THE EFFECT OF REDUCED OXYGEN LEVELS ON RESPIRATION, THE PRODUCTION OF VOLATILES, AND THE KEEPING QUALITY OF BROCCOLI (*BRASSICA OLERACEA VAR. ITALICA*)

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INTRODUCTION

The problems associated with the preservation of fruits and vegetables after harvest are closely tied up with the physiology of aging. Most fruits and vegetables at the right stage for harvest are past the grand period of growth and are, metabolically speaking, on the decline. At this stage of the life cycle the catabolic processes predominate while the synthetic processes are almost at a standstill. Consequently the energy for continuing the living system must be derived from the oxidation of available materials without any means for their renewal. This constitutes disintegration, and ultimately with the exhaustion of materials, death occurs. However, long before this point is reached the use of the stored foods in biological oxidations and the associated aging processes reduce the quality of the tissue for use as food.

For many years refrigeration has been used to reduce the rate of decline of fruits and vegetables after harvest. This method is extremely effective in lowering the rate at which biological processes occur. For example, if respiration is taken as an index of total oxidative activity, a reduction from 10.7 mg. CO_2 per kg. per hour at 60°F. to 1.4 mg. CO_2 per kg. per hour at 32°F. occurs with Jonathan apples (37). In terms of storage life the reduced temperature causes an increase from approximately three weeks at 60°F. to six months at 32°F. Smock (41) estimates that each day at room temperature takes approximately one week off the storage life of McIntosh apples at 32°F. Sweet corn has a respiration

rate of approximately 30 mg. CO_2 per kg. per hour at 32°F. and a rate of 220 mg. CO_2 per kg. per hour at 80°F. In terms of storage life in which edible quality is preserved by refrigeration, this adds two to three weeks (41). Similar differences are obtained with other fruits and vegetables. Refrigeration effects a reduction in all rate processes in all parts of the plant tissue. The result is a marked slowdown of catabolism which extends the life of the cells.

Altering the atmosphere in which the tissue is held is another method for prolonging the life of fruits and vegetables after harvest. Since respiration involves the absorption of O_2 and the release of CO_2, the rate of the respiration reaction may be lowered according to the law of mass action by reducing the O_2 in the atmosphere and raising the level of carbon dioxide. This method has been used in the so-called gas storage where an atmosphere of 10% CO_2 and 11% O_2 at 46°F. is commonly used for storing certain apple varieties in England (27). Also O_2 concentrations of 2% and CO_2 concentrations of 5% at 40°F. have been shown to prolong the storage life of some northeastern apple varieties (43). However, for many varieties of apples injurious effects are obtained with such atmospheres.

One of the interesting phenomena associated with post-harvest physiology of fruits and vegetables is the production of volatile products during storage. This is especially striking with fruits. Apart from causing a characteristic odor in a fruit or vegetable, the significance of the emanated gaseous products appears to be as end products in the type of metabolism taking place. With many fruits, the peak production of ethylene has been identified at a specific phase of its storage life. Ethylene appears to have a marked physiological action

in hastening the aging process and otherwise exhibits definite physiological effects on numerous plants. The specific origin of ethylene and its function has not been definitely determined. However, the effect of ethylene on ripening of apples and bananas, yellowing of citrus fruit, blanching of celery, and epinastic responses in many seedlings has been demonstrated many times.

Current problems in post-harvest physiology of fruits and vegetables center about a detailed clarification of the progression of the aging process from the time of harvest through senescence and death. A knowledge of the course of the reactions involved would greatly aid in discovering means for reducing their rates and might aid in preserving quality in fruits and vegetables over and above that accomplished with refrigeration. Also such knowledge might lead to methods which would retard senescence by altering the degenerative cycle associated with aging. As difficult as the resolution of such problems may appear at present, there is evidence from specific crops that the aging process may be varied by altering the atmosphere in which the tissue is held. A case in point is shown with green sprouting broccoli, which when held in a sealed atmosphere even at 75°F. will maintain its original fresh green color, whereas broccoli held in an open atmosphere at the same temperature will age and yellow rapidly. However, in this case the apparent halting of the aging process is far from a complete success, since the green broccoli which appears to be fresh and edible has an extremely objectionable odor and flavor.

Nevertheless, here a clue is offered as to what may be expected under modified atmosphere storage. Research in this direction may lead to an explanation of yellowing in broccoli and to the origin of the off-

flavors. It is in this specific area that the work reported here was attempted with the hope that some additional information might be obtained on the aging phenomena in broccoli after harvest.

REVIEW OF THE LITERATURE

Introductory Note:

The literature on post-harvest physiology in relation to the preservation of quality in fruits and vegetables, by methods other than refrigeration, may be divided into two phases. Phase one covers the early work which was essentially exploratory. In this period the concentrationsof O_2 and CO_2 in the atmosphere around fruits and vegetables were altered by sealing large containers and the gross effects were observed. In phase two some formulations and generalizations on the effects of variation of CO_2 and O_2 on respiration and ripening were made. The concept of controlled atmosphere storage of deciduous fruit was developed and the effect of normal and abnormal atmospheres on respiration and ripening was studied. The importance of ethylene was demonstrated, and its origin in the ripening process was established. Attempts were also made to identify volatiles other than ethylene that emanate from ripening fruit. Most of this work was done with the apple.

Review of Phase I:

More than 130 years ago Berard (1) reported that the life of fruit after harvest could be extended by picking a few days before it is ripe and storing in sealed jars. Peaches, plums, and apricots were held in good condition for 20 to 30 days, and pears and apples for three months.

Even at that early date Berard recognized the retarding effect on ripening of reduced O_2 in the atmosphere. He also observed that while fruits kept in the sealed containers would ripen well if removed within the recommended time, they would not ripen if held for a longer

period and subsequently returned to a normal atmosphere.

In 1907 Fulton (13) reported on experiments in which strawberries were stored in containers of varying degrees of tightness. He found that a moderately tight package lightly retarded mold and caused retention of the bright color of the fruit. Those packages that were sufficiently tight to allow the CO_2 of the storage air to reach a concentration of seven to 10% caused the fruit to develop a bad flavor.

Gore and Fairchild (18) found that the astringency of Japanese persimmons could be removed by three to five days storage in an atmosphere of carbon dioxide. This effect was presumed to be due to a transformation of tannins. During this process softening was retarded, but was resumed after the CO_2 storage period.

Hill (26) in 1913 studied the production of CO_2 in hydrogen, in nitrogen, and in air. The rate of softening of peaches was greatly decreased in CO_2, and also in hydrogen and in nitrogen. Peaches became discolored and acquired a bad flavor when O_2 was withheld from them. Ripe apples lost their color, texture and flavor by being kept for a sufficient length of time in oxygen-free gases. Good ventilation was recommended along with refrigeration for the storage of fruits.

Brooks, Cooley, and Fisher (8) found that high percentages of CO_2 for a short period or low percentages for a long period delayed the ripening of apples, but there was danger of injuring the flavor of the fruit. However, apples were held at 36°F. in an atmosphere containing two to three percent CO_2 for 20 weeks without injury. Attempts to use higher percentages of CO_2 over a prolonged period resulted in serious damage to the fruit. However, apples were held in 100% CO_2 for two to six days at 59°F. with no apparent injury.

In a comprehensive study on the post-harvest physiology of the apple, Magness and Diehl (30) used atmospheres containing CO_2 concentrations of five, 10, 20 and 50 % with 20% oxygen. They found that the softening of the apples was markedly inhibited, the retardation of softening varying with the CO_2 concentration used. Carbon dioxide concentration of five and 10% had no appreciable effect upon the flavor of the apples. In concentrations of 20% there was a slight flavor of fermentation. The fruit held in a concentration of 50% was entirely inedible. These experiments were carried out at 71.5°F. and were continued for a period of 10 to 11 days.

Brooks and associates (9) tested the effect of CO_2 in the atmosphere of various fruits and vegetables on the general quality and also the action of the gas in inhibiting spoilage organisms. It was found that the greatest limitation to the use of CO_2 was its effect on the flavor of the product. Peaches, apricots, strawberries and red raspberries were found to be very sensitive to carbon dioxide. Cherries, pears, plums, apples and oranges had a greater tolerance to the gas, while sweet corn and carrots stood high CO_2 concentrations with favorable results. The deleterious effect of CO_2 upon the flavor increased with an increase in temperature in a manner that indicated a relationship with general metabolic activities. Carbon dioxide was found to be especially effective in holding back the softening of fruit. The softening of warm peaches was as greatly checked with 25% or more of the gas as by a drop in temperature of 18°F. Botrytis and Rhizopus rot on strawberries were fairly well inhibited by 23% CO_2 and completely inhibited by 37 percent. Botrytis inoculations on Bartlett pears and Monilia on Italian prunes were held completely in check by 20 to 30% CO_2 and greatly inhibited by

12 to 15 percent.

Thornton (45) investigated the effect of CO_2 on various fruits and vegetables. Low concentrations of CO_2 (15 - 25%) were found to retard respiration, external color changes, and removed noticeable astringency found in some green fruits. High concentrations of CO_2 (50 - 80%) impaired flavor of all fruits and some vegetables, inhibited ripening, caused internal discoloration and breakdown in the apple, pear, peach and tomato. Generally CO_2 injury increased with increase in storage temperature. Thornton contended that these results indicated that O_2 content may be the controlling factor in CO_2 injury. These results are generally very similar to those of Brooks and associates (9).

In an attempt to determine the effect of the CO_2 content of the storage atmosphere on carbohydrate transformations, Miller and Brooks (32) subjected peaches, cherries, sweet corn and garden peas to CO_2 gas atmospheres and subsequently analyzed for sugars and hydrolyzable polysaccharides. The fruits and vegetables were held for one to six days in CO_2 concentrations of 35 to 47% at temperatures of 32°F., 41°F., 50°F., 68°F. and 77°F. The results showed no significant difference in percentage of reducing sugars, total sugar or acid hydrolyzable polysaccharides, with cherries or peaches at the lower temperatures. The rate of sugar loss in peas and sweet corn was retarded. At the higher temperatures the high CO_2 adversely affected the flavor of peaches, but not that of cherries. Exposure to the high CO_2 concentrations for two days did not affect the flavor of peas or sweet corn held at 59°F. and 77°F.

Thornton (47) treated various plant tissues with 50 to 70% CO_2 in the presence of O_2 at 77°F. and found that there was a decrease of acidity over the control. Storage of the tissues in air after the CO_2 treatment

resulted in restoration of the pH value at the same level as that of the control. The pH change in the potato tissue sap in the alkaline direction in the presence of CO_2 was found to be dependent on the presence of oxygen. In the absence of O_2 the change in pH was not in the alkaline direction but in the direction of increased acidity.

Summary of Phase I:

The work reviewed above is representative of the first mentioned phase. Different species of fruits and vegetables were shown to have varying tolerances to high CO_2 and low oxygen. The apparent advantage of high CO_2 was shown to be a retardation of ripening as exhibited by retention of hardness and green color in fruits. However, in the sensitive fruits and vegetables, and in all cases in which high concentrations were used, the flavor of the fruit was seriously affected, and in some varieties internal discoloration and breakdown occurred. The CO_2 injury increased with storage temperature which indicated that the injury was metabolic in nature. Most of the work was done with high concentrations of carbon dioxide. A range of 15 to 25% was considered a low concentration and ranges of 50 to 100% were used as high concentrations.

Generally very low O_2 (2.5%) and high CO_2 (above 25%) permanently injured the fruit so that normal ripening was never attained. Peaches were especially sensitive to carbon dioxide. Concentrations above 15% CO_2 were lethal to the peaches causing discoloration and death of the cells. There seemed to be a considerable difference among different commodities in their tolerance to CO_2 and in their O_2 requirements. Also there was apparently a difference in this respect at different maturity levels.

In this early work only minor emphasis was laid on O_2 concentration in relation to CO_2 concentration. What is clear from this work, however, is that the normal course of respiration and ripening was altered by a change in the atmosphere. This alteration appears to perform a desirable function at first, but apparently the retardation effects are not equally distributed to all the metabolic processes associated with ripening.

Review of Phase II:

Kidd and West (27) were the first to study quantitatively the CO_2 and the O_2 in the atmosphere as it affects post-harvest physiology in fruits. They formulated a practical method for lengthening the storage period of apples by modifying the surrounding atmosphere. It was demonstrated that the rate of ripening of the fruit after harvest is directly related to the amount of O_2, and inversely related to the amount of CO_2 in the atmosphere in which it is stored. It was further shown that CO_2 and O_2 within only a certain range of concentrations will allow ripening to progress and be completed in a normal manner. Atmospheres containing about 10% of CO_2 and 11% of O_2 at 46.5°F. were found to be the most effective concentrations for retarding the rate of ripening. Atmospheres containing less O_2 or more CO_2 were occasionally found to cause abnormal effects. Higher concentrations of O_2 and lower CO_2 lessened the retarding effect on ripening. In higher or lower temperatures the effect of the restricted atmosphere was lost. At the recommended temperature and concentrations of gases the storage life of some apple varieties was doubled from 140 days at 34°F. to 280 days at 46°F. in an atmosphere of 11% O_2 and 10% carbon dioxide. Failure of the lower temperatures with controlled atmosphere was attributed to the

accumulation of an excessive concentration of CO_2 in the tissue fluids while at higher temperatures O_2 was assumed to be the limiting factor for the tissues.

The effectiveness of gas storage was shown to be due to both the low O_2 and the high carbon dioxide. When O_2 was reduced to 11% while CO_2 was held to zero concentration the fruit had a reduced storage life as compared to the recommended concentrations of O_2 and carbon dioxide. The respiration of Bramley's Seedling apples at 46°F. in an atmosphere of nine percent O_2 and 12% CO_2 was approximately one half that in air storage at 46.5°F. The storage life in the controlled atmosphere was double that in air storage at the same temperature. These data indicate that the retardation of ripening and prolongation of storage life was associated with a proportionate slowing down of respiratory activities.

Since the early work of Kidd and West many workers in the United States have tried to use controlled-atmosphere storage to prolong the storage life of apples. Smock (43) has found that the storage life of McIntosh apples can be increased several months if they are kept in five percent CO_2 and 2.5% O_2 at 40°F. However, the varieties Jonathan, Golden Delicious, Rhode Island Greening and Cortland did not respond favorably to controlled-atmosphere storage.

