Controlled atmosphere-stored fruit removed from storage in May and placed at 32° F in air respired at a rate midway between the respiration rate of the control fruit at 32° F and the controlled atmosphere fruit in the 5 percent carbon dioxide and

2 percent oxygen atmosphere at 40°F. Fidler (15) explained that the immediate but temporary flush of carbon dioxide from fruit stored under high carbon dioxide conditions and then placed in air was due to the release of carbon dioxide dissolved in the cell sap. Smock (54) found controlled atmosphere-stored apples had four times as much carbon dioxide in the flesh as air-stored apples, but within 48 hours the carbon dioxide in the flesh of both was about equal. He conjectured that the oxygen levels reacted similarly.

Mattus (12) stored Bartlett pears at various levels of carbon dioxide and oxygen at 33°F and measured the ethylene evolution at 74°F after removal from storage. In October the 33°F regular air-stored fruit reached the highest peak of ethylene evolution, but after eight days at 74°F, the fruit of all treatments were evolving ethylene at about the same rate. In December, after 93 days storage, the ethylene evolution rate of the controlled atmosphere pears rose to a peak in its curve and then declined over the eight-day measuring period at 74°F. The regular air-stored control fruit had a higher initial rate, but the rate of ethylene evolution steadily declined until at the end of the eight-day period, the control fruit evolved less ethylene than any of the controlled atmosphere-stored pears.

## METHODS AND MATERIALS

The McIntosh apples for the experiments of the 1957-1958 storage season were grown in two areas; lot A was harvested from two trees in a commercial orchard near Sparta, Michigan on September 24, whereas lot B was picked on September 18 from a single tree in the Michigan State University orchard. The fruit of each lot was randomized, composited and placed in storage at 32° F within 12 hours after picking. Except where otherwise noted, the data in the 1957-1958 storage season were obtained for the fruit of lot A. The apples used for the 10- and 20-day delay treatments were stored at 32° F until placed in the controlled atmosphere storage chambers.

For the 1958-1959 storage season, the fruit of lot A, which was used for all experiments during this storage season, unless otherwise noted, was picked on September 26 in the same block that was used for the previous year's lot A but from two different trees. Lot B came from a single tree in a commercial orchard in the Belding area on September 19. Lot C was harvested from a single tree in a commercial orchard in the Sparta area on September 26. Lot D was harvested from a single tree on the University farm in East Lansing on September 17. All fruit was composited and placed at 32° F within 24 hours after picking.

The control (check) fruit was stored in regular air in a refrigerated apple storage room (7 ft. x 14 ft. x 10 ft.) at 32-33° F in the Horticulture building. In 1957 the controlled atmosphere (CA) fruit was sealed into the controlled

atmosphere chambers on October 8, after which the temperature was raised to 38°F. On October 23 the carbon dioxide level reached 3 percent where it was maintained until November 17. It was then increased to 5 percent and was maintained at this level for the remainder of the storage season. The required oxygen level of 3 percent was attained October 17. In the 1958-1959 season the controlled atmosphere chambers were sealed on September 29 and the temperature was raised to 38°F. The carbon dioxide level reached 3 percent on October 6 and was maintained there until November 21 when it was raised to 5 percent for the remainder of the storage period. The oxygen level reached 4 percent by October 21 and was then lowered to 3 percent for the storage season.

The arrangement of controlled atmosphere equipment is shown in Figure 1. Carbon dioxide and oxygen levels in chamber A were determined daily with an orsat gas analyzer and with a very few exceptions, the levels of carbon dioxide and oxygen were held within 0.5 percent of the desired levels throughout the two storage seasons. Periodic checks of the composition of the atmosphere in experimental chambers C (Figure 1) revealed that the levels of carbon dioxide and oxygen in the chambers were within a few tenths of 1 percent of the levels in master chamber A.

Flesh firmness of the apples was determined with a Magness-Taylor pressure tester using a 7/16 inch diameter tip, which was applied to the

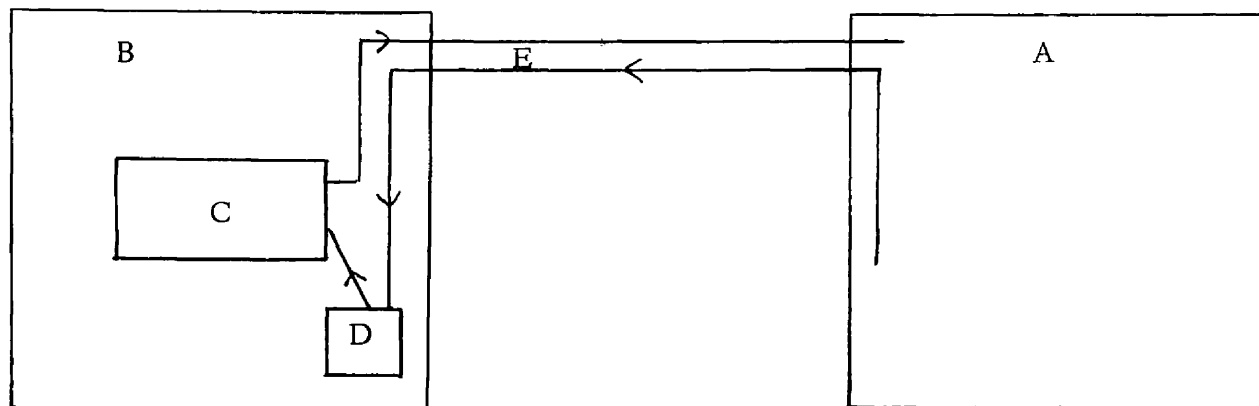


Figure 1. A 38-bushel capacity sealed chamber (described by Pflug et al. (49) ) located at A served as the master chamber for establishing and maintaining the controlled atmosphere. Metal chambers (C) with dimensions of 15 x 17.5 x 40 inches and sealed with Plexiglass covers contained 2 bushels of fruit in a separate refrigerated room, B. The atmosphere of A was circulated at a rate of 10 changes per hour through the 12 chambers C by a series of six auto fuel pumps (D) and the connecting 1-inch insulated pipes (E). This system enabled the periodic removal of fruit samples from C without disruption of the atmospheres.

pared green cheek and the opposite red cheek of each fruit. Twenty to thirty apples of 2 1/2 to 3 inches diameter were tested for each treatment. The Cornell color chart (60) was used to determine ground color of the skin. The soluble solids content of the composite juice of the fruit was measured with a Ziess Opton hand refractometer.

A panel of five to six persons, experienced in evaluating fruit quality, was utilized for judging appearance, texture, flavor and ripeness characteristics of the apples. The following tabulation sheet was used by the judges in making and recording their evaluations:

|                        |                    |                     |                       |
|------------------------|--------------------|---------------------|-----------------------|
| Plate _____            |                    | Date: _____         |                       |
| <u>Appearance</u>      | <u>Texture</u>     | <u>Flavor</u>       | <u>Ripeness</u>       |
| ___ fresh              | ___ hard           | ___ fresh (acid)    | ___ fresh             |
| ___ firm ripe (bright) | ___ crisp          | ___ slightly acid   | ___ firm ripe         |
| ___ ripe               | ___ melting        | ___ sweet           | ___ ripe              |
| ___ dull               | ___ slightly mealy | ___ ripe            | ___ slightly overripe |
| ___ unmarketable       | ___ mealy          | ___ lacking         | ___ overripe          |
|                        |                    | describe off flavor |                       |

Numerical evaluation of the observations was made by assigning the highest quality under each of the headings a value of five, the next highest quality was assigned a value of four, etc. Whole fruits were used the first year, whereas in the second year the apples were peeled, cored and quartered for the flavor, texture and ripeness evaluations. This was necessary to eliminate judgments influenced by wide differences in the skin characteristics of the apples. All lots were judged simultaneously the first year. In the second year,

the appearance of whole apples from the four different orchards was judged as follows: six plates were made up from each lot of apples; three plates consisted of apples from the CA treatment of the lot, and three using the apples from the regular air storage treatment of the lot. These six plates were arranged into two groups: one group contained two plates of CA stored apples, and one plate of air stored apples, while the other group contained two plates of air stored apples, and one plate of CA stored apples. Since there were four lots of apples, there was a total of 24 plates in the test. The judges were instructed to record which plate in each three-plate group was different from the other two plates in the same group. Thus, each judge made two comparisons of the CA apples with the regular air-stored apples for each lot. Since six judges were involved in the evaluation, twelve comparisons in each lot were made.

Apple skin toughness was measured by two methods. The first method consisted of puncturing the skin of intact fruit with a Chatillon<sup>1/</sup> dynamometer with a 0.8 mm wire tip. The pressures required to puncture the skin at four points around the circumference were recorded. With the second method, a portion of the peel was removed from the fruit and placed in a beaker of water. The epidermal tissues then were carefully scraped free of cortical tissue and

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<sup>1/</sup> Manufacturer: John Chatillon and Sons, 85-93 Cliff Street, New York 38, N. Y.

placed between the halves of a gum-rubber lined strap hinge through which had been drilled a hole to accommodate the 5/16-inch diameter tip of a Magness-Taylor pressure tester. The hinge was held tightly together with a pair of Vise-Grips. The pressure needed to break the skin was recorded to the nearest one-third pound. The quantity of cell wall material of the cortex tissue was determined for apples which had been peeled and cored. Twenty gram samples of cortical tissue were ground in 200 ml of 80 percent ethanol in a Waring blender for approximately 10 minutes. The ground material and ethanol were transferred quantitatively to a 500 ml Erlenmeyer flask and boiled for 30 minutes. The boiled material was then filtered on a Buchner funnel fitted with a tared number five filter paper and washed three times with 80 percent ethanol. The filter papers and residue, which was made up of all polysaccharides (pectin substances, starch if present, hemi-cellulose, cellulose, and lignin if present) and very small amounts of protein, were dried for 48 hours at 75°C and weighed.

The Claypool and Keefer (11) colorimetric method for carbon dioxide evolution was used for all respiration determinations. Each new solution of 0.0025N sodium bicarbonate and 0.0002 percent phenol red was calibrated at the two storage temperatures of 75° and 33° F. Carbon dioxide gas was not removed from the air supply before it was passed over the fruit, but a correction factor for the 0.03 percent carbon dioxide in the air was subtracted

from the colorimeter solution calibration data before the final calibration curve was drawn. Air flow rates were adjusted between 250 and 400 ml of air per minute at the beginning of each respiration study at 75°F to bring the color of the colorimeter solution into the straight portion of the calibration curve. Usually four kilograms of fruit were placed in each respiration jar, but two and three kilogram samples were sometimes used. All treatments were run in duplicate.

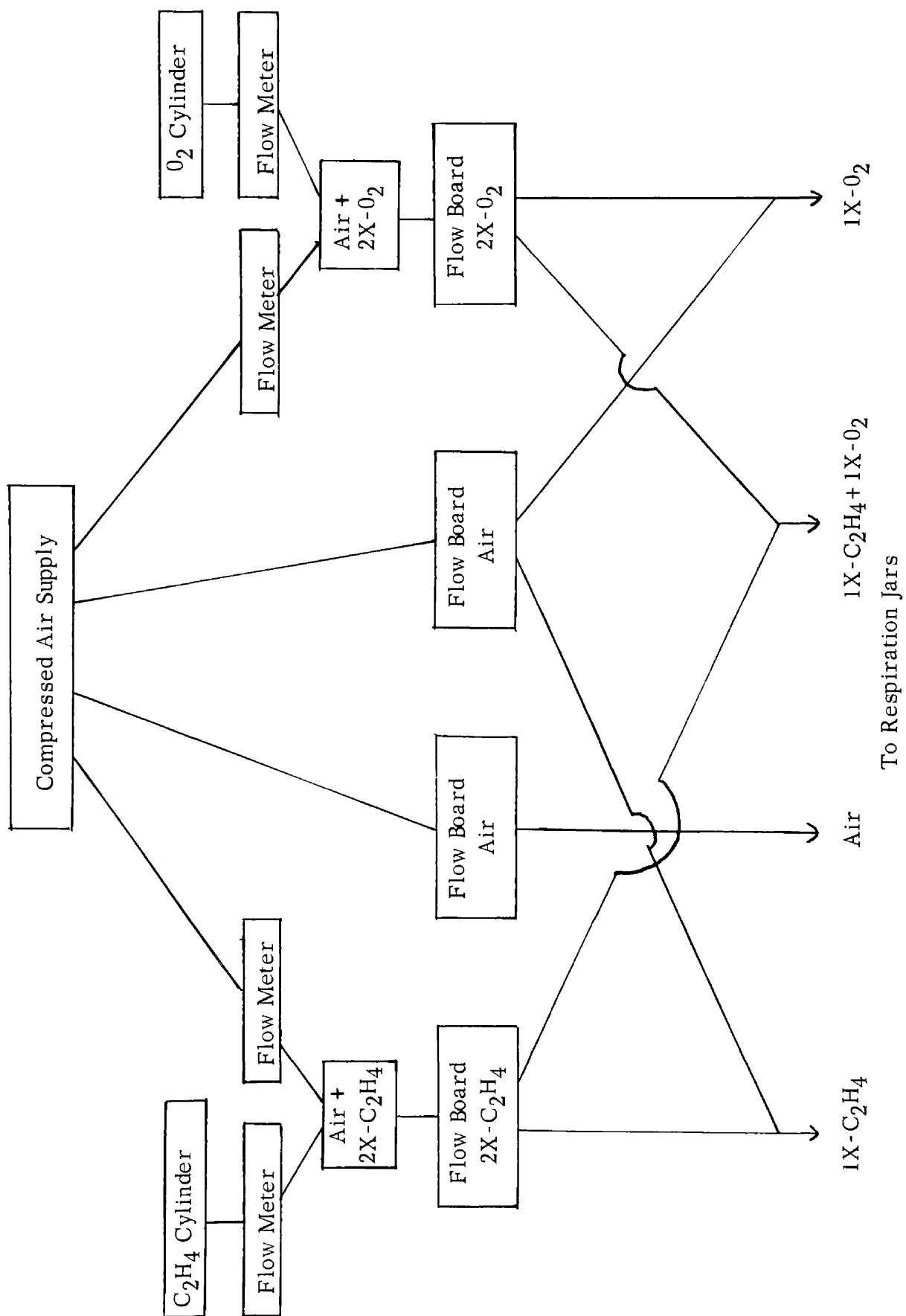
Figure 2 illustrates the arrangement of equipment used for the study of the effect of ethylene and high concentrations of oxygen on respiration. Four kilograms of fruit in duplicate samples were used at 75°F.

