# METABOLISM OF PEARS IN MODIFIED ATMOSPHERES

by

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# METABOLISM OF PEARS IN MODIFIED ATMOSPHERES

## INTRODUCTION

Preservation of fruits in the fresh state during the post-harvest period prior to marketing basically involves retardation of physiological and biochemical changes associated with senescence. In commercial practice, refrigeration has been relied upon to maintain a low level of metabolism for keeping the fresh product in marketable condition during the storage period. Use of temperature control alone, however, has several unfavorable aspects which tends to limit its effectiveness in obtaining optimum preservation of fruit quality. Certain kinds of fruits, for example, are susceptible to low temperature injury and must be stored at a temperature which tends to shorten storage life and reduce quality. In addition, some physiological disorders and changes affecting quality have not always been adequately controlled, even when fruit is stored at lowest permissable temperature. This is particularly true with reference to deterioration in certain characteristics which affect eating quality. Furthermore, the maximum reduction in rate of metabolism which can be obtained by refrigeration is limited by the temperature at which freezing of the fruit tissue occurs. Apples and pears, for example, normally are stored at 30° F., a temperature which is only approximately 2° above the freezing point of the

fruit. In view of these limitations, further improvements in preventing deterioration in quality during storage must be obtained by control of factors other than temperature.

Modifications in the oxygen and carbon dioxide composition of the storage atmosphere have been observed to result in greater reduction in respiratory activity and quality deterioration than can be obtained by cold storage alone. Application of this principle to commercial practice has shown considerable promise and is currently in a rapid stage of expansion. During the past 5 years, commercial controlled atmosphere storage capacity in the Pacific Coast area has increased from none to more than 1,500,000 boxes. It has been estimated (55) that the controlled atmosphere storage capacity in the entire United States will increase to approximately 16 million boxes during the next decade.

Research on the fundamental aspects of modified atmosphere storage has not kept pace with the rapid commercial expansion. Actually, experimentation has been limited largely to determination of optimum atmospheres and other requirements for the various commercially important varieties of apples and pears. Very little attention has been directed towards determining the metabolic changes induced by modified atmospheres, which are associated with improved storage and fruit quality. This investigation was

undertaken in an attempt to determine some of the basic alterations in the intermediary metabolism of pears which might account for these observed differences.

# REVIEW OF LITERATURE

There are a number of factors which must be considered in studying the effect of modified atmospheres on the metabolism of fleshy organs such as fruits. First of all, alterations in the concentrations of oxygen and carbon dioxide in the external atmosphere can result in pronounced quantitative changes in respiratory activity. Since these effects are presumably exerted at the site of the respiratory mechanism within the cells, then other factors including the rate of diffusion from the atmosphere into the tissues, the concentration of oxygen and carbon dioxide in the inter-cellular spaces, and the absorption coefficients of the cell fluids for these gases must be taken in account. In addition to these aspects, the changes occurring in the nature of respiration when the concentration of oxygen becomes critical requires that attention be given to the phenomena of fermentation, the Pasteur effect as well as to the critical oxygen concentration in relation to these processes. Furthermore, since it is reasonable to assume that the quantitative and qualitative changes in respiration will be reflected in those phases of intermediary metabolism which are directly connected to the respiratory processes, the magnitude and the nature of these changes and their possible effect on fruit quality must be taken into

account.

1. Effect of Carbon Dioxide and Oxygen Concentration on Respiratory Activity.

Carbon dioxide. The early literature on the effect of $CO_2$ on respiration of plant tissues is interesting from the viewpoint that the principles which evolved from these investigations form the basis upon which the use of modified atmosphere storage has developed. Mangin in 1915 (35) showed that carbon dioxide at a concentration of 5 percent retarded the respiration rate of germinating seeds. In 1915, Kidd (29) found that step-wise increase of $CO_2$ from 0 to 50 percent resulted in progressive decreases in rate of respiration as measured by either $CO_2$ output or $O_2$ absorption. Later Kidd et al. (32) in search for a method to control cold temperature injury of apples when stored at low temperatures found that the respiration rate of these fruits like seeds was retarded by $CO_2$. Similar effects on pears were reported by Trout in 1930 (52). Critical studies on the effect of added $CO_2$ on the respiration of fruits are limited, probably due to the difficulties involved in methodology. Only a few experiments have been carried out where the effects of $CO_2$ have been studied separately from those of oxygen. Kidd and West (30) treated Sturmer Pippin apples in the pre- and mid-climacteric

periods with 5 and 10 percent $CO_2$ in the presence of 20 percent $O_2$ at 10° C. They found that the climacteric was depressed by $CO_2$ in the post- but not in the pre-climacteric period and that the greater effect was obtained with the 10 percent concentration. Probably the most critical studies on the effect of $CO_2$ on respiration have been conducted by Young, Romani and Biale (62) who studied the effects of various concentrations of $CO_2$ in the presence of 5, 10 and 21 percent of $O_2$ on the respiration of avocados, bananas and lemons. In the avocado, $CO_2$ had no effect on respiration prior to the climacteric but delayed its onset and reduced the respiratory peak. The induction of the climacteric was likewise delayed in the banana but the rate of respiration at the peak was unaffected by $CO_2$ treatment. With lemons, $CO_2$ markedly stimulated respiration, the effect being greater with the 10 percent treatment.

Effect of external oxygen concentration. Biale (5) points out that the effect of $O_2$ on respiration may be different for fruits with and without a climacteric pattern. He lists 12 fruits with a climacteric including apple, pear, plum, peach, tomato, avocado, banana as well as a number of other tropical species. Among the non-climacteric fruits are cherry, strawberry, grape, melon,

orange, lemons, grapefruit and cucumber. Biale (3) found for a fruit having a climacteric pattern such as the avocado, that when the external $O_2$ was lowered from normal, there was a marked decrease in rate of $CO_2$ output and the climacteric was delayed. The maximum rate of respiration in 5 percent $O_2$ was approximately one-half that in air. Concentrations of $O_2$ above 21 percent had only slight effects on respiration. The effects of lowered $O_2$ levels become less with decrease in temperature from 15° to 5° C. Claypool and Allen (9) found the Wickson plum to increase in respiration rate with increasing partial pressures of oxygen. Their data with regard to relation between effect of $O_2$ concentration and temperature are especially interesting. At temperatures under 50° F., respiration at $O_2$ tensions below 21 percent was approximately proportional to the $O_2$ concentration. At 50° there was a slight divergence from this pattern, as shown by the similar rates of respiration by the 1 and 2.5 percent $O_2$ treatments. At temperatures above 50° there was increasingly less difference between $O_2$ treatments in the range of 1-10 percent and more effect from those treatments above 21 percent. At 77°, there was a complete reversal of the effects of the 1, 2.5, 5 and 10 percent treatments, indicating that at higher temperatures oxygen was limiting to the point where anaerobic $CO_2$ production became evident. In an

investigation with Bartlett pears, Allen and Claypool (1) found that the rate of respiration at 32° and 37° F. was proportional to the $O_2$ concentration. Respiratory activity of fruit stored in 2.5 percent oxygen was approximately one-half that of similar fruit in a normal atmosphere. With lemons, a non-climacteric type of fruit, Biale and Young (4) found an immediate increase in $CO_2$ production following treatment with concentrations of oxygen higher than in air. This increase in respiration was considered to be a response to treatment rather than a true climacteric, because it was a function of $O_2$ concentration and was not related to fruit maturity. At $O_2$ tensions below 21 percent, a reduction in respiratory activity was observed.

2. Rate of Diffusion and Composition of Internal Atmosphere.

Little information is available on factors affecting the diffusion of oxygen and carbon dioxide into fruits. However, the presence of numerous lenticels on the epidermis and the continuity of the connecting intercellular air spaces in parenchymatous fruit tissue provide a more or less unrestricted pathway for in and out diffusion of gases. Reeve (45) found intercellular air spaces in apples to occupy approximately 18-22 percent of the total volume.

That diffusion takes place largely through the epidermis, at

least in fruits with a closed calyx, is shown by the very marked effects which artificial skin coatings have on the internal $CO_2$ and $O_2$ concentration (17). Hackney (15) found the resistance of the tissues to diffusion of gases tended to increase during storage and that this change appeared to be reflected in a decrease in internal $O_2$ concentration and respiration. That the internal atmosphere may differ widely from the external atmosphere with reference to $CO_2$ and $O_2$ concentrations has been observed by a number of workers. Magness (34) found that the internal $O_2$ concentration in Newton apples ranged from approximately 14 to 3 percent and $CO_2$ from 3 to 14 percent when held at different temperatures from 2° to 30° C. Trout et al. (51) also found the composition of the internal atmosphere to vary with temperature. At 7° C. the internal $O_2$ and $CO_2$ contents of Granny Smith apples were 17 and 2 percent, respectively, but 2 and 17 percent at 29° C. In a general way the internal $O_2$ and $CO_2$ concentrations also tend to vary with rate of respiration.

One of the difficulties involved in the determination of $CO_2$ and $O_2$ concentrations in fruit tissues is differentiating between the gases in the intercellular atmosphere and those dissolved in the cell fluids. This is particularly true with $CO_2$, which has a high absorption coefficient in water (1.712 at 0° C. and 0.878 at 20° C.)

and can also exist in the "bound" form as carbonates or bicarbonates. According to Burton (8) the $CO_2$ content in the intercellular spaces of potato tubers is small in comparison to the "bound" form.

3. Qualitative Changes in Respiration in Relation to Oxygen and Carbon Dioxide Concentration.

In storing fruits in modified atmospheres, the changes in the nature of respiration which may become apparent under conditions where oxygen is limiting or carbon dioxide excessive are of vital concern. According to the modern viewpoint, $CO_2$ production in respiration of plants may come from three sources: (1) aerobic respiration, (2) anaerobic respiration (fermentation, anaerobic $CO_2$ production) and (3) a combination of the two. Under normal $O_2$ supply, aerobic respiration occurs with a R.Q. of 1.0, indicating the complete oxidation of a carbohydrate to $CO_2$ and $H_2O$. In an atmosphere devoid of oxygen (in nitrogen) $CO_2$ is produced only by fermentation (anaerobic $CO_2$ production) with the simultaneous formation of ethyl alcohol. If, however, oxygen is present in low concentrations, respiration and fermentation may occur simultaneously as indicated by an increase in the R.Q. With increasing concentrations of oxygen, fermentation will cease. The external concentration of oxygen at which fermentation is extinguished is

known as the extinction point (E. P. ) or as the critical oxygen concentration. Since the cell has the capacity for both types of $CO_2$ production, obviously it also must possess some mechanism by which fermentation is suppressed in the presence of oxygen. This is known as the Pasteur effect and has been defined as the action of the oxygen in supressing the accumulations of the products of fermentation. Considerable confusion and controversy have existed regarding the definition and nature of the Pasteur effect, a discussion of which is beyond the scope of this review. An excellent treatment of the subject has been presented recently by Turner (53).

In commercial controlled atmosphere storage practice, the $O_2$ concentration is generally maintained at comparatively low levels (2-5 percent). Since products of fermentation conceivably could be injurious over a prolonged storage period, information on critical oxygen levels is of primary importance. The term "extinction point of anaerobic respiration" denoting the critical or threshold $O_2$ concentration below or above which the rate of $CO_2$ evolution increases, was introduced by Blackman and Parija (6). Subsequent investigations have shown that this value varies according to species, temperature, maturity and other factors. Kidd and West (31) did not detect any alcohol in immature fruits at 0. 5 percent oxygen, while ripe apples produced appreciable quantities even

in air. Biale (4) found with lemons that E. P. was not a sharp and constant value but changed during the storage period and varied from sample to sample; however, it was limited to the range of 0.5 to 5 percent oxygen at 15° C. Similar observations were reported for oranges. With avocados the E. P. was much less distinct (3). He believed this difference could be explained by the fact that avocados have a low anaerobic $CO_2$ production while in lemons and oranges it is high. That temperature has a profound effect on critical oxygen levels is indicated by the work of Claypool and Allen with the Wickson plums (9). They found $CO_2$ production to be lower in 1 and 2.5 percent than in 5 percent oxygen at 32° F. but at 77° this order tended to be reversed, indicating that fermentation was contributing to the total $CO_2$ production in the fruits held at 1 and 2.5 percent oxygen at the higher temperature.

Little information is available on the possible injurious effects of fermentation products in fruits. Apples in the preclimacteric stage apparently will survive as long as six weeks in nitrogen without apparent damage (13). Smock (46) states that apples subjected to less than one percent oxygen during storage may develop an injury to the skin and the outer cortex tissues. Hansen (18), however, found that Anjou pears stored in an atmosphere of one percent oxygen for a period of approximately eight

months showed no injury and ripened normally with good quality.

4. Effects of Modified Atmospheres on Intermediary Metabolism.

Interest in the response of fruits to modified atmospheres has been confined almost exclusively to effects on respiration from the physiological and biochemical viewpoint and on the marketing quality aspects from the horticultural standpoint. The middle ground of how the intermiediary phases of metabolism which are linked to respiration may be influenced by alterations in $CO_2$ and $O_2$ concentrations and subsequently expressed as changes in fruit quality has received very little attention. That specific phases of metabolism other than respiration are affected by modifications in the storage atmosphere have been recognized in a few cases. Young et al. (61) in discussing their data on effects of $CO_2$ and $O_2$ on fruit respiration suggested that "reduced oxygen might be expected to delay the induction of the climacteric by decreasing the available ATP for synthesis, and increased carbon dioxide might delay the formation of a specific amino acid necessary for synthesis of an inhibitor of either new enzyme synthesis or another pathway." Allentoff et al. (2) have shown $C^{14}O_2$ fixation in malic acid and in the three key amino acids, glutamic, aspartic, and alanine. $CO_2$

fixation also occurs in pears (58). Development of superficial scald on apples (17) and pears (14) is also retarded or inhibited by storage in modified atmospheres. Production of ethylene and other volatiles is retarded in modified atmospheres (12, 19, 44). Smock (46) found that apples deteriorate less rapidly at warm temperatures after removal from controlled atmospheres storage. Mattus (37) found this to be true with Bartlett pears and that the fruit also respired at a much reduced rate when transferred to a temperature favorable for ripening. These data suggest that alterations in metabolism during storage are brought about by the modified atmospheres and the effects tend to persist in the fruit even after removal for marketing and consumption.

# MATERIALS AND METHODS

## 1. Variety, Source, and Storage Treatments.

Two varieties of commercially grown pears were used in these experiments. Bartlett pears were obtained from the Mid-Columbia Experiment Station at Hood River and the Anjou pears from the Medford Valley. The fruit was picked at approximately the mid-point of commercial harvest, and the experiments extended over the 1959-1960 and 1960-1961 seasons.

Composite samples were prepared from 5 boxes of fruit and divided into a sufficient number of 15-fruit lots to provide samples for analyses before and after ripening. One half of these lots were placed in controlled atmosphere cabinets maintained at 2 percent oxygen and 3 percent carbon dioxide at 30° F. This particular atmosphere was used because it had been shown previously to provide optimum storage and ripening conditions. Each cabinet was fitted with a $CO_2$ scrubber consisting of a 5-gallon jar containing 15 percent NaOH solution and a small air pump for circulating the atmosphere in the chamber through caustic soda solution. The pump operated on a time clock, which permitted maintaining the desired $CO_2$ concentration by adjusting the duration of the scrubbing

periods per day. Respiration of the fruit in the air tight cabinets reduced the $O_2$ level to 2 percent within 8-10 days after sealing. This concentration was maintained thereafter by admission of air through the scrubber inlet tube when the $O_2$ level became less than 2 percent. Since fruit was removed from the cabinets at periodic intervals during storage, it was necessary to re-establish the modified atmosphere as rapidly as possible. This was accomplished by flushing the cabinet with compressed nitrogen gas for 4-6 hours after re-sealing.

The remaining lots of pears of each variety were stored at 30° F. according to conventional commercial practice, without alteration in the $O_2$ and $CO_2$ contents of the atmosphere.

One sample of each variety was analyzed at time of storage. Further analyses were made thereafter at monthly intervals. In 1960-61, lots of fruit were analyzed also after ripening at 70-72° F.

2. Determinations of Chemical and Physiological Changes during Storage and Ripening.

Pressure test. Pressure tests, as an index of flesh firmness, were made with a Balauf tester on pared samples, using 2 punches per fruit.

Chemical analyses.

1. Preparation of samples. Wedge shaped sectors were cut from each of 15 fruits in a lot to provide a total weight of 300-350 grams. After peeling, the slices were ground through a food chopper and thoroughly mixed with a spoon. Duplicate 50-gram samples were weighed into 600 ml Pyrex beakers, covered with 270 ml of 95 percent alcohol and heated to boiling. After cooling, the samples were transferred quantitatively to a Waring blendor, ground at high speed for 3 minutes, then filtered through Whatman #2 filter paper into 500 ml volumetric flasks, finally washing the residues 4-5 times with 80 percent alcohol. The extracts were adjusted to volume with 80 percent alcohol. Suitable aliquots of these solutions after removal of alcohol were used for determination of sugars, alcohol soluble nitrogen, total amino nitrogen, free amino acids and organic acids.

2. Soluble solids and total acidity of juice. Approximately 50 ml of juice expressed from the ground tissues was centrifuged at 1500 RPM for 15 minutes. Percent soluble solids was determined with a hand refractometer. Titratable acidity was determined on duplicate 10 ml aliquots by titration to pH 7.0 with a Beckman pH meter. Total acidity was calculated as malic acid.

3. Sugars. Somogyi's phosphate sugar reagent (47) was used for reducing sugar determinations. Five ml of the alcohol extracts were evaporated on a hot water bath at 65° C. until alcohol free. The residues were dissolved in water, filtered into a 100 ml volumetric flask and adjusted to volume with distilled water prior to analysis. Duplicate 10 ml aliquots were diluted to 50 ml and reducing sugars determined on 5 ml aliquots. Total sugars were determined similarly after inversion with invertase solution. Sucrose was calculated as the difference between total and reducing sugars multiplied by the factor 0.95.

4. Pectic substances. Pectic substances in the alcohol insoluble residues were determined according to the spectrophotometric procedures of Dieze and Rouse (11) using sodium hexametaphosphate and sodium hydroxide solutions as extractants. Sodium hexametaphosphate soluble pectic substances are the low methoxyl content pectinates of the polyvalent cations, magnesium and calcium. The polyphosphates have a sequestering effect on the calcium and magnesium, thus solubilizing the low methoxyl pectinates. Sodium hydroxide soluble pectic substances include protopectin; the calcium and magnesium pectinates are not removed by sodium hexametaphosphate extraction. A standard curve

(Figure 1) was prepared, using anhydro-galacturonic acid. Pectic substances were expressed as percent anhydrogalacturonic acid.

5. Organic acids. The individual organic acids contained in the extracts were separated by ion-exchange chromatography according to the method of Hulme and Wooltorton (21). The principle of the method and practical details of its application to Krebs' cycle and other organic acids in the tobacco leaf have been described by Palmer (42). Briefly, the suitably purified solutions of organic acids from the alcohol extracts are first passed through a column of Dowex 50W-X4, $H^+$ form, to remove the free amino acids. The solutions plus washings were then passed through a column of a strongly basic anion exchange resin (Dowex I-X8, 200-400 mesh, acetate form). The adsorbed organic acids were eluted with 100 ml 2.5 N acetic acid, 60 ml 6 N acetic acid, 200 ml of 6 N formic acid and collected in 4 ml increments in 18 x 150 mm test tubes on a fraction collector. The volatile acids used for elution were then removed by heating the test tubes on a hot plate at 65° C., while a stream of air was blown on the surface of the liquid by use of the apparatus shown in Figure 2. When evaporation was complete, the residue in each test tube was dissolved in 2 ml $CO_2$-free distilled water and titrated with 0.01 N NaOH

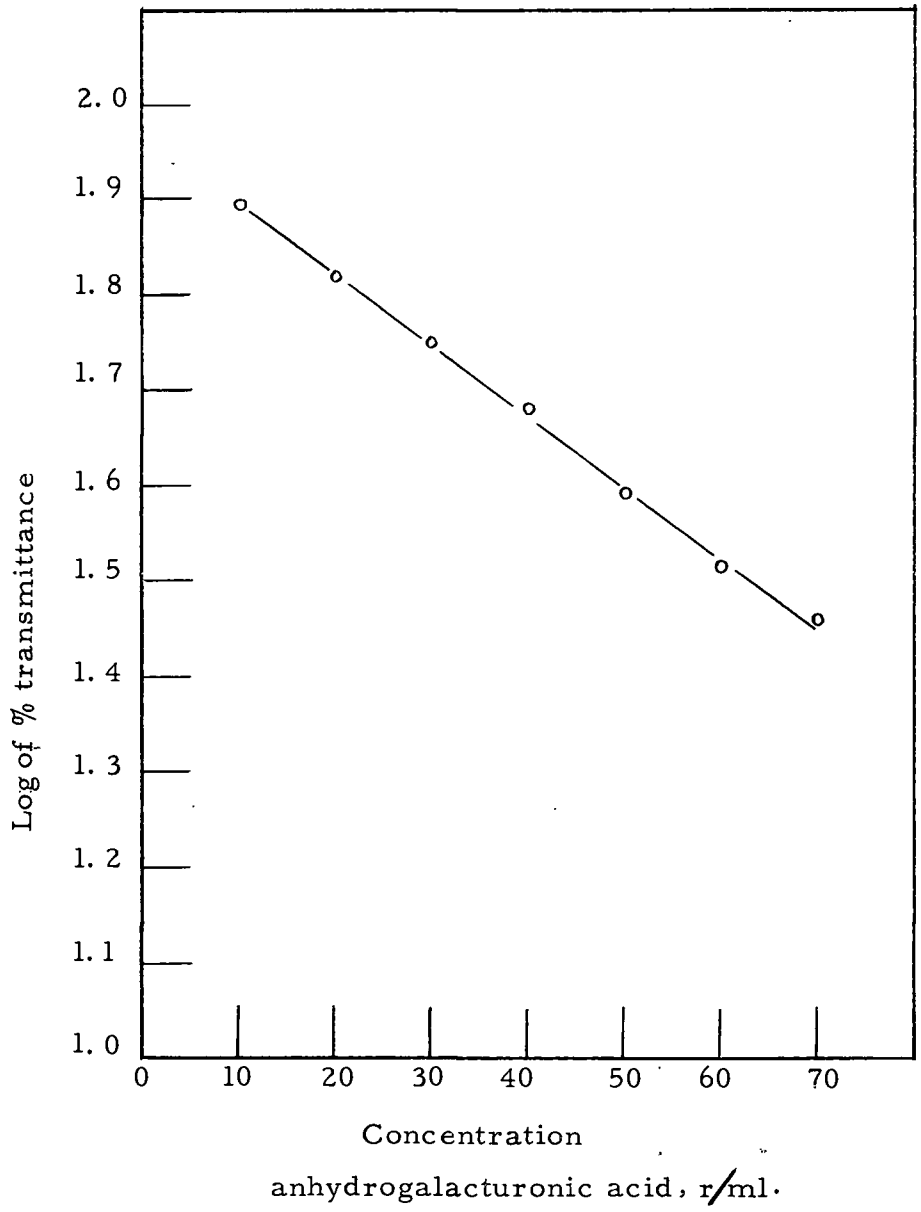
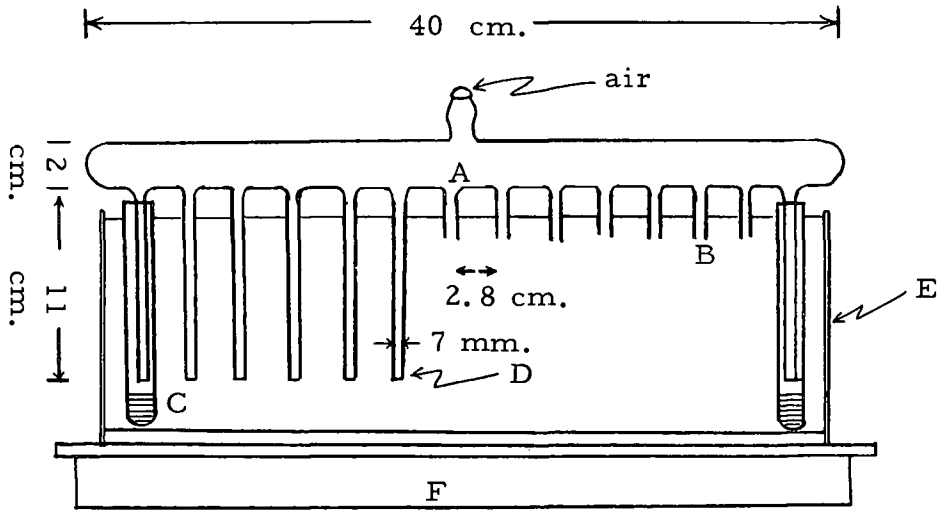



Figure 1. Standard curve of galacturonic acid at 525 mμ.




A. Header of manifold

B. One of 14 outlet tubes of manifold

C. Test tube in place during evaporation

D. Openings of outlet tube precisely tooled to 1 mm. diameter

E. Outline of test tube rack

F. Hot plate

The manifold outlets are spaced to fit into the row of test tubes which in turn are held in the metal test tube rack. The header of the manifold rests on the lips of the test tubes.

Figure 2. A diagram of evaporating apparatus for organic acid fraction test tubes.

(carbonate free), using phenol red as indicator. A chromatograph obtained with a mixture of pure known organic acids is shown in Figure 3. The identity of the specific acids eluted in the various fractions was determined by comparing with known acids and also by paper chromatography. For this latter purpose, the contents of the test tubes comprising each peak on the chromatograph were combined and evaporated under reduced pressure at 65° C. The concentrate was then passed through a Dowex 50W-X4, 50-100 mesh, $H^+$ form, ion-exchange column to remove $Na^+$. Aliquots of 10-100 microliters were then spotted on Whatman #1 filter paper and developed with N-butanol : formic acid : water solvent (40:8:60, v/v). After drying, the papers were sprayed with a mixed acid-base indicator (0.04 per cent brom-thymol blue and plus 0.04 per cent brom-cresol green in 80 per cent ethanol, adjusted to pH 8.5 with 0.01 N NaOH).