Mattus (31) reported that Bartlett pears stored in an atmosphere of 9% CO_2 and 3.5% O_2 at 33°F. were kept 206 days compared to 84 days in air at the same temperature. Respiration was shown to be considerably reduced in the controlled atmosphere.

Claypool and Allen (10) studied the effect of varying O_2 levels on the respiration of Santa Rosa plums, Primrose peaches, Bartlett pears, Thompson Seedless and Tokay grapes, and other fruit. During a 10 day

holding period at 65°F. and an O_2 level of 10%, a lower respiration rate than in air resulted for apricots, plums, Primrose peaches, Bartlett pears and Thompson Seedless grapes. This decrease was approximately in proportion to the O_2 reduction. A variety of sweet cherry showed reduced respiration only when the O_2 concentrations of 30 to 100% failed to show any higher respiration than when held in air.

In general 2.5% O_2 at 65°F. reduced metabolic activity so that there was little difference between the ripeness of the fruit after a 10 day period at 65°F. and those held at 40°F. Reduction in respiration rate was reflected in the general reduction in coloring and softening of the fruit.

In another study Claypool and Allen (11) held plums, pears and peaches in atmospheres containing 2.5, 5, 10, 15, and 21% O_2 in the absence of CO_2 and also in atmospheres of 2.5, 5, 10, 15, 25, 40 and 60% CO_2 with corresponding O_2 levels of 20.5, 20, 19, 18, 16, 12.5 and 8 percent. In each series of atmospheres the fruits were held at both 40°F. and 65°F. At 40°F. ripening was retarded no more in modified atmospheres than in air. However, after removal to 65°F. for color development in normal air the check lots developed color somewhat more rapidly than those previously held in high carbon dioxide. Comparable lots held at 65°F. showed marked differences. The lots in 2.5% O_2 showed retardation of color development. Carbon dioxide concentrations of 5 to 15% had some retarding effect on color development, whereas concentrations of 25 to 60% CO_2 prevented the normal development of color, and in some cases were lethal to the fruit.

Review of Respiration:

Respiration of mature and aging tissue has been studied by many

investigators usually in conjunction with ripening experiments (3,4,27,28,34). Biale (2), in his review of the literature on post-harvest physiology and biochemistry, discusses the different methods used to investigate respiration in aging tissue. Most respiration studies report the CO_2 produced by a kilogram of tissue per 24 hours.

Kidd and West (29) studied respiration of apples throughout the growing season. The apples were harvested at various stages of growth from the three-gram apple stage to the fully grown 120-gram apple stage. The apples of each harvest were held in respiration chambers at 10, 12, 18 and 22.5°C. until physiological breakdown or attack by mold occurred. The course of respiration followed four phases. The first phase soon after harvest showed a rapid fall in respiration. The second phase showed a levelling off but the slow and steady fall continued. This was followed by phase three during which there was a sharp rise to a maximum. Phase four showed a rapid drop and a levelling off. Kidd and West have termed these phases as preclimacteric (phases one and two), climacteric (phase three) and post climacteric. The climacteric as defined by Biale (2) is the marked and sudden rise in respiration prior to senescence. This senescent rise in respiratory activity will also occur while the apple is still attached to the tree. Kidd and West believe, and have some data to show, that there is no marked difference in the time of occurrence of the climacteric between apples gathered when weighing only a few grams (around twenty) at temperatures comparable to those in the orchard, and corresponding fruit allowed to grow to full size on the tree. At low temperatures the climacteric is prolonged and drawn out while at high temperatures it occurs very quickly (190 days at 2.5°C. compared to seven days at 22.5°C.). The difference between

early and late varieties is in the time of occurrence of the climacteric. This may be related to ethylene production which is believed to hasten the occurrence of the climacteric. In the absence of O_2 the climacteric does not occur, while raising O_2 concentration above air values brings the climacteric on at an earlier time. Moderate concentrations of CO_2 in the atmosphere (up to 10%) postpones the onset of the climacteric. Kidd and West attribute the climacteric to a change in state in the protoplasm which has a high temperature coefficient. Ethylene production by the fruit associated with this change in the protoplasm, stimulates respiration and causes the occurrence of a climacteric.

Smock and Gross (44) found respiration to be very high in apple fruit soon after setting. The rate declined quite rapidly during the season and reached a minimal value shortly before the normal harvest date for most varieties. Respiration then continued to rise to a maximum value (the climacteric) and then declined during senescence. This is essentially the same drift in respiration as found by Kidd and West. Smock and Gross further found that while the climacteric was very obvious at high temperatures of 60-75°F., it was not too well observed in all varieties at low temperatures. In general the varieties of apples with a short storage life had a higher climacteric peak than medium or long storage varieties. The long keeping varieties had a lower peak and the decline in rate following the peak was more gradual.

Blackman and Parija (4) considered the respiration of apples or any other fruit and vegetable after harvest as that of an isolated starving organ. In such a condition there is a change in the organization of the tissues, so that hydrolysis of reserve and semi reserve substances proceed at a rapid rate. This leads to a greater production

of substrate for respiration and consequently to an increased production of carbon dioxide. When this senescent change has completed itself, respiration drops due to the natural starvation condition that is present in an isolated plant organ. However, the work of Kidd and West (28) demonstrated that only about 16 to 20% of the carbohydrate reserve was exhausted at the time of death of the apples held in storage. This is opposed to the starvation concept as developed by Blackman and Parija.

Blackman (3) introduced the concept of the extinction point in respiration. In his study in which the O_2 concentration was varied in the atmosphere of Bramley's Seedling apples, he found the CO_2 production increased on either side of a 5% O_2 concentration. The extinction point is considered to be the concentration of O_2 at which CO_2 production is a minimum. Above this concentration CO_2 is increased in aerobic respiration, while below this minimum CO_2 production is increased in anaerobic respiration.

Platenius (36) studied the effect of O_2 concentration on the respiration of certain vegetables. He found that any decrease of atmospheric O_2 produced a corresponding drop in respiration but the relationship was not linear. An increase of O_2 at low O_2 levels in the atmosphere caused a much greater increase in respiration than it did at levels close to normal. Also the response to O_2 change was more pronounced in the beginning of the storage than subsequently. The magnitude of the response varied with different types of tissues. Platenius recognized a threshold concentration of O_2 below which anaerobic respiration takes place. This is Blackman's extinction point referred to previously. The position of the extinction point differed with time showing that the tissue became more tolerant to low O_2 concentration as the storage period was lengthened.

The role of O_2 in respiration was considered to be two-fold: (1) as a reactant in normal aerobic respiration and (2) as an inhibitor of anaerobic respiration (Pasteur effect). The Pasteur effect comes into play at the threshold value (extinction point) below which anaerobic respiration takes place. If anaerobic respiration is high, the production of CO_2 increases below the extinction point and at the extinction point the total CO_2 production is at a minimum. However, if anaerobic respiration is very low it is difficult to determine the extinction point since the plant tissue will fail to show a secondary rise in CO_2 production. Different tissues vary considerably in their tolerance to low O_2 concentrations. An O_2 concentration of less than 10% maintained normal aerobic respiration in spinach and lima beans while shelled peas did not tolerate levels below four percent. Tolerance to low O_2 concentration increases with the aging of the tissue and with lowering of temperature.

Volatile Emanations from Stored Fruit:

The aroma associated with fruits and vegetables is essentially due to the gases that are emitted in their metabolism. Brooks and Cooley (7) found that apple scald, the sub-surface browning of the tissue found in apples during storage, could be significantly reduced by oiled paper wraps or oiled shredded paper placed throughout the apple box. By absorbing the gases as they are produced, the oiled paper reduces the quantity of volatiles and prevents injury to the apple tissue.

Of all the volatiles emanating from plant tissue in storage, ethylene holds the dominant position. Crocker (12) has reviewed the history of ethylene and has stated that many and perhaps all plant tissues produce ethylene. Gane (14,15) was the first to chemically identify

ethylene as a by-product of apples in storage. An interesting sideline noted by Gane was that the plant tissues sensitive to ethylene or the volatile products from apples are pea seedlings, radish, tomato seedling and potato seedlings, while barley, corn, oats, wheat and other cereal seedlings are insensitive to ethylene. This is very similar to the relative sensitivity of monocots and dicots to 2-4D and related herbicides. The similarity of the action of ethylene to phytohormones suggests that ethylene may be classified along with other plant hormones. Gane estimated that ethylene production was approximately one c.c. during the life history of the apple fruit.

Hansen (24) studied the production of ethylene and the respiration of five varieties of apples ranging in season of maturity from midsummer to late fall. Generally ethylene production followed, but lagged behind, the curve for CO_2 production throughout the life of the fruit. Varieties of summer apples were found to produce more ethylene during ripening than late-maturing fall varieties. The maximum rates of ethylene production at 68°F. in ml. per kg. per 24 hours were as follows: Astrochan; 11.38; Red June; 9.27; Gravenstein; 5.16; and, Delicious; 1.77. The rate of ethylene production increased rapidly after picking in the summer-maturing apples but slowly in Delicious, a late variety. Apples stored at 32°F. produced ethylene at approximately 1/8 to 1/11 the rate at 68°F. which gives a Q_{10} of four or five. The early-maturing varieties showed a distinct climacteric rise in respiration, but this was not apparent in the late varieties, Delicious or Newtown apples.

Emanations from ripe pears have been found by Hansen and Hartman (25) to increase respiration and ripening of newly picked Bartlett, Comice

and Anjou pears. The greatest effect was obtained with fruit picked early in the season, and the least effect with fruit picked at post-mature stages. The production of ethylene, as indicated by leaf epinasty, appeared to increase during the period of ascending respiratory activity.

Smock (42) found that as few as 1% of the total number of apples in storage can supply enough stimulatory emanations to cause increased ripening of the other fruit. The stimulatory emanation is presumed to be ethylene. Post-climacteric apples, which produce more ethylene, are more potent sources of the stimulatory effect while the maturity of the lot being stimulated will determine the intensity of stimulation. Only pre-climacteric fruit is affected by ethylene stimulus.

The climacteric of Bartlett pears at 65°F. was found to occur approximately seven or eight days earlier than does the maximum for emanation of volatiles. Gerhardt and Ezell (16) showed that increase of volatile production was closely related to the presence of scald and other physiological storage disorders.

Mattus (31) found that after removal from 33°F. storage a considerably higher amount of organic volatiles other than ethylene was produced in air as compared to pears held in controlled atmosphere storage of 9% CO_2 and 3.5% oxygen. Ethylene was also somewhat higher in air while respiration was considerably reduced with the low oxygen. Kidd and West (27) state that low O_2 inhibits the production of ethylene and consequently reduces the ripening effect of ethylene.

In studies on the effect of ethylene on chemical changes in the plant Hansen (23) applied ethylene at definite periods in the ripening of pears. He found that as a result of ethylene treatment the rate of

starch digestion, the concentration of total and reducing sugars, and the transformation of protopectin to pectin all increased, but there was no change in titratable acidity. Apparently ethylene increased the hydrolytic reactions. The magnitude of the response was determined by the maturity of the fruit.

In another study, Hansen (22) found ethylene effective in increasing the rate of softening in fruits. Gooseberries, peaches, prunes, pears and lemons all showed this effect. The softening effect of ethylene is believed to be due to hydrolysis of protopectin to soluble pectin.

In a recent study, Hall (19) determined the ability of different substrates to yield ethylene after addition of a crude enzyme extract expressed from apples. Arabinose, ethanol, and some pectins appeared to be the best substrates for ethylene production. These data apparently corroborate the work of Hansen, which demonstrates that ethylene production in pears is associated with softening due to hydrolysis of protopectins to soluble pectins. Since hydrolysis to pectins yield arabinose, and the production of ethylene is generally assumed to be associated with the softening or ripening of fruit, this hypothesis, although not definitely proven, appears to follow logically from the available data.

Summary of Phase II:

In this phase particular attention was paid to the various ramifications of the respiration process. By reducing O_2 and increasing CO_2 concentrations in the atmosphere, respiration was retarded and formulation of a practical method for gas storage was developed. The concept of a climacteric in respiration was introduced and ethylene was dis-

covered as a natural product emanating from fruit in storage.

The course of respiration in apples after harvest was shown to pass through a pre-climacteric, climacteric and post-climacteric phase. The pre-climacteric is characterized by a fall in respiration followed by a levelling off, the climacteric by a sharp and rapid rise to a maximum, and the post-climacteric by a rapid drop and levelling off. The difference between early and late varieties of apples was related to the occurrence of the climacteric which took place earlier and had a higher peak in apples with a short storage life. The occurrence of a climacteric is believed to be due to a change of state of the protoplasm preceding death.

Blackman's concept of respiration in the apple as that of a starved isolated organ was disputed by Kidd and West who found that only 16 - 20% of the carbohydrate reserves were exhausted at the end of the storage life.

Respiration studies under conditions of low O_2 concentrations revealed an extinction point. The extinction point is defined as the level of O_2 concentration at which aerobic respiration ceases to exist. On either side of the O_2 concentration at the extinction point there is an increase of carbon dioxide. The position of the extinction point becomes lower after each day of storage. This indicates that aging tissue becomes more tolerant to low O_2 concentrations.

Ethylene in very small quantities was recognized as a volatile emanating from ripening fruit. The course of ethylene production was similar to that of carbon dioxide. However, ethylene production reaches its maximum value slightly later than the respiratory climacteric. Apple varieties with a short storage life were shown to produce more

ethylene than those that have an extended life in storage. Ethylene was suggested as the stimulus which brings on the climacteric in ripening fruit. A recent theory on the origin of ethylene associates the hydrolysis of pectins and the utilization of arabinose as a substrate in respiration to the production of ethylene.

Present Status of These Problems:

At present a third phase in post-harvest physiology may be recognized. Present problems are very similar to earlier ones in that basic information is still lacking as to the details of the mechanism of action of reduced CO_2 or O_2, or increased O_2 or CO_2 in the atmosphere. Particularly, an attempt is being made to explain the course of the post-harvest respiratory drift in the light of current concepts of respiration.

PURPOSE OF THE EXPERIMENT

The purpose of this experiment was to determine the factors associated with the yellowing of broccoli, especially at temperatures above 70°F. Broccoli will remain perfectly green at 70°F. when held in a sealed container (Fig. 1). However, the sealed atmosphere causes a buildup of undesirable odors and also has a deleterious effect on the flavor of the vegetable. The possibility of retaining the green color of the broccoli without the accompanying harmful effects caused by the restricted atmosphere of a tight package presented itself.