The effect of apple volatiles on respiration was studied by periodically placing a jar of apples between the calibrated flow board and the jar of apples to be treated with apple volatiles. The carbon dioxide evolved by the apples which supplied the volatiles was flushed from the jars for 24 hours before respiration measurements were made.

Respiration quotients were calculated from the composition of the air in the respiration jars immediately after the apples in the respiration jars had been flushed with normal air and then again after the bottles had been sealed for six hours. The oxygen and carbon dioxide contents were measured quantitatively with an orsat gas analyzer.

Polyphenolase activity was measured by the manometric procedures

Figure 2. Diagram of equipment arrangement for supplying ethylene and high concentrations of oxygen to apples for respiration studies.



described by Hackney (20). Catechol was used as the substrate and O-phenylenediamine was added with the substrate to remove the quinone which was oxidized from the catechol. A phosphate buffer of pH 5.5 (25) was prepared as the bathing solution for the tissue slices in the reaction vessels. All tests were made in duplicate.

The aldehyde evolution from duplicate four-kilogram fruit samples was determined by the methods outlined by Milton and Walters (44). The iodine solution was prepared and standardized according to Leiniger and Stone (41).

Ethylene evolved from three- to four-kilogram samples in duplicate was collected and determined manometrically by the method of Young, Pratt and Biale (69). One modification, however, was that 18 ml single side-arm reaction vessels were used for the release of ethylene from the aliquots instead of the specially designed reaction vessels described.

## RESULTS

### Flesh Firmness

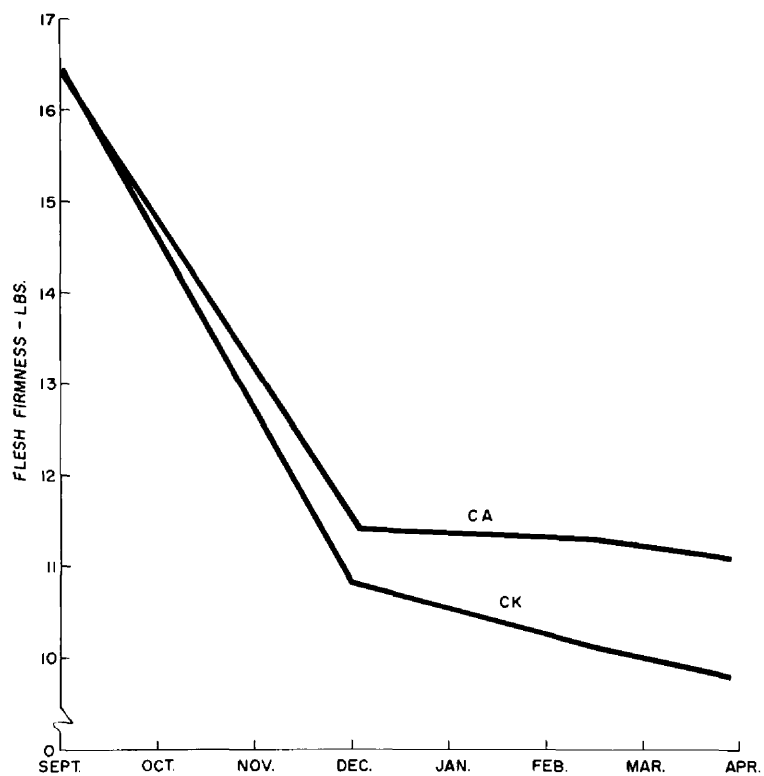
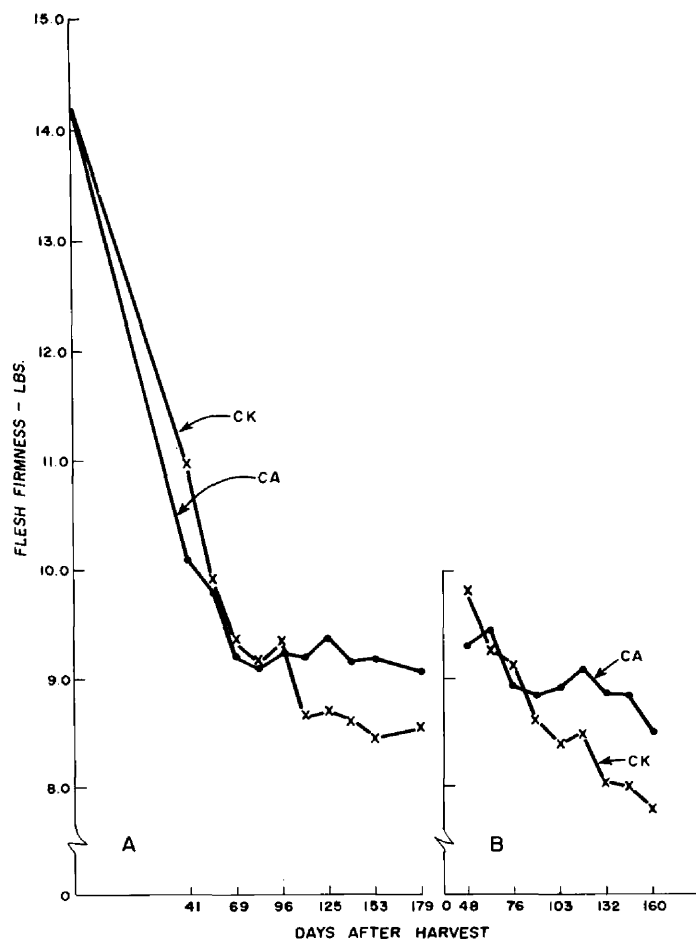
It has been demonstrated (21) that comparable measurements of flesh firmness with the pressure tester are best attained with apples of uniform temperatures. Tests of fruit firmness upon removal from storage, therefore, were made after warming the apples to 75°F. This was accomplished by holding the fruit at this temperature in normal air for one day before pressure testing.

The changes in flesh firmness of the apples at intervals during the 1957-1958 storage period are shown in part A of Figure 3. During the first half of the storage season the Check apples were more firm than the CA apples. At the midpoint of storage, however, the flesh firmness of the CA apples leveled off slightly above 9.0 pounds and then remained fairly constant, whereas the Check apples continued to decline in pressure to 8.5 pounds before leveling off.

The same general trend of flesh softening was evident in the 1958-1959 storage season, as shown in Figure 4, with the CA fruit being more firm than the Check fruit at three months. Since measurements were not made until after three months of storage in the second year of study, it is not known if the CA apples softened more rapidly than the Check apples during the early part of the storage period.

Figure 3. Flesh firmness of Check and CA apples at various storage removal dates during the storage season of 1957-1958. A - after one day at 75° F; B - after seven days at 75° F.

Figure 4. Flesh firmness of Check and CA apples at harvest and after storage to December, February, and March of the 1958-1959 storage season.



In commercial practice, apples are sometimes held at 33° F for several days before the CA rooms are sealed. For this reason, samples of apples were held at 33° F for 10 and 20 days before placement into the CA chambers in the fall of 1957. The effect on flesh firmness of these delays is shown in Table I. Storage for 10 and 20 days in regular air at 33° F before placing under controlled atmosphere conditions had little effect on the flesh firmness in late December. When measured in late January, the apples held at 33° F in regular air for 10 and 20 days before placement into CA storage, were slightly softer than apples stored in the CA chambers immediately at harvest time.

TABLE I

The Effect of Delaying the Placement of Apples into CA Storage on Flesh Firmness (Pounds) After Storage

| Storage Treatment               | Removed from Storage<br>December 29, 1957 |                             | Removed from Storage<br>January 26, 1958 |                             |
|---------------------------------|-------------------------------------------|-----------------------------|------------------------------------------|-----------------------------|
|                                 | After 1<br>day at<br>75° F                | After 7<br>days at<br>75° F | After 1<br>day at<br>75° F               | After 7<br>days at<br>75° F |
| CK - immediate storage at 33° F | 9.3                                       | 8.4                         | 8.7                                      | 8.1                         |
| CA - immediate storage at 38° F | 9.2                                       | 9.0                         | 9.4                                      | 8.8                         |
| 10-day delay at 33° F           | 9.3                                       | 8.7                         | 8.4                                      | 8.4                         |
| 20-day delay at 33° F           | 9.4                                       | 8.6                         | 9.1                                      | 8.6                         |

The changes in flesh firmness of CA and Check apples during seven days at 75° F after removal from storage in the 1957-1958 storage season are given in part B of Figure 3 and in Table II. The patterns of softening for both CA and Check apples, as shown in Figure 3-B, were similar to the firmness patterns at the time of removal from storage. Fruit held in CA for 153 days and then one week at 75° F were slightly more firm than the Check apples at the time of removal from storage at 153 days. The amount of flesh firmness loss (see Table II) of CA and Check apples during the seven-day holding periods was extremely variable. The average loss in flesh firmness during the 75° F holding periods was 0.4 pounds for the CA apples and 0.6 pounds for the Check apples. The average differences in loss of flesh firmness were not significant.

Apples were harvested from four orchards and samples from each orchard were placed in the CA chambers and in regular air storage in September, 1958. The apples were removed from storage in March and tested after one and seven days at 75° F after eight weeks at 33° F. The CA apples were firmer than the Check apples at all examinations. The data, presented in Table III, revealed pronounced differences in the effect of storage treatment on flesh firmness loss of the apples from the four orchards during the seven days holding period at 75° F. This loss in flesh firmness was as little as 0.2 and 0.4 pounds and as great as 1.2 and 1.3 pounds for the CA and

TABLE II

Flesh Firmness (Pounds) of Apples After Storage for Various Lengths of Time in the 1957-1958 Season

| Date of Removal<br>From Storage | CA Apples                           |                                      |                                         | Check Apples                        |                                      |                                         |
|---------------------------------|-------------------------------------|--------------------------------------|-----------------------------------------|-------------------------------------|--------------------------------------|-----------------------------------------|
|                                 | Firmness<br>After 1 Day<br>at 75° F | Firmness<br>After 7 Days<br>at 75° F | Firmness<br>Loss During<br>75° F Period | Firmness<br>After 1 Day<br>at 75° F | Firmness<br>After 7 Days<br>at 75° F | Firmness<br>Loss During<br>75° F Period |
| Nov. 4, 1957                    | 10.1                                | 9.3                                  | 0.8                                     | 11.0                                | 9.8                                  | 1.2                                     |
| Nov. 18, 1957                   | 9.8                                 | 9.4                                  | 0.4                                     | 9.9                                 | 9.3                                  | 0.6                                     |
| Dec. 2, 1957                    | 9.2                                 | 8.9                                  | 0.3                                     | 9.4                                 | 9.2                                  | 0.2                                     |
| Dec. 17, 1957                   | 9.1                                 | 8.8                                  | 0.3                                     | 9.1                                 | 8.7                                  | 0.4                                     |
| Dec. 30, 1957                   | 9.2                                 | 8.9                                  | 0.3                                     | 9.3                                 | 8.4                                  | 0.9                                     |
| Jan. 13, 1958                   | 9.2                                 | 9.1                                  | 0.1                                     | 8.6                                 | 8.5                                  | 0.1                                     |
| Jan. 27, 1958                   | 9.4                                 | 8.8                                  | 0.6                                     | 8.7                                 | 8.1                                  | 0.6                                     |
| Feb. 10, 1958                   | 9.2                                 | 8.9                                  | 0.3                                     | 8.6                                 | 8.1                                  | 0.5                                     |
| Feb. 24, 1958                   | 9.2                                 | 8.5                                  | 0.7                                     | 8.5                                 | 7.8                                  | 0.7                                     |
| Average                         |                                     |                                      | 0.4                                     |                                     |                                      | 0.6                                     |

$t' : .05 = 1.74$ ; differences in firmness loss during 7 days at 75° F = 1.39.

TABLE III

Flesh Firmness (Pounds) of Apples from Four Orchards Before and After Storage to March, 1959

| Orchard | Before<br>Storage | Storage Plus<br>1 Day at 75°F |      | Storage Plus<br>7 Days at 75°F |     | Loss During<br>6 Days at 75°F |     | Storage Plus<br>8 Weeks at 33°F |     |
|---------|-------------------|-------------------------------|------|--------------------------------|-----|-------------------------------|-----|---------------------------------|-----|
|         |                   | Ck                            | CA   | Ck                             | CA  | Ck                            | CA  | Ck                              | CA  |
| A       | 16.5              | 8.8                           | 11.1 | 8.4                            | 9.9 | 0.4                           | 1.2 | 8.9                             | 9.9 |
| B       | 14.9              | 8.3                           | 9.5  | 7.0                            | 8.6 | 1.3                           | 0.9 | 8.2                             | 9.2 |
| C       | 15.1              | 8.6                           | 9.6  | 7.5                            | 8.6 | 1.1                           | 1.0 | 8.2                             | 9.2 |
| D       | 16.0              | 9.1                           | 9.3  | 8.1                            | 9.1 | 1.0                           | 0.2 | 9.0                             | 9.6 |
| Average | 15.6              | 8.7                           | 9.9  | 7.8                            | 9.1 | 1.0                           | 0.8 | 8.6                             | 9.5 |

Check apples, respectively. There was no significant difference in the average values for flesh firmness loss during this period.

#### Panel Evaluations of Quality

Quality evaluation of apples removed from CA and regular air storage at two-week intervals during the 1957-1958 storage season are presented in Figure 5. No marked distinctions between storage methods were detected by the panel for fruit examined immediately upon removal from storage or after holding at a high temperature until approximately 100 days of storage had elapsed. Thereafter the CA apples consistently were judged to be of better appearance, texture, flavor, and considered to be less ripe than the Check apples.

The effect of delays in placing the apples into CA storage on the appearance, texture, flavor, and ripeness of the apples when taken from storage in late December and late January is shown in Table IV. The apples stored immediately in CA, with only one exception, were superior to the Check apples at all examinations. This exception was for appearance on December 30, 1957. The appearance, texture, flavor, and ripeness evaluations of all samples decreased during the 75° F holding periods, but the average loss in quality of the Check apples was greater than the average loss in quality of the CA apples during these periods.

Figure 5. Panel ratings of CA and Check apples removed from storage at intervals throughout the 1957-1958 season, and held one and seven days at 75° F.