6. Total alcohol soluble and insoluble nitrogen. Total nitrogen in the alcohol extracts and residues was determined by the A.O.A.C. Kjeldahl method (33). For the former determination, 25 ml aliquots of the extracts were evaporated under reduced pressure at 65° C. and the residues transferred by washing to Kjeldahl digestion flasks. For the latter determination, the dry residues were weighed on an analytical balance and transferred to

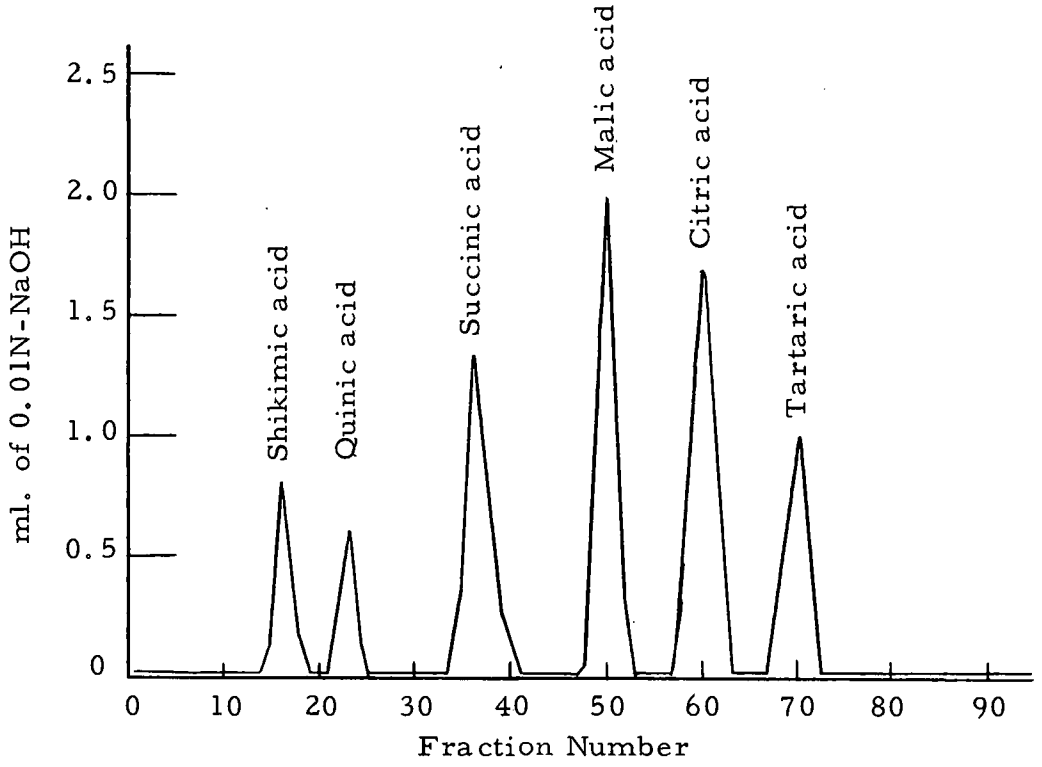



Figure 3. "Flowing" chromatogram of a known mixture of organic acids obtained by gradient elution.
(4 ml. fractions)

Kjeldahl flasks quantitatively.

7. Paper chromatography of amino acids. Amino acids which were adsorbed on the Dowex 50W-X4 column prior to removal of organic acids, were eluted by 3-4 washings with 3 N $NH_4OH$ solution. The combined elutes were then evaporated under reduced pressure at 65° C. until no odor of ammonia could be detected. The concentrated solutions were transferred quantitatively to 25 ml volumetric flasks and diluted to volume.

Amino acids were separated by 2-dimensional paper chromatography following the general procedures outlined by Block et al. (7). A 400 microliter aliquot of the extract was spotted on one corner of a 18 x 22 inch sheet of Whatman #1 filter paper, which was then suspended in a chromatography cabinet. After sealing, 30 ml of 3 per cent $NH_4OH$ solution was added for saturation of the chamber. The first dimensional solvent used was phenol : water (920:360, w/v) prepared from Mallinkrodt Gilt Label phenol. The solvent was kept in a dark bottle and stored in the refrigerator when not in use. After approximately 12 hours, 90 ml phenol-water solvent was added to each trough and the chromatogram developed for 22-23 hours. To prevent loss of fast moving acids such as proline, the paper was removed before the solvent front had reached the lower edge. After thorough drying in a ventilated hood, the

paper was replaced in the cabinet and developed in the second dimension with freshly prepared N-butanol:water:acetic acid (250:250:60, v/v), using 110-120 ml of solution in the trough. After 36 - 40 hours, the papers were removed and dried. The spots were detected by spraying the papers with 0.3 percent ninhydrin in 95 percent alcohol, then heating in an oven at 70-75° C. for 15 minutes. All spots were purple except proline (yellow), asparagine (brown), and beta-alanine (deep blue). Identity of the specific amino acids on the chromatograph was determined by comparing with a chromatograph of known amino acids (Figure 4).

8. Total amino nitrogen. Total amino nitrogen in the extracts was determined by the photometric method of Yemm and Cocking (61), using a Klett photometer at a wave length of 570 mμ.

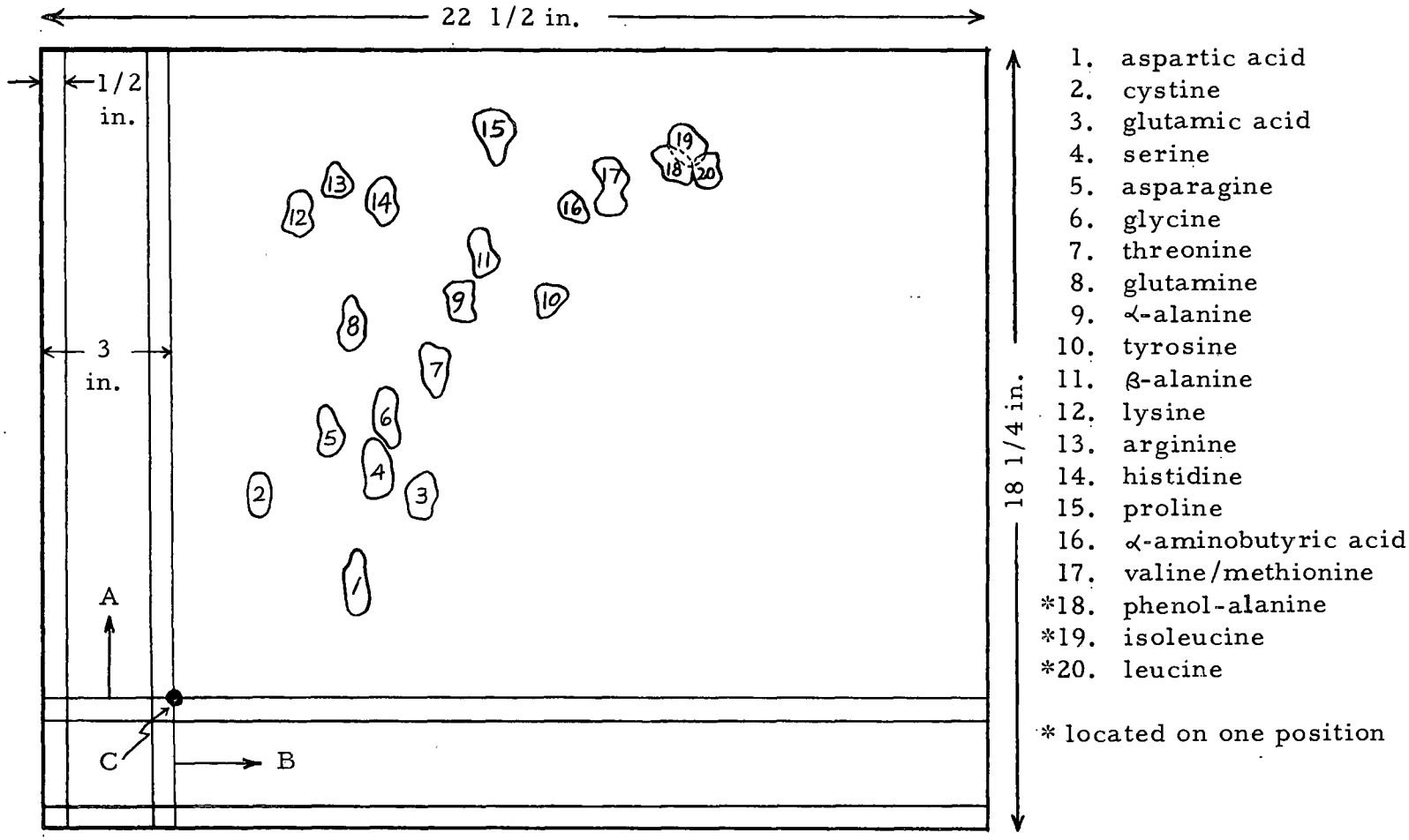



Figure 4. Two dimensional paper chromatogram of known amino acids.

A. phenol:water; B. N-butanol:water:acetic acid

C. original spot

# RESULTS

## 1. Effect of Modified Atmospheres on Physical and Chemical Changes During Cold Storage, 1959 - 1960.

The experiments conducted the first season (1959-1960) were exploratory in nature to determine what particular phases of fruit metabolism were affected by modification in the atmosphere during the storage period.

The abbreviations CS (conventional storage in unmodified atmosphere) and CAS (controlled atmosphere storage in modified atmosphere) are used in presentation and discussion of the results.

Firmness: Pressure test of both CS and CAS Bartlett pears decreased approximately 3 pounds during the first month in storage (Table 1). In the 3 following months, additional decreases of 1.5 pounds occurred in both lots. Thereafter, however, no change in the pressure test was found in the Bartlett pears stored in the modified atmosphere, but the samples in conventional storage continued to soften. Thus, after 6 months, the pressure test was 1.7 pounds higher for the CAS fruit. Anjou pears reacted similarly (Table 2). The pressure test tended to decrease at a similar rate for 5 months in fruit stored under both treatments. During the remaining 3 months, however, the CS fruit continued to soften

Table 1. Effect of controlled atmosphere storage on changes in firmness, soluble solids, pH and total acids in Bartlett pears, 1959 - 1960.

| Months in storage | Pressure test(lbs) | | Soluble solids(%) | | Juice | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | pH | | acids (mg./100 ml.) | |
| | CS | CAS | CS | CAS | CS | CAS | CS | CAS |
| 0 | 19.7 | 19.7 | 10.6 | 10.6 | 4.21 | 4.21 | 294.979 | 294.979 |
| 1 | 15.6 | 15.9 | 12.1 | 12.4 | 4.45 | 4.32 | 248.996 | 301.497 |
| 2 | 15.4 | 15.5 | 12.2 | 12.4 | 4.40 | 4.25 | 264.481 | 298.705 |
| 3 | 15.5 | 15.2 | 12.4 | 12.4 | 4.41 | 4.39 | 243.527 | 259.358 |
| 4 | 14.8 | 14.4 | 12.6 | 12.5 | 4.50 | 4.30 | 175.544 | 260.523 |
| 5 | 14.0 | 15.0 | 12.5 | 12.3 | 4.50 | 4.20 | 165.068 | 235.161 |
| 6 | 13.1 | 14.8 | 12.1 | 12.2 | 4.60 | 4.20 | 146.326 | 228.743 |

Table 2. Effect of controlled atmosphere storage on changes in firmness, soluble solids, pH and total acids in Anjou pears, 1959 - 1960.

| Months in storage | Pressure test lbs. | | Soluble solids % | | Juice | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | pH | | acids (mg./100 ml.) | |
| | CS | CAS | CS | CAS | CS | CAS | CS | CAS |
| 0 | 14.4 | 14.4 | 13.1 | 13.1 | 4.2 | 4.2 | 300.3 | 300.3 |
| 1 | 13.0 | 12.9 | 13.5 | 13.1 | 4.3 | 4.2 | 265.4 | 266.6 |
| 2 | 12.9 | 12.8 | 13.1 | 13.1 | 4.4 | 4.3 | 255.2 | 274.2 |
| 3 | 13.0 | 12.8 | 13.1 | 13.1 | 4.3 | 4.2 | 214.2 | 240.3 |
| 4 | 13.0 | 13.0 | 13.0 | 13.1 | 4.1 | 4.0 | 207.9 | 230.8 |
| 5 | 12.7 | 12.8 | 13.0 | 13.6 | 4.3 | 4.2 | 188.4 | 223.5 |
| 6 | 11.8 | 12.5 | 13.0 | 13.1 | 4.6 | 4.5 | 148.5 | 191.2 |
| 7 | 11.3 | 12.7 | 12.5 | 13.0 | 4.5 | 4.4 | 147.3 | 187.1 |
| 8 | 11.4 | 12.6 | 12.5 | 15.5 | 4.6 | 4.3 | 133.7 | 225.7 |

while the CAS fruit showed no further decrease. After 8 months' storage, the pressure test of Anjou pears kept in controlled atmosphere was 1.2 pounds higher than that of similar samples in conventional storage.

Soluble solids: Total soluble solids in the juice of Bartlett pears from both the storage treatments increased approximately 1.5 per cent during the storage period (Table 1). In Anjou pears, soluble solids content tended to remain at about 13 per cent, except for an increase in the CAS fruit during the final month of storage (Table 2).

pH and titratable acidity of juice: The pH of the expressed juice of both varieties was lower in the CAS than in the CS treatment throughout the storage period. Storage in the controlled atmosphere greatly retarded acid loss in both varieties (Table 1, 2). In CS Bartlett pears, mg of acid/100 ml of juice decreased from 295 mg to 146 mg, a loss of 149 mg during the storage period. In the CAS treatment, however, the concentration decreased from 295 mg to 229 mg, a loss of only 66 mg (Figure 5). Anjou pears at time of storage contained 300 mg total acid/100 ml of juice. At the termination of storage period, there were 134 and 226 mg in the CS and CAS samples, respectively (Figure 6).

Sugars, Starch and Pectic substances: According to the data (Tables 3, 4) there were no well-defined differences in

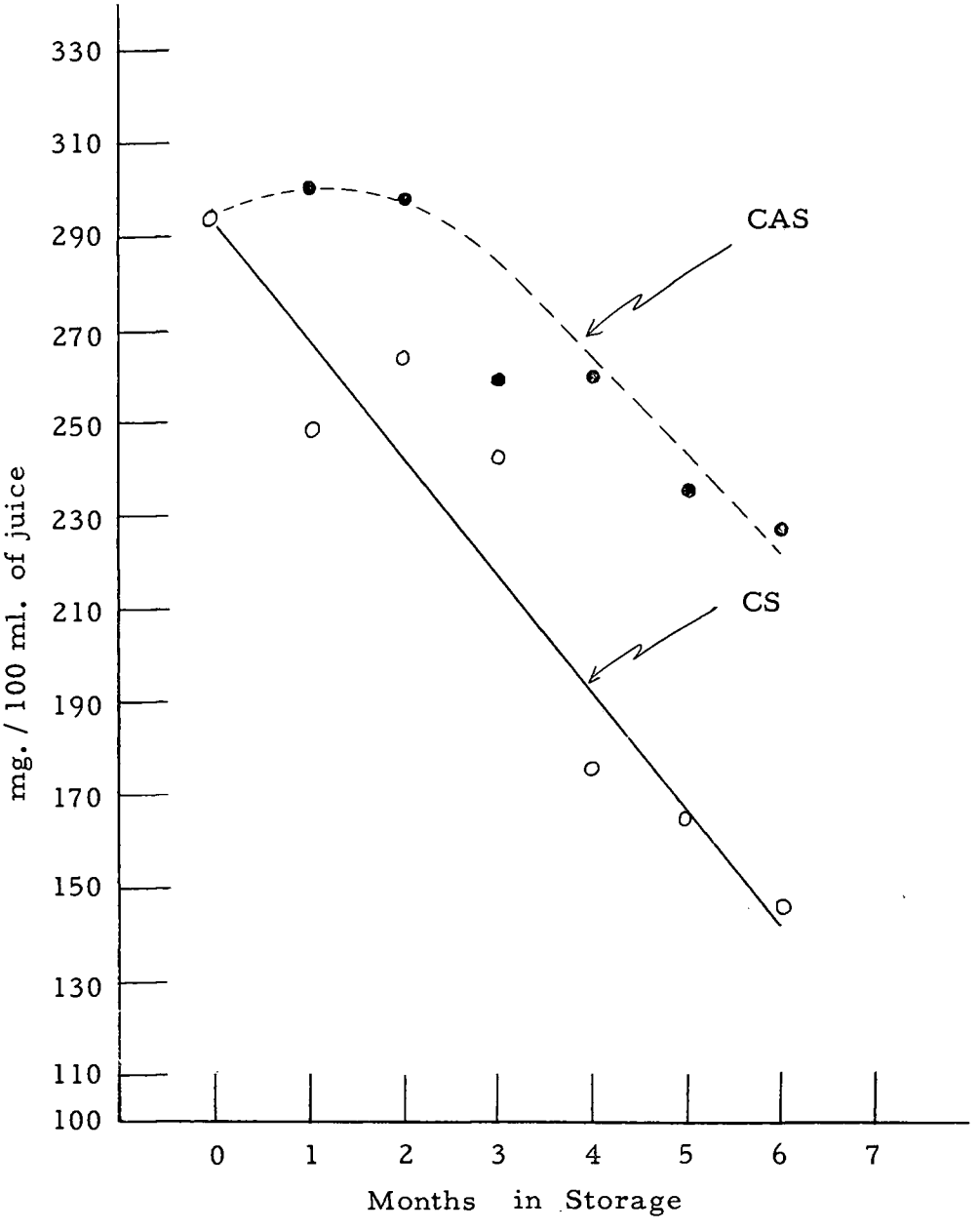



Figure 5. The effect of storage on total acid in juice of Bartlett pears.

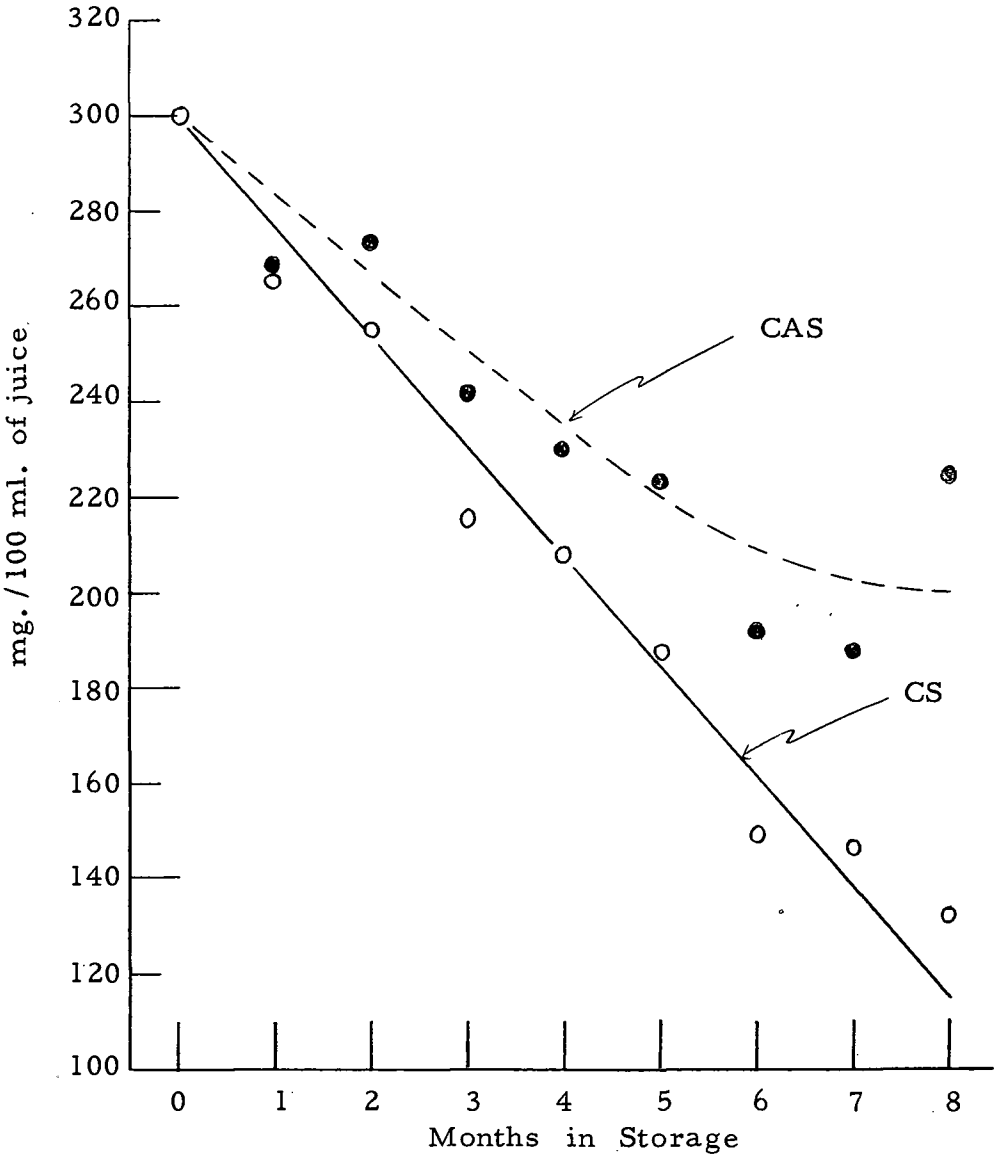



Figure 6. The effect of storage on total acids in juice of Anjou pears.

Table 3. Changes in sugars and pectic substances in Bartlett pears during conventional and controlled atmosphere storage, 1959 - 1960.

| Months in storage | Total sugars (% fresh wt.) | | Reducing sugars (% fresh wt.) | | Sucrose (% fresh wt.) | | Pectic substances (% dry wt.) | |
|---|---|---|---|---|---|---|---|---|
| | CS | CAS | CS | CAS | CS | CAS | CS | CAS |
| 0 | 7.53 | 7.53 | 5.83 | 5.83 | 1.62 | 1.62 | 15.35 | 15.35 |
| 1 | 8.15 | 7.97 | 6.54 | 6.30 | 1.52 | 1.59 | 23.47 | 22.39 |
| 2 | 8.22 | 7.89 | 7.52 | 7.17 | 0.67 | 0.68 | 23.04 | 23.09 |
| 3 | 8.27 | 8.16 | 7.90 | 7.67 | 0.35 | 0.46 | 22.22 | 22.64 |
| 4 | 8.13 | 8.25 | 7.90 | 7.67 | 0.22 | 0.54 | 22.86 | 24.21 |
| 5 | 8.12 | 8.05 | 7.24 | 6.48 | 0.83 | 1.55 | 23.91 | 24.79 |
| 6 | 8.12 | 8.02 | 7.82 | 7.70 | 0.29 | 0.30 | 24.71 | 24.78 |

Table 4. Effect of controlled atmosphere storage on sugars and pectic substances in Anjou pears, 1959 - 1960.

| Months in storage | Total sugars (% fresh wt.) | | Reducing sugars (% fresh wt.) | | Sucrose (% fresh wt.) | | Pectic substances (% dry wt.) | |
|---|---|---|---|---|---|---|---|---|
| | CS | CAS | CS | CAS | CS | CAS | CS | CAS |
| 0 | 7.90 | 7.90 | 6.97 | 6.97 | 0.88 | 0.88 | 18.59 | 18.59 |
| 1 | 8.03 | 7.99 | 7.14 | 7.27 | 0.96 | 0.68 | 18.94 | 20.37 |
| 2 | 7.97 | 8.10 | 6.73 | 6.96 | 1.18 | 1.09 | 19.50 | 20.03 |
| 3 | 7.63 | 7.27 | 6.76 | 6.94 | 0.83 | 0.31 | 21.56 | 21.26 |
| 4 | 7.59 | 7.58 | 6.32 | 6.37 | 1.20 | 1.13 | 20.53 | 20.05 |
| 5 | 7.72 | 7.60 | 6.38 | 7.26 | 1.27 | 0.33 | 21.98 | 22.62 |
| 6 | 7.87 | 7.76 | 7.39 | 7.10 | 0.46 | 0.63 | 21.24 | 21.67 |
| 7 | 7.80 | 7.64 | 6.43 | 6.66 | 1.11 | 0.94 | 20.00 | 23.16 |
| 8 | 7.44 | 8.38 | 6.30 | 7.46 | 0.96 | 0.87 | 20.98 | 21.63 |

concentrations of total and reducing sugars or sucrose between fruits from the 2 storage treatments in both varieties (Figures 7, 8, 10, 11). Starch, as indicated by the iodine test, disappeared during the first month in storage in both varieties in both treatments. Pectic substances, likewise, showed no difference in response to differential treatment (Figures 9, 12).

Alcohol soluble and insoluble nitrogen: In Bartlett pears (Table 11), alcohol soluble nitrogen tended to fluctuate considerably in concentration during the storage period. In CS Anjou pears (Table 12), this fraction increased from about 26 mg/100 gm F. W. to 28 mg during the first 4 months then decreased to 18 mg at the end of 8 months' storage. In CAS Anjou pears, there was an approximately similar increase, but during the last 4 months the concentration increased to 33.57 mg, nearly twice the amount found in the fruit in the CS treatment. Alcohol insoluble nitrogen tended to be lower in concentration in the CAS fruits of both varieties throughout the storage period. In general, this fraction tended to increase in CS fruit and to remain more or less constant in similar CAS fruit.