In the sealed package there is a reduction of O_2 content, an increase of CO_2, and also a buildup of volatile products. The test was designed to determine if the retention of the green color was due to the CO_2 in the sealed atmosphere, the low O_2, the presence of some volatile emanation or some other factor or factors.

One reference to broccoli storage in artificial atmospheres was found in the literature. Smith (40) stored broccoli at 32°F. and 38°F. under various O_2 and CO_2 levels. All the broccoli remained in good condition at these low temperatures for a considerable time. At 38°F. the broccoli in an atmosphere of 10% CO_2 and 5% O_2 kept best, and storage for 14 to 21 days was considered feasible. At 32°F. a storage life of four weeks was found feasible in an atmosphere containing 10% CO_2 and 10% oxygen.

It was found (38) that broccoli could be held in good condition for three to four weeks at 32°F. at normal atmospheric concentrations of O_2 and carbon dioxide. Therefore, gas storage at the lower temperatures

Fig. 1. Broccoli held in a tray open to the atmosphere and broccoli overwrapped with cellophane, one perforated and the other sealed, as indicated.

(32° and 38°F.) seems redundant. However, limited atmospheres at higher temperatures might prove to be beneficial.

MATERIALS AND METHODS

All of the broccoli used in these experiments was obtained at the Washington, D. C. wholesale market during the months of December, January, February, March, and April of 1951 and 1952. The produce was shipped from the Laredo, Texas region by rail, under standard refrigeration, and was part of the regular commercial pack of the Green Top brand. The broccoli was well iced in the crate and in the freight cars; so that the temperature of the commodity was approximately 40°F. during transit. The transit period from Laredo, Texas was usually seven days, and a day or two sometimes elapsed before the cars were unloaded. Generally when the broccoli was obtained for the test it was nine to 10 days from harvest in the field. The broccoli in each test was derived from a single crate from which stalks were selected for uniformity of maturity on the basis of appearance.

In order to determine the effect of a reduced O_2 atmosphere on retardation of yellowing, broccoli was subjected to atmospheres containing 10%, 5%, and 2.5% O_2; the balance of the atmosphere in each case was composed of nitrogen. In each test some of the broccoli was also subjected to atmospheres of normal air as a paired control comparison. Cylinders of compressed gas were used in all cases. The gas cylinders were made up to the desired O_2 concentration commercially and the concentrations were rechecked before they were put into use. In actual measurements the O_2 concentrations in the cylinders were found to be 11%, 6.5%, and 2.5% oxygen.

The plan was to determine respiration, ethylene, other volatiles

and organoleptic factors such as color, odor and appearance in the different oxygen atmospheres, in the presence and absence of carbon dioxide.

All the experiments were carried out in a constant temperature room in which the temperature was held at 75°F. ± 2°F. Except for the brief periods when reading were taken, the broccoli was kept in the dark. There were four treatments in each test throughout these experiments. These four treatments were replicated three times for each O_2 concentration.

Respiration:

In the respiration studies treatment one consisted of three levels of O_2; 2.5%, 6.5% and 11 percent. Treatment two was the "control" in which air was used. Treatment three was the treatment in which broccoli was held in a sealed atmosphere and in which CO_2 was continually absorbed as it was formed in respiration. The initial atmosphere in treatment three was fixed by purging to the concentration of O_2 under test. Treatment four was exactly the same as treatment three except that the CO_2 was allowed to build up as the O_2 was depleted. Treatments three and four represented the effect of CO_2 in a closed system along with the buildup of unknown volatiles. Each test ran for three days at 75°F., after which time the broccoli had reached the end of its salable life.

Desiccators of about 10 liter capacity were used as respiration chambers in all the treatments. The apparatus pictured in figure 2 was used in treatments one and two. This consisted of a gas cylinder with a two step pressure regulator, a gas washing bottle which contained a concentrated solution of KOH (20%), and a gas washing bottle containing water. The gas stream was fed into the respiration chamber at a rate

Fig. 2. Apparatus used for determining respiration in the presence of 2.5%, 6.5%, and 11% oxygen and in the air controls.

of approximately 100 c.c. per minute. The scrubbed and wetted gas was then passed through the respiration chamber and on through five aerator tubes which were contained in five large test tubes (75 c.c. volume) hooked in series. Each test tube contained 25 c.c. of approximately 2N KOH. Preliminary experiments indicated that five tubes containing 25 c.c. KOH of this normality were sufficient to absorb the CO_2 produced. Before the five test tubes were hooked into the gas line, the cylinder of gas with the required O_2 concentration was passed through the respiration chamber for approximately three hours to establish the desired atmosphere. The CO_2 released in the respiration chamber was continuously passed through the alkali absorption line for 24 hours and then replaced by another series of five tubes with renewed KOH. Over a three day period three samples were thus obtained at 24, 48 and 72 hours.

Respiration was determined by titrating the alkali with standard HCl of approximately 2N concentration in the double titration method described by Haller (20) and Magness and Diehl (30), in which the alkali is titrated to the phenolphthalein end point and then to the methyl orange end point. The difference between the phenolphthalein and methyl orange end points is equivalent to the CO_2 absorbed. Respiration is expressed as mg. of CO_2 per kg. of broccoli per hour.

Respiration in treatment three was studied by starting out with an atmosphere of the desired O_2 concentration obtained by sufficient flushing of the desiccator until the desired concentration was obtained, and allowing respiration to proceed in the closed system. Carbon dioxide was removed in the system by absorption with KOH of approximately 2N concentration. The apparatus is pictured in Fig. 3. It can be

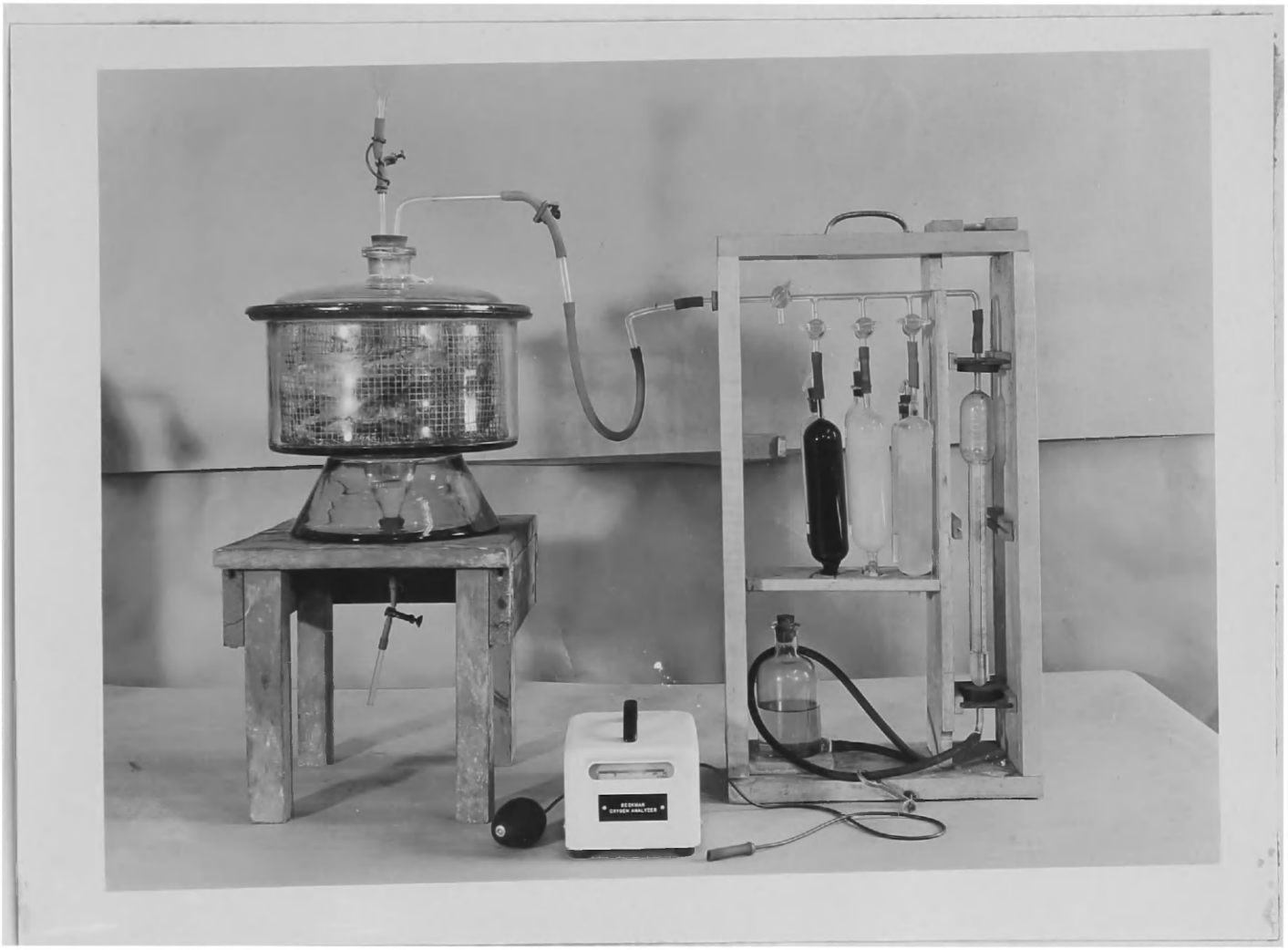

Fig. 3. Apparatus used for respiration studies in the sealed treatments in which the carbon dioxide was either allowed to accumulate or was absorbed.

(The Orsat apparatus is at the right; the Beckman-Pauling oxygen analyzer is shown in the bottom center.)

seen from the photograph that there is an upper funnel in which the KOH is introduced and a lower funnel in which the KOH reservoir is maintained. The KOH in the bottom funnel was drained and renewed every 24 hours over the three day period. At the time of KOH renewal, O_2 readings were taken with a Beckman - Pauling oxygen analyzer or with an Orsat apparatus when working at the low O_2 levels. Carbon dioxide was also determined at the same time with the Orsat apparatus. Carbon dioxide was determined by titration with standard HCl in the double titration method described above.

The apparatus in treatment four was exactly the same as that in treatment three except that respiration was allowed to take place in the presence of the CO_2 produced. Three separate desiccators made up the one day, two day and three day samples. The initial atmosphere containing the desired O_2 level was introduced in each of the three desiccators and respiration was allowed to proceed in the presence of the CO_2 produced. In the one day sample the KOH of approximately 2N concentration was introduced after 24 hours to absorb the CO_2 produced during the first day. The alkali was introduced after 48 hours for the two day sample and after 72 hours for the three day sample. In each case the alkali was allowed to remain in the chamber approximately 24 hours in order to absorb the carbon dioxide. Readings on the concentration of CO_2 and O_2 in the atmosphere of the respiration chamber were made before the addition of the alkali and before the alkali was drained. In order to obtain a sample of gas for analysis from the respiration chamber it was sometimes necessary to bring the pressure of the atmosphere in the desiccator back to normal. This was done with nitrogen. The correction for the addition of nitrogen on the percentage of O_2

and CO_2 was calculated to be negligible and was disregarded.

Respiration was determined by titrating the alkali with standard acid as described above. In order to obtain the respiration that occurred for the second day in the presence of CO_2, the respiration value for the first day was subtracted from the value obtained for the second day. For the three day value the respiration for two days was subtracted from the value obtained for three days. Since all the data are comparative rather than absolute, the corrections for pressure changes in the respiration chambers were not made. The error involved is therefore constant throughout the treatments.

Broccoli samples for respiration in all four treatments weighed approximately 425 grams.

Determination of ethylene and "other" volatiles:

In this phase of the study large jars of 20 liter capacity were used as chambers in which to maintain an atmosphere of reduced O_2 at a constant level. Treatment 1A was similar to treatment 1 in the respiration studies with the exception that larger vessels were used and the gas was passed through an absorption apparatus, as shown in figures 4 and 5. Treatment 2A was exactly the same as treatment 1A, except that air was used. Treatments 3A and 4A were similar to treatments 3 and 4, except that the "other" volatiles and ethylene were absorbed as will be described below.

The three air tight chambers containing the broccoli samples represented the one day, two day and three day samples to be discussed in the next section under organoleptic evaluations. Following these jars was the aspirator bulb used as a pump to push the air through the absorption apparatus for volatiles and ethylene. The aspirator bulb was

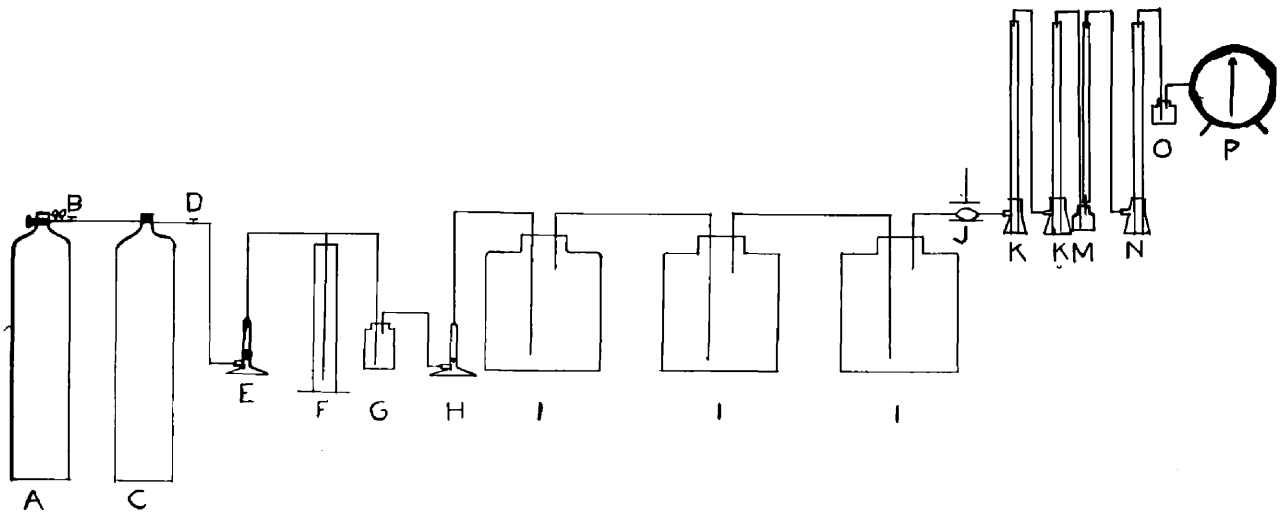

Fig. 4. Schematic diagram of apparatus used to determine ethylene and "other" volatiles in continuously flushed systems.