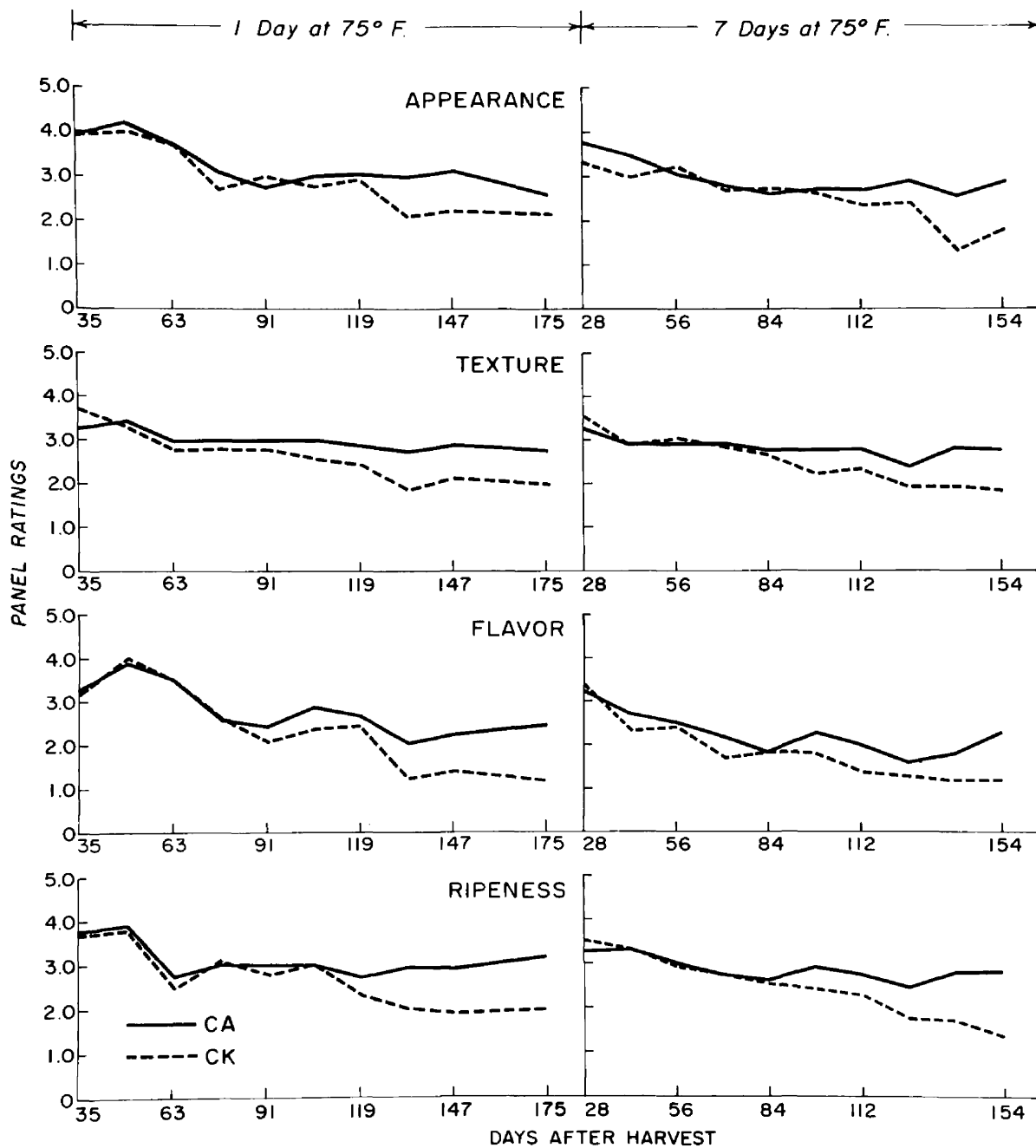


TABLE IV

The Effect of Storage Treatment and Holding Period at 75°F on Quality Rating<sup>a/</sup>

| Storage Treatment                             | Appearance |        | Texture |        | Flavor |        | Ripeness |        | Average |        |
|-----------------------------------------------|------------|--------|---------|--------|--------|--------|----------|--------|---------|--------|
|                                               | 1 day      | 7 days | 1 day   | 7 days | 1 day  | 7 days | 1 day    | 7 days | 1 day   | 7 days |
| <u>Removed from Storage December 29, 1957</u> |            |        |         |        |        |        |          |        |         |        |
| Ck - immediate storage at 33°                 | 3.0        | 2.6    | 2.8     | 2.2    | 2.1    | 1.8    | 2.8      | 1.9    | 2.7     | 2.1    |
| CA - immediate storage at 38°                 | 2.8        | 2.7    | 3.0     | 2.8    | 2.5    | 2.3    | 3.0      | 2.4    | 2.8     | 2.6    |
| 10-day delay at 33°                           | 2.8        | 3.0    | 3.0     | 2.6    | 2.0    | 2.0    | 3.0      | 2.6    | 2.7     | 2.6    |
| 20-day delay at 33°                           | 2.8        | 2.8    | 2.8     | 2.8    | 1.8    | 2.0    | 2.8      | 2.6    | 2.6     | 2.6    |
| <u>Removed from Storage January 26, 1958</u>  |            |        |         |        |        |        |          |        |         |        |
| Ck - immediate storage at 33°                 | 2.9        | 2.4    | 2.4     | 1.9    | 2.4    | 1.3    | 2.3      | 1.7    | 2.5     | 1.8    |
| CA - immediate storage at 38°                 | 3.0        | 2.9    | 2.9     | 2.4    | 2.7    | 1.6    | 2.7      | 2.5    | 2.8     | 2.6    |
| 10-day delay at 33°                           | 3.3        | 2.6    | 2.9     | 2.8    | 2.3    | 1.7    | 2.6      | 2.3    | 2.8     | 2.6    |
| 20-day delay at 33°                           | 2.7        | 2.5    | 2.8     | 2.3    | 2.0    | 1.5    | 2.4      | 2.5    | 2.4     | 2.2    |

<sup>a/</sup> See Methods and Materials for the basis of the ratings.

The panel ratings of texture, flavor, and ripeness of apples from the four orchards are tabulated in Table V. The CA apples from each orchard were of better texture and flavor and were less ripe than the Check apples from the same orchard. The beneficial effects of CA storage on each of the quality characteristics, according to the panel rating differences, were variable for the fruit of the different orchards. The importance of growing conditions on quality was indicated by the higher quality of Check apples than of CA apples from another orchard. For example, the CA apples from orchard C, although superior to the Check apples from the same orchard, were rated as inferior to the Check apples of orchard D. Also, the degree of response of apples to CA storage was variable. For instance, the panel found the CA apples from orchard C averaged only 0.6 rating points better than the Check apples from the same orchard; whereas they scored a difference of 1.4 rating points between the CA and Check apples from orchard B.

#### Condition

Comparisons of the condition of the apples from four orchards when removed from storage in March, 1959 may be made from the data of Table VI. The soluble solids contents of the extracted juice of the apples were not affected by storage treatment. There was greater skin shrivel of the Check apples than of the CA apples. After a holding period of one week at 75° F, none of the CA apples were affected with senescent core browning, whereas 20 to 95 percent

TABLE V

Panel Ratings of Texture, Flavor and Ripeness of Apples from Four Orchards Stored to April, 1959 and then Held for Four Days at 75°F

| Orchard | Texture |     |       | Flavor |     |       | Ripeness |     |       | Average |     |       |
|---------|---------|-----|-------|--------|-----|-------|----------|-----|-------|---------|-----|-------|
|         | CA      | Ck  | Diff. | CA     | Ck  | Diff. | CA       | Ck  | Diff. | CA      | Ck  | Diff. |
| A       | 3.2     | 2.6 | 0.6   | 3.4    | 2.9 | 0.5   | 3.8      | 3.0 | 0.8   | 3.5     | 2.8 | 0.7   |
| B       | 3.0     | 1.8 | 1.2   | 3.5    | 1.8 | 1.7   | 3.3      | 2.1 | 1.2   | 3.3     | 1.9 | 1.4   |
| C       | 2.1     | 1.5 | 0.6   | 1.8    | 1.2 | 0.6   | 2.1      | 1.4 | 0.7   | 2.0     | 1.4 | 0.6   |
| D       | 3.0     | 2.6 | 0.4   | 3.1    | 1.9 | 1.2   | 2.8      | 2.4 | 0.4   | 3.0     | 2.3 | 0.7   |
| Average | 2.8     | 2.1 | 0.7   | 3.0    | 2.0 | 1.0   | 3.0      | 2.2 | 0.8   | 3.0     | 2.1 | 0.9   |

TABLE VI

Condition of Apples from Four Orchards Held One and Two Weeks at 75°F Following Removal from Storage in March, 1959

| Orchard | Storage Treatment | Soluble Solids <sup>a/</sup> |  | Skin Shivel <sup>b/</sup> |  | Senescent Core Browning |             |          |  | Storage Scald |             | Mealy Breakdown |             |
|---------|-------------------|------------------------------|--|---------------------------|--|-------------------------|-------------|----------|--|---------------|-------------|-----------------|-------------|
|         |                   | 1 week (%)                   |  | 1 week (rating)           |  | 1 week (%)              | 2 weeks (%) | (amt)    |  | 1 week (%)    | 2 weeks (%) | 1 week (%)      | 2 weeks (%) |
| A       | CA                | 12.0                         |  | Slight                    |  | 0.0                     | 8.8         | Trace    |  | 0.0           | 0.0         |                 | 0.0         |
|         | Ck                | 12.0                         |  | Moderate                  |  | 25.0                    | 77.4        | Moderate |  | 74.3          | 95.2        |                 | 22.6        |
| B       | CA                | 10.5                         |  | Slight                    |  | 0.0                     | 30.4        | Trace    |  | 2.9           | 2.2         |                 | 0.0         |
|         | Ck                | 10.5                         |  | Mod-severe                |  | 95.0                    | 100.0       | Severe   |  | 91.4          | 100.0       |                 | 10.9        |
| C       | CA                | 11.5                         |  | Slight                    |  | 0.0                     | 16.7        | Trace    |  | 17.1          | 27.0        |                 | 0.0         |
|         | Ck                | 11.0                         |  | Slight                    |  | 20.0                    | 98.2        | Severe   |  | 62.9          | 94.6        |                 | 37.5        |
| D       | CA                | 11.3                         |  | Slight                    |  | 0.0                     | 10.7        | Trace    |  | 5.7           | 3.6         |                 | 0.0         |
|         | Ck                | 11.3                         |  | Moderate                  |  | 95.0                    | 98.1        | Severe   |  | 97.1          | 100.0       |                 | 5.6         |
| Average | CA                | 11.3                         |  | Slight                    |  | 0.0                     | 16.7        | Trace    |  | 6.4           | 8.2         |                 | 0.0         |
|         | Ck                | 11.2                         |  | Moderate                  |  | 58.8                    | 94.7        | Severe   |  | 81.4          | 97.5        |                 | 19.2        |

<sup>a/</sup> Soluble solids at harvest: A - 12.5%; B - 11.5%; C - 12.0%; D - 12.0%.

<sup>b/</sup> Skin shrink when apple was pressed between the thumb and forefinger.

of the Check apples were affected. After two weeks at 75° F, an average of 16.7 percent of the CA apples showed traces of senescent core browning and 94.7 percent of the Check apples were severely affected. The average percent storage scald of CA and Check apples increased slightly between the first and second weeks at a high temperature. After two weeks at 75° F, 97.5 percent of the Check apples showed evidence of storage scald and only 8.2 percent of the CA apples were scalded. At this time none of the CA apples were affected with mealy breakdown, whereas 5.6 to 37.5 percent of the Check apples showed this disorder.

In another experiment, in which the apples were removed from storage in March, 1959 and held at 75° F for 23 days, supplemental atmospheres were applied. Between the sixth and eighteenth days of this period the apples continuously received the following treatments: air, air plus ethylene, 37 percent oxygen, and 37 percent oxygen plus ethylene. All samples were flushed with normal air between the first and fifth days and between the nineteenth and twenty-third days of the test period. The effects of these post storage treatments on the amounts of storage scald, senescent core browning, and mealy breakdown are presented in Table VII. These treatments had no consistent effect on the storage scald of apples from the two storage treatments. The high oxygen treatments reduced the amount of senescent core browning

TABLE VII

Percent Storage Scald, Senescent Core Browning, and Mealy Breakdown of Apples Held for 23 Days at 75° F Following Removal from Storage in March, 1959

| Post Storage Treatment                             | Storage Scald |      | Senescent Core Browning |      | Mealy Breakdown |      |
|----------------------------------------------------|---------------|------|-------------------------|------|-----------------|------|
|                                                    | CA            | CK   | CA                      | CK   | CA              | CK   |
| Air                                                | 9.1           | 95.6 | 63.7                    | 98.6 | 0.0             | 18.2 |
| Air + C <sub>2</sub> H <sub>4</sub>                | 7.5           | 95.6 | 65.6                    | 92.7 | 1.5             | 16.2 |
| 37% O <sub>2</sub>                                 | 12.0          | 98.6 | 32.5                    | 86.9 | 0.0             | 3.1  |
| 37% O <sub>2</sub> + C <sub>2</sub> H <sub>4</sub> | 4.6           | 98.6 | 45.5                    | 86.1 | 0.0             | 1.7  |

of the CA apples and appeared to reduce the amount of senescent core browning in the Check apples. The high oxygen treatments tended to reduce the mealy breakdown of the Check apples, but no effect was evident on the CA apples since mealy breakdown was negligible. Mealy breakdown did not develop on apples of the same storage lot removed from storage at a later date when held at 75° F for only two weeks.

#### Skin Toughness

Puncture tests of the epidermal tissues of apples confirmed the common belief that CA apples have a tougher skin than comparable apples stored by regular means. The data obtained from the apples held for four days at 75° F and eight weeks at 33° F after storage appear in Table VIII. In all instances,

TABLE VIII

Skin Toughness of Apples from Four Orchards after Removal from Storage in March, 1959 and Tested after Four Days in Air at 75°F and after Eight Weeks in Air at 33°F

| Storage Treatment and Holding Period                                                  | Orchard A | Orchard B | Orchard C | Orchard D | Average |
|---------------------------------------------------------------------------------------|-----------|-----------|-----------|-----------|---------|
| <u>Magness-Taylor pressure tester: pounds to break the peeled skin with 5/16" tip</u> |           |           |           |           |         |
| CA - 4 days at 75°F                                                                   | 7.2       | 5.4       | 5.3       | 5.4       | 5.8**   |
| Regular Air - 4 days at 75°F                                                          | 3.9       | 4.9       | 4.0       | 4.5       | 4.4     |
| <u>Chatillon dynamometer: grams to break intact skin with 0.8 mm tip</u>              |           |           |           |           |         |
| CA - 4 days at 75°F                                                                   | 273       | 263       | 263       | 275       | 269**   |
| Regular Air - 4 days at 75°F                                                          | 208       | 225       | 220       | 239       | 223     |
| CA - 8 weeks at 33°F<br>plus 1 day at 75°F                                            | 242       | 240       | 229       | 250       | 240*    |
| Regular Air - 8 weeks at 33°F<br>plus 1 day at 75°F                                   | 207       | 212       | 210       | 225       | 215     |

the skins of the CA apples were significantly more resistant to puncture than the Check apples. Continued storage at 33°F in regular air for eight weeks tended to weaken the tissues and reduced the storage treatment differences.