2. Comparison of Quality and Chemical Composition of Pears Ripened After Conventional and Controlled Atmosphere Storage.

Quality: Bartlett pears at time of removal from CS after 4 months were yellow in color, indicating an advanced stage of senescence

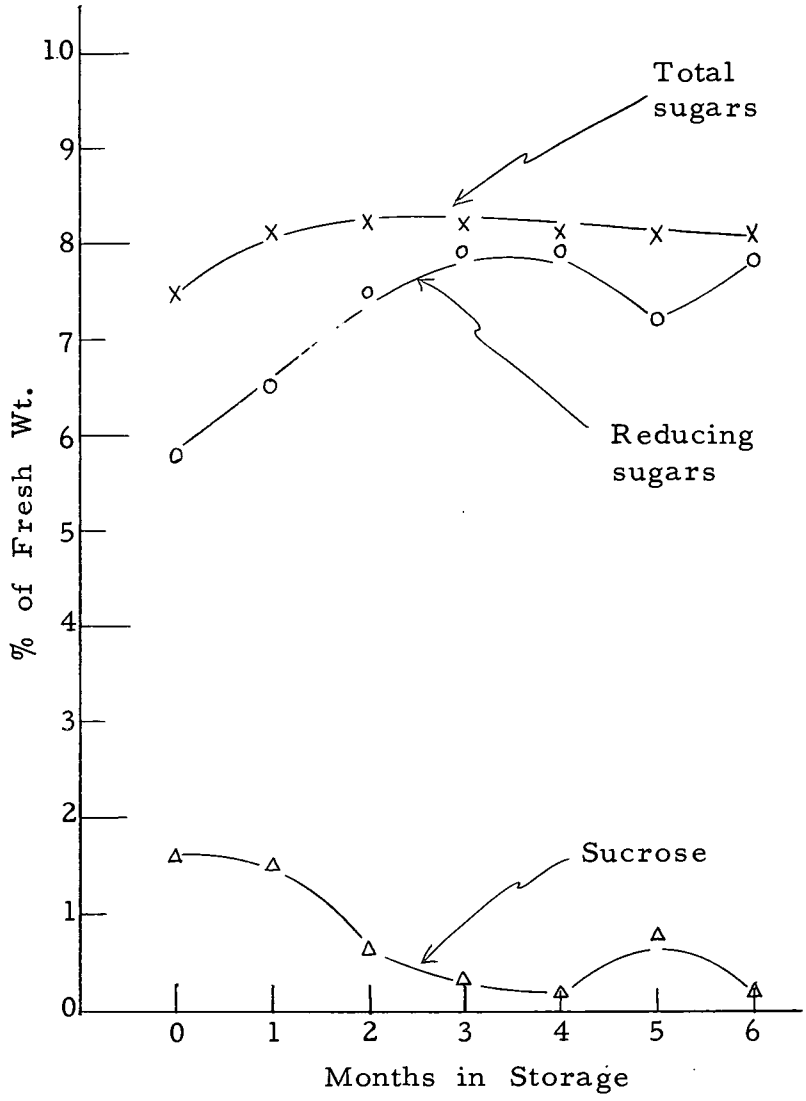



Figure 7. The effect of length of storage on sugars in Bartlett pears in conventional storage.

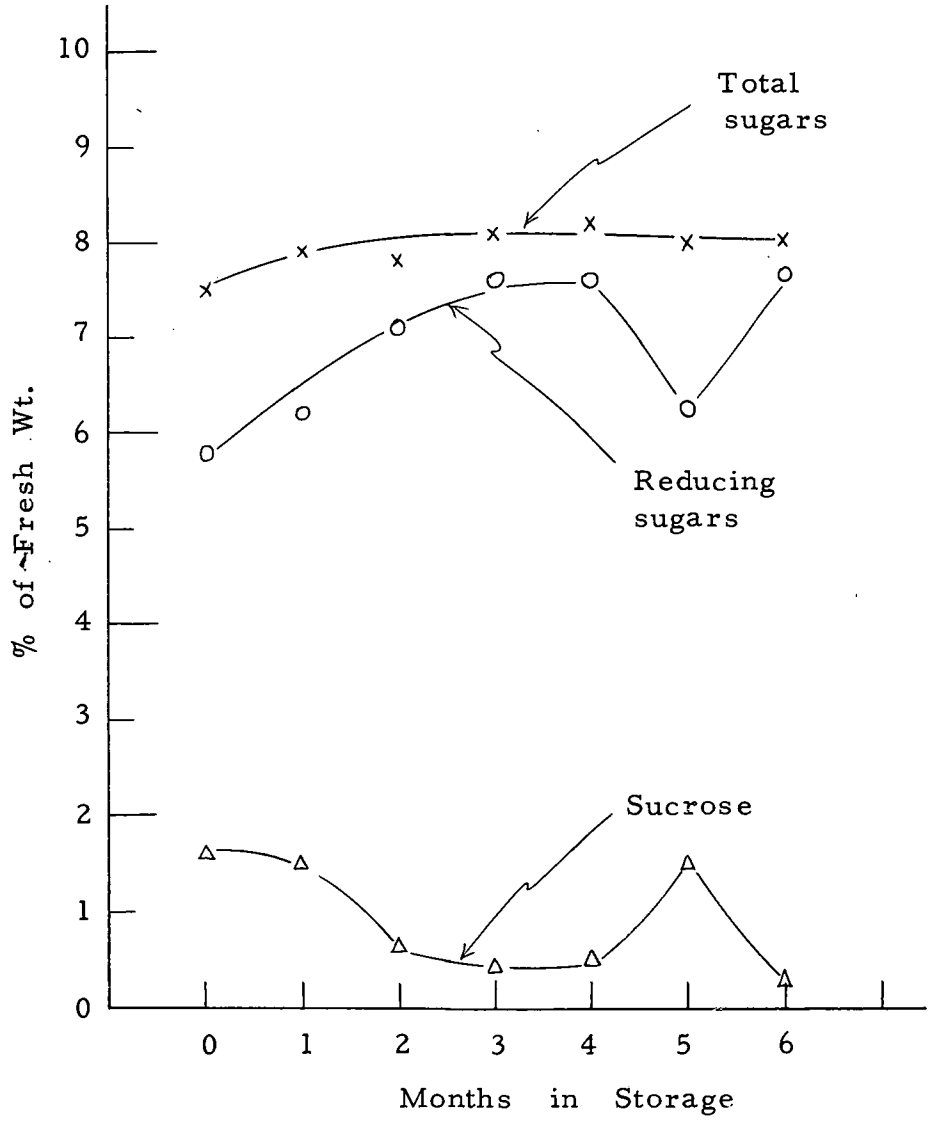



Figure 8. The effect of length of storage on sugars in Bartlett pears in controlled atmosphere storage.

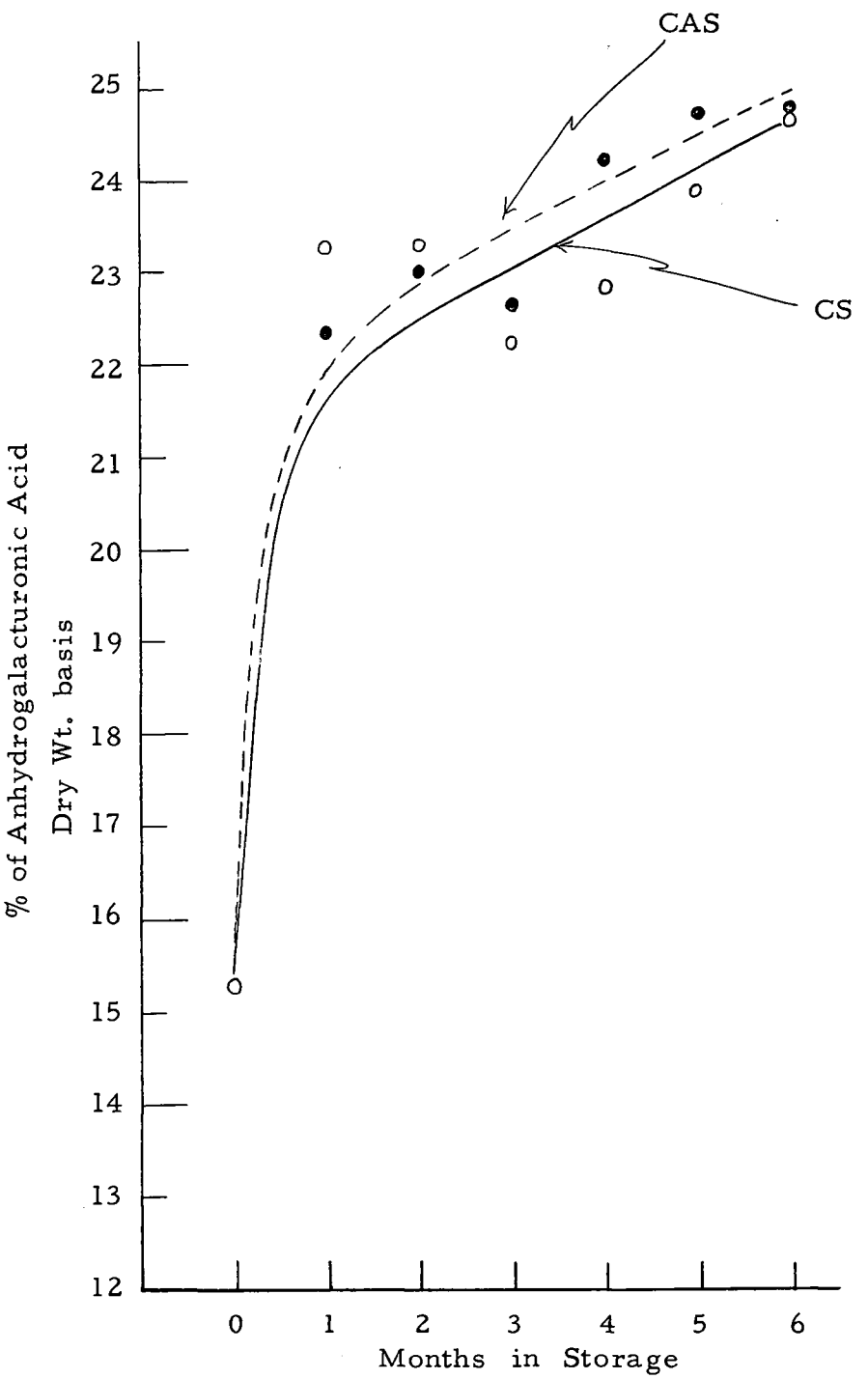



Figure 9. The effect of length of storage on pectic substances in residues of Bartlett pears.

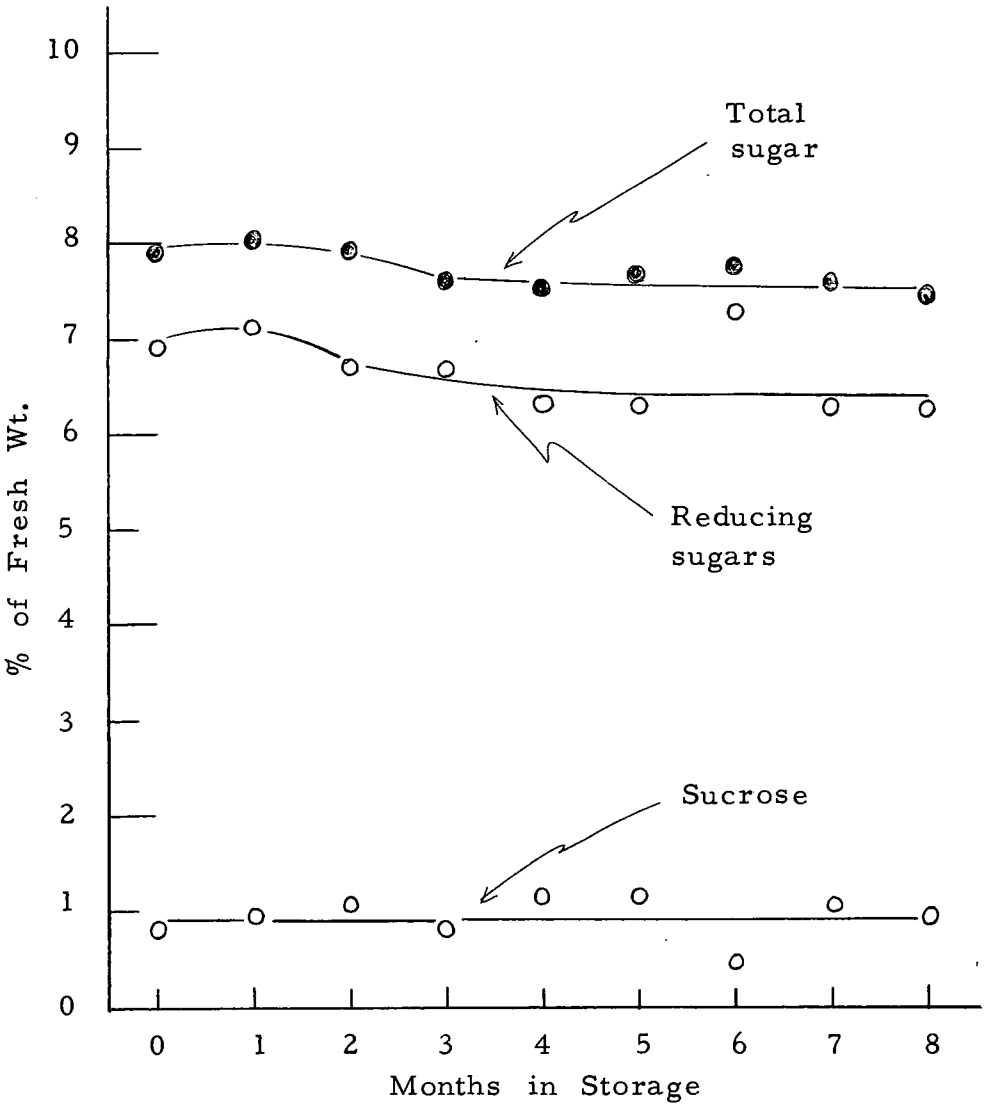



Figure 10. The effect of length of storage on sugars in Anjou pears in conventional storage.

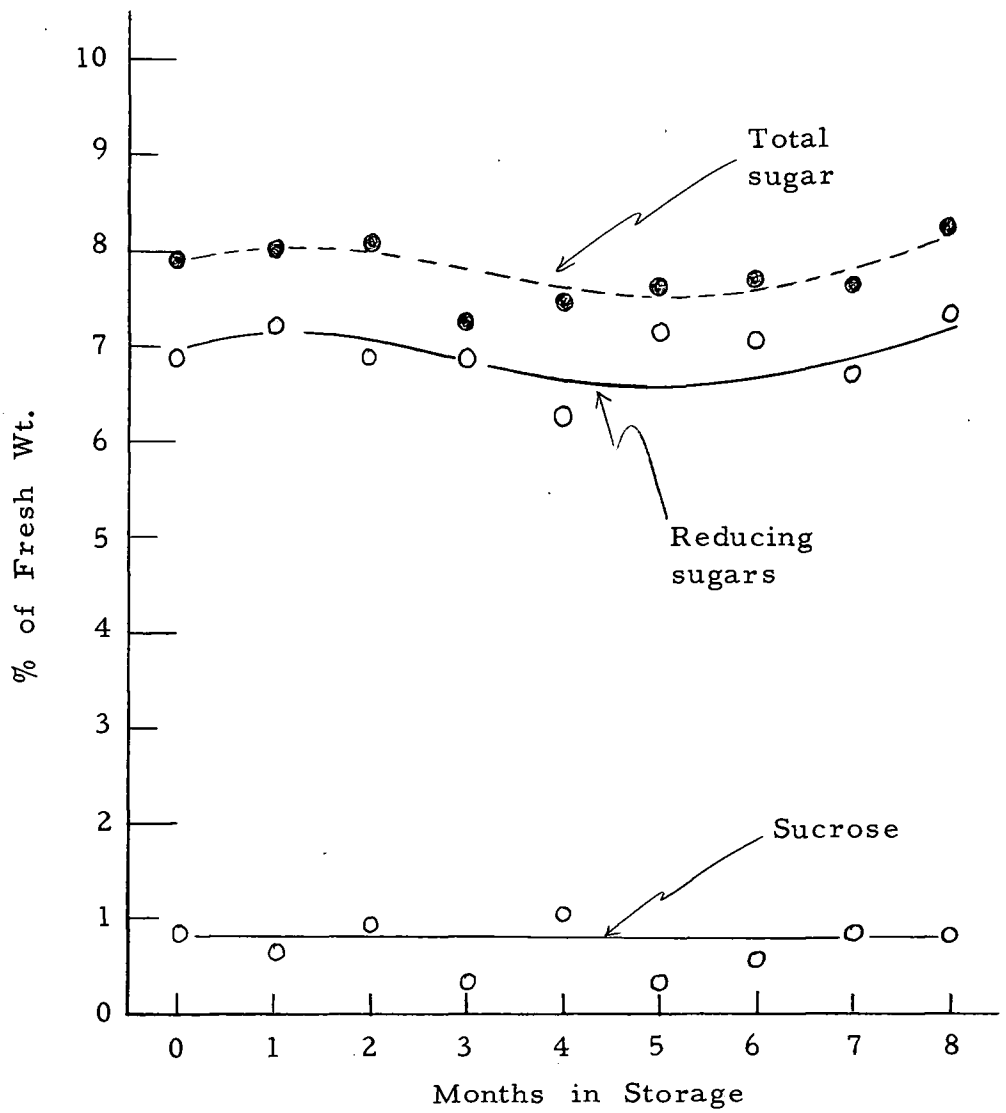



Figure 11. The effect of length of storage on sugars in Anjou pears in controlled atmosphere storage.

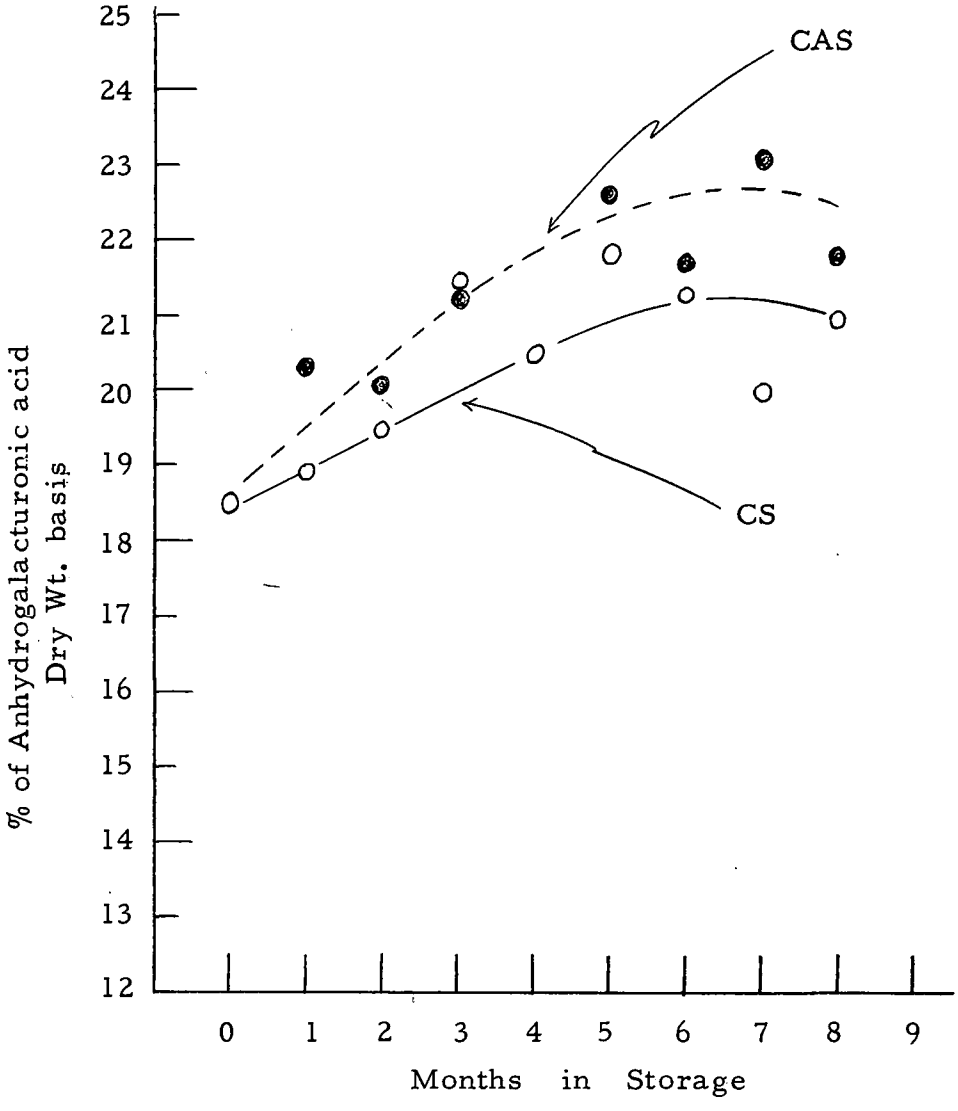



Figure 12. The effect of length of storage on pectic substances in residues of Anjou pears.

(Figure 13). These fruits failed to soften when transferred to a temperature favorable for ripening and developed scald as well as senescent core breakdown. Similar fruit from CAS was green to yellow-green and ripened normally with good eating quality. Anjou pears removed from CS after 6-7 months developed a dry, mealy texture during ripening, while the fruit from CAS ripened normally with good quality even after 8 months. Both Bartlett and Anjou pears which had been stored in CAS ripened at a slower rate and remained edible over a longer period of time than similar fruit from CS.

Data on CS and CAS Bartlett and Anjou pears ripened after various storage intervals are shown in Tables 5 and 6, respectively. In Bartlett pears no consistent differences were found in soluble solids, pH, sugars and pectic substances in fruits ripened after the 2 storage treatments. While total acid content was considerably higher in CAS Bartlett pears after 4 and 6 months of storage, only minor changes in concentrations were found during ripening of the fruit from either treatment. In Anjou pears, total acid content was consistently higher in the CAS fruits both before and after ripening, and in both treatments the concentration tended to increase slightly during ripening. Total sugars also tended to increase in concentration during ripening and were approximately one per cent higher in

Figure 13. A photograph illustrating change in color of Bartlett pears after 4 1/2 months in conventional and controlled atmosphere storage.

(A). Yellow color of CS fruits.

(B). Yellow-green color of CAS fruits.

Table 5. Effect of controlled atmosphere storage on soluble solids, total acids, sugars and pectic substances in Bartlett pears ripened after 4 and 6 months in storage, 1959 - 1960.

| Items | Units | Stored 4 months | | | | Stored 6 months | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | unripened | | ripened | | unripened | | ripened | |
| | | CS | CAS | CS | CAS | CS | CAS | CS | CAS |
| Soluble solids | % | 12.6 | 12.5 | 12.5 | 12.5 | 12.1 | 12.2 | 11.5 | 12.0 |
| Total acids | mg./100 ml. | 175.6 | 260.5 | 193.3 | 266.6 | 146.3 | 228.7 | 146.6 | 195.9 |
| pH | | 4.5 | 4.3 | 4.0 | 3.8 | 4.6 | 4.2 | 4.6 | 4.4 |
| Reducing sugars | % | 7.9 | 7.7 | 6.9 | 7.1 | 7.8 | 7.7 | 7.5 | 7.4 |
| Total sugars | % | 8.1 | 8.3 | 8.1 | 8.6 | 8.1 | 8.0 | 8.6 | 8.5 |
| Sucrose | % | 0.2 | 0.5 | 1.4 | 0.6 | 0.3 | 0.3 | 0.9 | 1.0 |
| Pectic subs. | % | 22.9 | 24.2 | 19.3 | 20.7 | 24.7 | 24.8 | 17.0 | 16.2 |

Table 6. Effect of controlled atmosphere storage on physical and chemical changes in Anjou pear, 1959 - 1960.

| Items | Units | Unripened | | Ripened | |
|---|---|---|---|---|---|
| | | CS | CAS | CS | CAS |
| | | 4 months' storage | | | |
| Soluble solids | % | 13.0 | 13.1 | 14.0 | 14.5 |
| Total acids in juice | mg./100 ml. | 207.9 | 229.8 | 229.1 | 236.3 |
| pH | | 4.1 | 4.0 | 4.0 | 4.0 |
| Reducing sugars | % | 6.32 | 6.37 | 8.44 | 8.37 |
| Total sugars | % | 7.59 | 7.58 | 8.61 | 9.06 |
| Sucrose | % | 1.20 | 1.13 | 0.15 | 0.62 |
| Pectic subs. | % | 20.53 | 20.05 | 18.00 | 17.43 |
| | | 7 months' storage | | | |
| Soluble solids | % | 12.5 | 13.0 | 11.5 | 13.5 |
| Total acids in juice | mg./100 ml. | 147.3 | 187.1 | 154.5 | 193.8 |
| pH | | 4.5 | 4.4 | 4.4 | 4.3 |
| Reducing sugars | % | 6.43 | 6.66 | 5.88 | 6.51 |
| Total sugars | % | 7.60 | 7.64 | 7.28 | 8.20 |
| Sucrose | % | 1.11 | 0.94 | 1.33 | 1.60 |
| Pectic subs. | % | 20.00 | 23.16 | 21.35 | 20.62 |
| | | 8 months' storage | | | |
| Soluble solids | % | 12.5 | 15.5 | 13.4 | 15.5 |
| Total acids in juice | mg./100 ml. | 133.67 | 225.7 | 155.4 | 193.8 |
| pH | | 4.6 | 4.3 | 4.5 | 4.2 |
| Reducing sugars | % | 6.30 | 7.46 | 7.37 | 8.22 |
| Total sugars | % | 7.44 | 8.38 | 8.17 | 9.22 |
| Sucrose | % | 0.96 | 0.87 | 0.76 | 0.95 |
| Pectic subs. | % | 20.98 | 21.63 | 15.74 | 20.18 |

the CAS samples in the ripe state.

3. Effect of Controlled Atmosphere Storage on Organic Acid and Nitrogen Metabolism of Pears During Storage and Ripening, 1960-1961.

Since the data obtained during the 1959-1960 season indicated that acid and nitrogen metabolism especially were affected by the changes in the storage atmosphere, a more detailed study of these particular phases was made in 1960-1961. Identity of specific organic acids occurring in Bartlett and Anjou pears was determined. In order to ascertain if specific acids were associated with the higher total acid concentration in the CAS fruits, ion-exchange chromatography was used to determine quantitative differences during the storage season. Analyses of alcohol soluble and insoluble nitrogen were continued. In addition, analyses of total amino nitrogen were included and the specific amino acids occurring during storage and ripening were determined by paper chromatography.

## Organic Acid Metabolism

Identification of organic acids in Bartlett and Anjou pears: Organic acids normally occurring in Bartlett pears throughout the storage period were identified as malic, citric, tartaric, shikimic, and quinic acids. Succinic acid (Figure 16a) also appeared in small

amounts (1-2 mg/100 g. F. W.) after 3 months' storage. In Anjou pears, the normally occurring acids were malic, tartaric, shikimic, and quinic. Citric and succinic acids appeared after 1-2 months in storage in trace amounts. Other acids such as pyruvate, oxaloacetate, etc. were not detected by the methods used.