A. Compressed gas cylinder
B. Pressure regulator
C. Gas cylinder used as reservoir
D. Needle valve
E. Flowmeter
F. 1 liter glass cylinder used as barostat
G. Water trap
H. Flowmeter
I. Jars used as chambers
J. Aspirator used as pump
K. Absorption tower for "other" volatiles (containing con. H_2SO_4)
M. Trap
N. Absorption tower for ethylene (60% $HClO_4$ + mercuric oxide)
O. Trap
P. Wet test gas meter

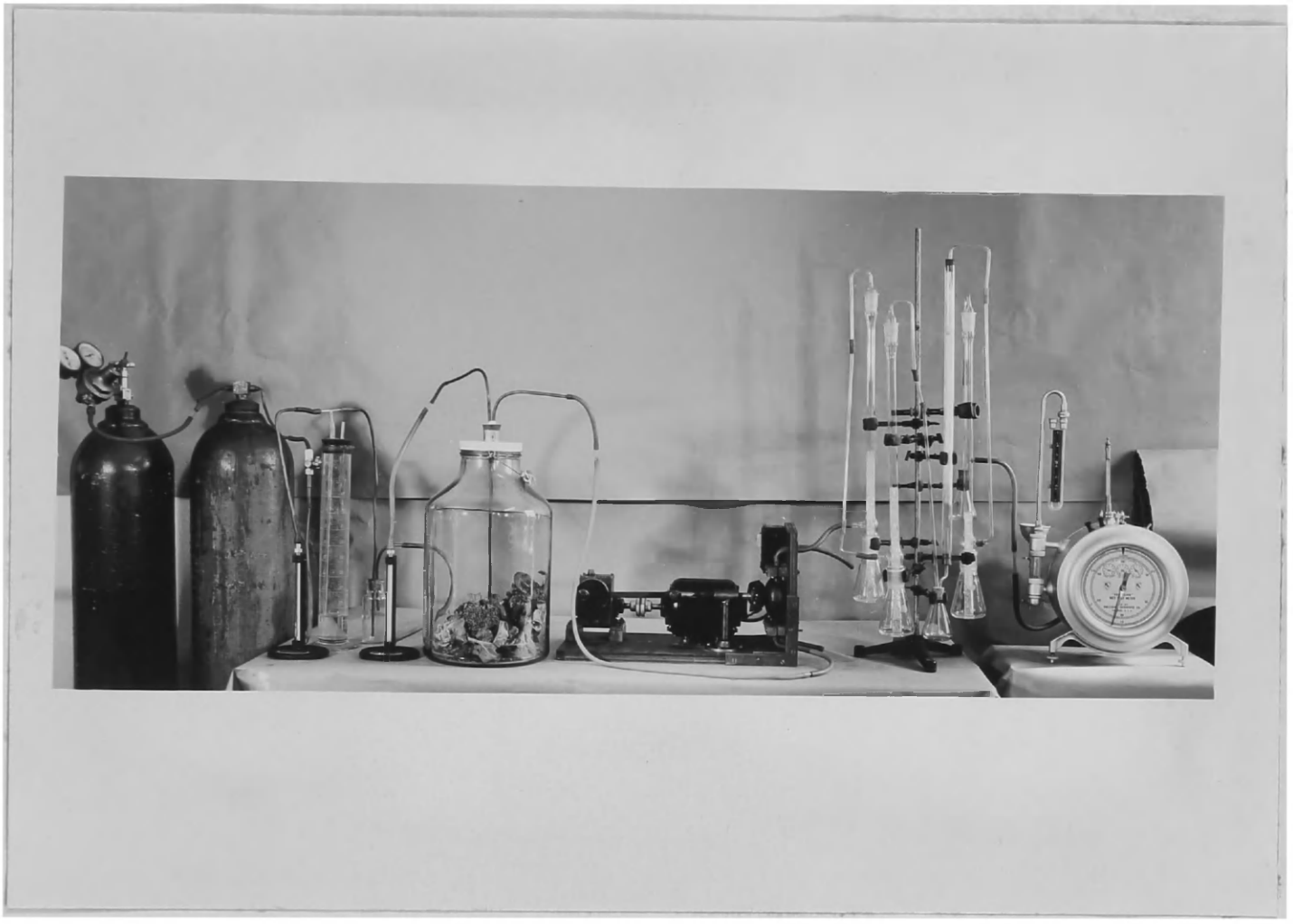

Fig. 5. Equipment used to determine ethylene and "other" volatiles in continuously flushed systems; only one jar shown. (See Fig. 4 for details).

compressed approximately once a second and drew air out of the jar and pushed it through the absorption system. The rate of flow was determined by adjustment of needle valves and length of stroke of the piston like rod on the aspirator pump. The rate of flow was adjusted to about 500 c.c. per minute and a record of the total amount of gas passing through the system was recorded on a wet test gas meter.

The setup for treatments 3A and 4A is pictured in figures 6 and 7. In these treatments the gas in the jars was initially brought to the desired O_2 level by continuous flushing. There were three jars, representing the one, two and three day samples, for each treatment. Each day a jar was connected to the exit end of the absorption apparatus on one end and the intake tube of the aspirator bulb at the other end. In this manner the atmosphere in the jar was circulated through the absorption apparatus and the "other" volatiles and ethylene were continuously extracted. The jars in treatment 3A had a beaker containing 300 c.c. of 10% KOH to absorb the CO_2 produced. In treatment 4A CO_2 was allowed to build up.

"Other" volatiles and ethylene were allowed to accumulate for one and two days in the two day and three day samples respectively before they were subjected to the absorption apparatus. Readings on the percentage of O_2 and CO_2 in the atmosphere before and after removal of ethylene and "other" volatiles were made at the start and end of an experiment. In treatments 1A and 2A approximately 750 grams of broccoli were used in each jar. In treatments 3A and 4A approximately 450 grams of broccoli were used.

The method for determining ethylene and other volatiles was that of C.R. Gross of the Department of Pomology, Cornell University (19). This

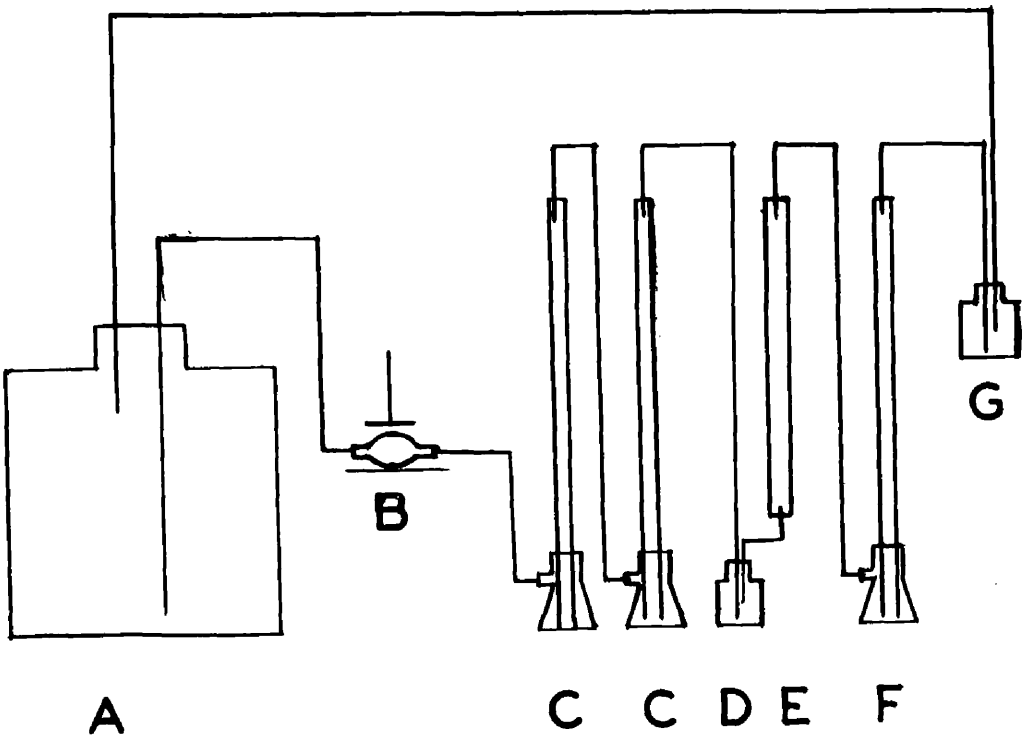

Fig. 6. Diagram of apparatus for the absorption of ethylene and "other" volatiles in sealed systems. In these treatments the atmospheres were circulated through the absorption apparatus.

A. Jar used as sealed chamber
B. Aspirator pump
C. Towers containing H_2SO_4 for absorption of "other" volatiles
D. Trap
E. Magnesium perchlorate absorbent
F. Tower containing perchloric acid for ethylene absorption
G. Trap

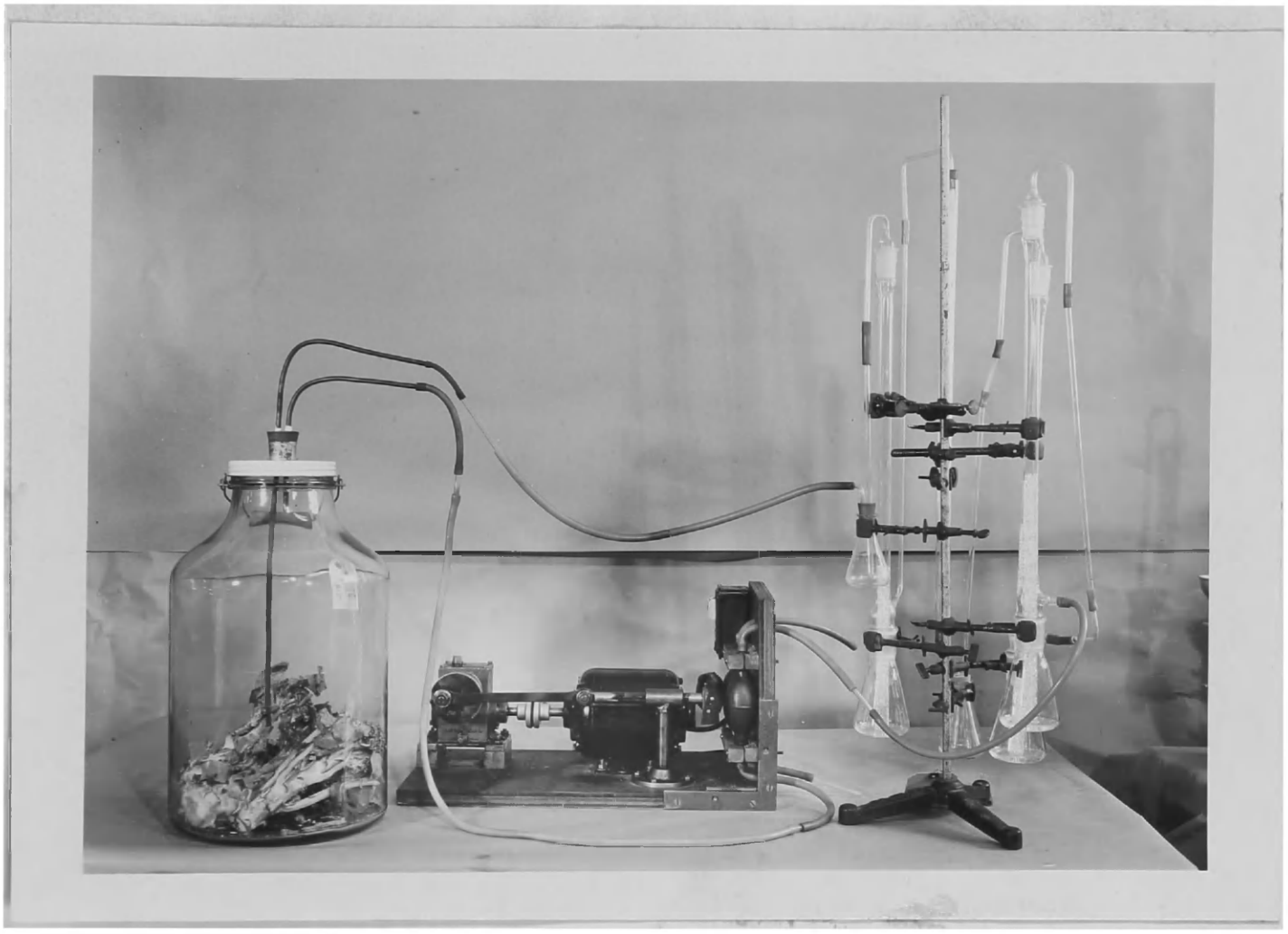

Fig. 7. Apparatus for the absorption of ethylene and "other" volatiles in sealed treatments. In these treatments the atmospheres were circulated through the absorption apparatus. (For details see Fig. 6.)

method combines an adaptation of the method Gerhardt and Ezell (17) for estimation of oxidizable volatiles, excepting ethylene, with the method developed by C. R. Gross for the determination of ethylene.

Volatiles other than ethylene were absorbed in concentrated sulfuric acid. In order to increase the absorptive surface the acid was drawn up on 90 grams of solid glass beads of four mm. diameter, in a modified Truog tower. The gas was then passed through a succeeding tower containing anhydrous magnesium perchlorate to absorb any volatiles other than ethylene that had not been absorbed by the concentrated sulfuric acid. Finally the ethylene was taken up by 15 ml. of a 60% perchloric acid and mercuric oxide mixture in a succeeding modified Truog tower assembly. This final tower with perchloric acid contained 65 grams of beads to increase the absorptive surface. The entire apparatus is illustrated in figures 4, 5, and 6, 7. Gross (19) has shown that the absorption of the volatiles other than ethylene by concentrated sulfuric acid was found to be highly efficient for apple volatiles other than ethylene. Also Gross' data showed that absorption of ethylene was quantitative in a perchloric acid solution activated with mercuric oxide.

Preliminary tests with broccoli showed that the method of Gross could be satisfactorily used as an empirical way of demonstrating ethylene and other volatiles emanating from broccoli.

The method of determining ethylene and "other" volatiles consisted in oxidation of ethylene with standard cerato perchlorate and oxidation of the "other" volatiles with standard cerato sulfate under standard conditions. The excess cerato perchlorate was back titrated with standard sodium oxalate and the excess cerato sulfate was back titrated

with standard ferrous sulfate. A blank was used to determine the volatiles and ethylene present in the absorbents. Ethylene was expressed as mg. of ethylene per kg. per day, since one ml. of 0.1N cerato perchlorate is equivalent to 0.3506 mg. of ethylene. Other volatiles are expressed as ml. of 0.1N cerato sulfate reduced per kg. per day.