The reliability of the dynamometer method as a measure of skin toughness was questioned, because it was thought that the firmness of the flesh under the epidermal tissues might affect the dynamometer reading. The data of Table VIII indicate it was as satisfactory as the pressure-tester method to detect the differences in skin toughness of the apples from the two storage treatments. Furthermore, dynamometer measurements of the skin toughness of Jonathan and Red Delicious apples showed the skins of these two varieties of apples were less resistant to puncture, while the flesh of these two varieties of apples was firmer than that of the McIntosh apples.

#### Cell Wall Material

The amounts of cell wall material in the cortical tissues of the CA apples and Check apples of similar size and appearance are given in Table IX. The CA apples had significantly less cell wall material than the Check apples when compared on a fresh weight basis, but the difference on a dry weight basis was not significant.

TABLE IX

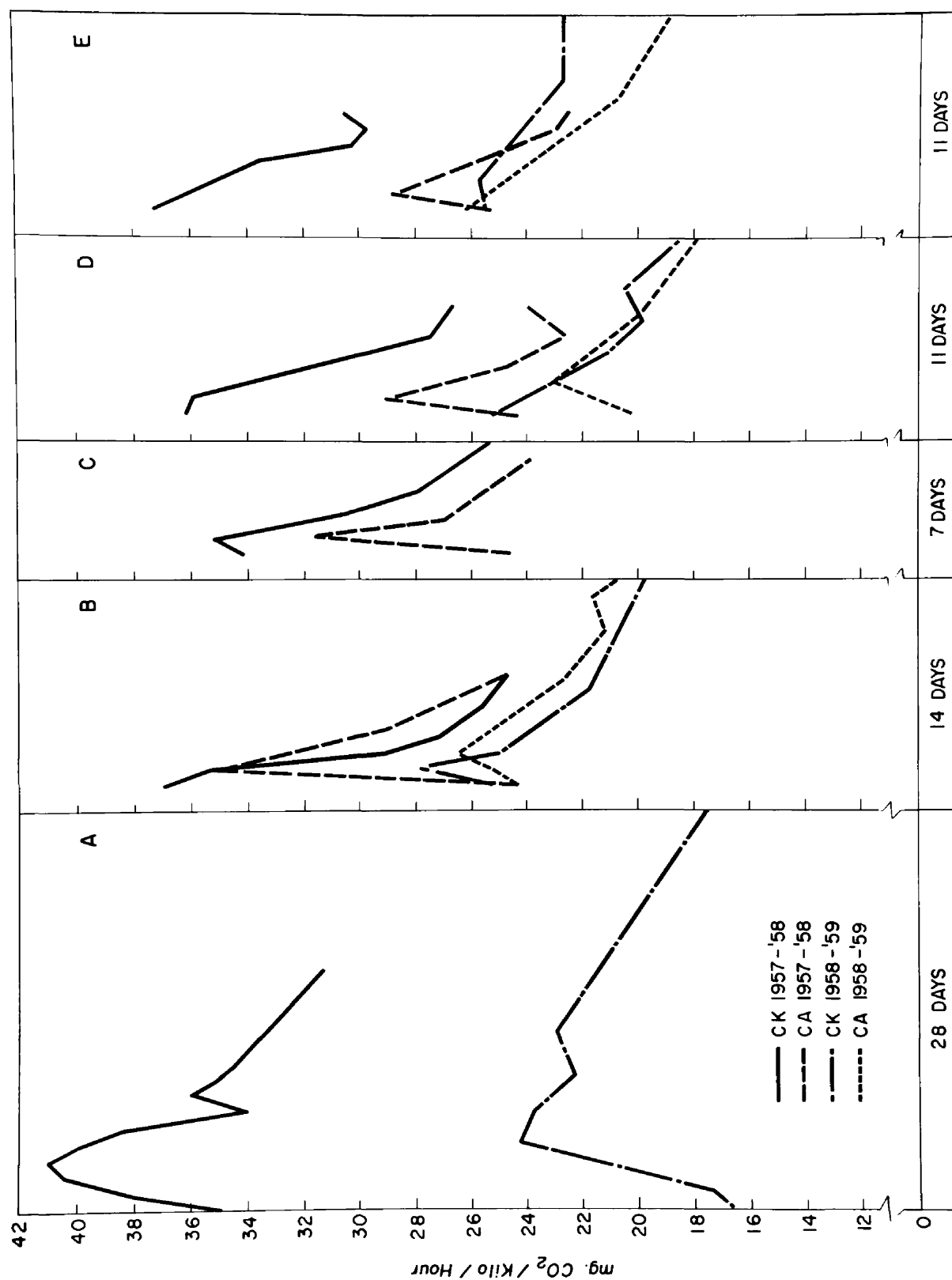
The Amount of Cell Wall Material in the Cortical Tissues of Apples Stored in CA Chambers and in a Regular Air Storage Room to March, 1959

| Storage Treatment | Percent of Fresh Weight |      |      |      |      | Percent of Dry Weight |       |       |       |       |
|-------------------|-------------------------|------|------|------|------|-----------------------|-------|-------|-------|-------|
|                   | Apple No.               |      |      |      |      | Apple No.             |       |       |       |       |
|                   | 1                       | 2    | 3    | 4    | Avg. | 1                     | 2     | 3     | 4     | Avg.  |
| CA chambers       | 1.58                    | 1.69 | 1.88 | 1.70 | 1.71 | 13.22                 | 13.81 | 13.72 | 12.94 | 13.42 |
| Air storage room  | 1.81                    | 1.90 | 1.91 | 1.98 | 1.90 | 13.42                 | 13.83 | 13.91 | 14.86 | 14.01 |
| F value           | 11.92*                  |      |      |      |      | 1.69                  |       |       |       |       |

#### Fruit Respiration at 75° F

The carbon dioxide evolution, respiration, curves at harvest time and after various storage durations for both seasons appear in Figure 6. As shown in Figure 6-A, the apples were harvested during the climacteric rise in respiration in both years, but the apples of the 1957 harvest respired at a much faster rate immediately after harvest than those of the 1958 harvest. The respiratory activity of the Check apples was of about the same intensity at each storage removal time throughout the first storage season (Figure 6-B, C, D, E), and the apples had similar steadily decreasing rates of respiratory activity for all of the seven-day holding periods. The CA apples showed a downward trend in respiration intensity as the first storage season progressed. At each removal date, the respiratory activity of the CA apples increased to a maximum at three to four days after

Figure 6. Carbon dioxide evolution of McIntosh apples in air at 75°F at harvest and upon removal from storage during the storage seasons of 1957-1958 and 1958-1959. A - Check fruit for the two seasons starting at harvest; B - CA and Check fruit after approximately one month of storage in both years; C - CA and Check fruit after approximately 1.5 months of storage in 1957; D - CA and Check fruit for both years stored 4.5 months, and E - for 6 months.



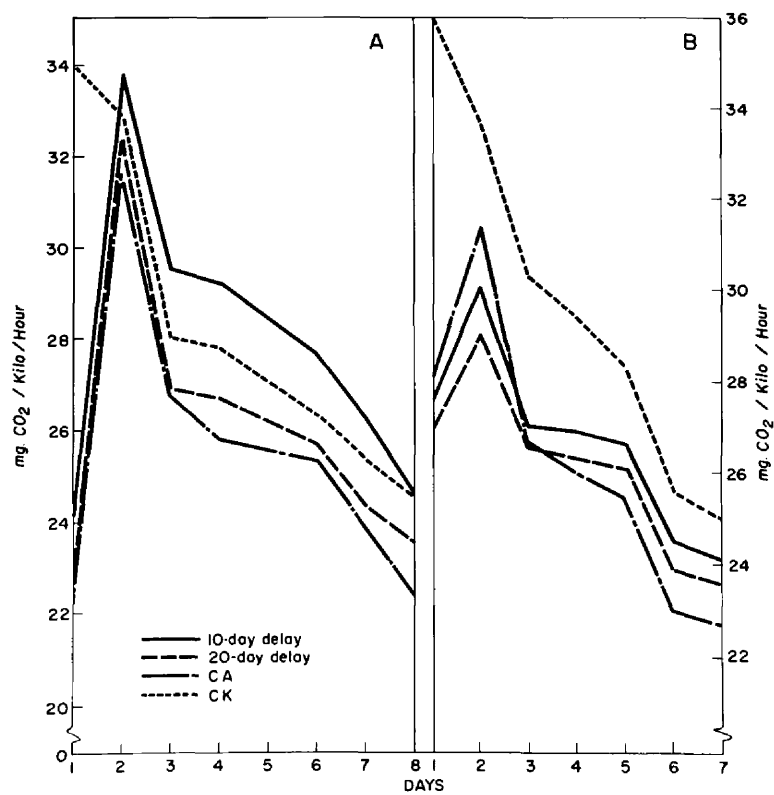
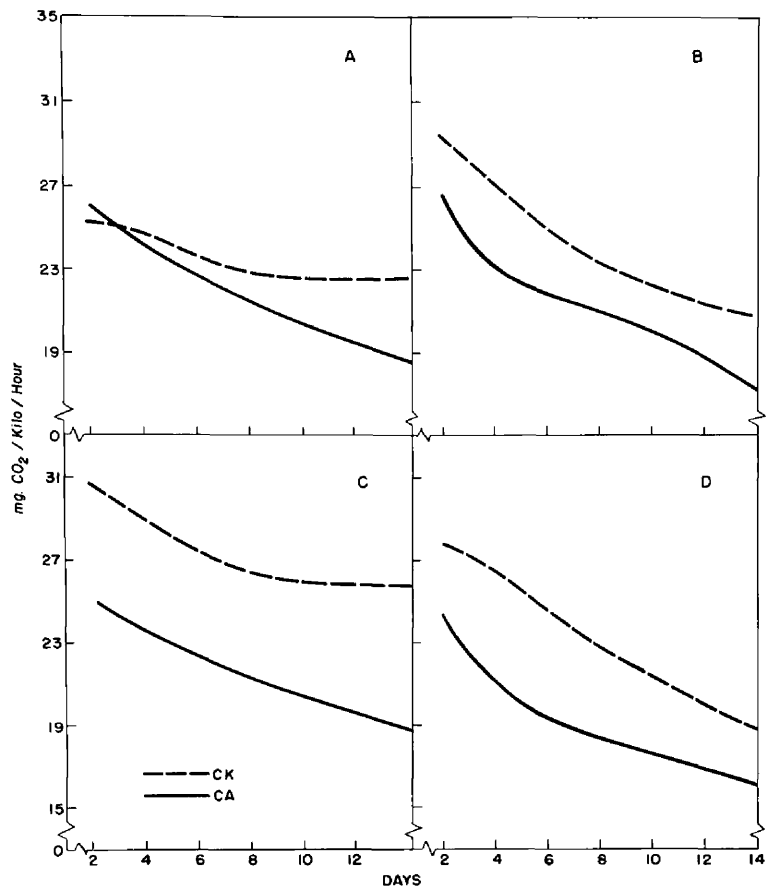
placement in air and then declined. The respiratory activity of the CA apples was markedly slower than that of the Check apples when tested after 100 days of storage (Figure 6-D, E and data for December, 1957 and January, 1958 not shown). In the 1958-1959 storage season the respiratory activity of the CA and Check apples at 75°F was similar until after the apples had been in storage for about 200 days. After 200 days of storage in this season, the respiration rates of apples of both storage treatments were at first equal, but dropped more quickly for the CA apples than for the Check apples when placed in air at 75°F.

Figure 7 depicts the respiratory activity in air at 75°F of the apples from four orchards which were stored to March, 1959. All CA apples respired at slower rates than comparable Check apples. The respiratory activity of all samples decreased during the two weeks of measurement.

The effect of delays in placing apples into CA chambers on the respiratory activity at 75°F is shown in Figure 8. In late December (Figure 8-A) the apples delayed in air storage for ten days respired faster than the Check apples; whereas, the apples stored immediately in the CA chambers and those placed in CA twenty days after harvest respired more slowly than the Check apples. In late January (Figure 8-B) all CA apples respired at slower rates than Check apples. At both times of measurement the respiration of the Check apples decreased daily throughout the seven-day holding period. The CA apples, how-

Figure 7. Respiration rates in air at 75° F of Check and CA apples from four orchards (A-D) after storage to March, 1959.

Figure 8. The effect on fruit respiration in air at 75° F of holding the fruit for ten and twenty days in regular air storage at 33° F prior to placement into CA storage in relation to continuous storage in air and in CA storage. A - fruit removed from storage December 30, 1957; B - fruit removed from storage January 27, 1958.



ever, always increased during the first two days when placed in air at 75°F before the decline in respiration became evident.