Quantitative changes in individual organic acids during storage: In Bartlett pears (Table 7), citric and malic were the predominant organic acids, with concentrations of 130.4 and 185.5 mg/100 gm. F. W. (Figure 15 a, b), respectively, at the beginning of storage. Both acids increased in concentration during the first month. Thereafter, citric acid content tended to decrease at approximately the same rate in both CS and CAS fruit, with final concentrations of 117.0 and 121.6 mg, respectively, at the end of 5 months. Thus, modified atmosphere storage appeared to have only minor effects on the metabolism of this particular acid. Malic acid decreased in concentration during the storage period, and except for the second and third months, was higher in the CAS samples. After 5 months, the concentrations of malic acid in the CS and CAS fruits were 93.3 and 135.4 mg, respectively, a difference of approximately 46 per cent. Tartaric acid (Figure 14c) in the CS samples increased from 8.54 to 15.47 mg/100 gm. F. W. the first month then decreased to 2.78 mg after 5 months' storage. In the CAS

Table 7. Changes in organic acid content of Bartlett pears during conventional and controlled atmosphere storage, 1960 - 1961. (Mg. / 100 gm. F. W.)

| Months' stored | Shikimic acid | | Quinic acid | | Citric acid | | Malic acid | | Succinic acid | | Tartaric acid | | Total acid | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CS | CAS | CS | CAS | CS | CAS | CS | CAS | CS | CAS | CS | CAS | CS | CAS |
| 0 | 6.90 | 6.91 | 11.90 | 11.90 | 130.4 | 130.4 | 185.5 | 185.5 | 4.09 | 4.09 | 8.54 | 8.54 | 347.3 | 347.3 |
| 1 | 37.32 | 30.00 | 11.78 | 10.07 | 183.1 | 187.5 | 203.5 | 217.9 | 0.00 | 0.00 | 15.47 | 21.26 | 451.1 | 466.7 |
| 2 | 25.11 | 32.56 | 12.24 | 12.05 | 180.9 | 179.9 | 188.6 | 145.8 | 0.00 | 0.00 | 9.20 | 13.67 | 416.0 | 383.9 |
| 3 | 5.71 | 7.35 | 12.96 | 16.70 | 157.1 | 201.2 | 190.1 | 172.6 | 1.92 | 0.00 | 10.35 | 13.24 | 378.1 | 411.1 |
| 4 | 3.49 | 9.24 | 11.49 | 19.89 | 145.2 | 160.0 | 104.1 | 177.6 | 1.29 | 2.62 | 4.63 | 9.78 | 270.2 | 399.1 |
| 5 | 7.34 | 5.92 | 16.62 | 12.07 | 117.0 | 121.6 | 93.3 | 135.4 | 1.26 | 2.12 | 2.78 | 8.67 | 238.3 | 285.7 |

Figure 14. Effect of length of storage on organic acids in pears, 1960-1961.

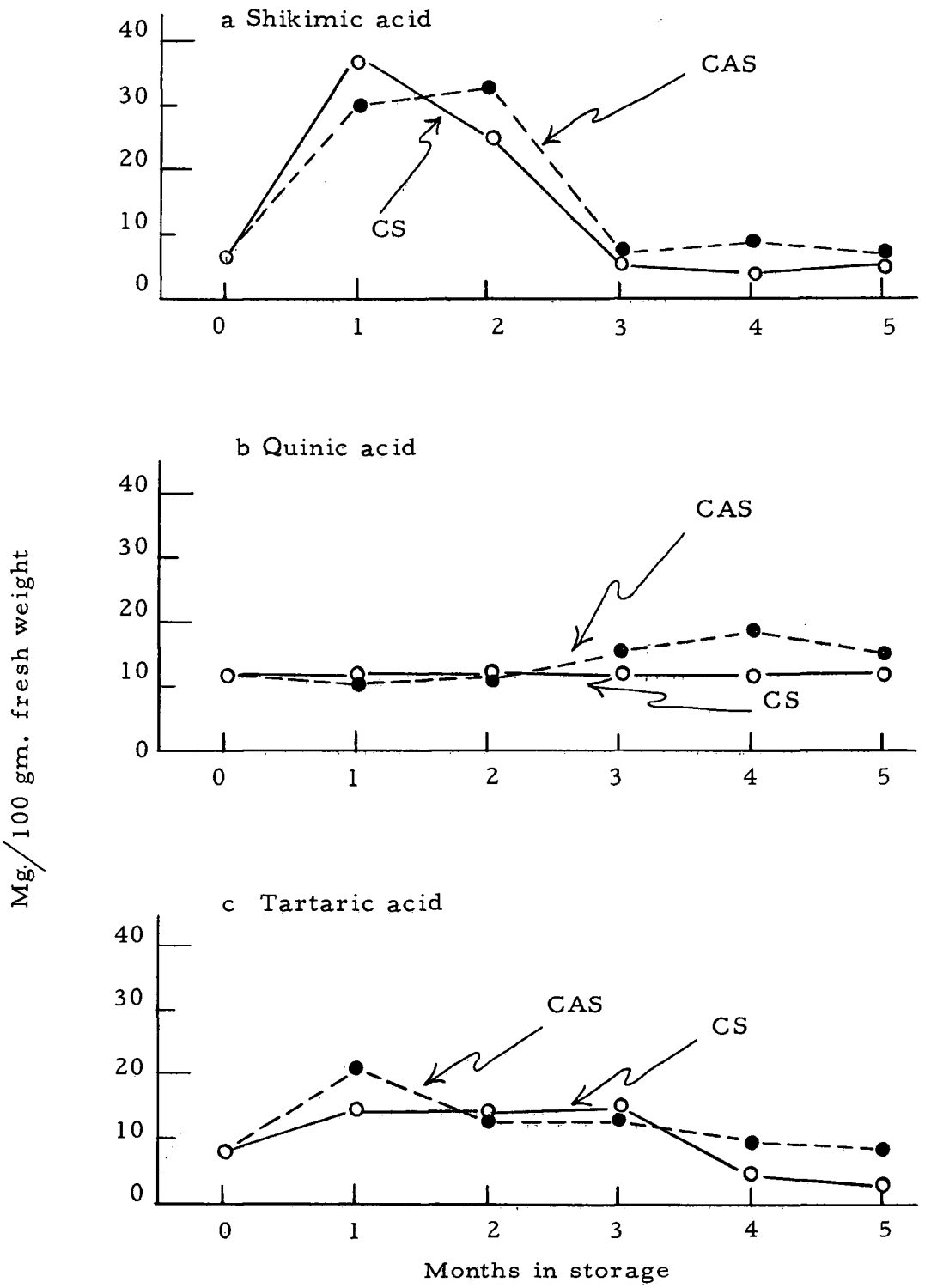


Figure 15. Effect of length of storage on organic acids in Bartlett pears, 1960-1961.

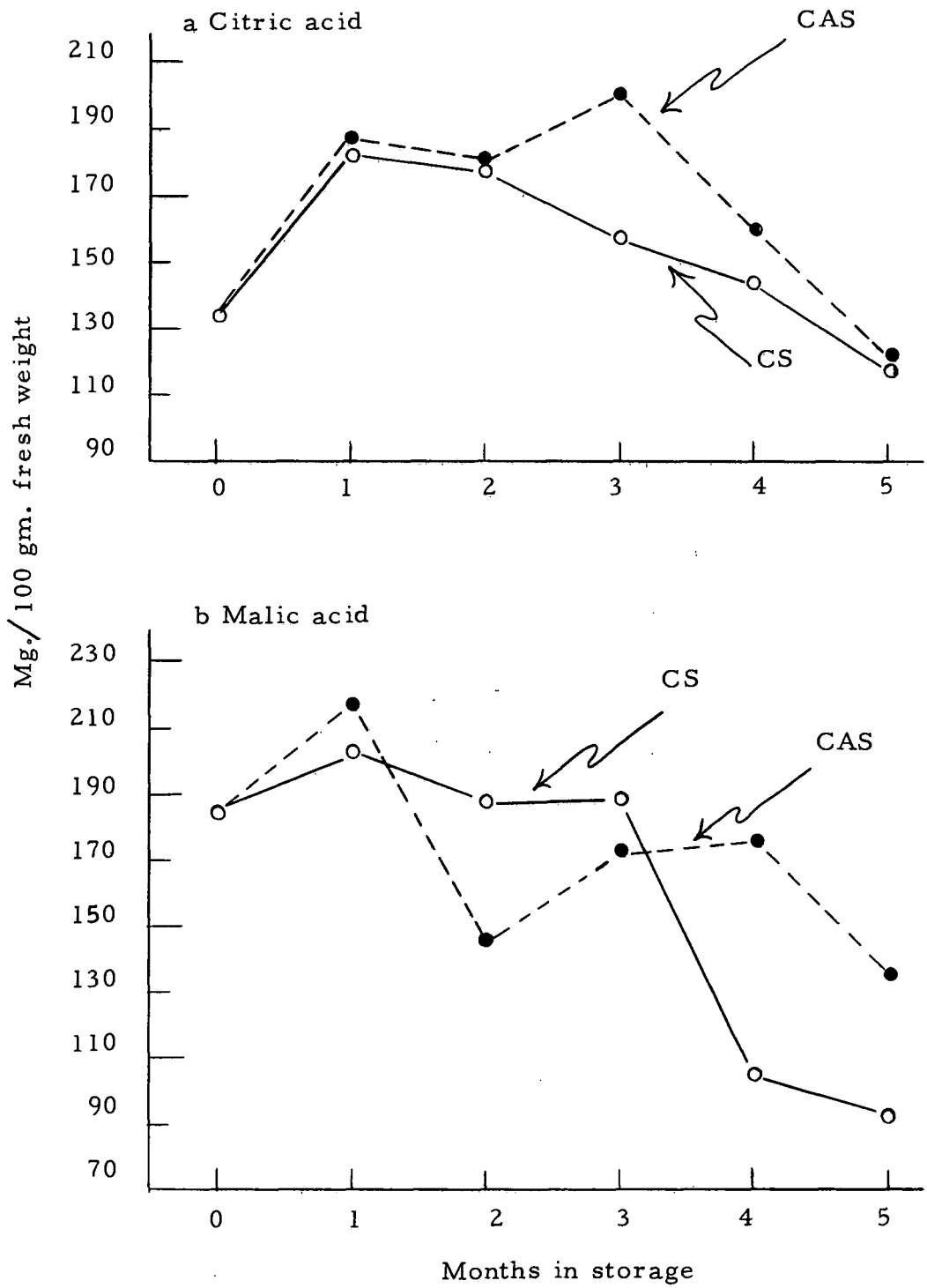
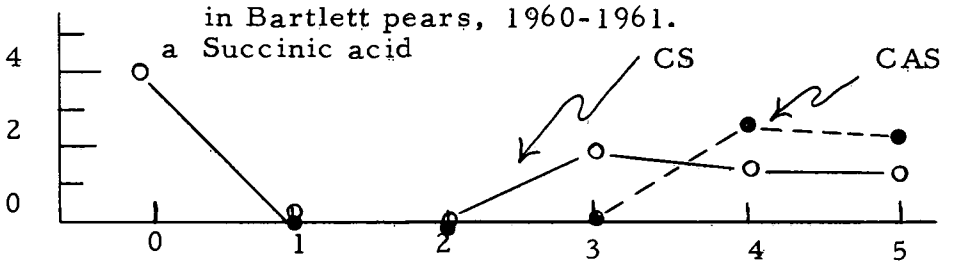
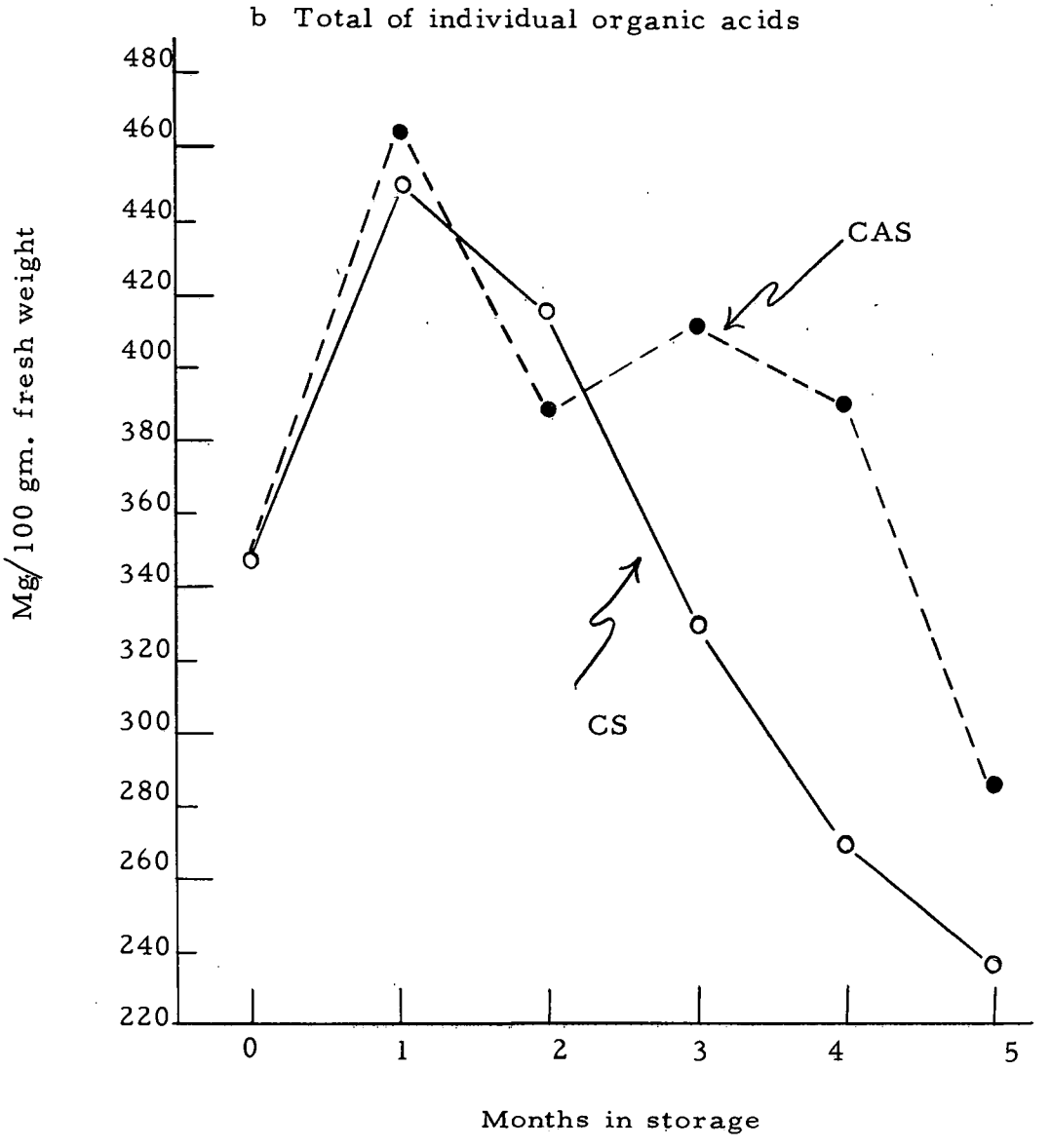


Figure 16. Effect of length of storage on organic acids in Bartlett pears, 1960-1961.



fruit, however, a slower rate of decline was observed, with 8.67 mg present at the end of 5 months. While quinic acid tended to fluctuate in concentration during storage, a more or less constant level was maintained, with no consistent differences between the two treatments (Figure 14 b). Shikimic acid showed a very large increase during the first month, then decreased subsequently to approximately the initial concentration (Figure 14 a). Differences due to treatment were not consistent.

In Anjou pears (Table 8), malic (Figure 18 b) was the principal organic acid, with a concentration of 295.0 mg/100 gm. F. W. at the time of storage. Thereafter, it tended to decrease in CS Anjou pears, with a final concentration of 166.4 mg at the end of 7 months. A slower rate of decrease for this acid was found in CAS fruit during the storage period, and a concentration of 212 mg was found at the end of storage, which was 78 percent more than in the CS fruit. Tartaric acid (Figure 18 a) in the CS pears, showed an overall decrease from 32.6 to 16.7 mg after 7 months' storage. In the CAS fruits, there was a similar rate of decrease for 4 months, followed by an increase to 35.5 mg at the end of 7 months. While shikimic acid and quinic acid tended to fluctuate in concentration (Figure 17 a, b) during storage, more or less constant levels were maintained, with no

Table 8. Changes in concentration of specific organic acids of Anjou pears during conventional and controlled atmosphere storage, 1960 - 1961.

(mg. / 100 gm. F. W.)

| Months stored | Shikimic acid | | Quinic acid | | Citric acid | | Malic acid | | Succinic acid | | Tartaric acid | | Total acid | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CS | CAS | CS | CAS | CS | CAS | CS | CAS | CS | CAS | CS | CAS | CS | CAS |
| 0 | 6.61 | 6.61 | 10.29 | 10.29 | 0 | 0 | 295.0 | 295.0 | 0 | 0 | 32.69 | 32.69 | 344.6 | 344.6 |
| 1 | 18.25 | 5.57 | 18.27 | 6.19 | 0 | 0 | 283.2 | 294.2 | 0 | 0 | 34.04 | 37.50 | 353.8 | 343.5 |
| 2 | 7.16 | 6.58 | 14.42 | 15.58 | 3.97 | 6.87 | 274.7 | 293.8 | 0 | 1.00 | 32.50 | 36.53 | 331.7 | 360.4 |
| 3 | 5.82 | 17.40 | 10.14 | 10.29 | 2.61 | 3.65 | 229.7 | 217.2 | 1.46 | 1.16 | 25.52 | 24.90 | 275.2 | 274.6 |
| 4 | 5.32 | 6.11 | 10.53 | 11.71 | 2.92 | 2.60 | 218.4 | 274.3 | 1.45 | 1.79 | 19.14 | 21.98 | 257.8 | 318.5 |
| 5 | 4.76 | 6.19 | 13.88 | 13.96 | 2.70 | 2.46 | 215.8 | 305.3 | 1.55 | 1.95 | 20.54 | 27.02 | 259.2 | 356.9 |
| 6 | 4.72 | 5.23 | 14.10 | 11.11 | 2.32 | 1.47 | 172.6 | 283.2 | 1.60 | 1.59 | 20.41 | 22.99 | 215.6 | 325.6 |
| 7 | 6.66 | 4.72 | 12.92 | 11.15 | 1.90 | 1.61 | 166.4 | 212.1 | 1.33 | 1.53 | 16.75 | 35.58 | 206.0 | 266.7 |

Figure 17. Effect of length of storage on organic acids in Anjou pears, 1960 - 1961.

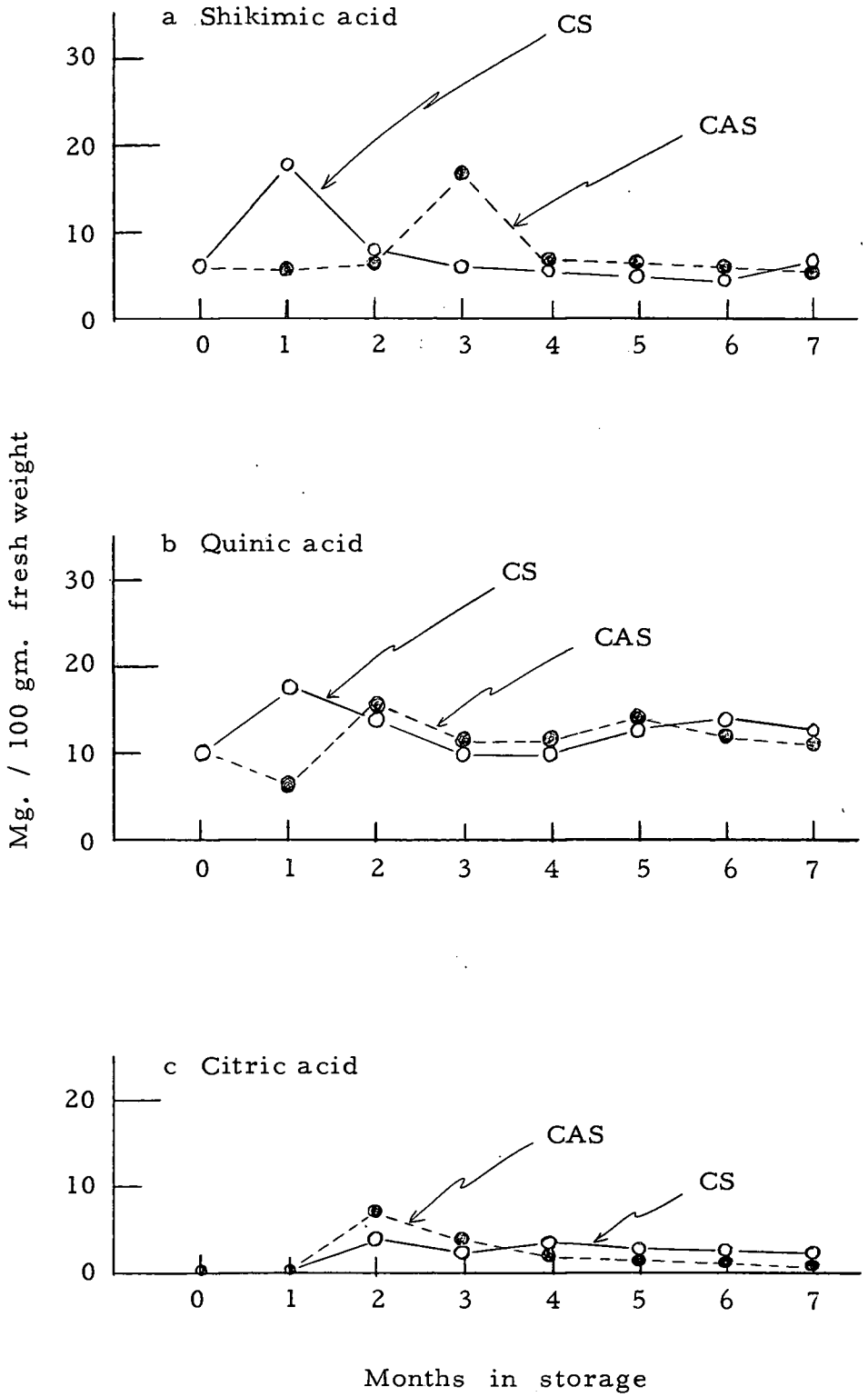
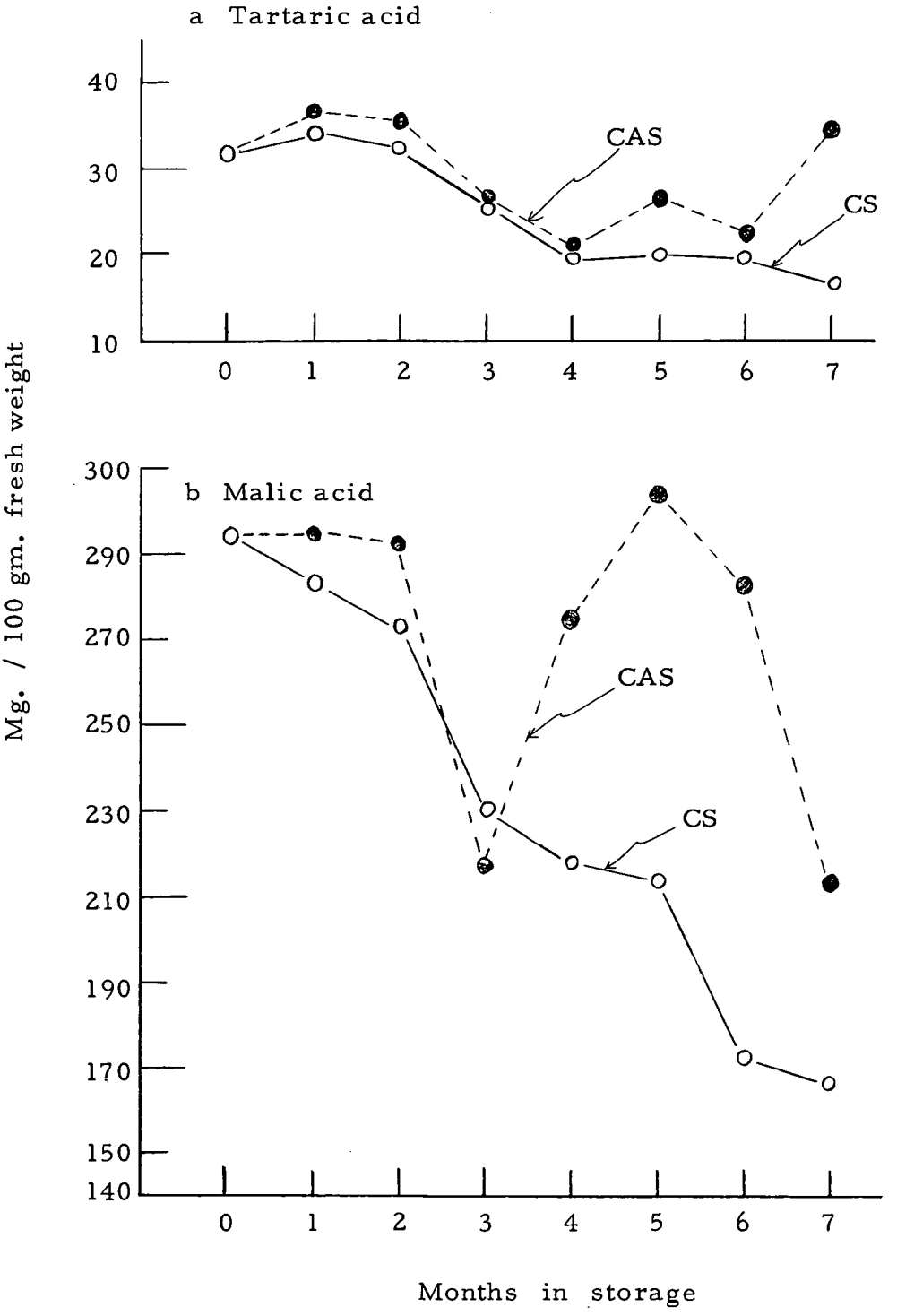


Figure 18. Effect of length of storage on organic acids in Anjou pears, 1960 - 1961.



consistent differences between the 2 treatments. Succinic acid appeared one month earlier in CAS than in CS fruit (Figure 19 a). No differences were observed between the 2 lots. Citric acid in both CS and CAS samples appeared after 2 months' storage with a concentration of 3.9 and 6.9 mg/100 gm. F. W., respectively. Thereafter, a slow rate of decline was observed in CAS and CS fruit, with a final concentration of 1.6 and 1.9 mg, respectively, at the end of 7 months' storage.