The use of the terms cerato perchlorate and cerato sulfate rather than ceric perchlorate and ceric sulfate is due to the present concept that the tetravalent cerium ion is predominantly anionic in its properties rather than cationic. Methods of standardizing cerate ion in sulfuric and perchloric acid media are thoroughly discussed by Smith (39) and Young (48).

Details of the Method:

To determine volatiles other than ethylene, 25 ml. of a standard solution of approximately 0.1N cerato sulfate was added to the 20 ml. of sulfuric acid containing the absorbed "other" volatiles. This solution was oxidized in an autoclave at 15 pounds pressure for three hours. A blank was prepared, using 20 ml. of sulfuric acid with zero volatiles absorbed, 90 grams of solid glass beads and 25 ml. of approximately 0.1N standard cerato sulfate, and was likewise oxidized in an autoclave. After cooling to room temperature the excess cerato sulfate was titrated with a standard ferrous sulfate solution of approximately 0.1N concentration using one drop of 1, 10 - ortho phenanthroline ferrous sulfate (ferroin) as an indicator. The difference in ml. of ferrous sulfate between the blank and experimental was equivalent to the "other" volatiles absorbed by the sulfuric acid.

Ethylene was determined by adding 25 ml. of cerato perchlorate to

the 15 c.c. of perchloric acid and mercuric oxide mixture and oxidized in a water bath at 55°C. for 45 minutes. A blank was prepared and oxidized in the same manner. After cooling to room temperature, 100 c.c. of water were added to the solution and the excess cerato perchlorate was titrated with a standard solution of approximately 0.1N sodium oxalate. Nitro-ortho phenanthroline ferrous sulfate (nitro ferroin) was used as indicator. The difference between the amount of oxalate needed to titrate the experimental and the blank is equivalent to the cerato perchlorate reduced by the ethylene.

In experimental runs it was found necessary to use two towers for absorption of the "other" volatiles. This was especially true in treatments three and four after the first day. The diagrams and photographs (Figs. 5, 6 and 7, 8) therefore show two absorption towers for other volatiles.

The absorption of volatiles was carried out overnight, usually over a period of 16 to 18 hours. The apparatus was then taken down and renewed for the next day's determination. After each test, organoleptic examinations were made on one jar from each of the treatments. The jars in treatments 3A and 4A were then replaced in the circulatory system by the next day's sample jar. Readings on O_2 and CO_2 were made before and after circulation through the absorption apparatus as described above.

Organoleptic Examinations:

Organoleptic examinations on the four treatments were made on the appearance, odor, color, injury, decay and taste of each day's sampling. These factors were rated on a scale of 1 to 5 as follows: 1 - very good, 2 - good, 3 - fair, 4 - poor, 5 - bad.

Each test for any one O_2 concentration was repeated three times in consecutive weeks.

RESULTS

Respiration in the Flushed Systems:

The data on respiration in the flushed systems, treatments one and two, are given in table I and figure 8. In view of the inherent variability of the material, no significance is attached to the slopes of the curves for treatments one and two. However, in every case, a reduced O_2 level in the atmosphere around the broccoli reduced the rate of respiration as compared to the normal air controls. There was no apparent adverse effect on the respiratory mechanism as a result of the reduced O_2 atmospheres.

Respiration in Sealed Systems:

Treatments three and four, in which respiration was carried out in sealed desiccators, showed an entirely different picture from the treatments in which O_2 was supplied at a constant level. In the closed systems O_2 was being continuously depleted and CO_2 was either absorbed as in treatment three or built up as in treatment four. The volatiles produced were built up in both treatments. Under these conditions respiration rapidly declined.

In comparison to treatments one and two the respiration in the sealed system was at a much lower level for all O_2 concentrations tested. The one day samples of the closed systems were in all cases closer to the three day samples of the comparable lot in the constant O_2 level treatments (see Fig. 8 and Table I). After the third day respiration was down to 57, 64 and 41 mg. CO_2 per kg. per day in the sealed systems in which CO_2 was absorbed. These initially had a concentration of 11%,

TABLE 1. Respiration rate of broccoli held at 75°F. under different oxygen levels in systems in which the oxygen level was maintained and in sealed systems in which oxygen was depleted. (Expressed as mg. CO_2 per kg. hr.)

Treatments**	Days	Oxygen Levels		
		11%	6.5%	2.5%
1	1	227*	302	207
2	1	300	346	304
3	1	163	163	115
4	1	198	210	172
1	2	261	286	193
2	2	335	345	330
3	2	98	81	70
4	2	19	15	23
1	3	198	242	176
2	3	254	264	223
3	3	57	56	41
4	3	0	6	3
	L.S.D. 5%	30	30	18
	L.S.D. 1%	42	42	25

* Each figure is the average of 3 replicates.

** Treatments:

1 - Flushed with reduced oxygen level.
2 - Flushed with air.
3 - Sealed; carbon dioxide absorbed.
4 - Sealed; carbon dioxide accumulated.

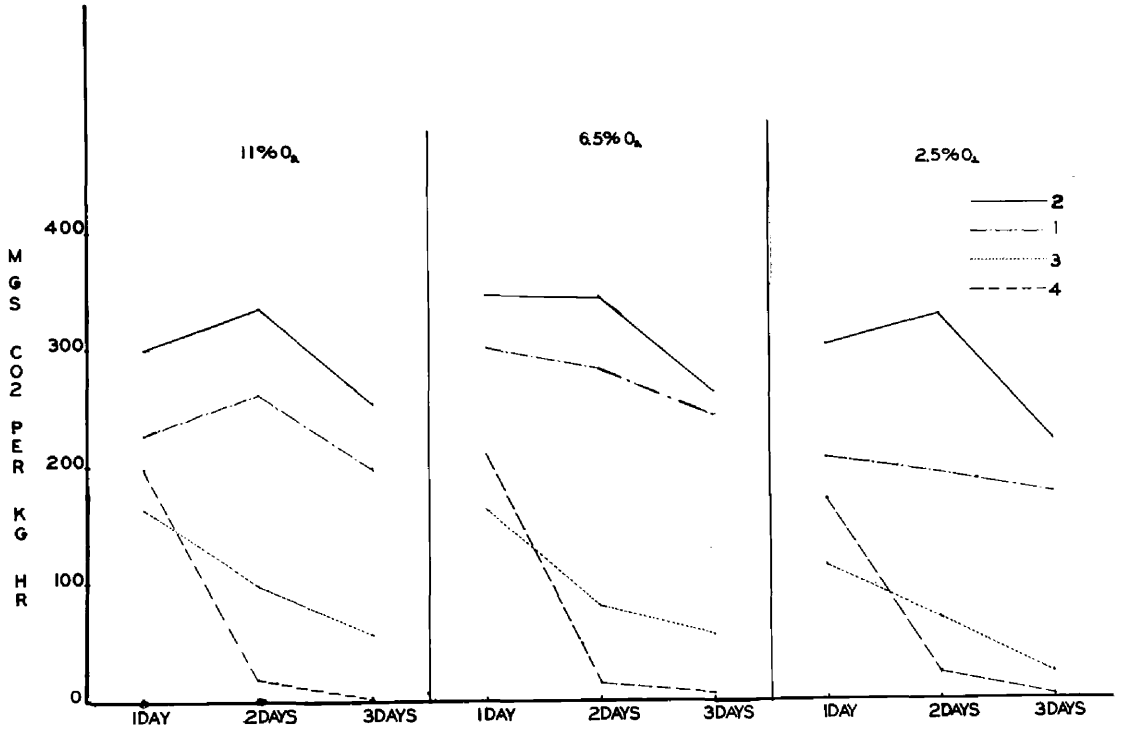

Fig. 8. Respiration rate of broccoli held at 75°F. under different oxygen levels and in air.

Treatments:

1. Constant reduced oxygen levels
2. Air control
3. Sealed system in which O_2 is depleted and CO_2 absorbed
4. Sealed system in which O_2 is depleted and CO_2 accumulated

6.5% and 2.5% O_2 respectively. In the sealed system in which CO_2 was allowed to build up, respiration was down to 0, 6, and 3 mg. CO_2 per kg. per day in the treatments which initially had a concentration of 11%, 6.5% and 2.5% oxygen. These values when compared to 198, 242 and 176 mg. CO_2 per kg. per day at 11%, 6.5% and 2.5% constant O_2 levels emphasize the amount of reduction in respiration that took place.

Of particular interest is the rate of decline in respiration per day. The system which initially had 11% O_2 and in which CO_2 was absorbed declined 40% from the first to the second day and 42% from the second to the third day. The comparable system in which CO_2 accumulated showed a 90% decline from the first to the second day and a 100% decline to zero respiration from the second to the third day. The sealed desiccator which initially had 6.5% O_2 declined 50% from the first to the second day and 37% from the second to the third day. The comparable lot in which CO_2 accumulated declined 92% from the first to the second day and 60% from the second to the third day. The sealed desiccator in which the initial O_2 concentration was 2.5% and in which CO_2 was absorbed declined 39% from the first to the second day and 41% from the second to the third day. The comparable lot in which CO_2 accumulated declined 87% both from the first to second day and from the second to the third day.

An important observation to be made from these data is that there is a significantly higher level of respiration in treatment three in which the CO_2 was absorbed than in the comparable treatments in which CO_2 was allowed to accumulate. This difference was very marked in every case. Whereas the total reduction in CO_2 production after three days was approximately 65% for the treatment in which CO_2 was absorbed, there

was an approximate reduction of more than 98% in the comparable treatment in which CO_2 accumulated. This was found in all the samples regardless of the original concentration of the oxygen.

The rate of decline in respiration was considerably lower in the systems in which CO_2 was absorbed. This was especially true during the first to second day interval when there was an extremely rapid drop in respiration. (See graph, Fig. 8). It is interesting to note that the percentage of total reduction was approximately the same in all three tests for any one treatment, in both systems. In the system in which CO_2 accumulated, the overall reduction in CO_2 produced was 100, 97 and 98% for the 11%, 6.5% and 2.5% initial O_2 concentrations. In the system in which CO_2 was absorbed the overall reduction in CO_2 production was 65, 66 and 64% at 11%, 6.5% and 2.5% O_2 respectively. Only approximately 2% or less of the CO_2 produced the first day was produced on the third day in the systems in which CO_2 accumulated, whereas in the systems in which CO_2 was absorbed approximately 35% as much CO_2 was produced the first day as was produced on the third day.

The cause of the rapid reduction in respiration was principally due to the reduction in the O_2 concentration. The effect of CO_2 accumulation may be observed from the data in table 2. From these data it may be seen that when the initial concentration of O_2 was 11%, the O_2 percentage was reduced after 24 hours to 2.0%, in the system in which CO_2 was absorbed. In the comparable system in which CO_2 accumulated, O_2 was reduced to 0.3%. Apparently the effect of the accumulation of CO_2, which was 12.4% in this case, was to increase the rate of respiration as evidenced by the reduced O_2 supply. This is also apparent from the values for mgs. of CO_2 produced during the first day (Table 1). These

TABLE 2. Daily change of carbon dioxide and oxygen in the atmospheres of sealed chambers in which broccoli was held at 75°F.

Oxygen Level	Treatments**	Hours	Desiccators		Jars		Desicc. CO_2/O_2 Ratio	Jars CO_2/O_2 Ratio
			Percent O_2	Percent CO_2	Percent O_2	Percent CO_2		
11%	3 & 3A	0	11.0	0.0	11.0	0.0		
		24	2.0*	1.3	4.5	0.5		
		48	1.0	0.4	1.2	0.2		
		72	0.5	0.0	0.1	0.2		
	4 & 4A	0	11.0	0.0	11.0	0.0		
		24	0.3	12.4	3.9	6.3	1.1	0.9
		48	0.2	13.1	0.9	10.0	1.2	1.0
		72	0.0	14.9	0.3	12.3		
6.5%	3 & 3A	0	6.5	0.0	6.5	0.0		
		24	0.1	0.3	1.7	0.6		
		48	0.0	0.1	0.1	0.3		
		72	0.0	0.1	0.0	0.1		
	4 & 4A	0	6.5	0.0	6.5	0.0		
		24	0.2	10.4	1.5	5.3	1.6	1.1
		48	0.0	12.2	0.0	8.1		
		72	0.0	16.9	0.0	9.4		
2.5%	3 & 3A	0	2.5	0.0	2.5	0.0		
		24	0.0	0.0	0.3	0.2		
		48	0.0	0.1	0.0	0.6		
		72	0.0	0.0	0.0	0.1		
	4 & 4A	0	2.5	0.0	2.5	0.0		
		24	0.1	6.2	0.4	3.5	2.6	1.7
		48	0.0	10.7	0.0	5.7		
		72	0.0	12.5	0.0	6.9		

* Each figure is a mean of 3 replicates.

** Treatments:

3 - Sealed systems in desiccators, with CO_2 absorbed.
3A - Sealed systems in jars, with CO_2 absorbed.
4 - Sealed systems in desiccators, with CO_2 accumulated.
4A - Sealed systems in jars, with CO_2 accumulated.

first day values for the systems in which CO_2 was accumulated are higher in every case than the first day values in the comparable systems in which CO_2 was absorbed.

The effect of initial CO_2 accumulation on O_2 concentration in the sealed systems with initial concentrations of 6.5% and 2.5% is not seen because the drop in O_2 concentration is very rapid when the initial concentration in the atmosphere is as low as in these tests. Apparently, however, there is an initial increase in respiration stimulated by the accumulation of carbon dioxide. This is followed by a decrease in both the amount and rate of respiration as accumulation becomes toxic. This phenomenon will be further discussed in the following sections.

Production of Volatiles other than Ethylene:

Study of the volatiles produced under conditions of a constant O_2 supply and in sealed systems in which O_2 was depleted, revealed a marked and consistent difference in the quantity of volatiles produced under the two types of storage.

At the end of the first day there was a considerable difference between the sealed, declining O_2 systems, and the open systems with a constant O_2 level. The sealed systems produced approximately four to six times the amount of volatiles produced by the open systems. The lower the O_2 percentage under test the greater was the difference between the sealed and open systems.