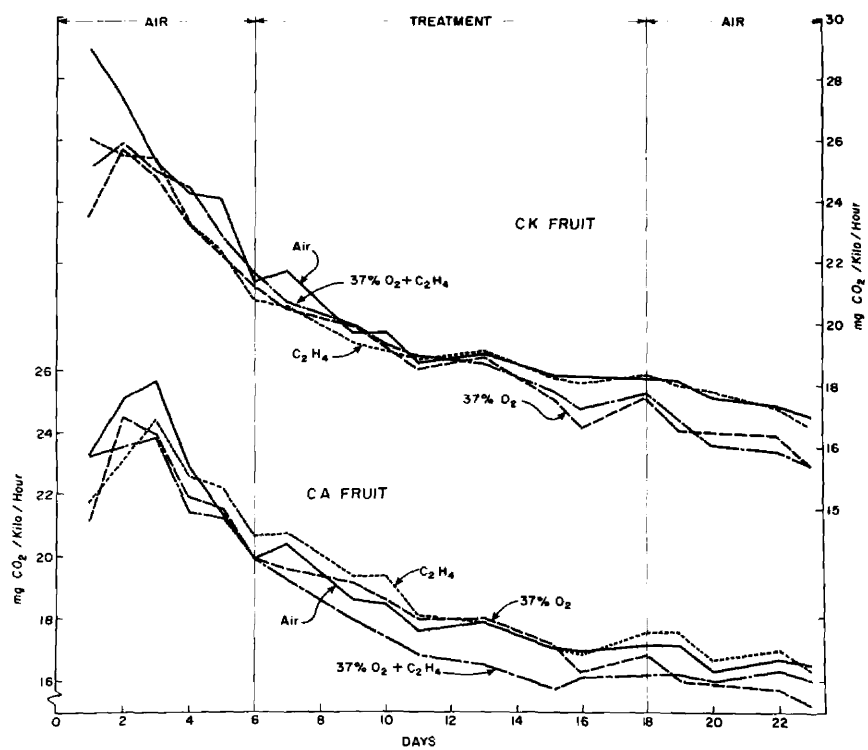
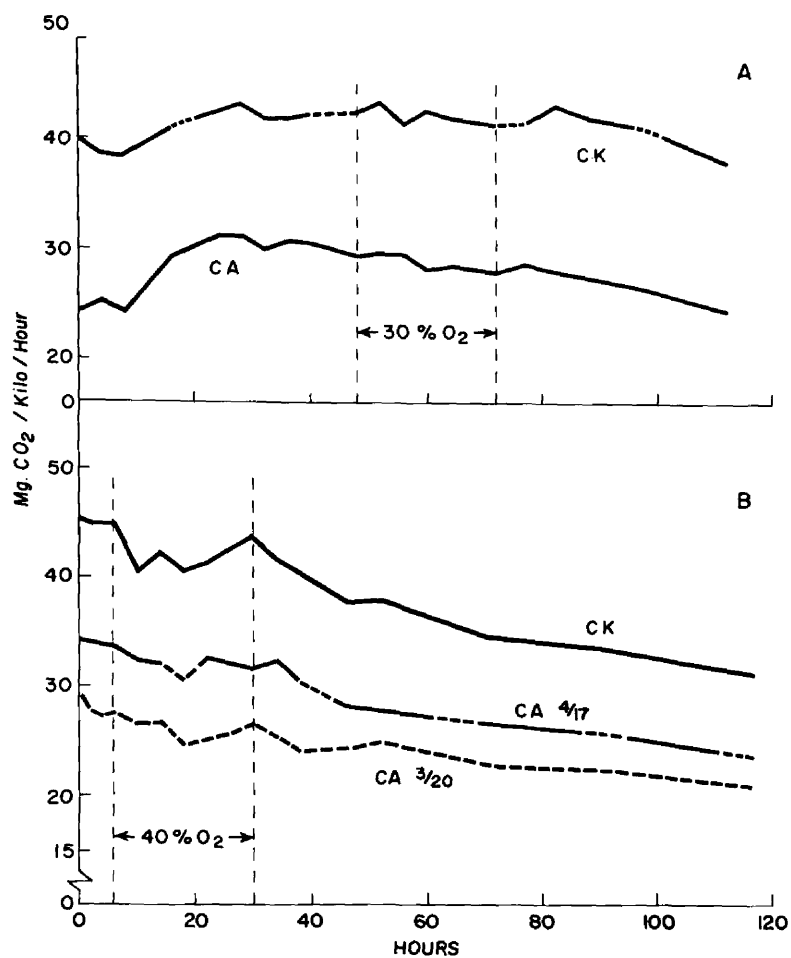
#### Effect of High Oxygen Tensions and Ethylene on Respiration at 75°F

Fruit removed from storage, placed in air at 75°F for four days and then exposed to high oxygen tensions were observed for respiratory activity. As shown in Figure 9-A, a 30 percent oxygen treatment for a period of 24 hours had no effect on the respiration of apples previously stored in either controlled atmosphere or regular air storage. Similarly, a 40 percent oxygen tension did not influence the fruit respiration (Figure 9-B). In this test a sample of the 1957-1958 lot A fruit was removed from the CA chambers on March 20 and placed in air at 33°F. Another CA sample and a Check sample of the same lot were taken April 17, and all three samples were placed in respiration jars at 75°F on April 17. On April 24 the atmosphere passed over the apples was changed from air to 40 percent oxygen for a period of 24 hours.

During the storage period of the second year the effects of ethylene (100 microliters per kilogram of fruit each hour), 37 percent oxygen, and a combination of these gases on the respiratory activity of fruit placed in air at 75°F following storage were measured and the respiration curves are shown in Figure 10. These treatments, administered continuously between the sixth and

Figure 9. Effect of high oxygen tension on the carbon dioxide evolution at 75° F of apples after removal from CA storage and regular air storage. A - Effect of 30 percent oxygen on the respiration of lot B apples after removal from storage in May, 1958. B - Effect of 40 percent oxygen on the respiration of lot A apples: "CA 3/20" removed from controlled atmosphere storage on 3/20/58; "CA 4/17" removed from CA storage on 4/17/58, and "CK" removed from regular air storage on 4/17/58.

Figure 10. Effect of ethylene, 37 percent oxygen, and a combination of the two gasses on the rate of carbon dioxide evolution at 75° F of CA and Check apples removed from storage in March, 1959.



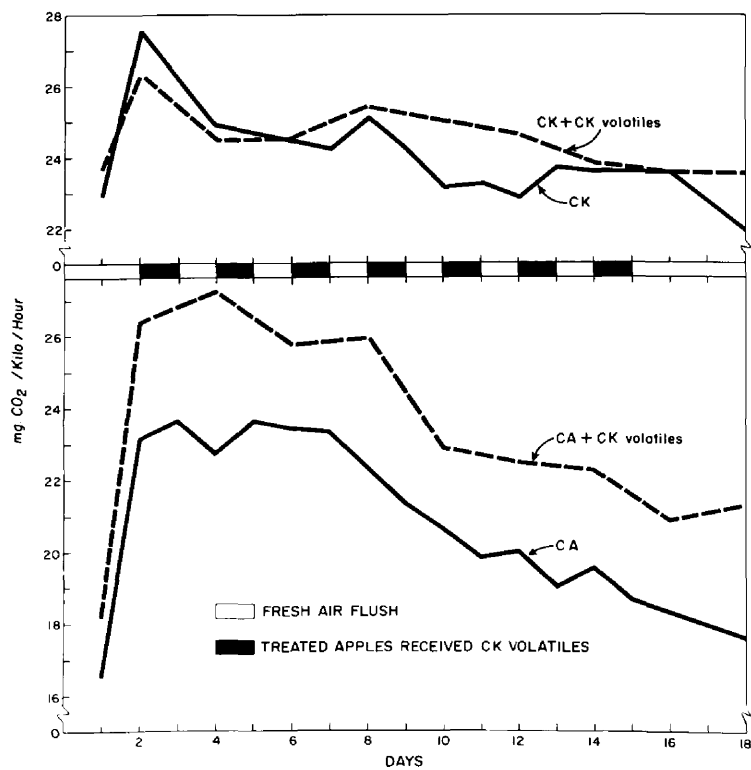
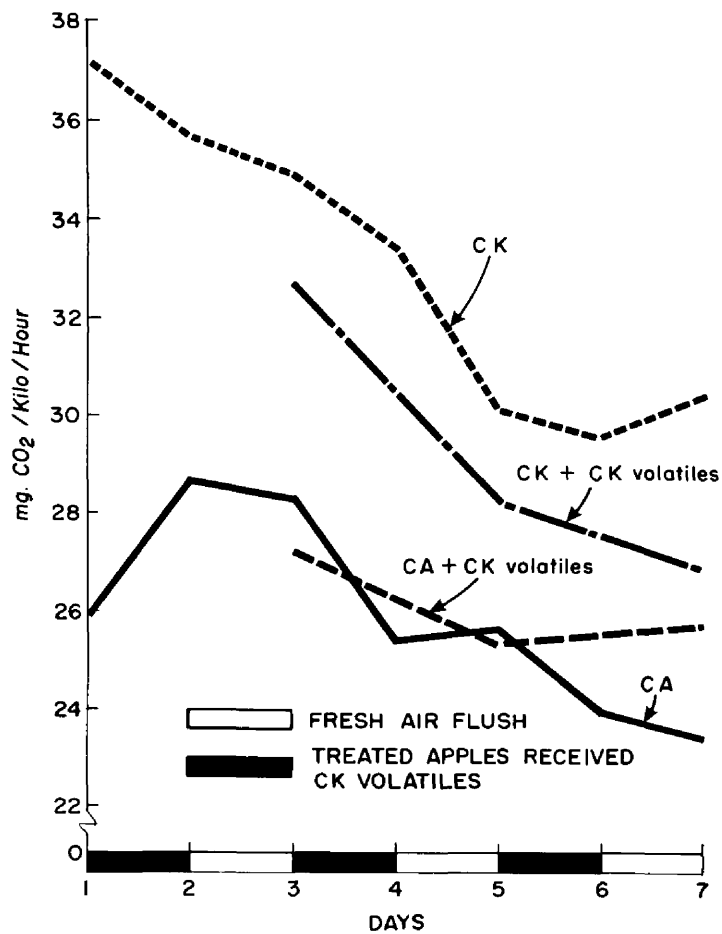
eighteenth days of the test period, did not markedly affect the respiration of the apples, regardless of the previous method of storage. High oxygen, with and without ethylene, appeared to have a slight retarding effect on the carbon dioxide evolution of all fruit, but further tests would be desirable to verify this.

#### Effects of Apple Volatiles on Respiration at 75° F

The effect of apple volatiles emanated by Check apples on the respiration of other Check apples and CA apples is shown in Figures 11 and 12. When tested in March, 1958 (Figure 11), the volatiles given off by the Check apples depressed the respiratory activity at 75° F of the Check apples, but increased the respiratory activity of the CA apples. When the test was repeated over a longer period of time in April, 1959 (Figure 12), the respiratory activity of the CA apples again was increased by the volatiles given off by the Check apples. This time, however, the volatiles did not markedly affect the respiratory activity of the other Check apples. Examination of Figure 12 reveals that the treated CA apples were respiring considerably faster than the untreated CA samples before the volatile treatment was applied. Although great differences in the respiratory peaks after removal from CA storage were frequently observed, it was unusual to find great differences in respiration after the peak value was passed. Since the duplicate samples of the treated CA apples respired at levels considerably above the duplicate samples of untreated CA

Figure 11. The effect of Check apple volatiles on the carbon dioxide evolution at 75° F of CA and Check apples removed from storage in March, 1958.

Figure 12. The effect of Check apple volatiles on the carbon dioxide evolution at 75° F of CA and other Check apples removed from storage in April, 1959.



apples, it is likely that there was a significant effect of volatiles on the respiration of the CA apples.

#### Respiration Quotient

The respiration quotients at 75°F, measured by carbon dioxide evolution and oxygen consumption of whole apples removed from CA and regular air storage are presented in Table X. All respiration quotients increased slightly between the first and second dates of measurement and remained fairly constant between the second and third measurements. Previous storage treatments did not markedly affect the respiration quotients.

#### Polyphenolase Activity

Polyphenolase activity was determined in March, 1958 and measurement of oxygen uptake by apple discs suspended in a buffer solution with the addition of catechol. Measurements were taken, one, three, five and nine days after the apples were removed from storage. The averages of the four Warburg manometric measurements are plotted in Figure 13. The addition of catechol (0-phenylendiamine was added with the catechol to inactivate the quinones oxidized from the catechol) to CA apple discs brought about a faster rate of oxygen absorption than did the addition of catechol to the Check apple discs. This indicated the polyphenoloxidase system of the Check apple discs was not as active as that of the CA apple discs.

TABLE X

The Respiration Quotients at 75° F of Apples Stored in CA and Regular Air Storage in the 1957-1958 Storage Season

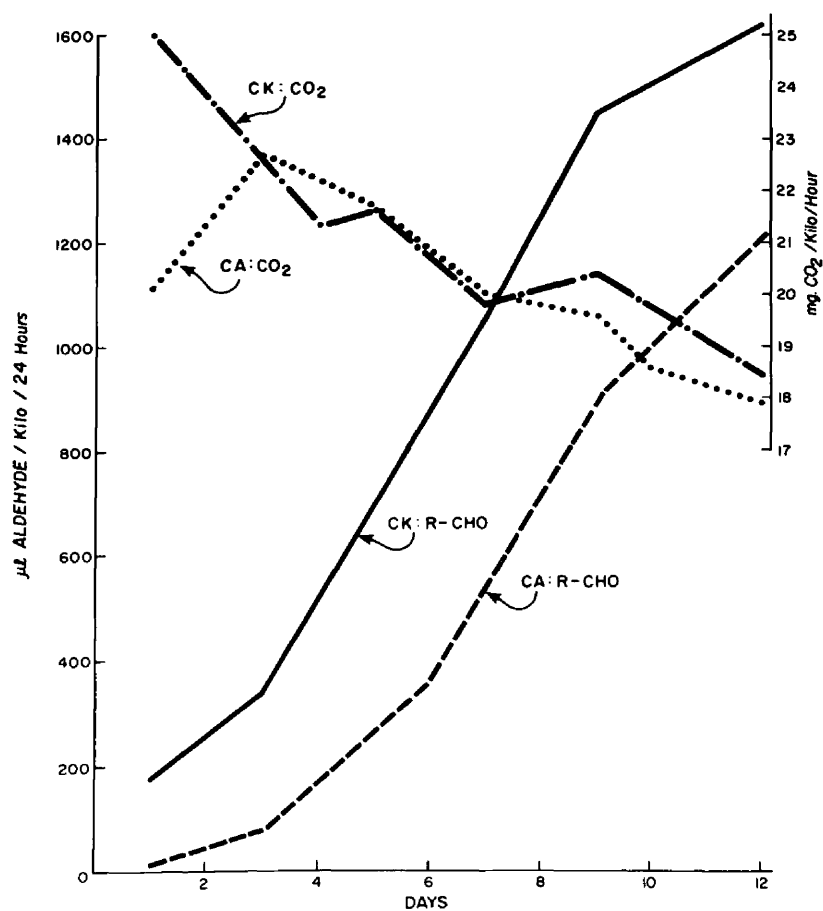
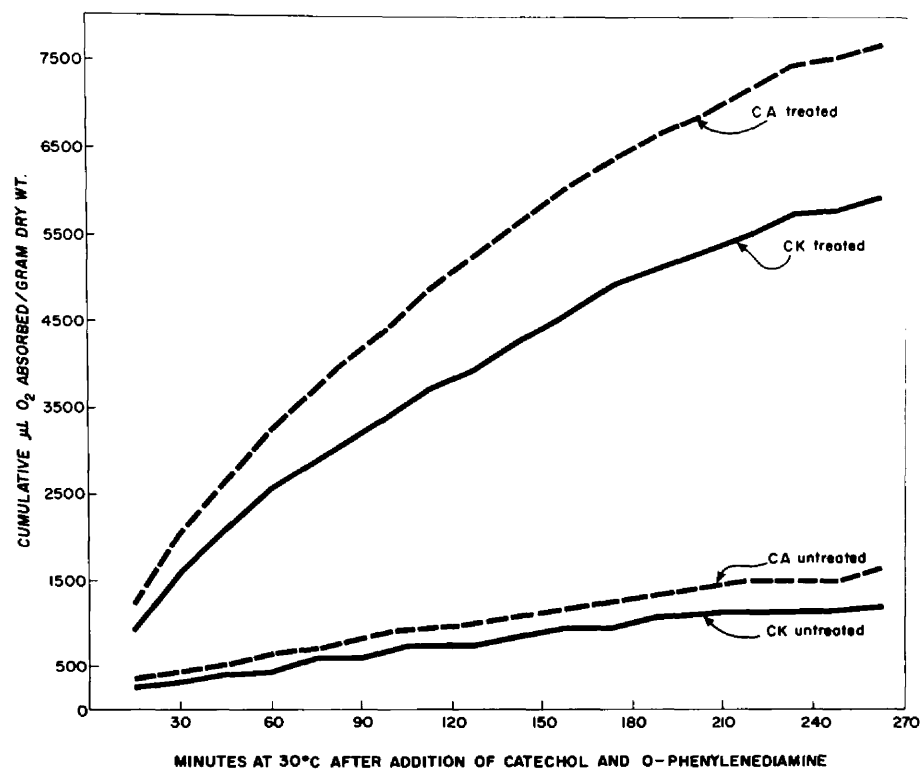
| Date of Measurement | Sample No. | CA - Mar. 20 <sup>a/</sup> | CA - Apr. 17 <sup>b/</sup> | Ck <sup>b/</sup> |
|---------------------|------------|----------------------------|----------------------------|------------------|
| April 18            | 1          | 1.2                        | 1.3                        | 1.4              |
|                     | 2          | <u>1.4</u>                 | <u>1.4</u>                 | <u>1.5</u>       |
|                     | Avg.       | 1.3                        | 1.4                        | 1.4              |
| April 21            | 1          | 1.9                        | 1.7                        | 1.7              |
|                     | 2          | <u>1.5</u>                 | <u>1.7</u>                 | <u>1.6</u>       |
|                     | Avg.       | 1.7                        | 1.7                        | 1.6              |
| April 23            | 1          | 1.6                        | 1.8                        | 1.8              |
|                     | 2          | <u>1.6</u>                 | <u>1.8</u>                 | <u>1.5</u>       |
|                     | Avg.       | 1.6                        | 1.8                        | 1.7              |