During ripening of Bartlett pears, quinic acid and tartaric acid tended to increase, while all other organic acids decreased in concentration (Table 9). This decrease during ripening was especially apparent in the CS samples ripened after 1, 2, 3, and 4 months' storage. Similar fruit from CAS maintained much higher levels of malic acid during the ripening processes. During ripening of Anjou pears, no changes were observed among shikimic, citric, succinic, and tartaric acids, but quinic acid tended to increase and malic acid to decrease in concentration (Table 10). More malic acid was maintained in CAS fruits than in similar samples from CS after ripening.

Figure 19. Effect of length of storage on organic acids in Anjou pears, 1960 - 1961.

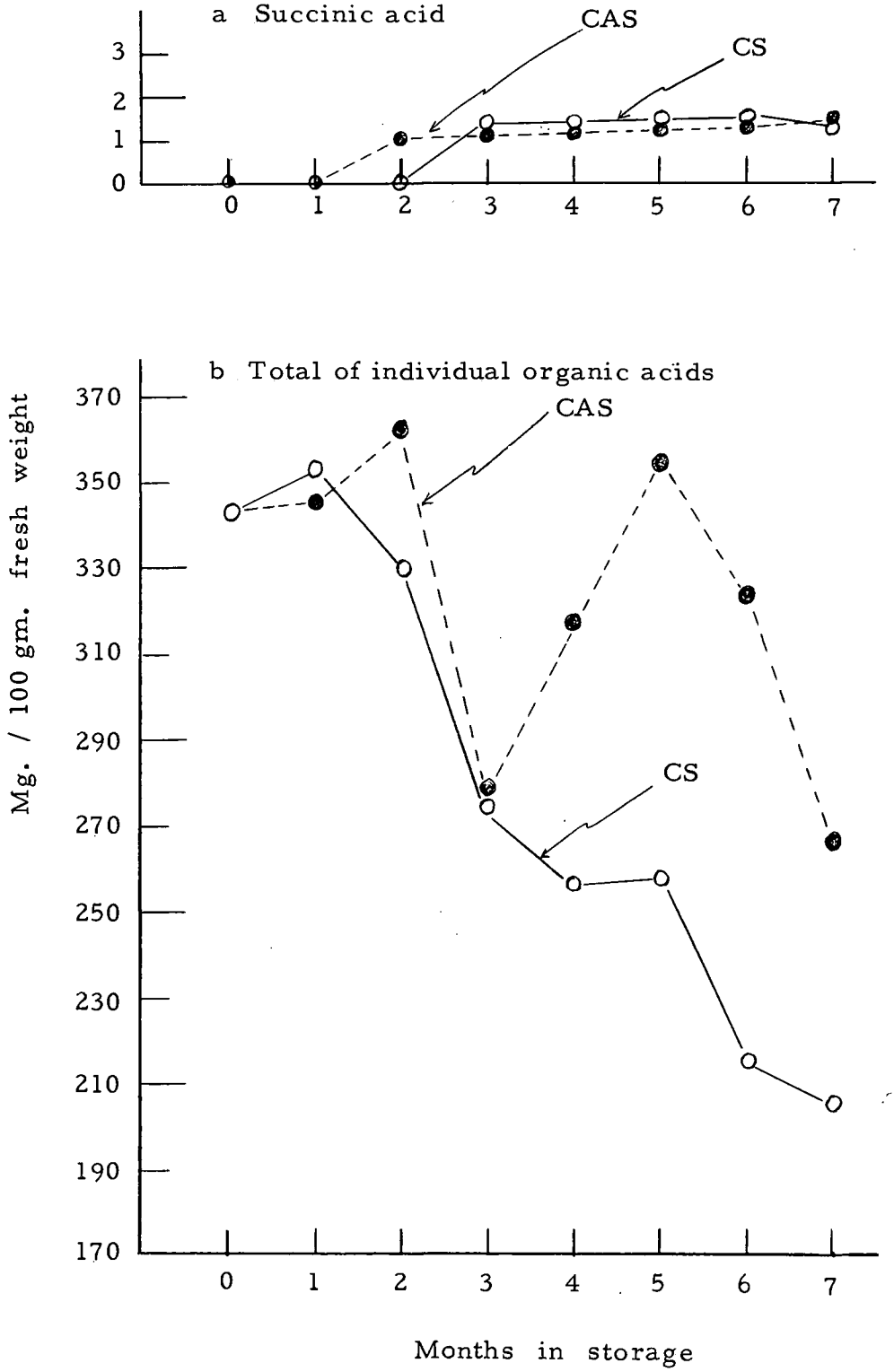


Table 9. Organic acid content of Bartlett pears ripened after conventional and controlled atmosphere storage, 1960-1961.

(Mg. / 100 gm. fresh weight)

| Months stored | Shikimic acid | | Quinic acid | | Citric acid | | Malic acid | | Succinic acid | | Tartaric acid | | Total acid | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CS | CAS | CS | CAS | CS | CAS | CS | CAS | CS | CAS | CS | CAS | CS | CAS |
| 0 | 6.18 | 6.18 | 11.64 | 11.64 | 173.9 | 173.9 | 159.3 | 159.3 | 2.02 | 2.02 | 18.94 | 18.94 | 372.1 | 372.1 |
| 1 | 6.47 | 10.84 | 20.86 | 22.15 | 139.2 | 183.7 | 93.4 | 129.1 | 2.02 | 1.62 | 14.37 | 23.16 | 276.5 | 370.7 |
| 2 | 25.40 | 26.67 | 26.11 | 21.86 | 171.2 | 195.1 | 91.8 | 119.2 | 2.45 | 2.29 | 22.87 | 20.54 | 340.0 | 385.7 |
| 3 | 14.79 | 27.37 | 25.81 | 26.30 | 160.1 | 194.1 | 68.7 | 146.8 | 3.51 | 2.73 | 18.57 | 13.37 | 291.6 | 410.8 |
| 4 | 11.19 | 6.29 | 19.38 | 18.93 | 93.6 | 137.1 | 59.5 | 146.5 | 2.78 | 1.89 | 18.08 | 25.51 | 204.6 | 336.3 |
| 5 | 6.43 | 5.60 | 18.42 | 19.82 | 93.6 | 136.8 | 62.1 | 93.2 | 2.44 | 1.97 | 11.55 | 22.95 | 194.5 | 280.4 |

Figure 20. Effect of length of storage on organic acids in Bartlett pears after ripening, 1960 - 1961.

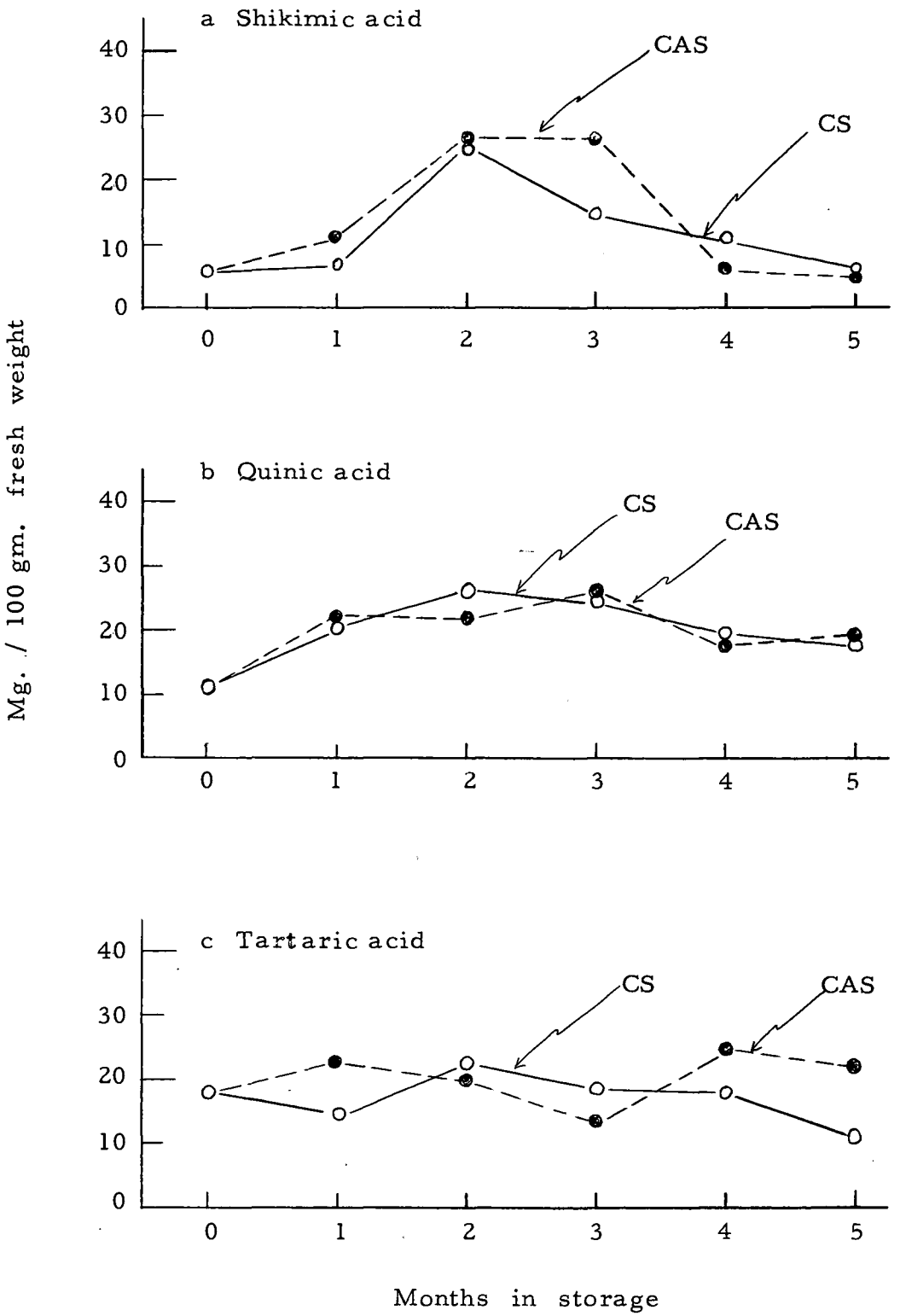


Figure 21. Effect of length of storage on organic acids in Bartlett pears after ripening, 1960 - 1961

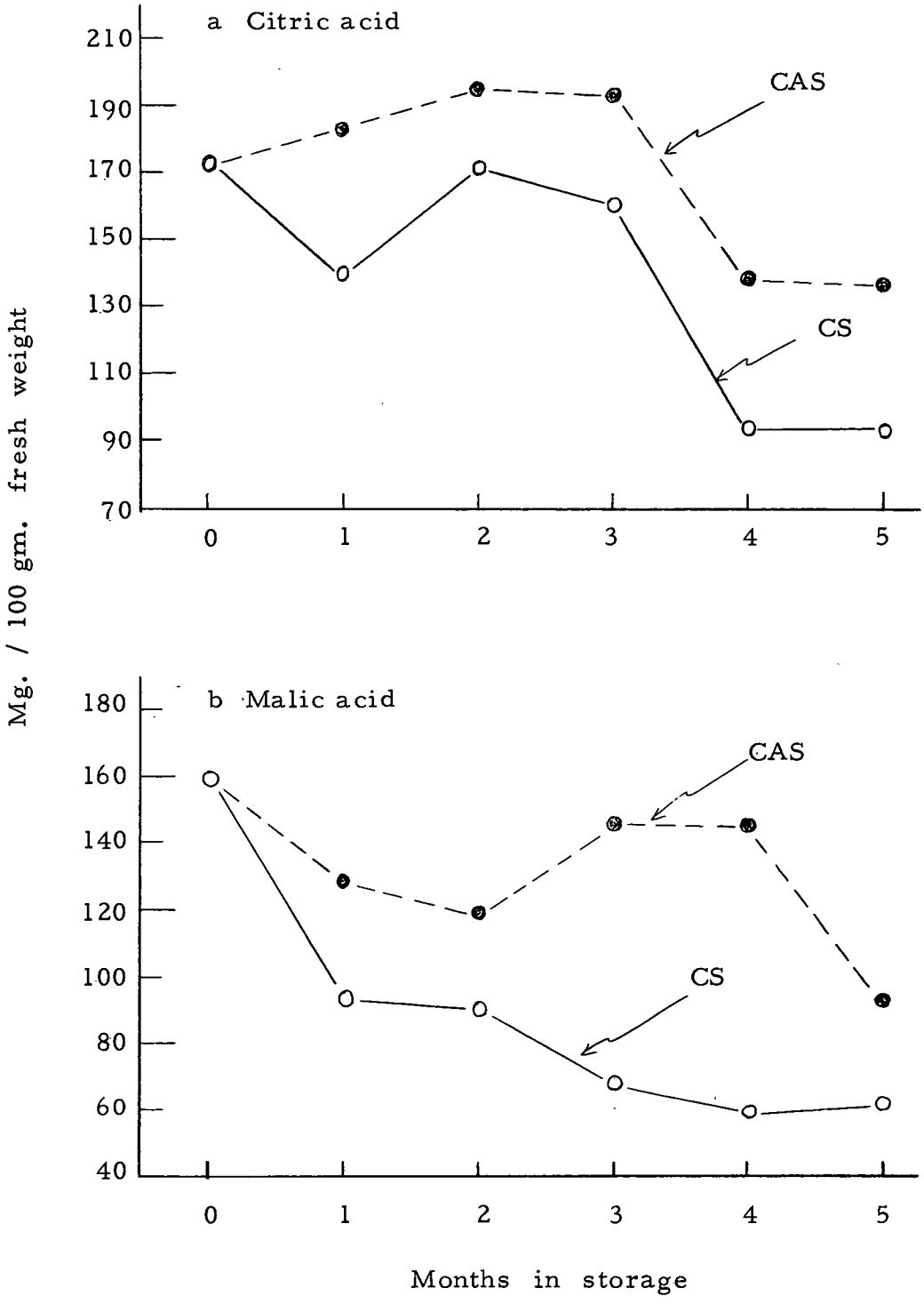


Figure 22. Effect of length of storage on organic acids in Bartlett pears after ripening, 1960 - 1961.

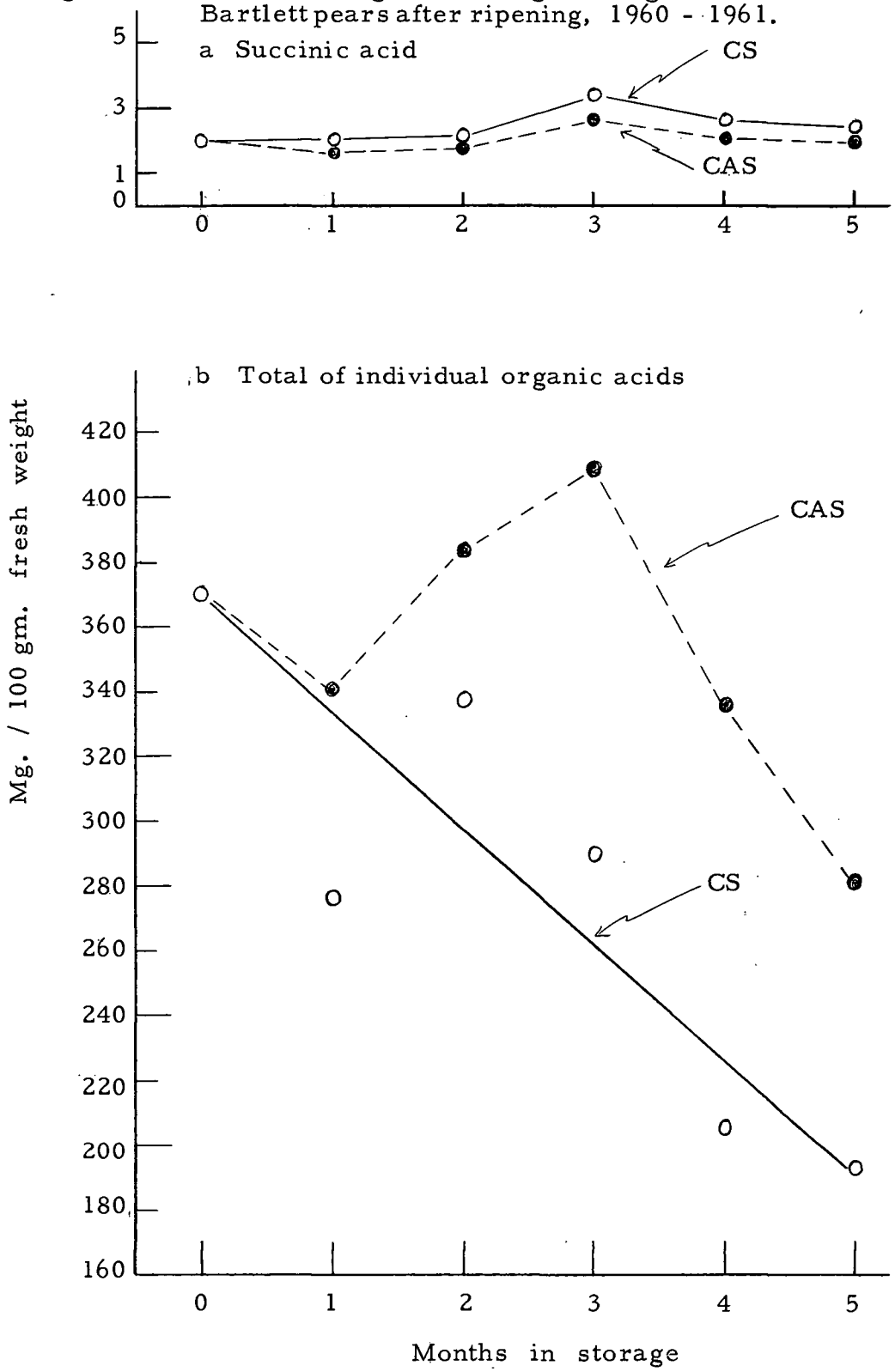
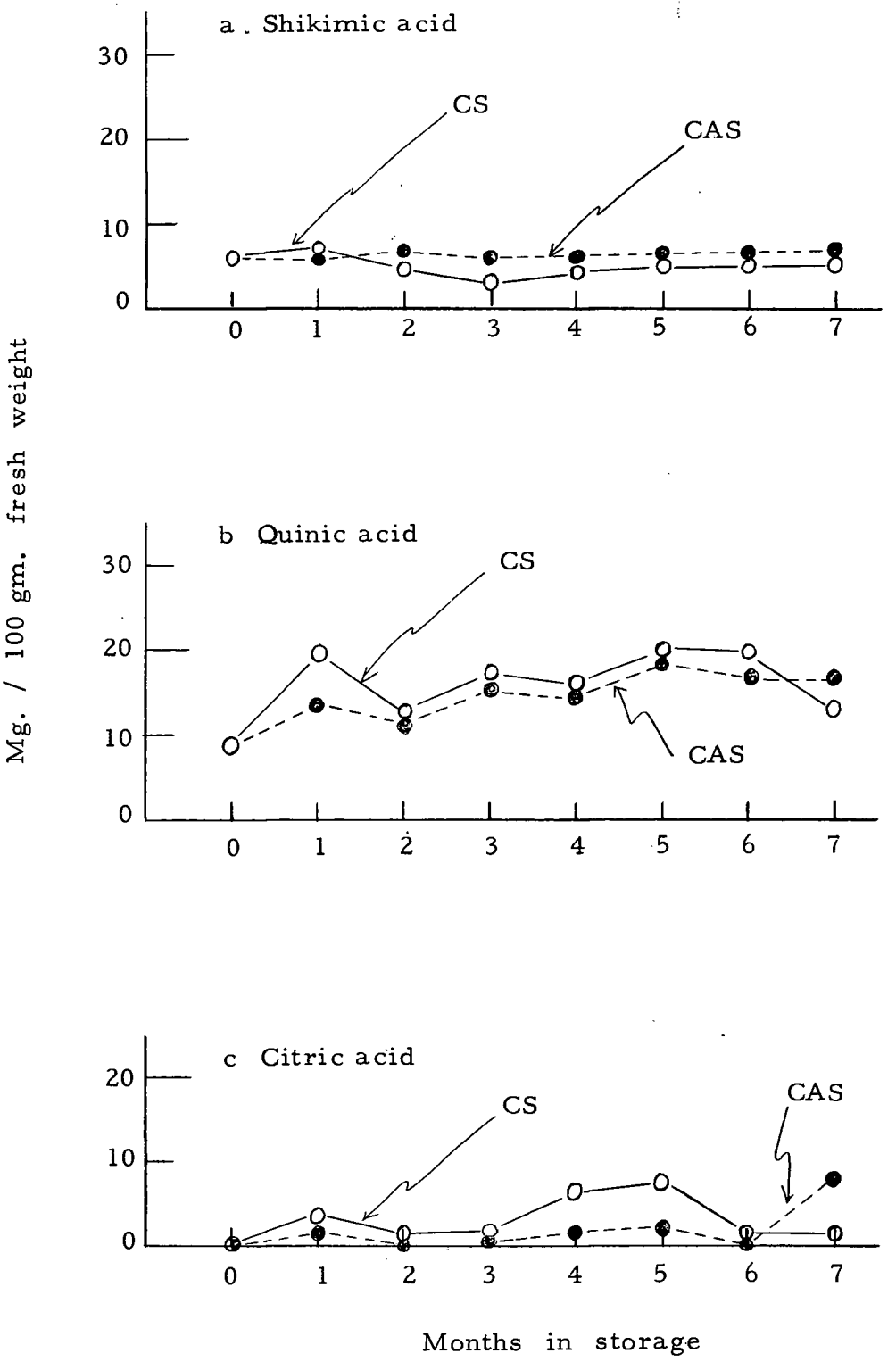


Table 10. Changes in concentration of specific organic acids of Anjou pears ripened after conventional and controlled atmosphere storage, 1960-1961.

(Mg. / 100 gm. F. W.)

| Months' stored | Shikimic acid | | Quinic acid | | Citric acid | | Malic acid | | Succinic acid | | Tartaric acid | | Total acid | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CS | CAS | CS | CAS | CS | CAS | CS | CAS | CS | CAS | CS | CAS | CS | CAS |
| 0 | 6.39 | 6.39 | 9.23 | 9.23 | 0 | 0 | 332.4 | 332.4 | 0 | 0 | 34.04 | 34.04 | 382.1 | 382.1 |
| 1 | 6.80 | 6.35 | 20.04 | 13.97 | 3.67 | 1.62 | 249.0 | 345.5 | 1.75 | 1.56 | 31.68 | 39.56 | 312.9 | 408.6 |
| 2 | 4.57 | 6.27 | 12.89 | 12.29 | 1.00 | 0 | 207.1 | 245.4 | 1.44 | 0 | 23.10 | 36.75 | 250.1 | 300.7 |
| 3 | 2.90 | 5.32 | 17.63 | 15.26 | 1.68 | 1.09 | 200.1 | 225.0 | 2.01 | 0 | 20.42 | 34.89 | 244.7 | 311.6 |
| 4 | 4.76 | 5.64 | 16.62 | 14.71 | 7.78 | 1.84 | 181.4 | 224.8 | 1.82 | 1.51 | 19.24 | 24.58 | 231.6 | 273.1 |
| 5 | 5.68 | 6.06 | 20.51 | 19.79 | 8.03 | 2.23 | 252.1 | 308.1 | 5.00 | 2.93 | 22.85 | 28.08 | 314.2 | 367.2 |
| 6 | 5.23 | 7.16 | 20.58 | 16.63 | 1.09 | 0 | 191.1 | 280.5 | 2.54 | 1.41 | 18.43 | 20.31 | 239.0 | 326.0 |
| 7 | 5.63 | 6.90 | 12.78 | 16.88 | 1.78 | 8.45 | 133.2 | 204.2 | 3.25 | 2.33 | 27.07 | 43.46 | 183.7 | 282.2 |

Figure 23. Effect of length of storage on organic acids in Anjou pears after ripening, 1960 - 1961.