The second day samples showed a greater difference between the open and sealed systems. The sealed systems of the 11% O_2 lots produced approximately six times as much volatiles as the open systems, the 6.5% O_2 lots approximately 30 times as much and the 2.5% O_2 lots approximately 40 times as much as the open systems.

A comparison of sealed and open samples on the third day showed still a greater difference between these treatments. The sealed systems of the 11% O_2 lots produced 15 times as much volatiles as the open systems; the 6.5% O_2 lots 50 times as much, and the 2.5% O_2 lots approximately 45 times as much as the open systems.

It is seen from these data that there is an enormous increase in volatile production in the sealed systems after the second day in the 11% O_2 lots, and after the first day in the 6.5% and 2.5% O_2 lots. This increase became greater on the third day and at the lower O_2 percentages (see table 3 and figure 9).

The volatile production in the open systems, though considerably lower than the sealed systems, showed a definite increase each day. There does not seem, however, to be much difference between the compressed air treatment and the reduced O_2 treatments. Generally the two open treatments are approximately at the same magnitude and increase proportionately from day to day. This leads to the conclusion that lowering the O_2 concentration down to 2.5% does not significantly decrease or increase emanation of volatiles from broccoli.

There was a difference in many cases between the volatiles in the sealed system in which CO_2 was absorbed and those in the sealed system in which CO_2 was allowed to accumulate. This difference was not evident in any of the first day samples or in the second day sample of the 11% O_2 level, but became very marked in the second and third day samples of the other O_2 levels.

The 11% O_2 treatment showed a considerable difference in the third day sample. The 6.5% O_2 and 2.5% O_2 treatments showed a considerable difference, especially in the two day samples between the CO_2 accumulated

TABLE 3. "Other" volatiles produced by broccoli held at 75°F. under different oxygen levels in sealed systems in which oxygen was depleted and in systems in which the oxygen level was maintained. (Volatiles expressed as ml. of 0.1N cerato-sulfate reduced per kg. of tissue per 24 hours.)

Treatments**	Days	Oxygen Levels		
		11%	6.5%	2.5%
1A	1	1.8*	1.4	2.7
2A		1.4	1.5	3.3
3A		5.6	9.5	16.9
4A		6.1	9.8	19.9
1A	2	3.8	4.2	6.2
2A		4.8	3.6	7.1
3A		24.9	77.1	160.4
4A		24.1	146.9	374.2
1A	3	27.1	12.0	12.4
2A		20.8	11.2	20.6
3A		322.1	504.9	502.6
4A		429.5	696.6	868.7

* Each figure is the mean of 3 replicates.

** Treatments:

1A - Flushed with reduced oxygen level.
2A - Flushed with air.
3A - Sealed; carbon dioxide absorbed.
4A - Sealed; carbon dioxide accumulated.

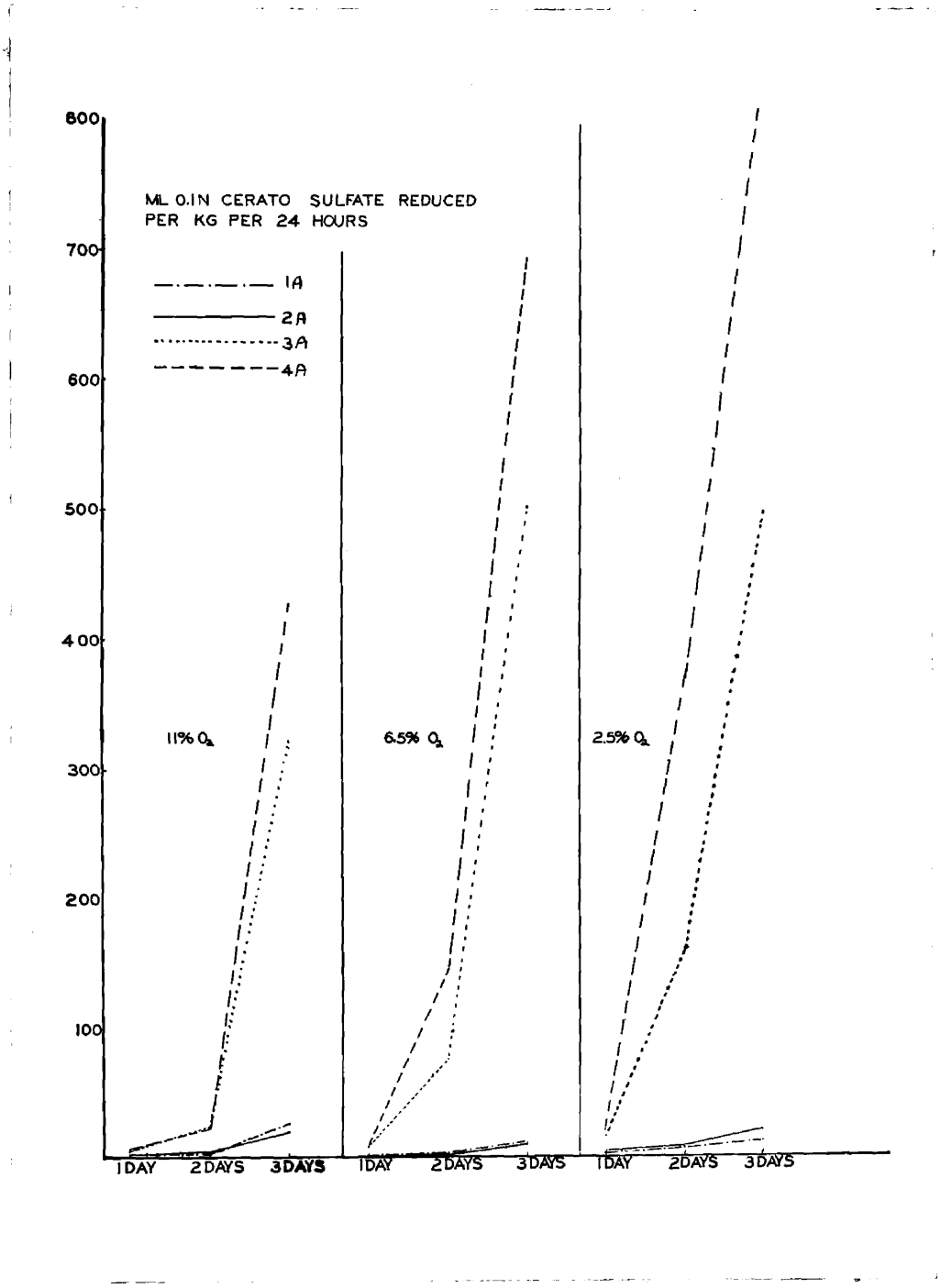

Fig. 9. "Other"volatiles produced by broccoli held at 75°F. under different oxygen levels in sealed systems in which the oxygen was depleted and in systems in which the oxygen level was maintained.

Treatments:

1A. Constant reduced O_2 levels
2A. Air control
3A. Sealed system in which O_2 is depleted and CO_2 absorbed
4A. Sealed system in which O_2 is depleted and CO_2 accumulated

and CO_2 absorbed systems. Differences as high as 47% and 57% were found as can be seen from the data in table 3. On the third day the 6.5% O_2 and 2.5% O_2 treatments still showed a difference of volatile production of 27% and 42% respectively between CO_2 accumulated and CO_2 absorbed systems. The evidence is not clear as to whether this difference is due to the dissimilarity of CO_2 in the atmosphere affecting the production of "other" volatiles, or to the partial absorption of "other" volatiles by the KOH used to absorb respiratory CO_2 in the CO_2 free system. If the latter is the case, then the drop in percentage difference on the third day for the 6.5% O_2 and the 2.5% O_2 lots can be explained by assuming that the absorption efficiency of the KOH for volatiles reached a peak at two days and thereafter declined.

The sudden and rapid rise of "other" volatiles in the closed system is associated with the decline in O_2 concentration in the jar. In the 2.5% O_2 samples, where almost zero percent O_2 was reached in a short time, there was a steady rise of volatile production in straight line fashion from the first to the third day. With an initial concentration of 6.5% O_2 there was considerable departure from the straight line curve. The rate of volatile production from day one to day two was at a different rate than the rate from day two to day three. This can be seen from the slope of the curves in figure 9. The slope of the curves for 11% O_2 changed very significantly from day one to day two and from day two to day three. This is shown as a sudden rise of volatile production from day two to day three.

The reason for the sudden rise of volatile production in such great magnitude is associated with the depletion of the O_2 in the atmosphere and to the anaerobic or fermentation processes taking place in the

broccoli cells. This becomes obvious when the curves (Fig. 9) showing volatile production for 11% O_2 are compared to the curves for 6.5% O_2 and 2.5% oxygen. The 11% O_2 sealed lots did not have their O_2 reduced as rapidly as did those with initially lower O_2 levels. The break in the curve at day two probably coincides with the reduction of the O_2 to the level at which anaerobic respiration and the consequent production of volatiles in large quantities takes place. At a lower O_2 level, 6.5% O_2, the break in the curve at day two occurs at a different level. Since anaerobic respiration has been taking place right along with the simultaneous production of volatiles in large quantities, the break in the curve probably signifies a change in rate of volatile production during the interval considered. At 2.5% O_2 anaerobic respiration probably occurs very soon and a break in the curve or change in rate is not apparent at the two day interval. The slight dip in the curve visible at the two day interval in the treatment in which CO_2 was absorbed may be related to an initially slower respiration rate which slightly retarded the O_2 depletion and consequently anaerobic respiration, which is coincident with increased volatile production. Where CO_2 was accumulated, the initial CO_2 concentration accelerated respiration (O_2 depletion) and consequently anaerobic conditions and increased volatile production were attained sooner.

Ethylene Production:

The magnitude of ethylene production appears from the data to be far below that of "other" volatiles. This is evident even though a direct comparison cannot be made since the expression of the quantities for ethylene are in mg. per kg. per day, while those for "other" volatiles are in ml. of 0.1N cerato sulfate reduced per kg. per day.

However, when the ethylene is expressed in ml. of 0.1N cerato perchlorate reduced per kg. per day, as calculated from the original data, the largest amount of ethylene had a value of 0.12 ml. whereas the lowest value for "other" volatiles was 1.4 ml. of 0.1N cerato sulfate per kg. per day. Most values for "other" volatiles ran considerably higher; values of 77, 160 and as high as 868 ml. of 0.1N cerato sulfate per kg. per day were obtained. Even though cerato perchlorate has a higher redox potential (1.7) than cerato sulfate (1.4), it appears that the production of "other" volatiles exceeded by far the production of ethylene. The orders of magnitude of ethylene and that of "other" volatiles suggest that whereas the production of "other" volatiles was greatly affected by anaerobic respiration, the production of ethylene was not affected to the same extent.

The quantity of ethylene appears to be quite small in terms of absolute values. However, when these values are compared to the values obtained for ethylene production with various fruits, it appears that broccoli produced a large amount of ethylene.

Crocker (12), Nelson (33), Hansen (24) and others list the ethylene production of various fruits as follows: Bartlett pears 4.06 - 5.6 mg. per kg. per day at 0°C., Anjou pears 0.71 - 0.97 mg. per kg. per day and bananas 0.1 - 0.2 mg. during the entire ripening period. Compared to these figures broccoli produced a considerable amount of ethylene.

In the tests in which 11% O_2 was used there generally appeared to be a greater production of ethylene in the sealed systems than in the open ones. The compressed air, and 11% O_2 open lots both produced about the same amount of ethylene, and the amount produced appeared to be more or less constant during the three day period. Ethylene production

in the sealed systems was higher. This was especially true of the sealed system in which CO_2 accumulated. In this lot ethylene production on the third day was considerably higher than in the lot in which CO_2 was absorbed (see table 4 and figure 10).

In the tests with 6.5% O_2 the results were very similar to those in which 11% O_2 was the reduced O_2 level. The sealed treatment in which CO_2 accumulated showed an increase of ethylene production from day to day, and was consistently higher than its comparative treatment in which CO_2 was absorbed. On the third day the greatest increase in ethylene production occurred in the sealed system with accumulated carbon dioxide.

The open systems showed slight increases from day to day and the air lots appeared to have slightly greater values.

In the 2.5% O_2 lots the data for ethylene production again showed the same pattern. Values for ethylene production appeared to be considerably higher for all treatments after one day and increased from day to day in all treatments. The open systems showed a lower ethylene production than the sealed systems. Again the compressed air lots showed a trend towards higher values over the open treatment with 2.5% O_2 during the second and third days. The closed system where CO_2 accumulated had considerably higher ethylene production every day over all the other lots. On the third day the ethylene production in the CO_2 accumulated lot was about seven times as high as its value on day one and was more than eightfold greater than the open systems and more than fivefold greater than its comparison in which CO_2 was absorbed.

It is interesting to note that these data are at variance with that given by Crocker (12), Kidd and West (29) and others stating that

TABLE 4. Ethylene produced by broccoli, held at 75°F. under different oxygen levels in sealed systems in which oxygen was depleted and in systems in which the oxygen level was maintained. (Ethylene is expressed as mgs. per kg. per 24 hours.)

Treatments**	Days	Oxygen Levels		
		11%	6.5%	2.5%
1A	1	0.20*	0.07	0.16
2A		0.16	0.30	0.13
3A		0.70	0.47	0.66
4A		0.77	0.73	1.10
1A	2	0.20	0.13	0.21
2A		0.27	0.27	0.29
3A		0.63	0.63	0.73
4A		0.63	0.90	3.07
1A	3	0.20	0.30	0.56
2A		0.33	0.37	0.83
3A		0.33	0.43	1.46
4A		1.10	1.23	7.53
	L.S.D. 5%	0.43	0.35	1.02
	L.S.D. 1%	0.60	0.49	1.44

* Each figure is the mean of 3 replicates.

** Treatments:

1A - Flushed with reduced oxygen level.
2A - Flushed with air.
3A - Sealed; carbon dioxide absorbed.
4A - Sealed; carbon dioxide accumulated.