<sup>a/</sup> Removed from CA storage March 20, 1958 and stored in air at 33° F until placed at 75° F April 17, 1958.

<sup>b/</sup> Removed from storage April 17, 1958 and immediately placed at 75° F.

Figure 13. The oxygen absorption of CA and Check apple discs in the presence of buffer solution and of buffer solution plus catechol and 0-phenylenediamine. These discs were from apples removed from storage in March, 1958.

Figure 14. Aldehyde, expressed as acetaldehyde, and carbon dioxide evolution in air at 75°F of CA and Check apples stored to February 14, 1959.



Contrary to the relative respiration rates of whole apples in the respiration jars at 75°F, the untreated CA apple discs respired faster than the untreated Check apple discs in the Warburg reaction vessels at 30°C.

#### Aldehyde Evolution at 75°F

The simultaneous evolution of carbon dioxide and aldehydes at 75°F by CA and Check apples stored to the middle of February, 1959 are shown in Figure 14. The respiratory activity of the CA apples increased to a peak on the third day at 75°F and then decreased, whereas the respiratory activity of the Check apples decreased immediately and continuously. After three days at 75°F, the respiration rates of the CA and Check apples were approximately equal.

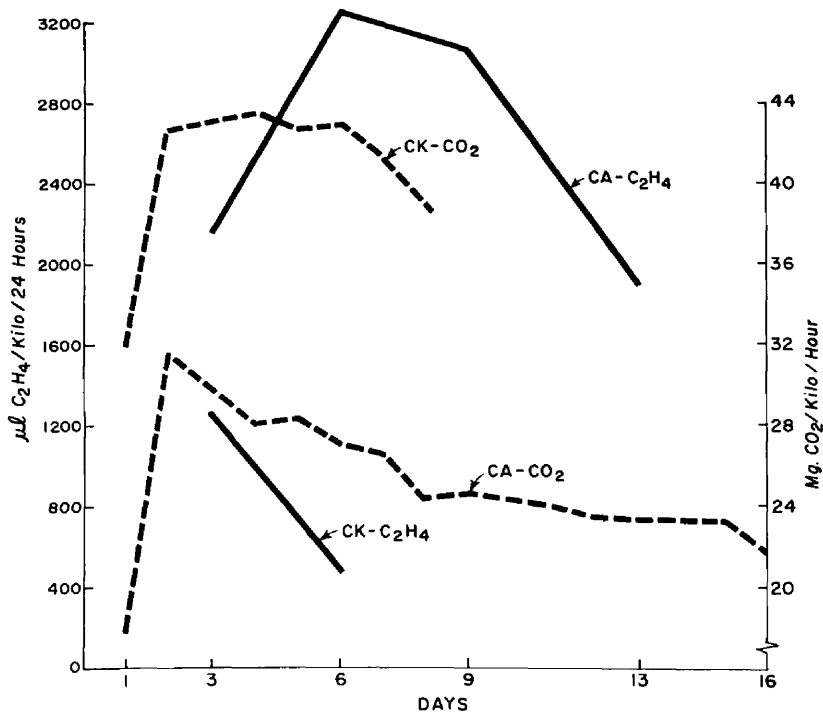
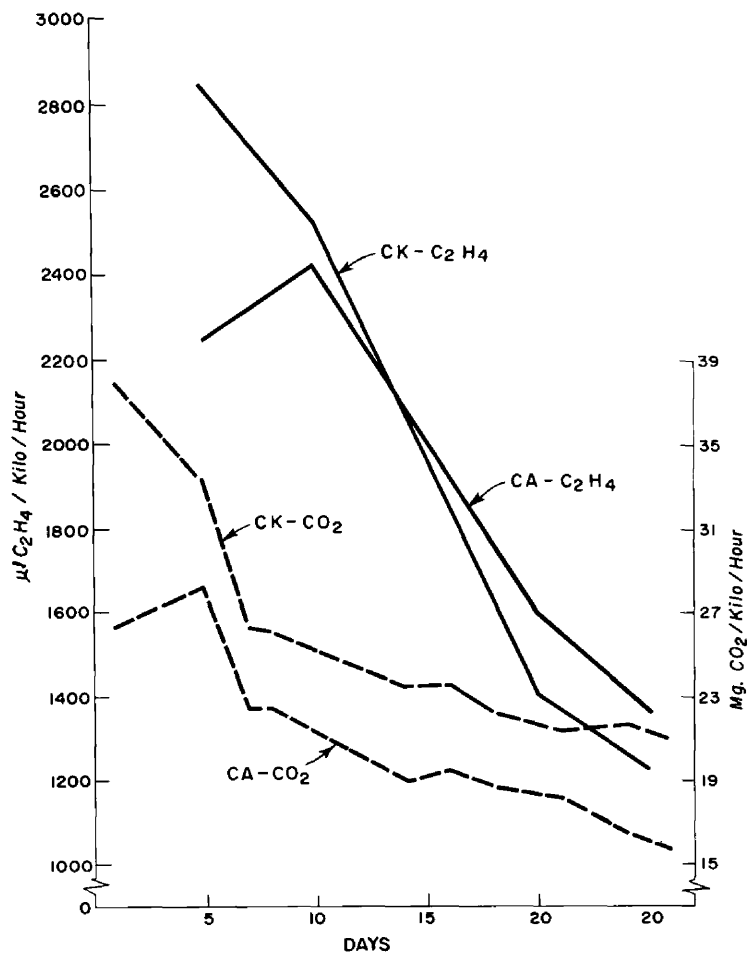
The rates of aldehyde evolution of both storage samples increased rapidly during the twelve-day test period at 75°F, with the increase being slightly faster for the Check apples than for the CA apples. The average increase in aldehyde production in microliters per kilogram of fruit per day was 120.7 for the Check apples as compared to 100.3 for the CA apples. Similar results had been obtained in a comparable test conducted two weeks prior to this one.

### Ethylene Evolution at 75° and 33° F

Ethylene production at 75° F, together with carbon dioxide evolution, for CA and Check apples removed from storage in February, 1958, is shown in Figure 15. The respiratory activity of the Check apples decreased daily throughout the test period; the CA apples increased to a maximum at five days and then declined. The CA apples respired at a slower rate than the Check apples on any specific day during the test period. The rate of evolution of ethylene by the CA apples reached a peak after ten days at 75° F and then declined rapidly. Ethylene was evolved by the Check apples at a rate that was initially higher than the CA apples, but the rate decreased rapidly at each measurement so that by the end of the 21-day test period the rate of ethylene evolution of the Check apples was slightly slower than the rate of evolution by the CA apples. Similar measurements for lot B, removed from storage in May, 1958, appear in Figure 16. The CA apples of this lot respired at a much lower rate than the Check apples. The initial rate of ethylene evolution of the Check apples was considerably lower than that of the CA apples and it decreased rapidly from this initially lower rate. Ethylene evolution by the CA apples rose to a peak at six days after removal from storage and then declined during the remainder of the 13-day test period. Even with this decline, the CA apples continued to evolve ethylene at a markedly faster rate than the initially high rate measured for the Check apples.

Figure 15. Ethylene and carbon dioxide evolution in air at 75° F of apples from lot A after removal from storage in CA and regular air to February, 1958.

Figure 16. Ethylene and carbon dioxide evolution in air at 75° F of apples from lot B after removal from CA and regular air storage to May, 1958.

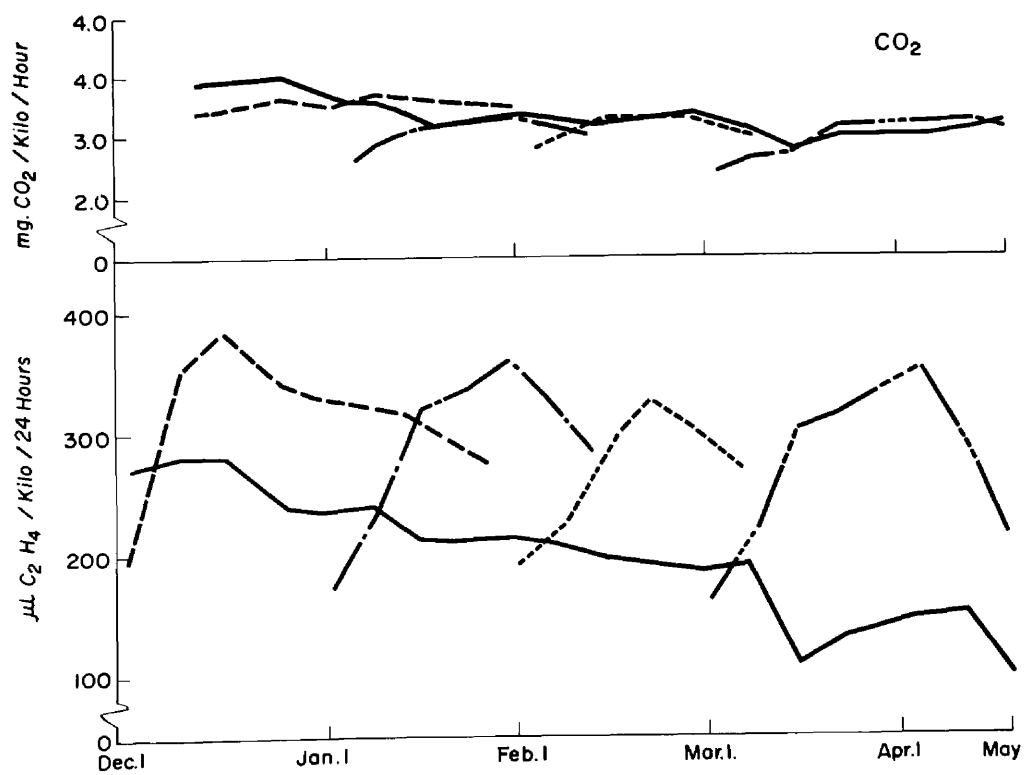
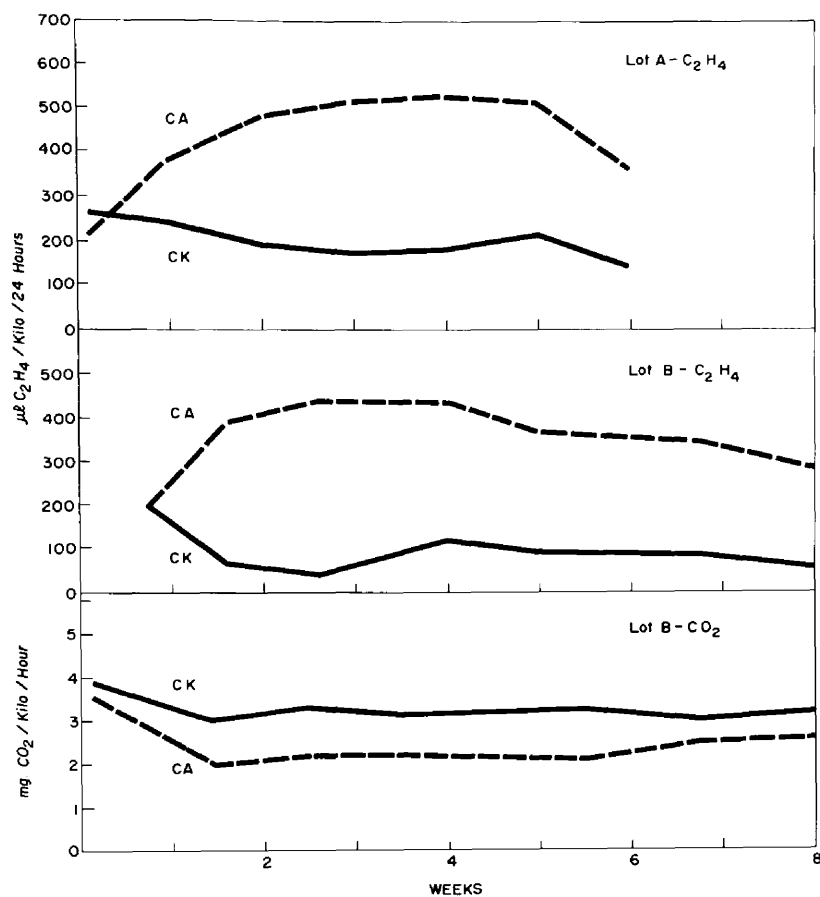


The ethylene evolution in air at 33°F by the CA and Check apples from two orchards (lots A and B) was measured in March and May, 1958, respectively. As shown in Figure 17, the initial rates of ethylene production for the apples of lot A from the two storage treatments were approximately equal, but after two weeks at 33°F the CA apples produced ethylene at rates twice that of the Check apples. The rates of ethylene evolution did not change appreciably between the second and fifth weeks and then dropped slightly between the fifth and sixth weeks just prior to the conclusion of the experiment. Also, the initial ethylene evolution rates of the apples of lot B, measured in May, 1958, were initially the same. Again, after two weeks at 33°F the CA apples were evolving more than twice as much ethylene as the Check apples and the CA apples continued at this higher level of ethylene evolution throughout the eight weeks of measurement. At eight weeks the CA apples were evolving three times as much ethylene, but only three-fourths as much carbon dioxide, as the Check apples.