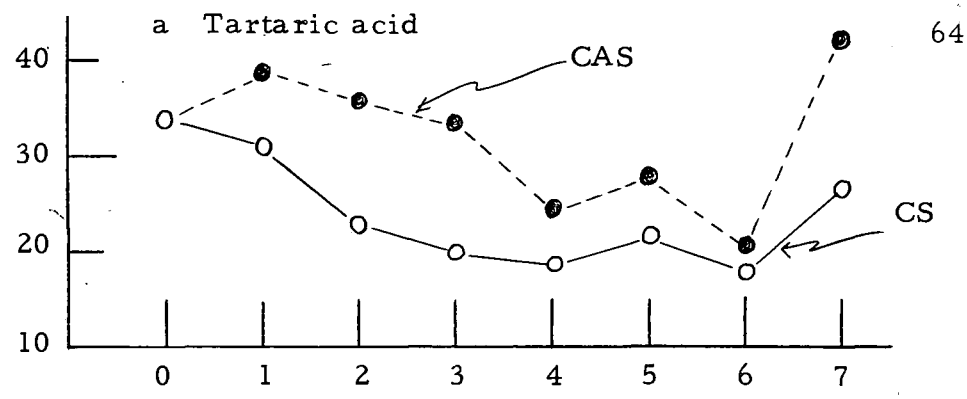
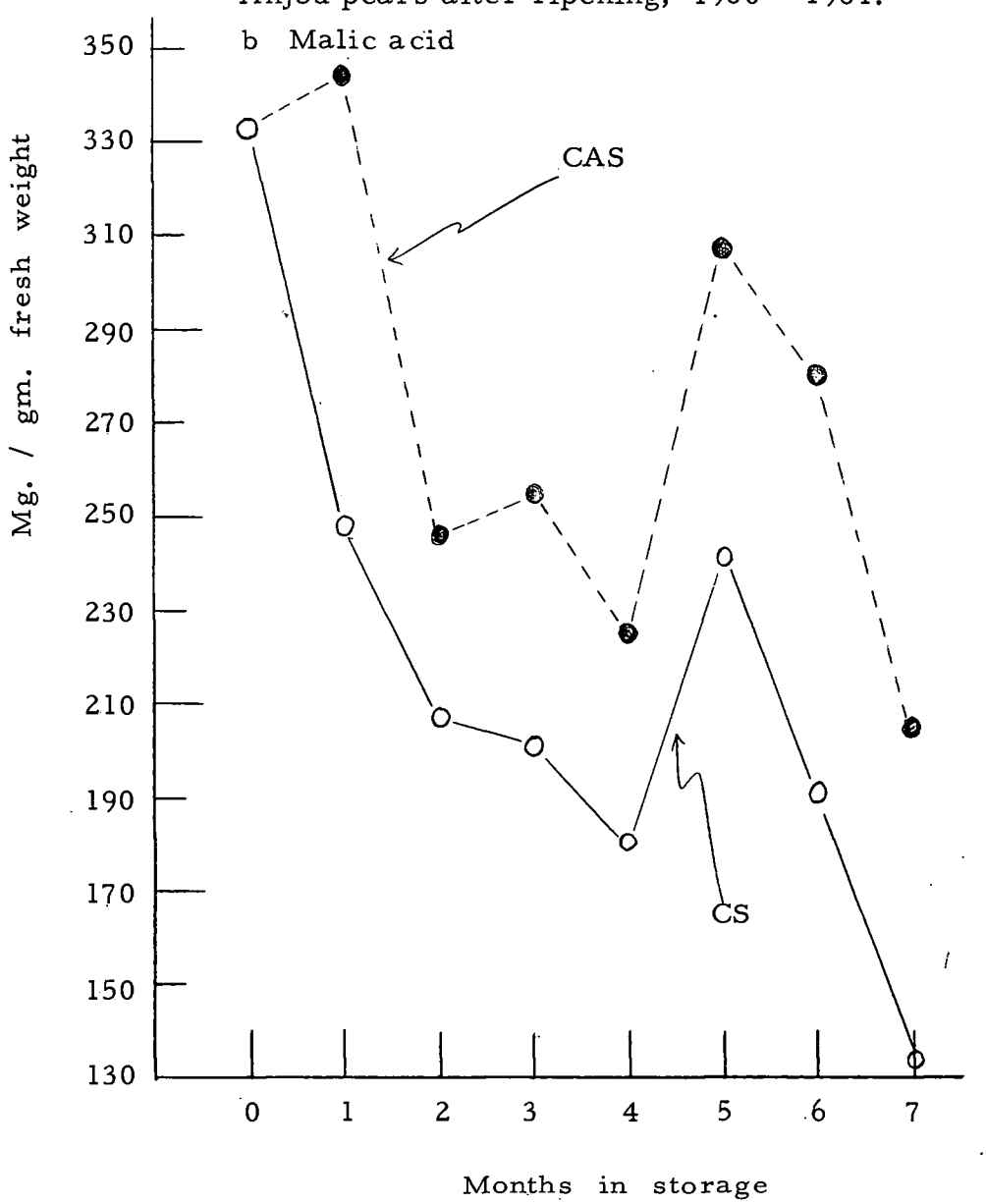



Figure 24. Effect of length of storage on organic acids in Anjou pears after ripening, 1960 - 1961.



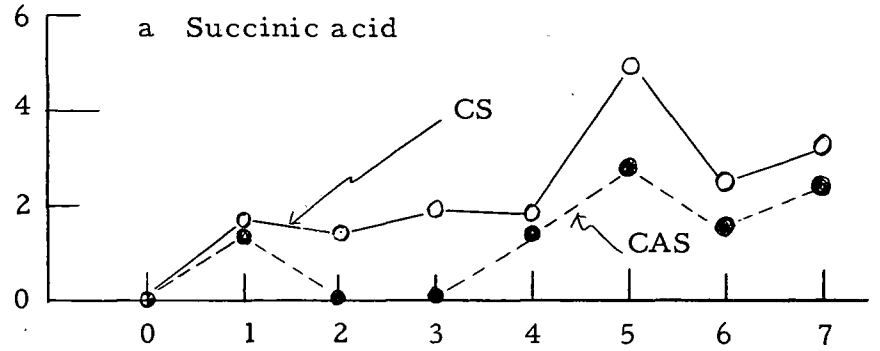
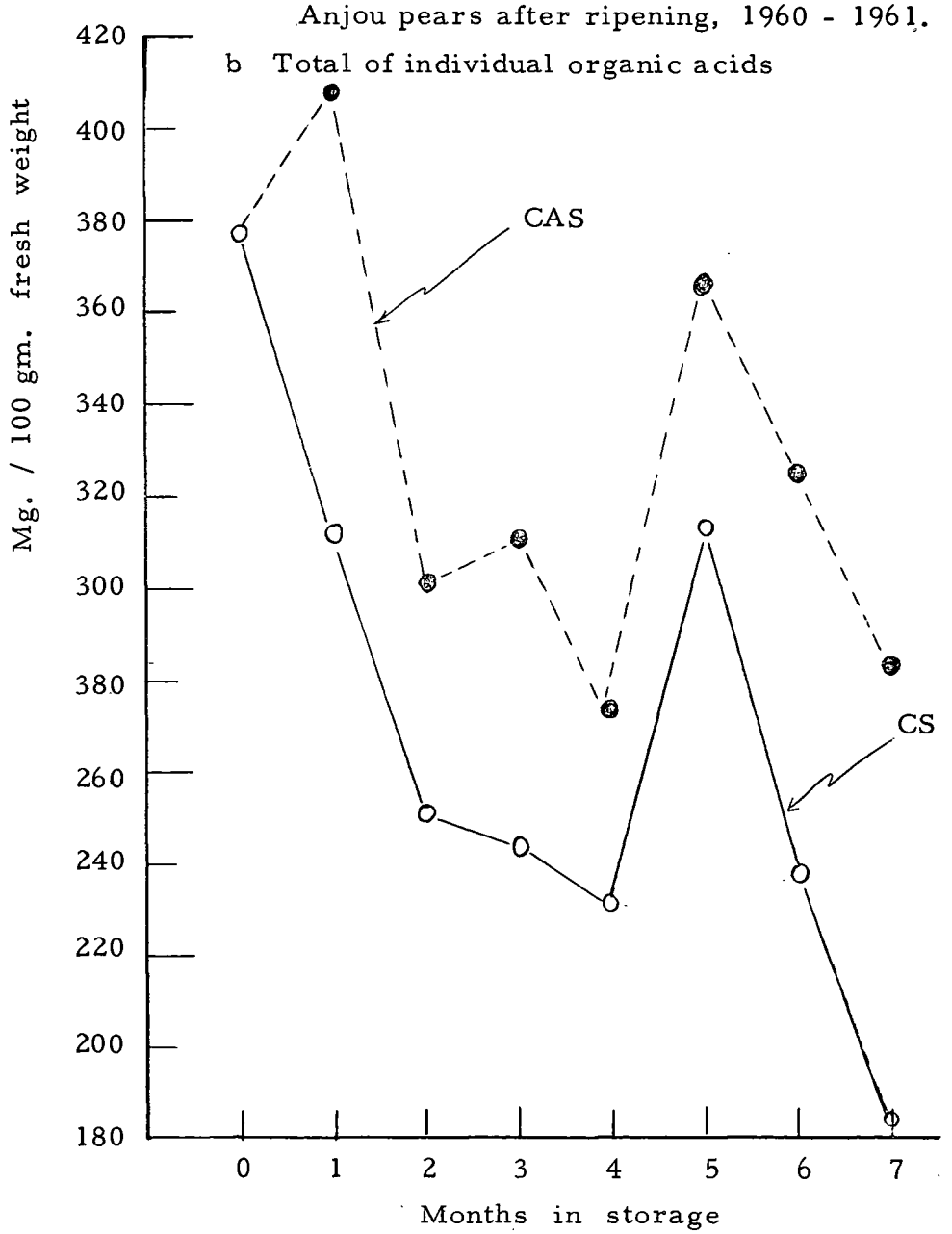



Figure 25. Effect of length of storage on organic acids on Anjou pears after ripening, 1960 - 1961.



## Changes in Nitrogen Metabolism During Storage and Ripening

Alcohol soluble and insoluble nitrogen: Changes occurring in the alcohol soluble and insoluble nitrogen fractions during storage were similar to these observed the previous season. Alcohol soluble nitrogen in CS Bartlett pears (Figure 26 a ) increased from 20.21 to 24.72 mg/100 gm F. W. during the first month, then increased gradually to 27.44 mg. during the remainder of the 5 months' storage period. In the CAS fruit, however, this fraction increased to 31.82 mg the first month, with a further increase to 34.55 mg at the end of 5 months. The content of alcohol soluble nitrogen was approximately 20 per cent higher throughout the storage period in the CAS fruit.

In Anjou pears, alcohol soluble nitrogen (Figure 28 a) did not tend to increase during storage as in the Bartlett variety; however, the concentration was higher in the CAS fruit throughout storage. Alcohol insoluble nitrogen, as in the Bartlett pears (Figure 27 a), was maintained at a lower level in the CAS fruit (Figure 29 a).

During ripening a marked difference in the metabolism of the alcohol soluble and insoluble nitrogen in the CS and CAS fruits was indicated by the data obtained (Table 13). Alcohol soluble nitrogen tended to decrease in both kinds of pears when ripened, while alcohol insoluble nitrogen increased, indicating an active net synthesis of protein during ripening. In the CS pears, however, the difference in concentration of protein nitrogen before and after ripening gradually decreased during the storage period until little if any difference was found in fruit ripened late in the season (Figures 30, 31). These data indicate a progressive loss during storage in ability of the CS pears to synthesize protein. In CAS fruit, however, alcohol insoluble nitrogen tended to increase during ripening, even after prolonged periods of storage, indicating that no similar loss in protein synthesizing capacity occurred in CAS pears.

Total amino nitrogen: Total amino nitrogen in CS Bartlett pears increased from 8.65 to 13.49 mg during the first month of storage, but decreased thereafter to approximately the initial value at the end of 5 months (Table 14). In the CAS treatment, a similar pattern was followed; however, amino nitrogen content was maintained at a higher level during storage (Figure 32 a).

Table 11. Effect of controlled atmosphere storage on total alcohol soluble and insoluble N in Bartlett pears.

| Months in storage | Total alcohol soluble N mg/100 gm. F. W. | | Total alcohol insoluble N % dry weight | |
|---|---|---|---|---|
| | CS | CAS | CS | CAS |
| | ------ Before ripening, 1959 - 1960--------- | | | |
| 0 | 20.90 | 20.90 | 0.57 | 0.57 |
| 1 | 27.53 | 23.39 | 0.79 | 0.79 |
| 2 | 22.77 | 24.52 | 0.77 | 0.66 |
| 3 | 22.56 | 23.64 | 0.89 | 0.72 |
| 4 | 26.62 | 17.57 | 0.74 | 0.95 |
| 5 | 20.74 | 23.65 | 0.94 | 0.73 |
| 6 | 20.27 | 23.37 | 0.91 | 0.68 |
| | ------Before ripening, 1960 - 1961 -------- | | | |
| 0 | 20.21 | 20.21 | 0.55 | 0.55 |
| 1 | 24.72 | 31.82 | 0.77 | 0.77 |
| 2 | 25.94 | 31.61 | 0.88 | 0.61 |
| 3 | 26.33 | 32.35 | 0.94 | 0.72 |
| 4 | 26.57 | 29.76 | 0.97 | 0.79 |
| 5 | 27.44 | 34.45 | 0.98 | 0.90 |
| | -----After ripening, 1960 - 1961 --------- | | | |
| 0 | 15.93 | 15.93 | 0.95 | 0.95 |
| 1 | 25.08 | 25.06 | 1.09 | 0.98 |
| 2 | 23.18 | 21.24 | 1.08 | 0.99 |
| 3 | 20.39 | 22.87 | 1.05 | 1.06 |
| 4 | 22.53 | 28.74 | 1.08 | 1.07 |
| 5 | 29.10 | 35.06 | 0.92 | 1.13 |

Figure 26. Total alcohol soluble nitrogen in Bartlett pears, 1960 - 1961.

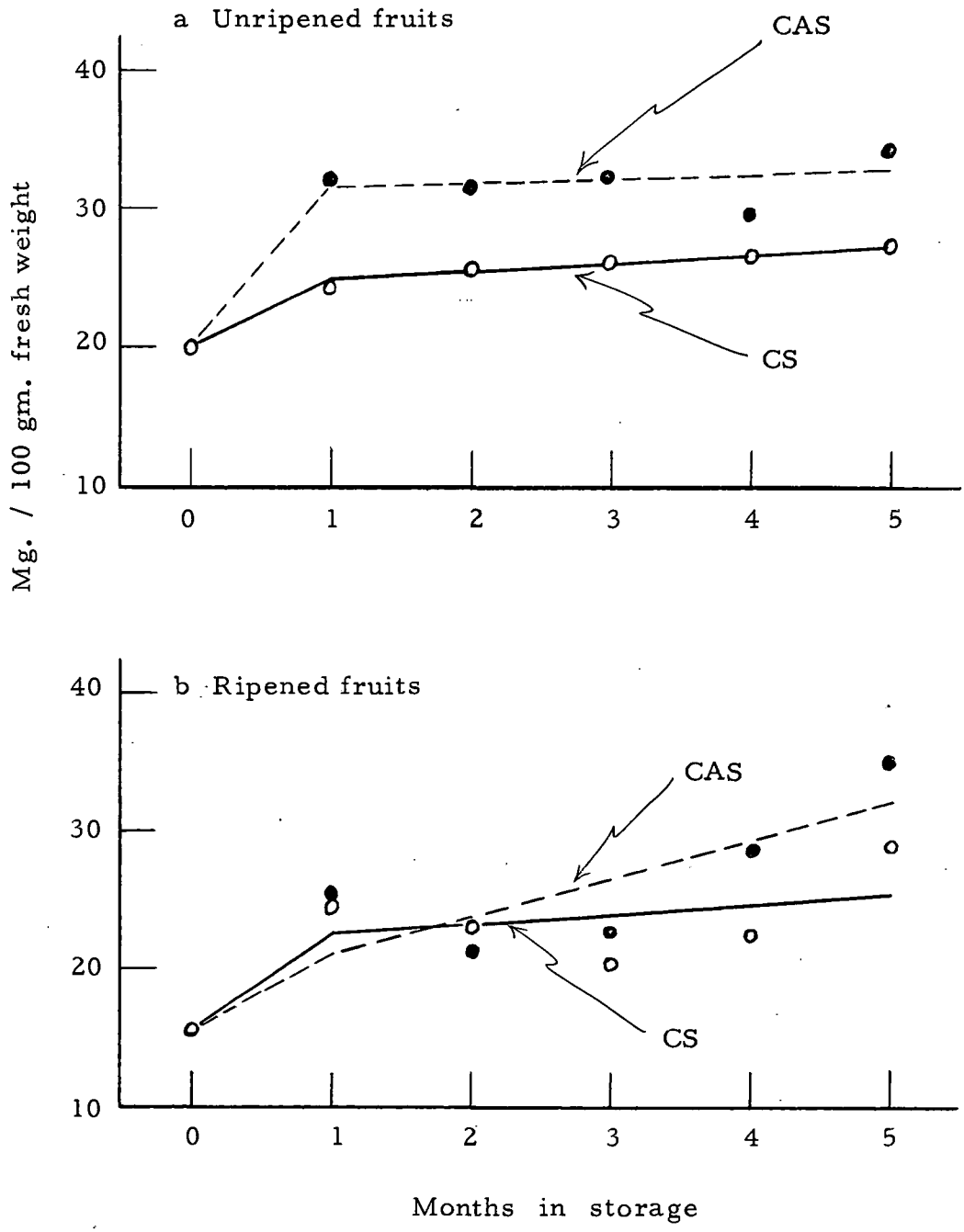


Figure 27. Total alcohol insoluble nitrogen in Bartlett pears, 1960 - 1961.

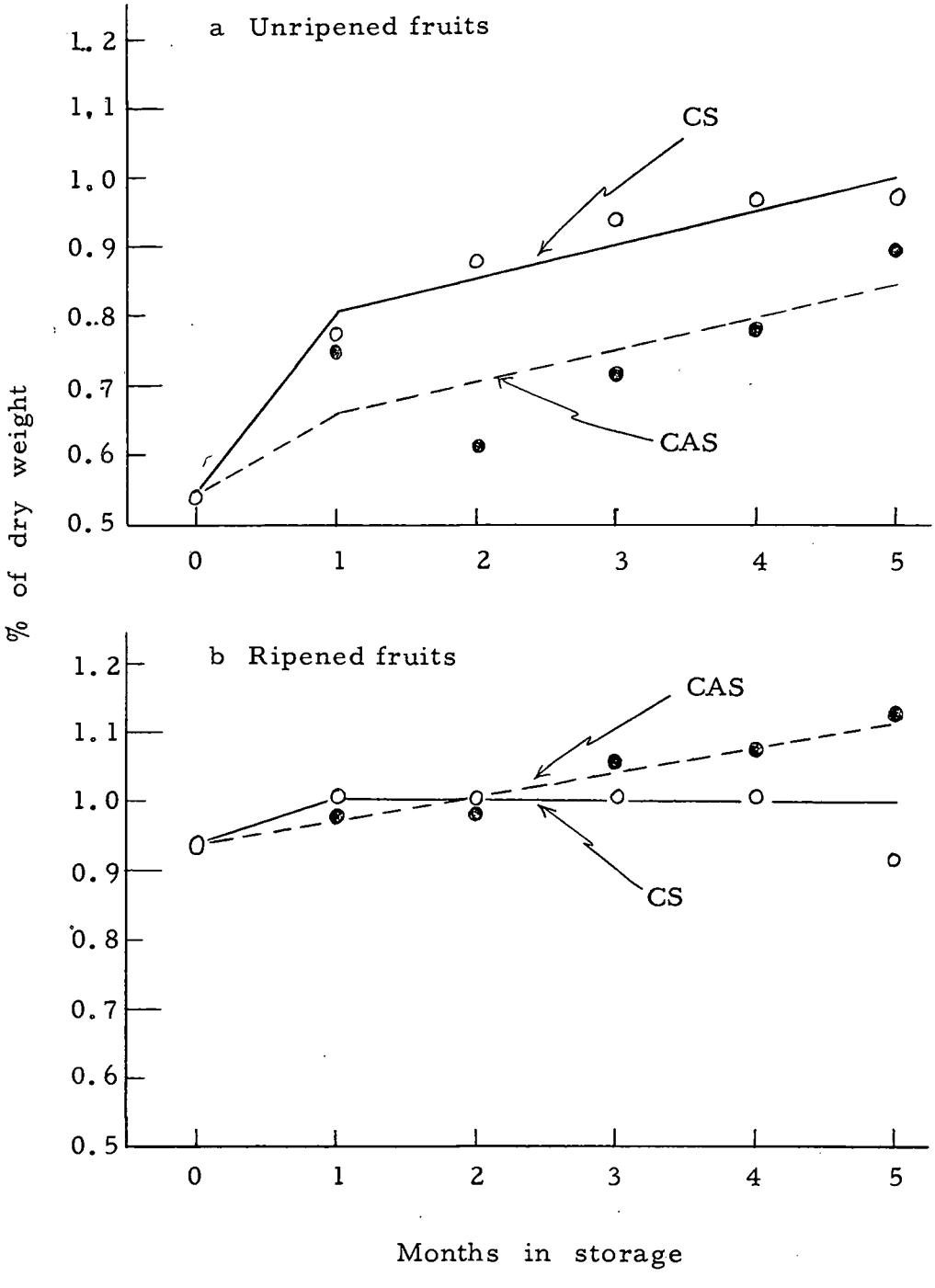


Table 12. Effect of controlled atmosphere storage on total alcohol soluble and insoluble N in Anjou pears before and after ripening.

| Months in storage | Total alcohol soluble N (mg./100 gm. F.W.) | | Total alcohol insoluble N (% dry weight) | |
|---|---|---|---|---|
| | CS | CAS | CS | CAS |
| | Before ripening (1959-1960) | | | |
| 0 | 25.94 | 25.94 | 0.75 | 0.75 |
| 1 | 29.45 | 25.58 | 0.81 | 0.78 |
| 2 | 31.34 | 27.29 | 0.84 | 0.73 |
| 3 | 30.87 | 27.16 | 0.95 | 0.65 |
| 4 | 28.38 | 27.83 | 0.86 | 0.69 |
| 5 | 25.27 | 30.67 | 0.83 | 0.68 |
| 6 | 25.54 | 32.97 | 0.86 | 0.71 |
| 7 | 25.03 | 32.43 | 1.04 | 0.65 |
| 8 | 17.97 | 33.57 | 1.06 | 0.59 |
| | Before ripening (1960-1961) | | | |
| 0 | 30.04 | 30.04 | 0.71 | 0.71 |
| 1 | 23.42 | 25.45 | 0.81 | 0.65 |
| 2 | 30.88 | 33.01 | 0.90 | 0.74 |
| 3 | 26.59 | 31.64 | 0.97 | 0.71 |
| 4 | 27.88 | 33.48 | 1.03 | 0.73 |
| 5 | 20.99 | 33.07 | 1.25 | 0.80 |
| 6 | 20.43 | 29.54 | 1.24 | 0.72 |
| 7 | 30.11 | 35.77 | 1.09 | 0.77 |
| | After ripening (1960-1961) | | | |
| 0 | 34.98 | 34.98 | 0.87 | 0.87 |
| 1 | 21.56 | 28.69 | 1.03 | 0.88 |
| 2 | 25.78 | 33.60 | 1.13 | 0.98 |
| 3 | 22.21 | 31.94 | 1.29 | 1.01 |
| 4 | 23.34 | 27.71 | 1.11 | 0.99 |
| 5 | 23.51 | 24.02 | 1.18 | 0.97 |
| 6 | 24.72 | 29.87 | 1.14 | 1.06 |
| 7 | 24.03 | 31.00 | 1.21 | 1.05 |

Figure 28. Total alcohol soluble nitrogen in Anjou pears, 1960 -1961.

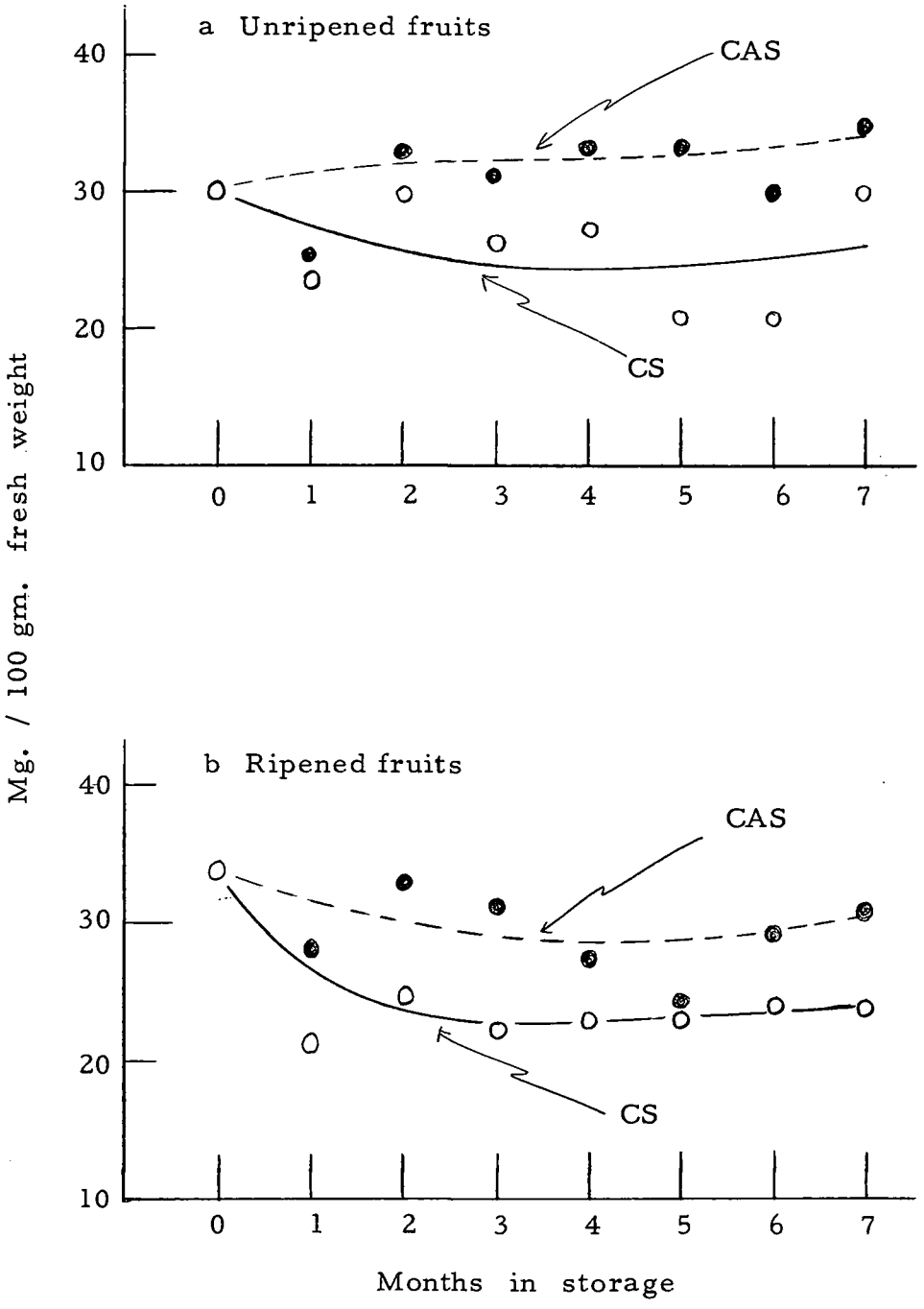


Figure 29. Total alcohol insoluble nitrogen in Anjou pears, 1960 - 1961.