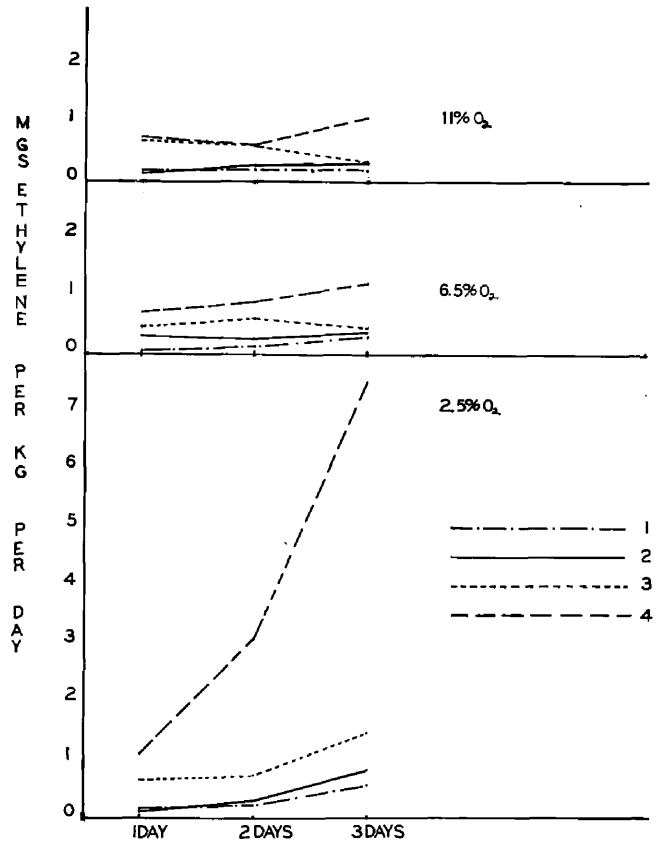

Fig. 10. Ethylene produced by broccoli held at 75°F. under different oxygen levels in sealed systems and in systems in which the oxygen level is maintained.

Treatments:

1A. Constant reduced oxygen levels
2A. Air control
3A. Sealed system O_2 depleted and CO_2 absorbed
4A. Sealed system O_2 depleted and CO_2 accumulated

ethylene production is inhibited under anaerobic conditions. In the data reported herein, however, the greatest production of ethylene and the trend towards higher ethylene occurred in the closed systems in which O_2 was depleted. The greatest production of ethylene, 7.53 mg. per kg. per day, which is a sizeable amount in comparison to ethylene production in other materials, occurred in the closed system under conditions which produce the greatest degree of anaerobiosis.

The Dynamics of Oxygen and Carbon Dioxide in the Sealed Systems:

The changing atmosphere in the closed systems in relation to O_2 and CO_2 determined the rate of respiration, the production of "other" volatiles and possibly also ethylene production.

The chambers used for respiration studies were desiccators of approximately ten-liter capacity. It can be seen from the table 2 and figure 11 that O_2 was depleted very rapidly in these chambers.

In the 11% O_2 test, after the first day, the value of O_2 in the CO_2 accumulated system was only 0.3% while in the CO_2 absorbed system it was 2.0%. After the second day it was 0.2% in the CO_2 accumulated system and 1.0% in the CO_2 absorbed system. After three days the O_2 value was zero percent for the accumulated system and 0.5% for the CO_2 absorbed lot. Carbon dioxide accumulated very rapidly in the CO_2 accumulated system during the first day and less rapidly thereafter. After the first day the CO_2 percentage was 12.4 and thereafter 13.1 and 14.9 at the end of the second and third day.

In the 6.5% O_2 test and in the 2.5% O_2 test, O_2 depleted almost to zero after one day and subsequently zero values were obtained at the end of the second and third day. There was apparently little difference in O_2 depletion between the CO_2 accumulated and CO_2 absorbed systems in

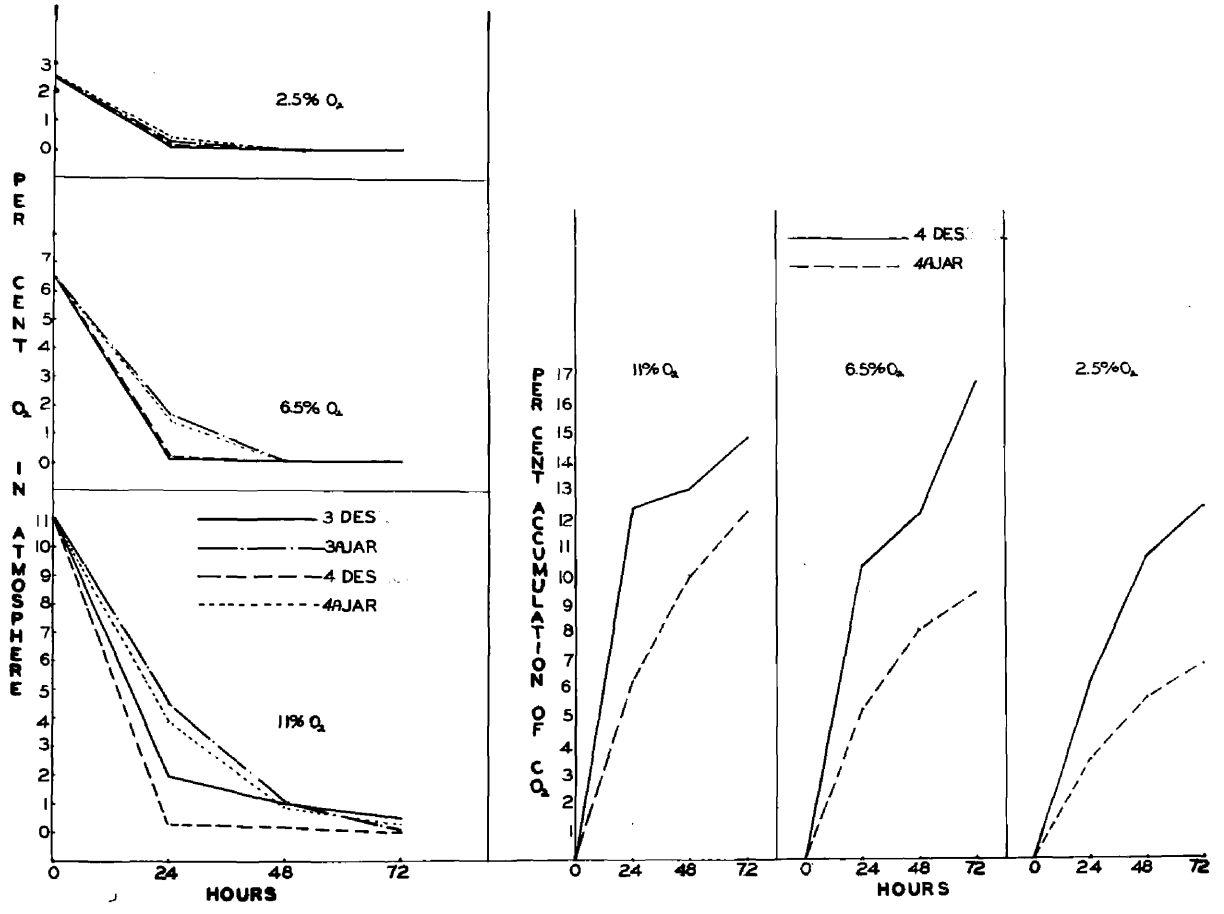

Fig. 11. Daily changes of carbon dioxide and oxygen in the atmospheres of sealed chambers in which broccoli was held at 75°F. with initial oxygen concentrations of 2.5, 6.5, and 11 percent.

Treatments:

3 des. - Sealed desiccator in which O_2 is depleted and CO_2 absorbed. Volume is approximately 10 liters.

3A jar - Sealed system in which O_2 is depleted and CO_2 absorbed. Volume is approximately 20 liters.

4 des. - Sealed desiccator in which O_2 is depleted and CO_2 accumulated. Volume is approximately 10 liters.

4A jar - Sealed system in which O_2 is depleted and CO_2 accumulated. Volume is approximately 20 liters.

both the 2.5% and the 6.5% O_2 tests.

In the 6.5% O_2 test CO_2 accumulated to 10.4%, 12.2% and 16.9% at the end of the first, second and third days respectively. In the 2.5% O_2 test CO_2 accumulated to 6.2%, 10.7% and 12.5% respectively.

Apparently CO_2 accumulation initially had some effect on the rate of O_2 depletion and this is associated with its effect on respiration rate. However, the effect can only be seen in the 11% O_2 test in which the initial CO_2 increase apparently increased the rate of O_2 depletion. This may be seen from the respiration graphs (Fig. 8) and from the larger percentage of O_2 remaining throughout the three day period in the CO_2 absorbed system as shown in table 2. At the lower initial O_2 concentrations O_2 was depleted so rapidly during the first day that the reduced effect on O_2 loss was not observed. However, the respiration curves (Fig. 8) show the higher initial respiration of the CO_2 accumulated system.

Another explanation may be advanced for the initially higher respiration in the CO_2 accumulated system. The manner of making the respiratory CO_2 determination allows for a considerably longer period of exposure to the alkali than does the CO_2 absorbed system. This longer period for CO_2 absorption may account for the apparent initially higher respiratory rate. However, the O_2 readings corroborate the first premise that an initially higher respiratory rate was stimulated by CO_2 in the CO_2 accumulated system.

The jars used for chambers in which broccoli was held for organoleptic and volatile determinations had a capacity of approximately 20 liters, roughly twice that of the desiccators. Since the weight of broccoli was approximately the same in the jars and desiccators it

would be expected that the O_2 depletion should be approximately half that in the desiccators. This exact ratio of two to one was not found except in one case. Generally O_2 remained higher and CO_2 accumulation was reduced in the jars as compared to the desiccators for all tests. This phenomenon is believed to be simply a matter of the larger quantity of O_2 in the jars and the greater volume of the jars as compared to the desiccators.

Regardless of whether the O_2 and CO_2 in the jars or desiccators are considered, the rate of O_2 depletion and CO_2 accumulation is greatest during the end of the first to the second day for all the tests at all initial O_2 concentrations. This is also apparent from the curves for respiration and may be assumed to be coincident with the start of anaerobic processes which finally reach a much reduced level during the third day.

Organoleptic Examinations:

The primary objective of the organoleptic examinations was to determine the effect of the lowered O_2 treatments and the sealed atmosphere on the general quality of the broccoli under test. The appearance, color, odor, decay, raw taste and salability were the principal factors observed.

Of all these, color was considered the most interesting since it was found in previous studies (38) that broccoli remained green in a sealed atmosphere for many days, whereas it rapidly yellowed in an open atmosphere. These conditions were obtained in sealed and perforated packages. Figure 1 illustrates this point. The color value for the treatments was determined by observation. At the end of each day the broccoli in each treatment was judged on a scale of one to five. A

value of one indicated very good color, two indicated good, three indicated fair, four indicated poor and five indicated very poor color or completely yellowed broccoli. Tables 5, 6, and 7 outline the results obtained at the different O_2 levels.

These data indicate that at all O_2 concentrations and in all lots, the color value was satisfactory after one day at 75°F. After two days at 75°F. the broccoli under compressed air atmospheres were all rated poor (4) having become yellow in color. Those under a constantly lowered O_2 atmosphere, whether at 11% O_2, 6.5% O_2 or 2.5% O_2, all appeared to be somewhat better than the air group, but almost all were nevertheless beyond the stage where they could be considered of an acceptable green color. A few stalks, however, did remain fairly green. After three days at 75°F. all the samples in air were completely yellowed. Those samples in the reduced O_2 concentrations, though less yellow, were approximately in the same condition.

The broccoli in the sealed jars, however, all retained good color throughout the three day period no matter what the initial O_2 concentration. There appeared to be only a slight difference in flavor of the broccoli in the sealed jars in which CO_2 accumulated as against those in which CO_2 was absorbed. This difference was apparent at the 11% O_2 concentration and to a lesser extent at the 6.5% O_2 concentration.

From these data it appears that maintainance of green color in broccoli is associated with very low O_2 concentration, perhaps in the zero range of concentration. Reduction of the O_2 concentration to 2.5% had only a slight beneficial effect on the maintainance of the green color. After three days the broccoli yellowed even in the 2.5% O_2 atmosphere, but it did appear to be slightly greener than the control

TABLE 5. Summary of organoleptic evaluation for 3 replicates of broccoli held at 75°F. in the 11 percent oxygen test. Evaluations are in terms of numerical ratings of 1 to 5*.

Days	Treatments**	Appearance	Color	Odor	Decay	Taste	Salability
1	1A	2.8	2.7	1.4	1.0	2.1	2.8
	2A	2.8	2.7	1.4	1.0	2.1	2.8
	3A	2.8	2.7	1.3	1.0	1.9	2.8
	4A	2.5	2.3	1.3	1.0	2.4	2.7
2	1A	3.8	3.8	1.9	1.0	3.7	4.5
	2A	4.5	4.5	2.2	1.0	3.7	4.7
	3A	3.8	3.3	2.6	1.0	3.2	4.1
	4A	3.1	2.8	3.3	1.0	3.0	3.7
3	1A	5.0	5.0	3.7	2.8	5.0	5.0
	2A	5.0	5.0	3.3	2.5	5.0	5.0
	3A	3.6	3.3	4.0	1.2	4.3	4.3
	4A	2.7	2.5	3.7	1.0	4.3	4.0
L.S.D. 5%		0.5	0.3	0.9	0.4	1.2	0.2
L.S.D. 1%		0.7	0.4	1.3	0.5	1.7	0.3

* Numerical Ratings:

1 - Very good
2 - Good
3 - Fair
4 - Poor
5 - Very poor

** Treatments:

1A - Flushed reduced O_2 level.
2A - Flushed air.
3A - Sealed, CO_2 absorbed.
4A - Sealed, CO_2 accumulated.

TABLE 6. Summary of organoleptic evaluation for 3 replicates of broccoli held at 75°F. in the 6.5% oxygen test.
Evaluations are in terms of numerical ratings of 1 to 5*.

Days	Treatments**	Appearance	Color	Odor	Decay	Taste	Salability
1	1A	2.2	2.2	1.2	1.0	2.1	2.4
	2A	2.3	2.0	1.0	1.0	2.1	2.5
	3A	2.2	2.0	1.2	1.0	2.1	2.3
	4A	2.2	2.2	1.2	1.0	2.1	2.3
2	1A	3.6	3.5	1.0	1.0	3.6	4.1
	2A	4.0	4.0	1.0	1.0	3.6	4.1
	3A	2.8	2.8	1.2	1.0	3.0	3.0
	4A	2.4	2.3	2.1	1.0	3.0	3.4
3	1A	4.7	4.7	2.6	2.6	4.0	5.0
	2A	5.0	5.0	2.9	3.0	4.0	5.0
	3A	3.1	2.7	3.3	3.2	3.0	4.2
	4A	2.8	2.7	3.6	3.0	3.0	4.2
L.S.D. 5%		0.4	0.4	1.0	0.8	1.1	0.6
L.S.D. 1%		0.6	0.6	1.3	1.1	1.5	0.8

* Numerical Ratings:

1 - Very good
2 - Good
3 - Fair
4 - Poor
5 - Very poor

** Treatments:

1A - Flushed reduced O_2 level.
2A - Flushed air.
3A - Sealed CO_2 absorbed.
4A - Sealed CO_2 accumulated.