Further observations of ethylene production at 33°F, made on the CA and Check apples of lot A of the second season, are summarized in Figure 18. The check apples showed a steady decrease in ethylene evolution throughout the storage season. The ethylene evolution of the CA apples, which were also removed from storage at monthly intervals, was initially lower than that of the Check apples, but increased to a peak at three to five weeks and then

Figure 17. Ethylene production in air at 33°F of CA and Check apples from lot A after storage to March, 1958, and ethylene and carbon dioxide production in air at 33°F of CA and Check apples from lot B after storage to May, 1958.

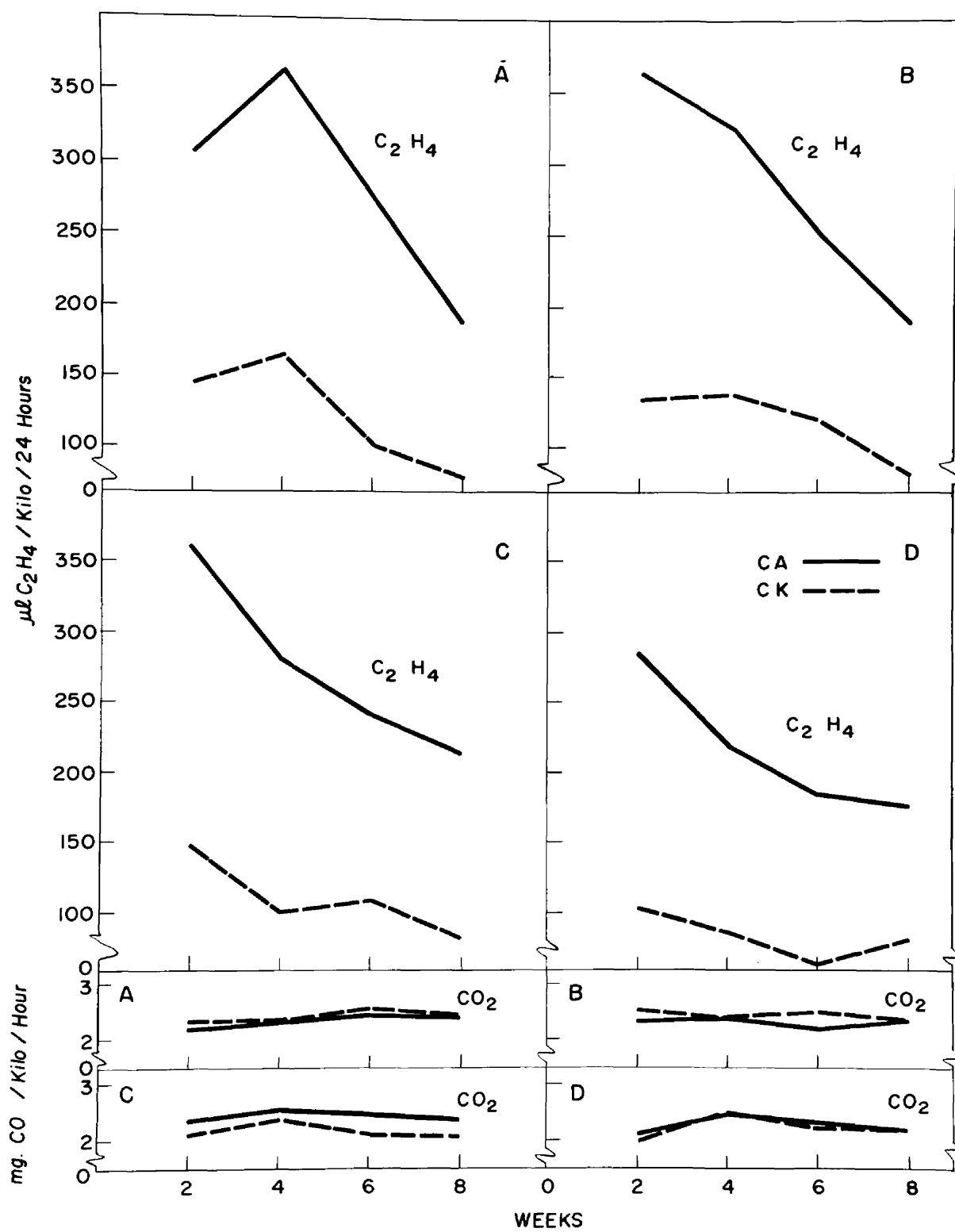
Figure 18. Ethylene and carbon dioxide evolution rates in air at 33°F of CA and Check apples after storage for various lengths of time during the 1958-1959 storage season. Solid line — apples stored in regular air; broken line ---- apples previously stored in CA chambers.



decreased for the remainder of the measurement period. After the first two weeks of each test period at 33°F, the CA apples evolved ethylene at rates considerably higher than the rates of the Check apples. The rates of ethylene production by the CA apples followed approximately the same curve after each of the monthly removals from the CA storage chambers, but since the rates of ethylene evolution by the Check apples decreased throughout the storage season, the difference in rates between the two storage treatments increased as the season progressed. The respiration rates of the samples removed from CA storage were initially lower than those of the Check apples, but after two weeks at 33°F there was very little difference between the respiration rates of the apples from the two storage treatments.

The ethylene and carbon dioxide curves at 33°F for apples from four orchards (A-D) appear in Figure 19. The apples were removed from the CA and regular air storage in April, 1959. The respiration rates of the CA and Check apples from each orchard were about equal during the period of measurement which extended from the second to the eighth week after the apples were removed from storage. Ethylene production by the apples from orchards B, C and D declined over the period of measurement, but the CA apples from orchard A increased in ethylene production between the first and second weeks of the test period, this was the third and fourth weeks after removal from CA

Figure 19. Ethylene and carbon dioxide evolution in air at 33° F of  
CA and Check apples from four orchards (A-D). All apples  
stored to April, 1959.



storage, and then declined. Since the test was started two weeks after the CA apples had been removed from the CA chambers, the initial rise in ethylene production of the CA apples from orchards B-D was not observed. The CA apples from each orchard evolved at least twice as much ethylene as the corresponding Check apples on any specific date after the beginning of the test period. At no time, even after eight weeks in air at 33° F, did the CA apples drop to a level of ethylene production that was lower than the highest measured level of ethylene production of the Check apples.

These ethylene studies in air at 33° F showed that CA-stored McIntosh apples evolved considerably more ethylene than comparable Check apples in both years. The ethylene production curves of the CA apples rose to a maximum value in two to five weeks after removal from CA storage and then declined. The rate of ethylene production after the maximum value declined very slowly the first year and more rapidly the second year of study. In March of both years the Check apples evolved approximately 200 microliters of ethylene per kilogram of fruit per day. In April, 1959, the Check apples from four orchards evolved approximately 150 microliters of ethylene per kilogram of fruit each day. In late April and May the Check apples of both years of study evolved approximately 100 microliters of ethylene per kilogram of fruit per day. Thus, in both years, the Check apples evolved ethylene at approximately equal rates at corresponding dates. The ethylene production be-

havior of CA apples did not correspond as closely from year to year. Two weeks after removal from CA storage in March or later, however, all CA apples evolved more than twice as much ethylene at 33° F as did the Check apples, and this marked difference in ethylene production was maintained for at least four to eight additional weeks at 33° F.

## DISCUSSION

The objectives of these studies were to define the residual effect of CA storage in respect to the post-storage physiological behavior of the fruit and to ascertain the time of inception of the residual effect. Data were gathered to also form the basis of theories on the causal factors responsible for the residual effect.

### The Measurable Effects of CA Storage on the Physiological Behavior of Apples After They Were Removed from CA Storage

Indirect measurements of physiological activity: - Other researchers have found that storage of apples under modified atmospheres has extended the shelf life of apples after they were removed from storage (36, 54, 1, 66, 51, 13). The results of the experiments described in the previous chapter show that the CA apples had firmer flesh than the Check apples upon removal from storage in January and later. Possibly the rapid softening of the apples stored in the CA chambers during the first part of the storage season occurred because the temperature within the CA chambers was maintained at 38° F throughout the season and the desired levels of carbon dioxide and oxygen were not attained until the fruit had been in the chambers for about two weeks. The Check apples, on the other hand, were stored at 33° F continuously. Temperatures lower than 38° F were not used in the CA chambers because lower tempera-

tures in CA storage have been found to increase the incidences of carbon dioxide injury (63) and of brown core in McIntosh apples (61).

The rates of softening of the CA and Check apples during the seven days of holding at high temperatures after storage were extremely variable (see Tables II and III) and not significantly different. Since the CA apples were firmer than the Check apples upon removal from storage, and the rates of softening were about equal after storage, it is logical that the Check apples would reach an unacceptable level of flesh firmness (57) sooner than the CA apples. Thus, the period of marketability of the CA apples was longer than that of Check apples, but the rate of softening was not affected appreciably. These findings were not in agreement with those of Smock (57), who reported that CA-stored McIntosh apples softened at high temperatures at considerably slower rates than regular air-stored McIntosh which had the same flesh firmness as measured immediately upon removal from storage. The lack of agreement between the data of this study and the data presented by Smock (57) may have been due to the considerably longer holding periods used by Smock.

The panel, which subjectively evaluated the appearance, texture, flavor and ripeness of CA and Check apples, found very little difference in the rates of quality loss of the CA and Check apples during the seven-day holding periods at a high temperature. The period of acceptable quality of CA apples was longer than that of the Check apples, but only because the quality upon removal from storage was higher.

Check apples had higher incidences of storage scald, senescent core browning, and mealy breakdown than CA apples at the end of the 75°F holding periods. It was impossible to ascertain whether this was a result of a residual effect of CA storage, or merely a result of the decreased or altered metabolic activity of CA apples while they were in the CA chambers. The differences in skin shrivel, as shown in Table VI, were probably due to differences in the relative humidity in the CA chambers and the regular air storage room, and not due to differences in the levels of carbon dioxide and oxygen. These differences in skin shrivel indicated that more water was lost from the Check apples than from the CA apples during storage. The greater loss of water from the Check apples undoubtedly accounted for the significantly less amount of cell wall material in the cortical tissues of the CA apples, when the comparison was made on the fresh weight basis, and the lack of significant difference in the amount of cell wall material when the comparison was made on the dry weight basis.

Heulin and Gallop (24) found less oil in the skin of Granny Smith apples stored under modified atmospheres than in the skin of Granny Smith apples stored in regular air at the same temperature. It is not known whether the difference in the skin toughness of the CA and Check apples after removal from storage was due to differences in oil content of the skin (if such differences were present), or due to differences in the temperature, or in the

levels of carbon dioxide and oxygen, or in the relative humidity of the environment of the two storage treatments. It was also impossible to ascertain from the data obtained whether or not the greater loss in skin toughness of the CA apples after removal from CA storage was a manifestation of a residual effect of CA storage.

None of the indirect measurements used in these studies revealed a marked effect of CA storage on the physiological behavior of apples after they were removed from the CA storage.

Direct measurements of physiological activity: - -Storage of apples in CA storage definitely had a depressing effect on the respiratory activity of the apples after they were removed from storage and placed in air. This effect of CA storage has been previously reported (54). The depressing effect of CA storage on subsequent respiration in air at 33° and 75° F was greater in the first year of study than in the second year. The volatiles given off by Check apples seemed to stimulate the carbon dioxide evolution of CA apples and either decreased or had no effect on the carbon dioxide evolution of other Check apples. The polyphenoloxidase activity of CA apple discs was higher than that of Check apple discs.

CA apples evolved considerably more ethylene than Check apples during the test periods at 33° or 75° F in air. Mattus (42) found this relationship was also present in Bartlett pears held in air at 74° F after removal from CA and regular air storage.

These physiological measurements indicated there were definite effects of CA storage upon the subsequent metabolic activity of the fruit. It may be concluded that these post-storage effects were a residual effect of CA storage.

The Time of Occurrence of the Residual Effect of CA Storage on the Physiological Behavior of Apples After They Were Removed from Storage

Marked differences in the respiration rates at 75° F in air of CA and Check apples occurred after three months of storage in the first year of study, but not until after six months of storage in the second year of study. The length of the CA storage period required to bring about an effect on ethylene production of apples after removal from storage was not determined, because no ethylene determinations at 33° and 75° F were made prior to March of the first year, and prior to December of the second year. At these two earliest dates of measurement in each year, however, this residual effect of CA storage was evident.

Since the other two positive indices of the residual effect, namely, the effect of Check apple volatiles on respiration of CA apples at 75° F in air and the high activity of the polyphenoloxidase system, were not measured before March, it was impossible to ascertain when these effects would have been first noted in the storage period.

Theories on the Causes of the Effects of CA Storage on the Physiological  
Behavior of Apples After They Were Removed from CA Storage

Correlation of the effects:--It would appear as though the residual effects of CA storage of high ethylene production and high polyphenolase activity were opposite to the residual effect of depressed respiration. It is possible, however, that a change in the environment surrounding the fruit might inhibit one biochemical reaction more than another, or even stimulate one reaction and not another. For instance, Kidd and West (34) found modified atmospheres had a greater retardation effect on the change in ground color from green to yellow than upon the extension of the storage life of Bramley's Seedling apples; Artsikhovskaya and Sokolova (4) found that lowering the oxygen concentration had no effect on the concentration of ethyl alcohol in the flesh of apple fruits, but increased the acetaldehyde concentration.