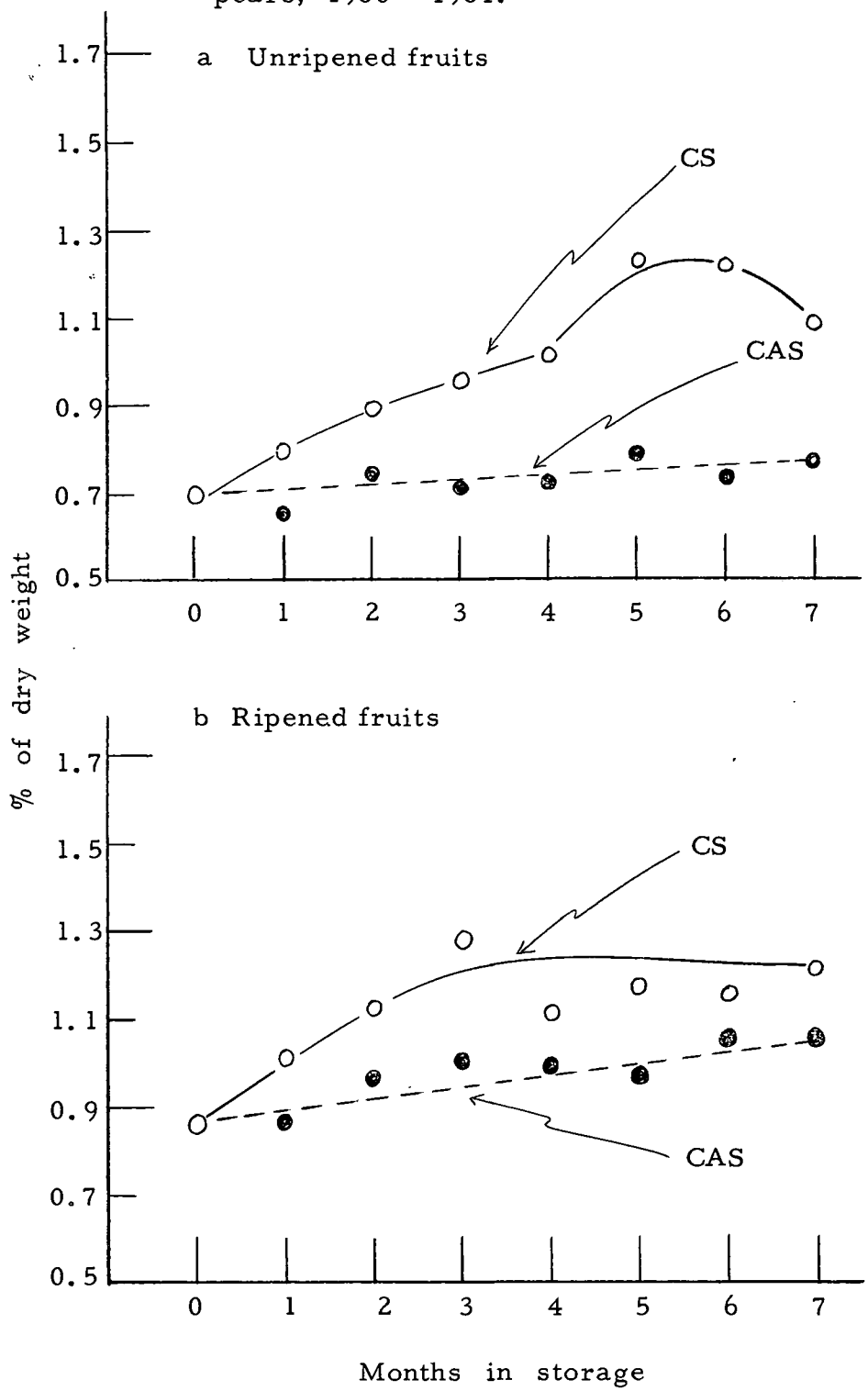


Table 13. Differences in alcohol insoluble nitrogen between ripened and unripened Bartlett and Anjou pears during conventional and controlled atmosphere storage, 1960-1961.

| Months in storage | Bartlett (%) | | Anjou (%) | |
|---|---|---|---|---|
| | CS | CAS | CS | CAS |
| 0 | 0.399 | 0.399 | 0.167 | 0.167 |
| 1 | 0.318 | 0.215 | 0.219 | 0.227 |
| 2 | 0.193 | 0.376 | 0.228 | 0.231 |
| 3 | 0.111 | 0.341 | 0.325 | 0.293 |
| 4 | 0.102 | 0.288 | 0.084 | 0.261 |
| 5 | -0.057 | 0.236 | -0.061 | 0.169 |
| 6 | - | - | -0.093 | 0.333 |
| 7 | - | - | -0.117 | 0.286 |

Figure 30. Differences in protein N between unripened and ripened Bartlett pears from conventional (CS) and controlled atmosphere storage (CAS).

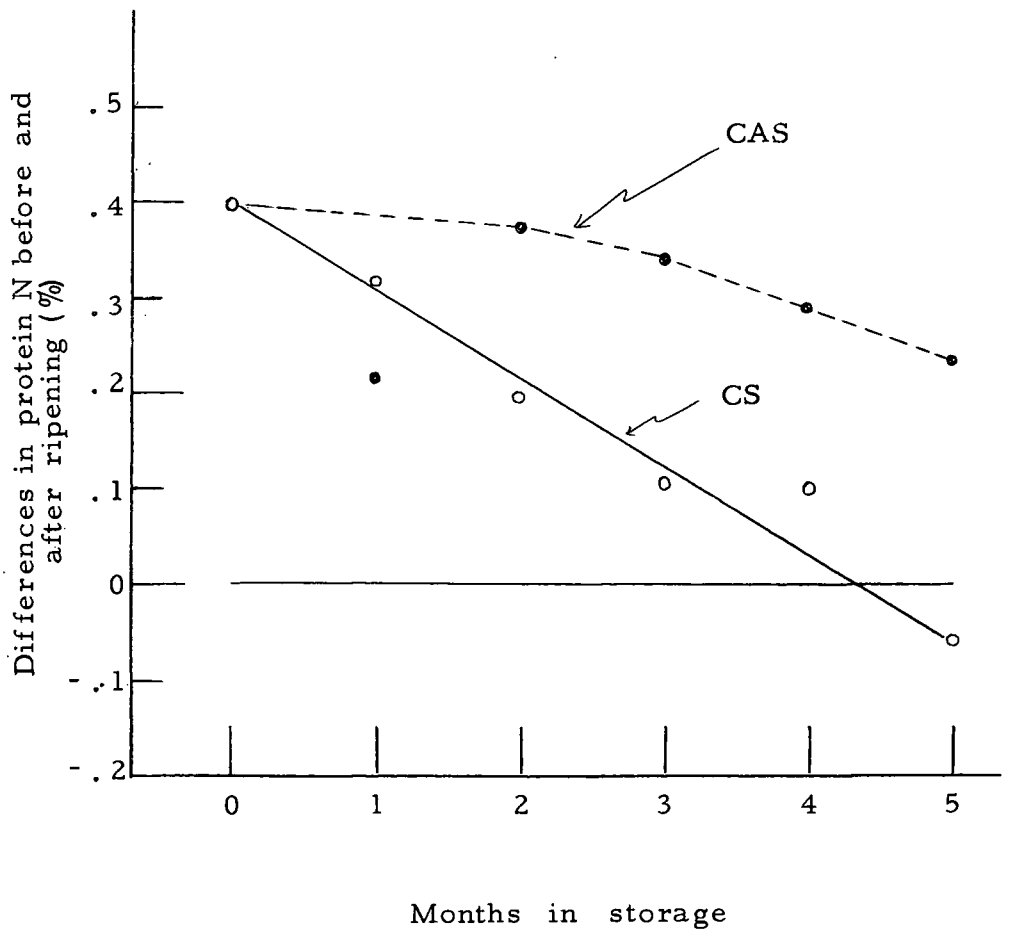


Figure 31. Differences in protein N between unripened and ripened Anjou pears from conventional (CS) and controlled atmosphere storage (CAS).

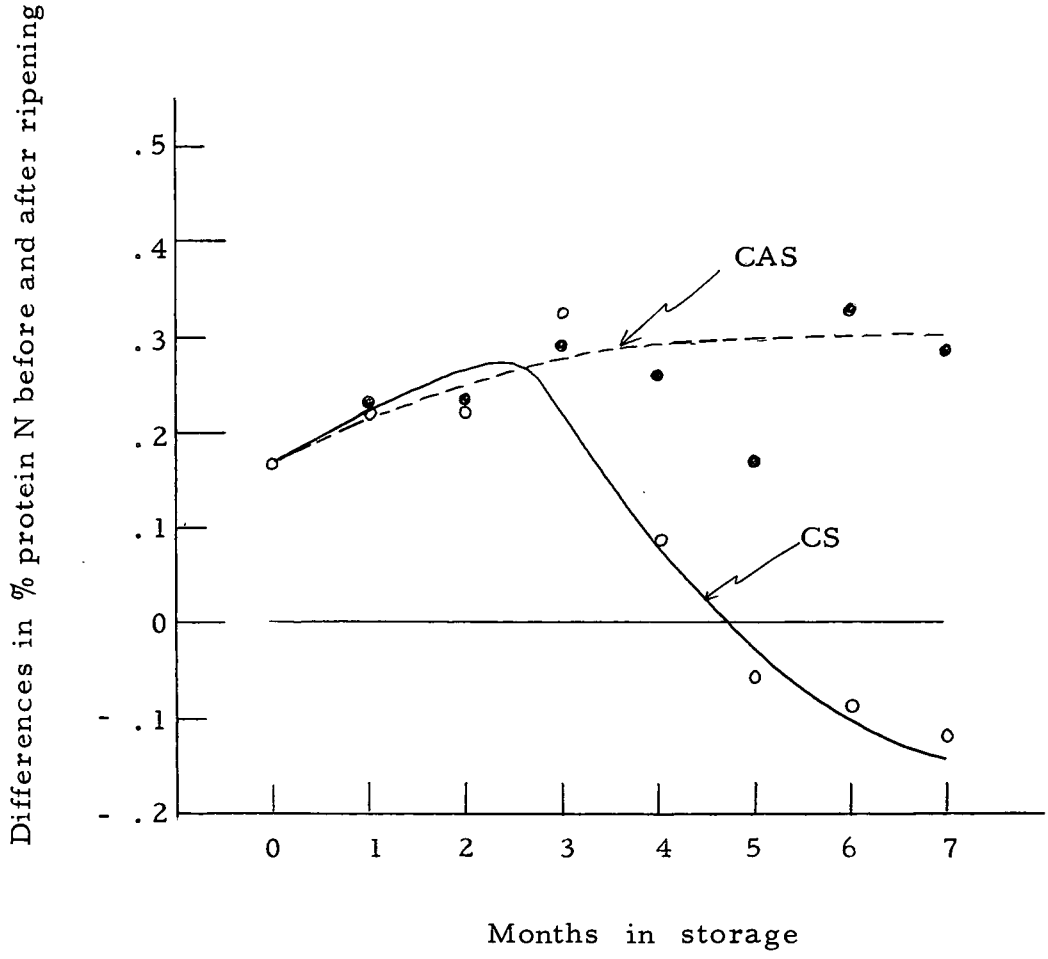
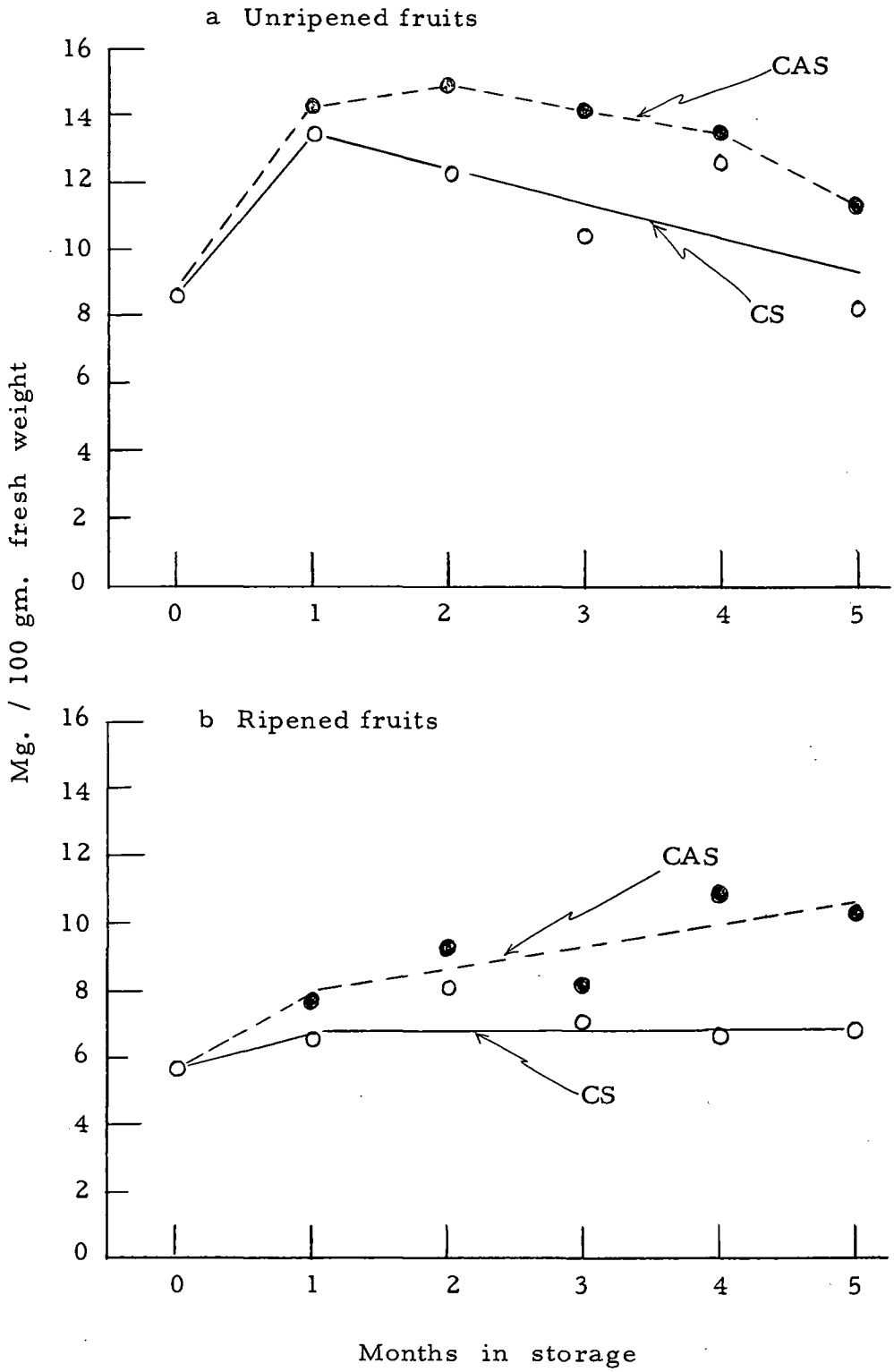


Table 14. Effect of controlled atmosphere storage on total amino nitrogen in Bartlett and Anjou pears, 1960 - 1961.

(Mg. / 100 gm. fresh weight)

| Months in storage | Unripened | | Ripened | |
|---|---|---|---|---|
| | CS | CAS | CS | CAS |
| | | Bartlett pears | | |
| 0 | 8.65 | 8.65 | 5.73 | 5.73 |
| 1 | 13.49 | 14.25 | 6.68 | 7.87 |
| 2 | 12.30 | 14.93 | 8.22 | 9.36 |
| 3 | 10.47 | 14.27 | 7.09 | 8.13 |
| 4 | 12.78 | 13.56 | 6.86 | 10.92 |
| 5 | 8.14 | 11.25 | 6.89 | 10.38 |
| | | Anjou pears | | |
| 0 | 6.47 | 6.47 | 4.84 | 4.84 |
| 1 | 5.71 | 6.51 | 6.12 | 6.36 |
| 2 | 6.01 | 6.42 | 5.15 | 7.79 |
| 3 | 7.74 | 7.52 | 7.52 | 6.41 |
| 4 | 7.01 | 9.95 | 3.29 | 6.07 |
| 5 | 7.18 | 10.92 | 7.89 | 6.66 |
| 6 | 6.40 | 13.84 | 7.24 | 12.80 |
| 7 | 9.47 | 14.23 | 6.28 | 10.12 |

Figure 32. Total amino nitrogen in Bartlett pears, 1960-1961.



In Anjou pears, the concentration remained approximately the same in both treatments (Table 14) during the first 3 months. Subsequently, amino nitrogen in the CAS fruit increased to approximately 2.2 times the amount originally present, while there was no further increase in the CS samples (Figure 33 a ) until the last month of storage. The final concentration was 9.47 and 14.23 mg/100 gm. F. W. in the CS and CAS treatments, respectively.

During ripening of Bartlett pears, amino nitrogen decreased in concentration in both treatments (Figure 32 b). In Anjou pears, however, there was no similar consistent pattern of changes in either CS or CAS fruit (Figure 33 b).

Identification of free amino acids: The amino acids occurring in Bartlett pears during storage and after ripening are shown in Tables 15 and 16. In the CS samples, 10 amino acids in the free state were identified at time of storage as aspartate and asparagine, glutamate, serine, threonine, alpha-alanine, histidine, alpha-amino-butyric acid, valine, and isoleucine/leucine. These present in highest concentration were aspartate and asparagine, glutamate and serine. After the first month of storage glutamine appeared on the chromatograph, but histidine disappeared (Figure 35). There were no changes in other amino acids during the second and third

months, with the exception of an increase in alpha-alanine. Proline was first identified after the fourth month and showed an increase in concentration the fifth month of storage. Alpha-amino-butyric acid was not present in the 5-month storage sample.

The specific differences in the amino acids in the CAS as compared to the CS Bartlett pears during storage were as follows. The amino acids which were identified throughout the storage period in CAS pears were glutamic and aspartic acids, serine, alpha-alanine, alpha-amino-butyric acid, valine, and isoleucine/leucine. Threonine as well as histidine disappeared after one month, but appeared again in the 3-month storage sample. Asparagine, likewise, was absent in the second month but appeared again in the final storage sample. In contrast to the CS samples, proline did not appear in the CAS fruit at any time during storage. Aspartic acid showed a large increase after second month.

CS Bartlett pears ripened at time of storage contained 12 amino acids:aspartate, glutamate, serine, threonine, alpha-alanine, arginine, histidine, proline, alpha-amino-butyric acid, valine, isoleucine/leucine, and one unidentified. The unknown amino acid as well as arginine and proline were not present in the fruit samples before ripening. The principal amino acids present in fruit ripened before storage were aspartic acid, proline, and isoleucine/leucine. In CS fruit ripened after one month in

Figure 33. Total amino nitrogen in Anjou pears, 1960-1961.

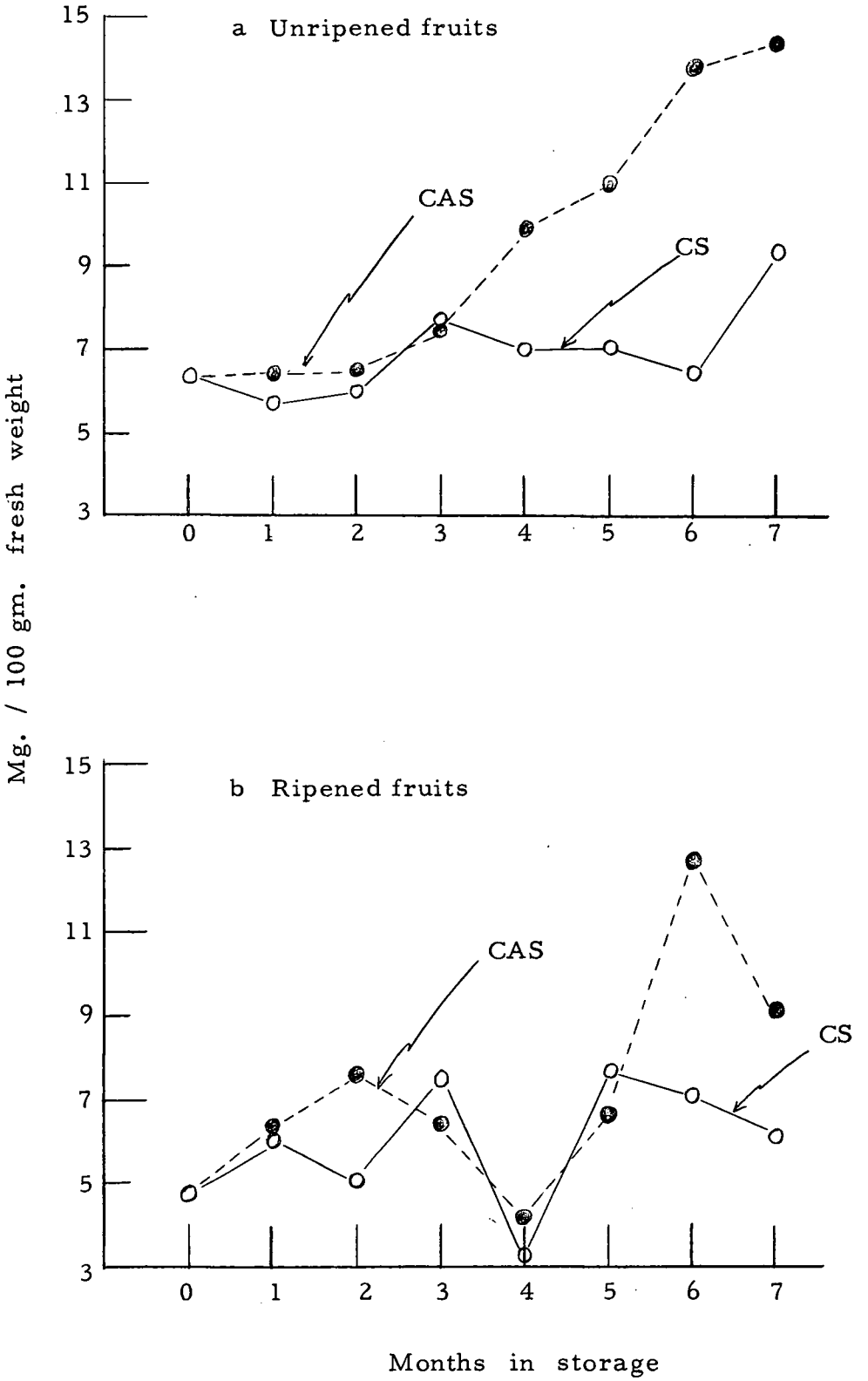


Table 15. Free amino acids in Bartlett pears during conventional and controlled atmosphere storage, 1960 - 1961.

| Amino acids | Before storage | CS | | | | | CAS | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 |
| Aspartic acid | x | x | x | x | x | x | x | x | x | x | x |
| Glutamic acid | x | x | x | x | x | x | x | x | x | x | x |
| Asparagine | x | x | x | x | x | x | x | - | - | - | x |
| Glutamine | - | x | - | - | - | - | - | - | - | - | - |
| Serine | x | x | x | x | x | x | x | x | x | x | x |
| Threonine | x | x | x | x | x | x | - | - | x | x | x |
| Alpha-alanine | x | x | x | x | x | x | x | x | x | x | x |
| Histidine | x | - | - | - | - | - | - | - | - | - | - |
| Proline | - | - | - | - | x | x | - | - | - | - | - |
| Alpha-amino-butyric acid | x | x | x | x | x | - | x | x | x | x | x |
| Valine | x | x | x | x | x | x | x | x | x | x | x |
| Isoleucine/leucine | x | x | x | x | x | x | x | x | x | x | x |
| Unknown spot | - | x | - | - | - | - | - | - | - | - | - |

x = present; - = absent; 1, 2, 3, 4, 5 = months in storage.

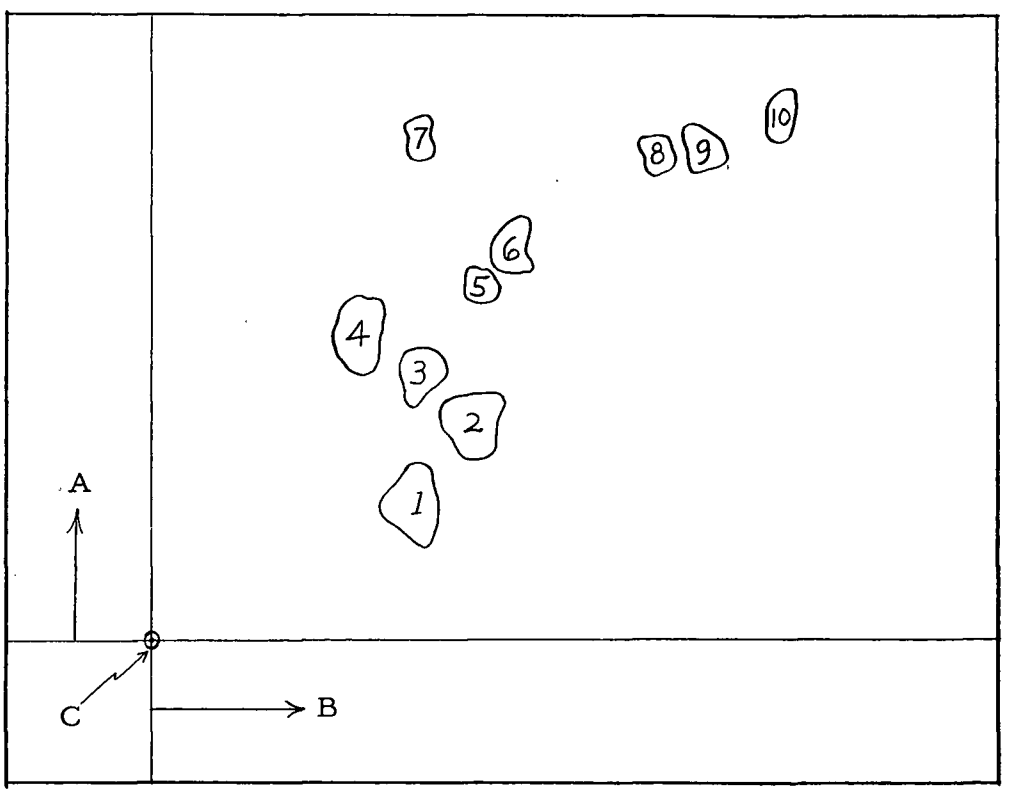



Figure 34. Amino acids in Bartlett pears before storage.