TABLE 7. Summary of organoleptic evaluation for 3 replicates of broccoli held at 75°F. in the 2.5 percent oxygen test. Evaluations are in terms of numerical ratings of 1 to 5*.

Days	Treatments**	Appearance	Color	Odor	Decay	Taste	Salability
1	1A	2.1	2.0	1.0	1.0	1.7	2.5
	2A	2.1	2.0	1.0	1.0	1.7	2.5
	3A	1.9	1.8	1.0	1.0	2.0	2.4
	4A	1.8	1.8	1.3	1.0	2.0	2.3
2	1A	3.5	4.0	1.0	1.2	3.0	3.8
	2A	4.5	4.5	1.1	1.2	3.7	4.7
	3A	2.1	2.0	2.7	1.0	2.8	3.3
	4A	2.1	2.0	3.2	1.0	2.8	3.8
3	1A	4.7	4.3	1.7	1.7	4.0	5.0
	2A	5.0	5.0	2.3	2.7	4.0	5.0
	3A	3.2	2.8	4.0	1.0	3.7	4.3
	4A	3.2	2.8	4.2	1.0	3.7	4.3
L.S.D. 5%		0.5	0.5	0.7	0.7	0.5	0.5
L.S.D. 1%		0.7	0.7	1.0	1.0	0.8	0.7

* Numerical Ratings:

1 - Very good
2 - Good
3 - Fair
4 - Poor
5 - Very poor

** Treatments:

1A - Flushed reduced O_2 level.
2A - Flushed air.
3A - Sealed CO_2 absorbed.
4A - Sealed CO_2 accumulated.

samples in air. The presence of high CO_2 had but slight effect in improving the green color over the comparative condition in which CO_2 was absorbed.

On the basis of appearance, color, and decay, the broccoli in the sealed systems appeared to be perfectly satisfactory; however, as the additional organoleptic data will show, the broccoli was unsalable principally on the basis of objectionable odors.

The data for all the organoleptic factors evaluated in terms of a one to five rating are shown in tables 5, 6, and 7, where the rating system is also outlined. All the treatments appeared satisfactory after the first day. After two days the samples in air were mostly poor and were not considered of good salable quality. The samples under lower O_2 concentrations did not fare much better except at 2.5% O_2 in which the appearance and general quality appeared to be somewhat superior to the air controls. After three days the samples in unsealed atmospheres were completely unsalable. In the reduced O_2 atmospheres of 6.5% and 2.5%, even though the broccoli appeared to show less yellowing than the broccoli in the compressed air atmospheres, the salability of the two lots were considered equally bad since they were past the edible and salable stage.

The sealed systems were generally of good to fair appearance even on the third day, but they all had an extremely offensive ammonia-like odor which was often fairly persistent even after the sealed atmosphere was opened. On this basis alone the broccoli was considered unsalable. Some of the broccoli stems in the 2.5% O_2 lots appeared blackish green. Also the raw taste was considered rather mild and flat but it was not thought to be objectionable.

Decay was very low during the first two days in all tests. On the third day there was a slight increase. However, the decay was never considered to be serious and in no way interfered with the studies.

DISCUSSION

Three factors are evident from these data:

1. Respiration, though reduced in level, is not critically affected by lowering the O_2 concentration to 11% O_2, 6.5% O_2 or 2.5% O_2, as long as these O_2 levels are maintained.

2. Respiration in the sealed systems is rapidly reduced to the point where CO_2 production is almost absent and there simultaneously occurs a tremendous increase in the emanation of "other" volatiles.

3. Yellowing of the buds is prevented in the sealed systems in the presence or absence of CO_2 and in the presence of high concentration of ethylene and "other" volatiles.

There seems to be no great disturbance to the respiratory cycle in broccoli by reducing the O_2 in the surrounding atmosphere even when the reduction is to almost one tenth that present in normal air. The rate of CO_2 production is lowered over that occurring in the air controls, but there is little adverse effect on the tissues and the metabolism of the plant appears to be perfectly normal. This indicates that O_2 concentration at least down to 2.5% is not limiting to broccoli tissue.

In the sealed systems, however, there is a rapid decline in respiration almost to zero and the tissues are subjected to anaerobic conditions. A rapid buildup of volatiles other than ethylene accompanies the reduction in respiration. Ethylene production as compared to that in the open atmospheres, also increases in the sealed atmospheres, especially at 2.5% O_2, but the amount of increase is no where near that

which occurs with the "other" volatiles.

There is no doubt that the production of these volatiles is associated with fermentation in the tissues, but the origin and nature of these volatiles and the changes in metabolism that takes place coincident with the production of these volatiles presents an intriguing problem.

What is known is that: 1. These volatiles are non ethylenic; 2. They are produced in tremendous quantities; 3. They have a pronounced odor; 4. They are products of anaerobic respiration.

If sugar is the substrate for respiration in broccoli, then under anaerobic conditions the lack of O_2 should not affect glycolysis - - the first phase in respiration. Glycolysis takes place anaerobically and is common to both aerobic and anaerobic respiration (5,6).

In the glycolytic phase the sugar substrate is enzymatically degraded to pyruvic acid through a series of phosphorylated intermediates, whereas in the terminal phase pyruvic acid is either oxidized to CO_2 and water under aerobic conditions, or reduced to ethyl alcohol and CO_2 under anaerobic conditions.

Under the conditions present in the sealed systems, the production of ethyl alcohol or acetaldehyde and CO_2 would be expected if the substrate in respiration were a sugar. However, as previously mentioned, very large quantities of volatile materials are emanated that have odors very unlike those of alcohols or aldehydes. The odors produced are putrid, ammonia like, sulfurous odors, which are generally associated with protein decomposition. Alcoholic odors were not detected. It is therefore believed that the origin of these volatiles stems from the degradation of proteins or the sulfur containing amino acids.

The question naturally arises as to whether these proteins are a substrate in the anaerobic respiration or are merely the results of breakdown of tissues concomitant with or following anaerobic conditions. The breakdown of protein materials may occur as the anaerobic conditions become so severe that the living protoplasm cannot be maintained in its normal state. If this is true, it would seem that the by-products of anaerobic respiration, ethyl alcohol and CO_2, should be present and the breakdown of proteins should not occur until the very late stages of anaerobiosis.

It is possible that the odor resulting from protein degradation is so penetrating that it obscures the alcoholic odors arising from fermentation. However, alcoholic odors are not evident even at the start of anaerobic processes, at the end of the first day. Furthermore, there appears to be no lag in time between the onset of anaerobiosis and the buildup of "other" volatiles. The appearance of "other" volatiles occurs concurrently with the disappearance of O_2 and increases at an extremely rapid rate between the second and third day. This seems to support the concept that the production of "other" volatiles does not occur as an aftermath to anaerobic respiration, but occurs simultaneously and is directly associated with the anaerobic metabolism.

Another point which supports this view is the physical condition of the tissue even after the third day in the sealed atmospheres. The appearance of the broccoli stems and buds did not show any evidences of breakdown in most samples, and after the second day when the broccoli was returned to the normal atmosphere the putrid proteinaceous odor was often dissipated, with an apparent return to aerobic respiration.

A theory of protein respiration could adequately explain the tremendous volume of "other" volatiles produced under anaerobic conditions.

If it is assumed that broccoli respires amino acids under normal atmospheric conditions, then under anaerobic conditions a large build-up of amino acid decomposition products containing sulfur and ammonia could be obtained. These may be H_2S, CS_2, NH_3 and various mercaptans. A system of proteolysis or aminolysis similar to glycolysis may be assumed in the normal respiration of broccoli. This series of reactions would normally occur under anaerobic conditions, and may end with the formation of a keto acid similar to pyruvic acid and ammonia depending on the alpha amino acid deaminated by the enzyme deaminase. For example:

$$\text{R-CH(NH}_2\text{)-COOH} \xrightarrow{\text{deaminase}} \begin{matrix}\text{R-C(=O)-COOH} + \text{NH}_3 \\ \text{R-C(OH)=COOH} + \text{NH}_3\end{matrix}$$

The breakdown of a sulfur-containing amino acid such as cysteine may yield mercaptans and H_2S.

$$\text{HSCH}_2\text{-CH(NH}_2\text{)-COOH} \xrightarrow{\text{deaminase}} \text{H}_3\text{C-C(=O)-COOH} + \text{NH}_3 \text{ or RHS}$$

The ammonia and mercaptans normally produced in a phase perhaps analogous to the glycolytic phase of respiration, might impart the characteristic pungent odor of broccoli. In the terminal phase of respiration the ammonia and the mercaptans are oxidized in gradual step by step reactions into water and carbon dioxide. The nitrogen and the sulfur are returned to the system in a cycle similar to the Krebs cycle, and are again incorporated into the sulfur containing amino acids. In this scheme hexose respiration could take place simultaneously with amino acid respiration and it is possible that the keto acids produced

in amino acid breakdown may be undifferentiated from pyruvic acid and taken up in the Krebs citric acid cycle.

However, when anaerobic conditions are present the breakdown of the amino acids reach the keto acid, ammonia and mercaptan stages without going any further in the cycle. The buildup of these products to high levels occurs in the tissues and may poison both the glycolytic reactions and the reactions in the Krebs cycle. The result is an extremely high production of sulfurous volatiles and the absence of alcohols and CO_2 which are generally associated with anaerobic respiration.

Although this theory may follow logically, there is little evidence in the literature on protein respiration in the presence of ample carbohydrate. Generally it is assumed that protein is the last substrate to be respired in starving tissue.

The CO_2 / O_2 ratio data is not sufficiently accurate in these tests to warrant the drawing of conclusions as to the substrate oxidized. Consumption of O_2 was measured, only in the sealed systems, under conditions in which anaerobiosis and the buildup of volatiles occurred very rapidly. The results of these values are therefore not too precise and yield values that are indefinite as to the type of substrate oxidized (see table 3).

The work of Platenius (35,36) provides some evidence of protein respiration in plants in the presence of excess sugars. In the normal respiration of asparagus, chemical analysis revealed that sugar losses accounted for only one-half to two-thirds of the total quantity of CO_2 evolved and that there was a definite decrease in protein nitrogen and a corresponding rise in soluble nitrogen. Therefore, it was assumed

proteins furnished about one-third of the substrate in asparagus respiration.

These data indicate that protein may be a substrate in the normal respiration of vegetables.

In order to provide additional evidence for the above theory on protein respiration, more work needs to be done to identify the "other" volatiles and to determine the changes of sugars and amino acids that occur during respiration.

Color Retention in Broccoli:

The yellowing of broccoli is principally an aging phenomenon and can be considerably retarded by reducing the rate of aging. Reduced temperatures (40° and 32°F.) have been shown to delay the yellowing of broccoli for long periods, i.e., three to four weeks, (38). Reduction of the O_2 in the atmosphere also appears to delay yellowing to a certain extent at 75°F. However, yellowing is not significantly delayed even in an atmosphere of 2.5% O_2, as compared to a normal 21% O_2 atmosphere.

When broccoli is confined in a sealed system in which O_2 is reduced very rapidly to anaerobic conditions, yellowing is completely prevented. In such a system there is a buildup of CO_2 and volatiles. These tests have shown that even in the absence of CO_2, yellowing is prevented. In the sealed systems, ethylene and "other" volatiles were removed from the atmosphere, but they were allowed to build up for one day and two days before being subjected to the absorption train. Therefore the broccoli was under an atmosphere of ethylene and "other" volatiles for one and two days. The effect of these gases on yellowing cannot be accurately stated, but it does appear that they do not directly cause or retard the yellowing of broccoli.

The data indicates that the retention of the green color, or the prevention of yellowing in broccoli is directly correlated to anaerobic metabolism and is independent of the CO_2 accumulation. The presence of ethylene and "other" volatiles in large quantities occurs as a result of the anaerobic metabolism and does not appear to have any relationship to the retention of the green color. However, the reduced O_2 supply appears to affect the enzymatic reactions which control the destruction of the chlorophyll. Apparently the mechanism of this process is intimately tied up with the terminal oxidases of the respiratory cycle and is inhibited by the lack of oxygen.

The elucidation of this mechanism will require a study of the effect of the various terminal oxidases on the chlorophyll pigments extracted from broccoli.

SUMMARY

Broccoli was subjected to atmospheres of 2.5%, 6.5%, and 11% O_2 in sealed systems in which O_2 was depleted, and in open systems in which the oxygen levels were maintained. There were two type of sealed systems, one in which CO_2 was absorbed and one in which CO_2 accumulated. The broccoli was held at 75°F. for three days during which time respiration, the production of "other" volatiles, and ethylene production were determined each day. Concurrent organoleptic examinations were also made.

Respiration was lowered in the reduced O_2 treatments which were continuously flushed. However, there was no apparent aberration of respiration.

In the sealed systems, respiration rapidly declined after the first day and it was apparent that anaerobic conditions prevailed. A slower decline was noted in the sealed system in which CO_2 was absorbed. In the sealed system in which CO_2 accumulated respiration declined to almost zero after three days. This was attributed to CO_2 toxicity.

The production of "other" volatiles in the sealed systems was 20 to 50 times as great as in the open systems. This difference was attributed to the accumulation of volatile decomposition products as a result of the anaerobic conditions present in the sealed systems. The origin of these volatiles was assumed to arise from the incomplete oxidation of the respiratory substrate. The odor of these volatiles appeared to be those of mercaptans, ammonia, and hydrogen sulphide. The sulfur-containing amino acids were assumed to be a substrate in the respiration

of broccoli, and the source of these volatiles. A mechanism of protein and amino acid respiration was postulated to account for the buildup of these volatiles.

Ethylene production was higher in the sealed systems than in the open systems. The quantity of ethylene produced, though considerable when compared to the quantity produced by other plant tissue, was, however, far below the quantity of "other" volatiles produced by broccoli.

Yellowing of the broccoli buds and stems was prevented in sealed systems regardless of whether CO_2 was absorbed or accumulated. However, the broccoli in the sealed systems was considered of poor quality because of the associated offensive odors. Keeping quality was therefore not significantly improved in the sealed systems or in the open systems with reduced oxygen atmospheres.

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