It does not seem unlikely, therefore, in these studies that the high carbon dioxide and low oxygen tensions surrounding the apples in the CA chambers, retarded the normal failure of the oxidative systems (62, 26) through its depressing effect on the rate of reactions in glycolysis and in the Krebs' cycle (66); and at the same time brought about an accumulation of metabolic intermediates or end-products through stimulation of such reactions as the carboxylation of pyruvic acid to form malic acid (2, 3). Accordingly, apples from CA storage would have had highly potentially active terminal oxidase system(s)

but at the same time might have had a low rate of respiration due to an accumulation of metabolic intermediate(s) and/or end-product(s) which directly or indirectly acted as inhibitor(s) to the normal metabolic pathways of respiration.

Respiration: - After removal from CA storage, most apples exhibited a rapid rise in carbon dioxide evolution rate before the rate started to decline. Fidler (15) stated there was release of carbon dioxide from the cell sap after the apples were removed from "gas" storage, and Smock (54) found the amount of carbon dioxide in CA apples and in regular air-stored apples was the same after 48 hours of exposure to air. The peak in carbon dioxide evolution after removal from CA storage occurred two to four days after the apples were removed from the CA chambers in this study. Smock (54) hypothesized the rise in carbon dioxide evolution was a normal respiratory curve. The respiration quotient (Table IX) of apples at 75° F one day after removal from CA storage was slightly higher than that of CA apples which had been removed from CA storage and stored in air at 33° F for one month prior to the respiration quotient determinations. This difference would be much greater if the rapid rise in rate of carbon dioxide evolution was due entirely to the release of carbon dioxide from the cell sap.

Delays in the occurrence of the normal respiratory climacteric of CA apples, as frequently observed here, have been previously observed (36, 54)

and attributed to the residual effect of the CA atmospheres. In these studies, as well as in the studies by Smock (54), the respiratory activity of CA apples rose to a maximum value after removal from CA storage and then declined. This corresponded to the respiratory pattern described for apples by Kidd and West (39, 35) as the climacteric rise, the climacteric, and the post climacteric decline of apples at harvest time. Nelson (46) found the ethylene production of harvested McIntosh apples reached a peak about five days after the respiratory climacteric when the apples were held in air at 68° F. The close association of the respiratory climacteric and the peak of ethylene production has been noted by many workers (46, 23, 58, 8). Since the cause and effect relationship between the normally occurring respiratory climacteric and the normal rates of ethylene production have not been established, the occurrence of the rise in ethylene production by CA apples after removal from storage cannot be considered as proof of a delayed respiratory climacteric. The suspected respiratory climacteric at 75° F in air for CA apples after removal from CA storage, and the associated rise in ethylene production at 75° F and 33° F cannot be overlooked, however.

The overall lower respiratory activity of apples from CA storage in comparison with apples from regular air storage has been attributed by Smock (54) to the possible effect of modified atmospheres on the respiratory enzymes. The results of this study indicate that if the lower respiratory activity was

caused by a partial or complete enzyme inactivation, some enzyme(s) other than terminal oxidase(s) was affected. The following four reasons may be cited: 1.) Burg and Thimann (9) concluded that the terminal oxidase for oxidative respiration of McIntosh apples was also the terminal oxidase for ethylene synthesis. The higher rate of ethylene evolution by the CA apples in this study indicated that the activity of the ethylene (respiration) oxidase was not reduced by CA storage. 2.) Discs of CA apple tissue, placed in a solution of citric acid and disodium phosphate, absorbed more oxygen than similar discs of Check apples. The most probable explanation of this phenomenon was that the CA apple discs metabolized the citric acid at a faster rate than the Check apple discs. This indicated that CA apples had a more potentially active respiratory electron transfer system and terminal oxidase. 3.) The higher rate of oxygen absorption by CA apple discs in the presence of catechol indicated the polyphenoloxidase system was potentially more active than that of the Check apples. Ulrich (64) pointed out that several investigators have found that polyphenoloxidase played a major role in the respiration of many types of matured fruits. 4.) The rate of evolution of acetaldehyde, the principal aldehyde of apple emanation, has been found to be an indicator of the rate of glycolysis (34, 27). The CA apples evolved less aldehydes than the Check apples at 75° F in air after removal from storage in February, 1959. Since the rates of carbon dioxide evolution were about equal,

the ratio of glycolysis to oxidative respiration was probably higher in the Check apples than in the CA apples.

Thus, the depressing effect of CA on the post-storage respiratory activity of apples was probably caused by either an inactivation of some enzyme(s) other than those of the principal oxidative respiratory electron transfer systems and their terminal oxidases, or the accumulation of metabolic intermediate(s) or end-product(s) which, directly or indirectly, acted as inhibitor(s) to the normal metabolic pathways of respiration.

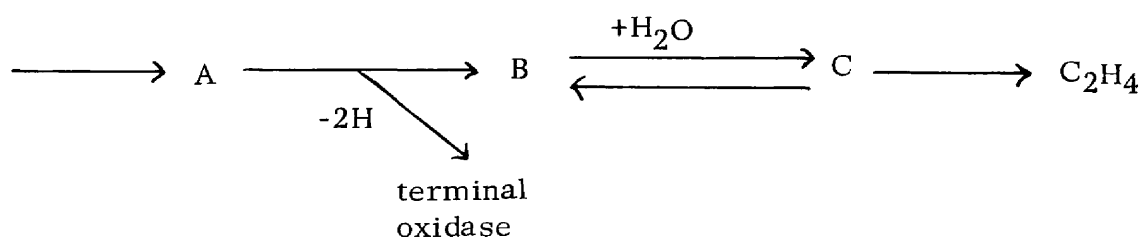
Effect of Check apple volatiles on the respiration of CA apples in air at 75° F: -- Chace (10) found that CA-stored Jonathan and McIntosh apples, after subjection to high concentrations of  $C^{14}$  labeled carbon dioxide at room temperature, evolved the labeled carbon dioxide for a longer period of time than regular air-stored apples. If the higher carbon dioxide values recorded in Figures 11 and 12 for the CA apples subjected to the volatiles of Check apples were merely an expression of the release of carbon dioxide absorbed from the emanation source, the carbon dioxide curve for the CA apples treated with Check apple volatiles should have decreased during the three-day air flush period after the volatile treatment was concluded. Since higher values of carbon dioxide evolution occurred during the three-day air flush period after the treatment was terminated for the volatile-treated CA apples, than for CA apples subjected continuously to air free of volatiles, it may be concluded

that the respiratory activity of the CA apples was stimulated by the volatiles. Some fraction other than ethylene must have been responsible since treatment with pure ethylene gas, in quantities similar to those evolved by Check apples at 75°F, did not stimulate respiration at this temperature.

The validity of these findings should be tested further with oxygen absorption studies being conducted simultaneously with carbon dioxide evolution measurements.

Ethylene evolution: -- Burg and Thimann (9) studying the physiology of ethylene formation in McIntosh apples found that tissue plugs extracted from various parts of the flesh had similar rates of ethylene production. The optimal temperature for ethylene production during their two hour periods of study was 32°C and the  $Q_{10}$  value between 10° and 25° was 2.8. Ethylene production and oxygen consumption were dependent, almost equally, upon oxygen tension; the half maximum value for both processes occurred in 1.5 to 2.0 percent oxygen and the maximum value for both occurred at about 10 percent oxygen. They suggested that the same terminal oxidase, characterized as cyanide-insensitive (see reference 22) and having a relatively high oxygen affinity, was active in respiration and ethylene production. An 80 percent carbon dioxide and 20 percent oxygen mixture of atmosphere surrounding the tissue slice inhibited ethylene production by 40 percent, but when the slices were returned to air the ethylene evolution rate returned to the value

of the control. Slices maintained in 100 percent nitrogen for four hours and then returned to air showed an accelerated rate of ethylene production for about 90 minutes before the rate dropped back to the rate of the control. This indicated that under anaerobic conditions a precursor accumulated which was converted to ethylene very rapidly under aerobic conditions. They concluded that the terminal step or steps in ethylene production were not oxidative because tissue slices placed in an atmosphere of 100 percent nitrogen continued to produce ethylene at a high rate for about 60 minutes. They found ethylene dissolved in tritiated water did not become labeled, whereas ethylene was labeled very rapidly when tritiated water was admitted into the apple tissue. This lead them to the conclusion that water was metabolically incorporated into the ethylene precursor at the positions which would ultimately lead to unsaturation. The following scheme for ethylene production was proposed:



Potter and Griffiths (50) and Fidler (15) found the rate of ethylene evolution of apples during storage under modified atmosphere conditions was slower than in air. Griffiths and Potter (19) reported the accumulation of volatiles in modified atmosphere storage rooms was the cause of the de-

creased rate of volatile production by the apples. Accordingly, one might theorize that the greater ethylene production by the CA apples after removal from CA storage was caused by the accumulation of an ethylene precursor, such as compound "A" of the Burg-Thimann scheme (9). On this basis, however, it would be difficult to account for the peak in ethylene production at 33°F in air to occur as late as five weeks after the apples were removed from the CA chambers rather than earlier. A more plausible explanation of the ethylene curves observed here for CA apples would be that the factors which normally bring about this type of curve in apples shortly after harvest also brought about this type of curve in CA apples after they were removed from the CA chambers.

## CONCLUSIONS

1. After removal from controlled atmosphere storage at 38°F, McIntosh apples evolved ethylene for more than eight weeks at 33°F in air at higher rates than apples which had been stored in regular air storage at 33°F for the same length of time.

2. These studies confirmed the findings of others that storage in controlled atmospheres had a depressing effect on the respiratory activity of apples after the apples were removed from storage and placed in air at high temperatures.

3. The polyphenoloxidase system of controlled atmosphere-stored apples was potentially more active than that of regular air-stored apples. This was demonstrated by the greater rate of oxygen absorption by controlled atmosphere-stored apples in the presence of catechol.

4. The electron transfer system associated with oxidative respiration was potentially more active in controlled atmosphere-stored apples than in regular air-stored apples. This greater potential activity was demonstrated directly by the greater oxygen absorption of controlled atmosphere-stored apple discs in citric acid buffer solution and indirectly through the Burg-Thimann scheme for ethylene synthesis and comparative rates of glycolysis.

5. The volatiles given off by regular air-stored apples appeared to have

stimulated the respiratory activity of controlled atmosphere-stored apples at 75° F, but had no effect on the respiratory activity of other regular-stored apples. Since ethylene gas treatments did not stimulate the respiratory activity of these apples, some compound other than ethylene in the volatiles emanated by the regular air-stored apples was responsible for the stimulation.

6. Apples stored under controlled atmosphere conditions at 38° F had tougher skins than comparable apples stored in regular air at 33° F.

7. These studies also confirmed the findings of others that apples stored under controlled atmosphere conditions were firmer, were of higher quality, and were in better condition upon removal from storage than comparable apples stored in regular air. These more favorable characteristics were maintained by the controlled atmosphere-stored apples through the holding periods.

8. When measured after removal from storage, controlled atmosphere storage appeared to have had no effect on the quantity of cell wall material in the cortical tissues and had no effect on the respiratory quotients of apples in 75° F air.

## SUMMARY

Harvested McIntosh apple fruits were placed into storage under controlled atmosphere conditions of 5 percent carbon dioxide and 3 percent oxygen at 38° F and into regular air storage at 33° F. Comparisons were made of the physiological behavior of the apples after periodic removal from storage during the storage seasons of 1957-1958 and 1958-1959. The flesh firmness of the apples as measured by pressure tests decreased more rapidly in the controlled atmospheres than in regular air during the first few months of storage. After approximately 100 days of storage and thereafter, however, the controlled atmosphere apples were firmer than regular air-stored apples. The rates of softening of controlled atmosphere-stored and regular air-stored apples during the seven days of holding at high temperatures were extremely variable and not significantly different. Quality differences according to storage method also were detectable at this time by a panel of six persons. They judged the controlled atmosphere-stored apples to have better appearance, flavor and texture, and to be less ripe than comparable regular air-stored apples. However, they found very little difference in the rates of quality loss of the controlled atmosphere-stored and regular air-stored apples during the seven-day holding periods at a high temperature. Marked reductions in storage scald, mealy break-

down, and core browning associated with senescence were characteristic of the CA storage method.

The epidermal tissues of the controlled atmosphere-stored apples were found to be more resistant to puncture than the skin tissue of regular air-stored apples at the time of removal from storage and after eight weeks of holding in air at 33° F. Storage treatment had no significant effect on the total amount of cortical cell wall material of the apples.

A marked depressing effect of controlled atmospheres on post-storage respiration rate of 75° F became apparent after three months of storage in the first season and after six months in the second year. Ethylene treatments had no effect on the respiratory activity of apples stored to March, 1959, whereas the volatiles given off by regular air-stored apples appeared to stimulate the post-storage respiratory activity of controlled atmosphere-stored apples at 75° F. Post-storage treatment with 37 percent oxygen for a period of twelve days at 75° F had a slight depressing effect on the respiratory activity of the apples stored under both storage treatments until March. Storage treatment had no marked effect on the respiratory quotient.

Measurements of polyphenoloxidase activity of apple discs in Warburg reaction vessels by measurement of oxygen absorption after the addition of catechol showed the activity was higher in controlled atmosphere-stored apple discs than in discs from regular air-stored apples.

Controlled atmosphere-stored apples evolved aldehydes at slower rates than regular air-stored apples, but the patterns of increase in aldehyde production of both storage samples were similar during the twelve days of measurement at 75° F. These measurements were made in February, 1959, when the respiratory activity of apples from both storage treatments was equal.

The post-storage rates of ethylene production at 33° F in air by the controlled atmosphere-stored apples followed approximately the same curve throughout the season, but the rates of ethylene production by the regular air-stored apples decreased throughout the second storage season. Controlled atmosphere-stored apples, removed from storage in March of both years, evolved ethylene at rates twice those of regular air-stored apples after three to five weeks at 33° F, and this difference in rates of ethylene evolution was maintained throughout the balance of the six- to eight-weeks test periods.

Measurement of carbon dioxide and ethylene evolution indicated the respiratory climacteric in controlled atmosphere-stored apples occurred after the apples were removed from storage. Comparative rates of ethylene evolution, aldehyde evolution, and catechol and citric acid oxidation after removal from storage, indicated the principal terminal oxidase systems of controlled atmosphere-stored apples were potentially more active than those of the regular air-stored apples.

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