A. phenol: water; B. N-butanol:water:acetic acid;
C. original spot

1. aspartic acid
2. glutamic acid
3. serine
4. asparagine
5. threonine
6. alpha-alanine
7. histidine
8. alpha-amino-butyric acid
9. valine
10. isoleucine/leucine

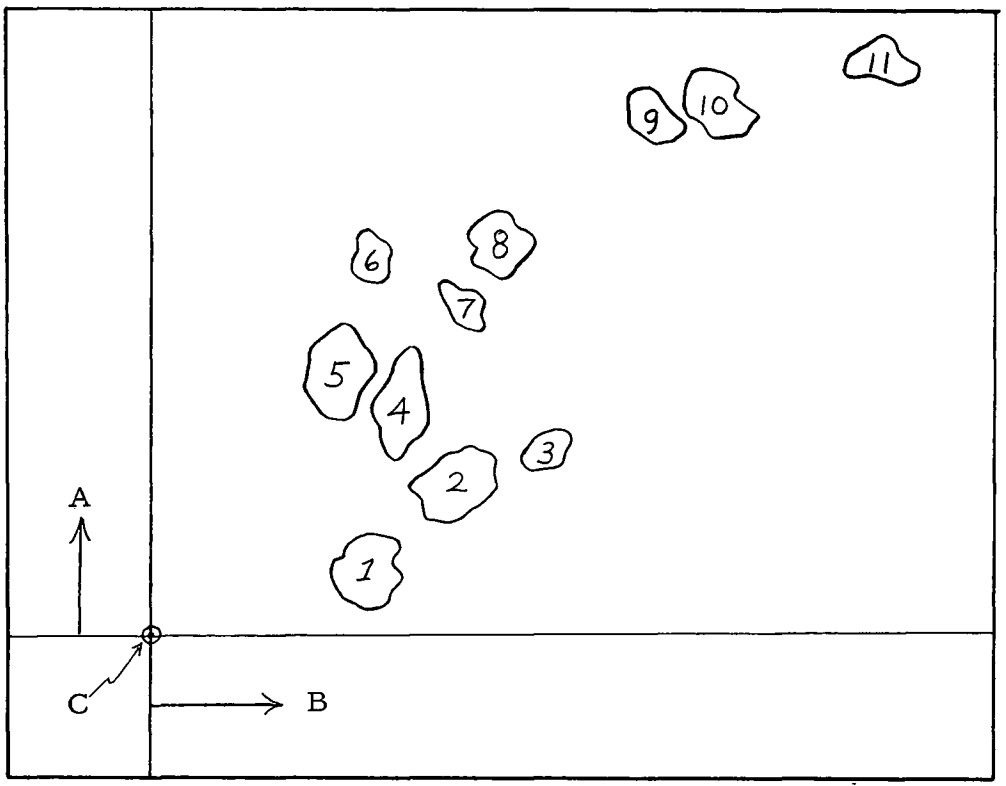



Figure 35. Amino acids in Bartlett pears after 1 month in CS.

A. phenol: water
B. N-butanol:water: acetic acid
C. original spot

1. aspartic acid
2. glutamic acid
3. unidentified spot
4. serine
5. asparagine
6. glutamine
7. threonine
8. alpha-alanine
9. alpha-amino-butyric acid
10. valine
11. isoleucine/leucine

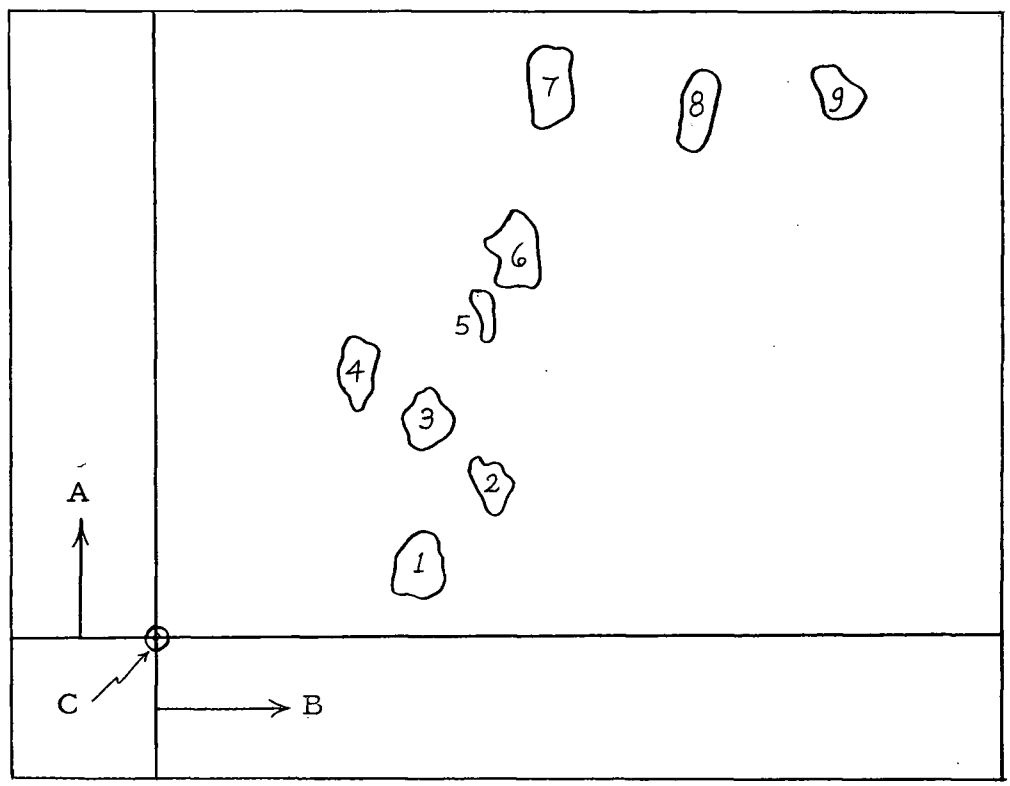



Figure 36. Amino acids in Bartlett pears after 5 months in CS. A. phenol:water; B. N-butanol: water: acetic acid; C. original spot.
1. aspartic acid, 2. glutamic acid, 3. serine, 4. asparagine, 5. threonine, 6. alpha-alanine, 7. proline, 8. valine, 9. isoleucine/leucine.

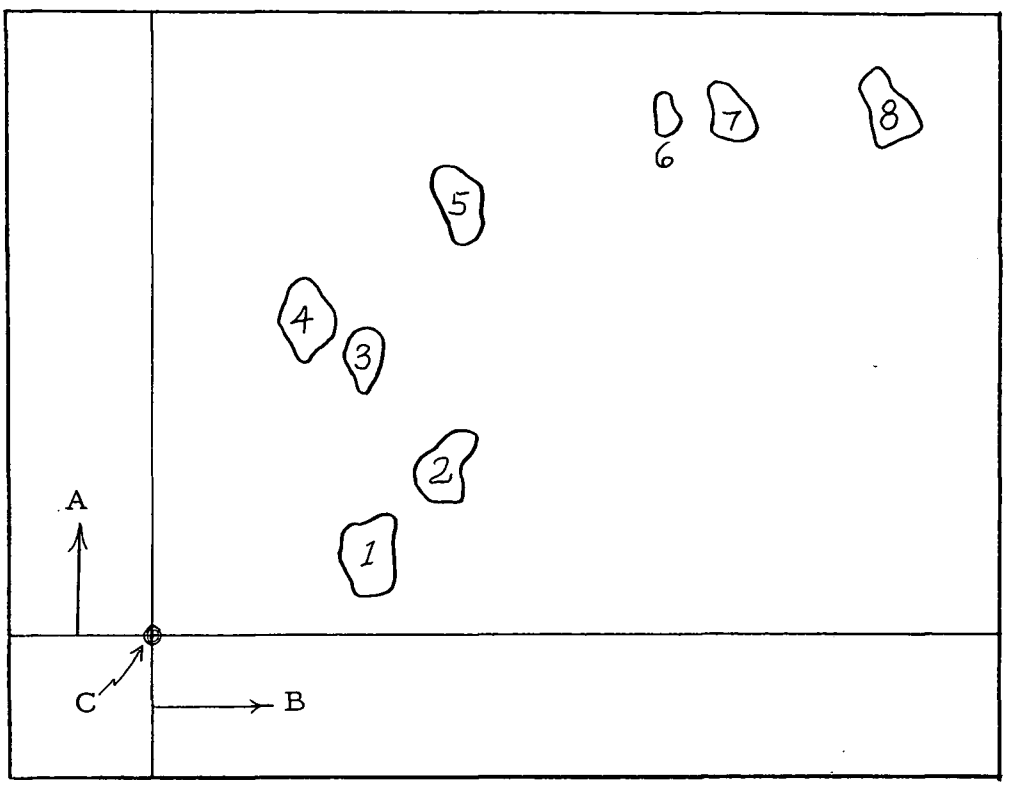



Figure 37. Amino acids in Bartlett pears after 1 month in CAS.

A. phenol:water B. N-butanol:water:acetic acid
C. original spot

1. aspartic acid
2. glutamic acid
3. serine
4. asparagine
5. alpha-alanine
6. alpha-amino-butyric acid
7. valine
8. isoleucine/leucine

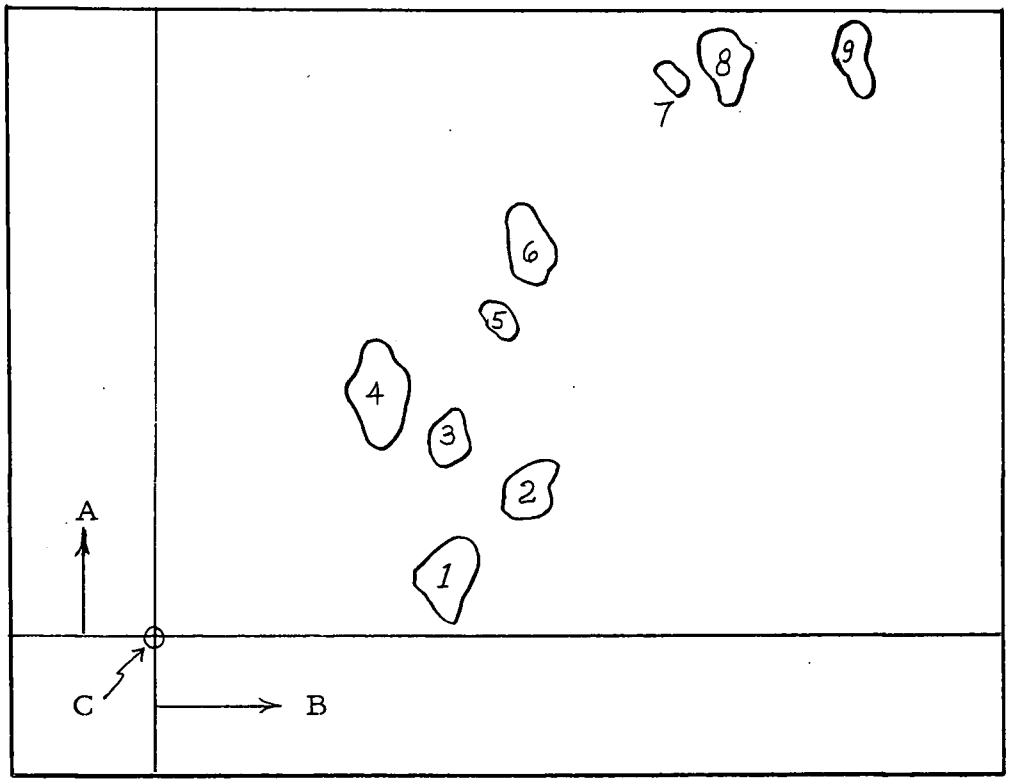



Figure 38. Amino acids in Bartlett pears after 5 months in CAS.

A. phenol: water B. N-butanol: water: acetic acid
C. original spot

1. aspartic acid 2. glutamic acid 3. serine
4. asparagine 5. threonine 6. alpha-alanine
7. alpha-amino-butyric acid 8. valine
9. isoleucine/leucine

Table 16. Changes in free amino acids in Bartlett pears ripened after conventional and controlled atmosphere storage. 1960 - 1961.

| Amino acids | Before storage | CS | | | | | CAS | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 |
| Aspartic acid | x | x | x | x | x | x | x | x | x | x | x |
| Glutamic acid | x | x | x | x | x | x | x | x | x | x | x |
| Asparagine | x | x | x | x | x | x | x | x | x | x | x |
| Serine | x | x | x | x | x | x | x | x | x | x | x |
| Threonine | x | x | x | x | x | x | x | x | x | x | x |
| Alpha-alanine | x | x | x | x | x | x | x | x | x | x | x |
| Arginine | x | - | - | - | - | - | - | - | - | - | - |
| Histidine | x | - | - | - | x | x | - | - | - | x | x |
| Proline | x | x | x | x | x | x | x | x | x | x | x |
| Alpha-amino-butyric acid | x | x | x | x | x | x | x | x | x | x | x |
| Valine | x | x | x | x | x | x | x | x | x | x | x |
| Isoleucine/leucine | x | x | x | x | x | x | x | x | x | x | x |
| Unknown spot | x | - | - | - | - | - | - | - | - | - | - |

x = present; - = absent; 1, 2, 3, 4, 5 = months in storage.

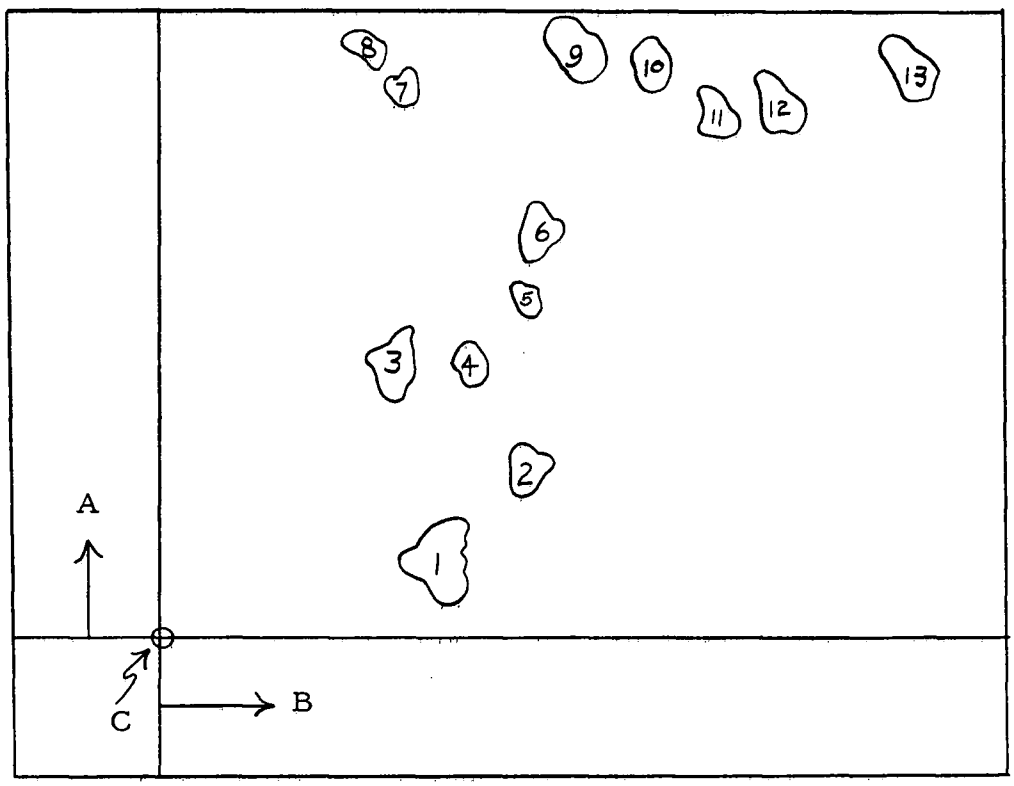



Figure 39. Amino acids in Bartlett pears ripened before storage.

A. phenol:water B. N-butanol:water:acetic acid
C. original spot

1. aspartic acid 2. glutanic acid 3. asparagine
4. serine 5. threonine 6. alpha-alanine
7. histidine 8. arginine 9. proline
10. unknown spot 11. alpha-amino-butyric acid
12. valine 13. isoleucine/leucine

storage, arginine, histidine and the unidentified amino acid disappeared (Figure 40). Thereafter, alpha-alanine increased in concentration and histidine reappeared in the fruit ripened after 4 and 5 months in storage.

Changes in the amino acids in ripe CAS Bartlett pears were similar to those described for the CS fruit.

The amino acids occurring in the Anjou pears during storage and after ripening are shown in Tables 17 and 18. In CS Anjou pears at time of storage, 10 amino acids were identified. These were aspartate and asparagine, glutamate, serine, threonine, alpha-alanine, histidine, proline, valine, and isoleucine/leucine. These are the same amino acids found in Bartlett pears before storage except that proline was present instead of alpha-amino-butyric acid. However, this particular amino acid appeared after one month (Figure 45). Histidine disappeared after one month and asparagine after 2 months. Aspartic acid, alpha-alanine and proline increased in concentration throughout storage. Beta-alanine was detected at the end of the storage period (Figure 46). In CAS Anjou pears, 3 amino acids in addition to those in CS samples appeared after one month. These were identified as cystine, beta-alanine with one unknown. After the second month, asparagine and cystine disappeared, while alpha-alanine and

proline increased.

No changes were found in the identity of amino acids in pre-storage CS pears after being ripened. Asparagine decreased in concentration and could not be detected in fruit ripened after 5 months, but reappeared on the chromatographs of samples ripened after 7 months. Alpha-amino-butyric acid appeared in fruit after 4 months and beta-alanine after 7 months. Aspartic acid and proline increased throughout the storage period after ripening.

Changes in the amino acids in CAS Anjou pears during ripening were similar to those found in the CS fruit. Specific differences observed were: (1) alpha-amino-butyric acid appeared after two instead of four months and (2) histidine disappeared after the third instead of the first month in storage.

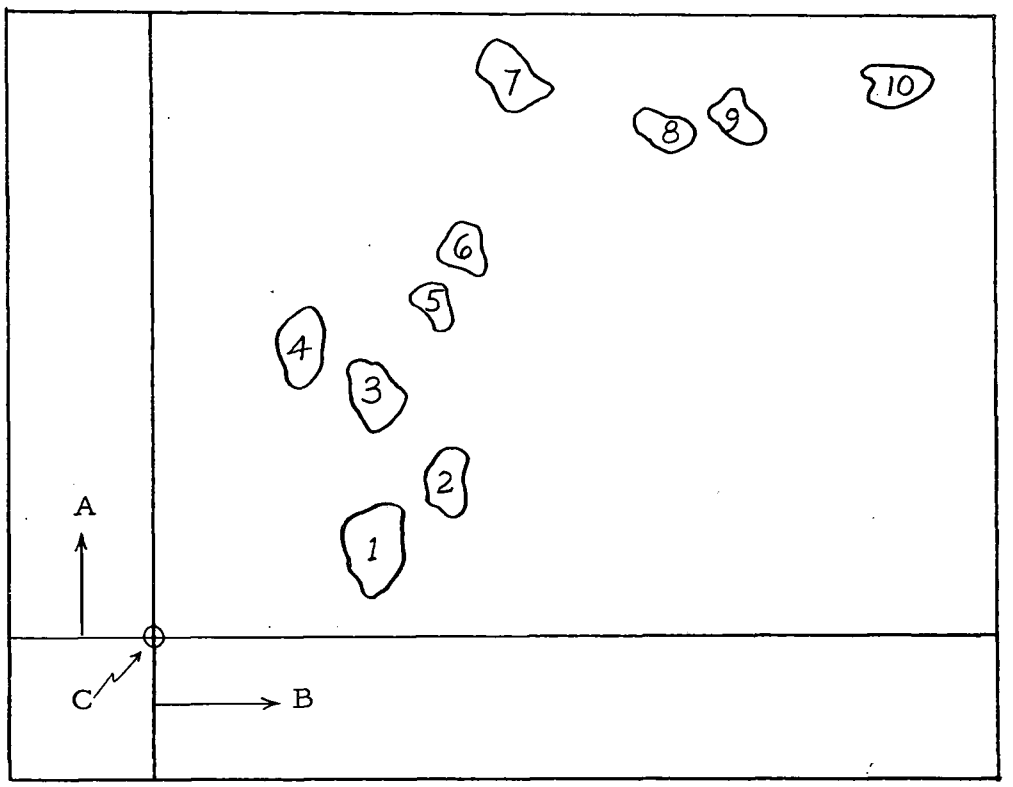



Figure 40. Amino acids in Bartlett pears ripened after 1 month in CS.

A. phenol:water
B. N-butanol:water:acetic acid
C. original spot

1. aspartic acid
2. glutamic acid
3. serine
4. asparagine
5. threonine
6. alpha-alanine
7. proline
8. alpha-amino-butyric acid
9. valine
10. isoleucine/leucine

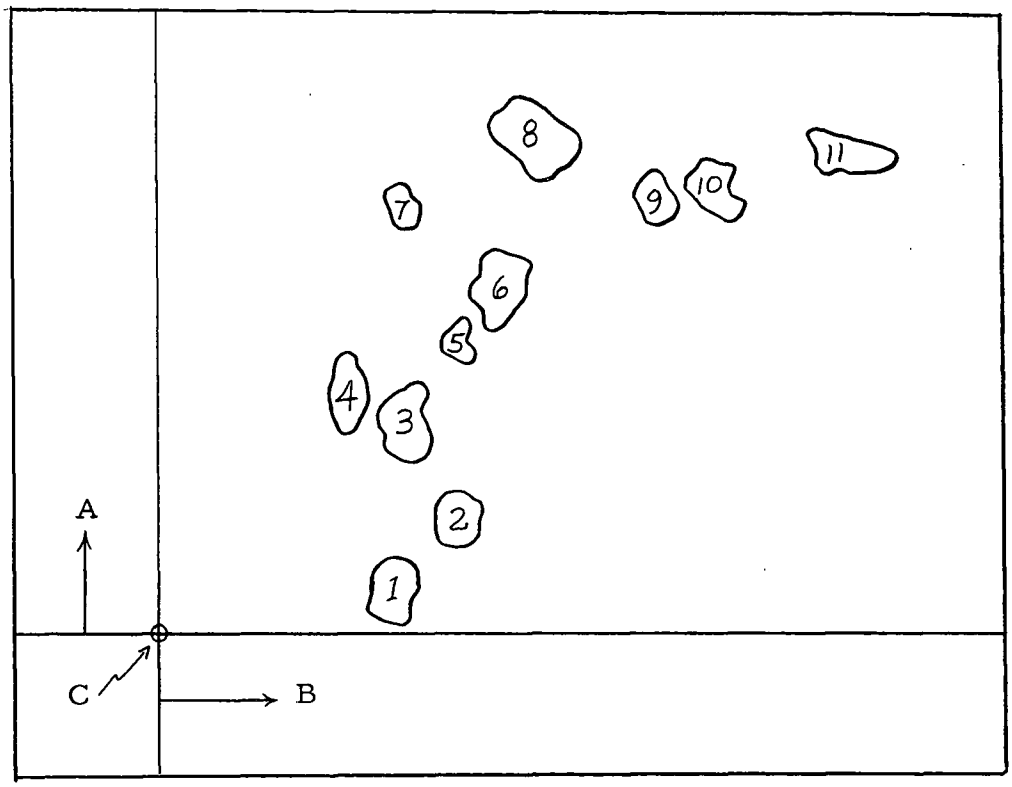



Figure 41. Amino acids in Bartlett pears ripened after 5 months in CS.

A. phenol: water
B. N-butanol:water:acetic acid
C. original spot

1. aspartic acid
2. glutamic acid
3. serine
4. asparagine
5. threonine
6. alpha-alanine
7. histidine
8. proline
9. alpha-amino-butyric acid
10. valine
11. iosleucine/leucine

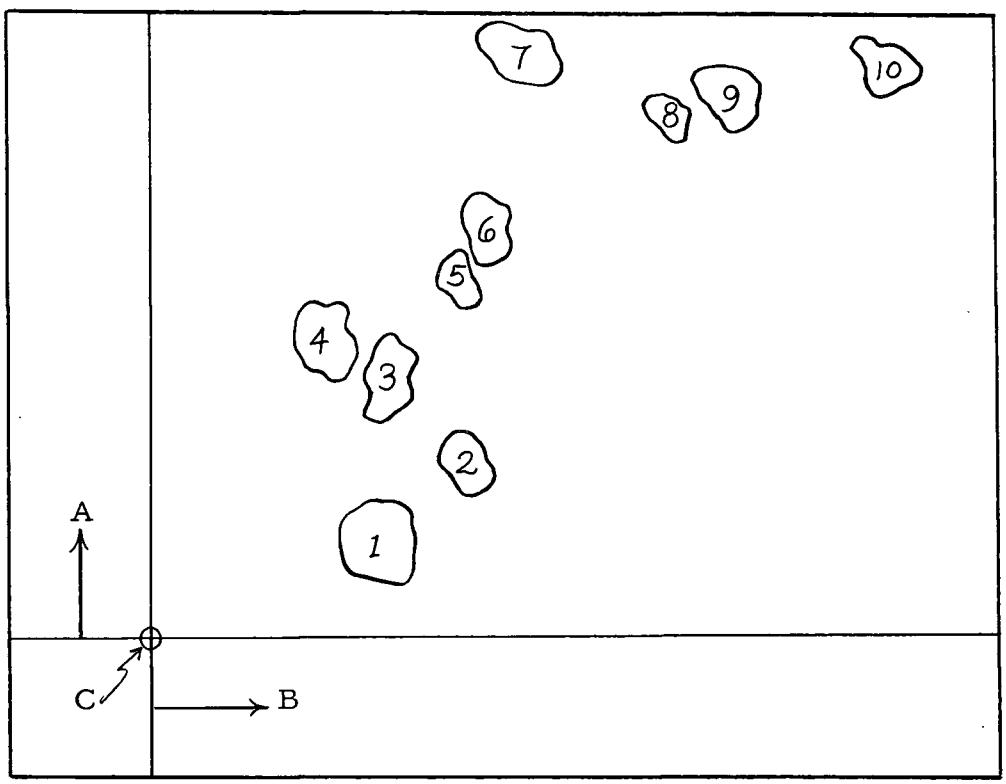



Figure 42. Amino acids in Bartlett pears ripened after 1 month in CAS.

A. phenol:water
B. N-butanol:water:acetic acid
C. original spot

1. aspartic acid
2. glutamic acid
3. serine
4. asparagine
5. threonine
6. alpha-alanine
7. proline
8. alpha-amino-butyric acid
9. valine
10. isoleucine/leucine

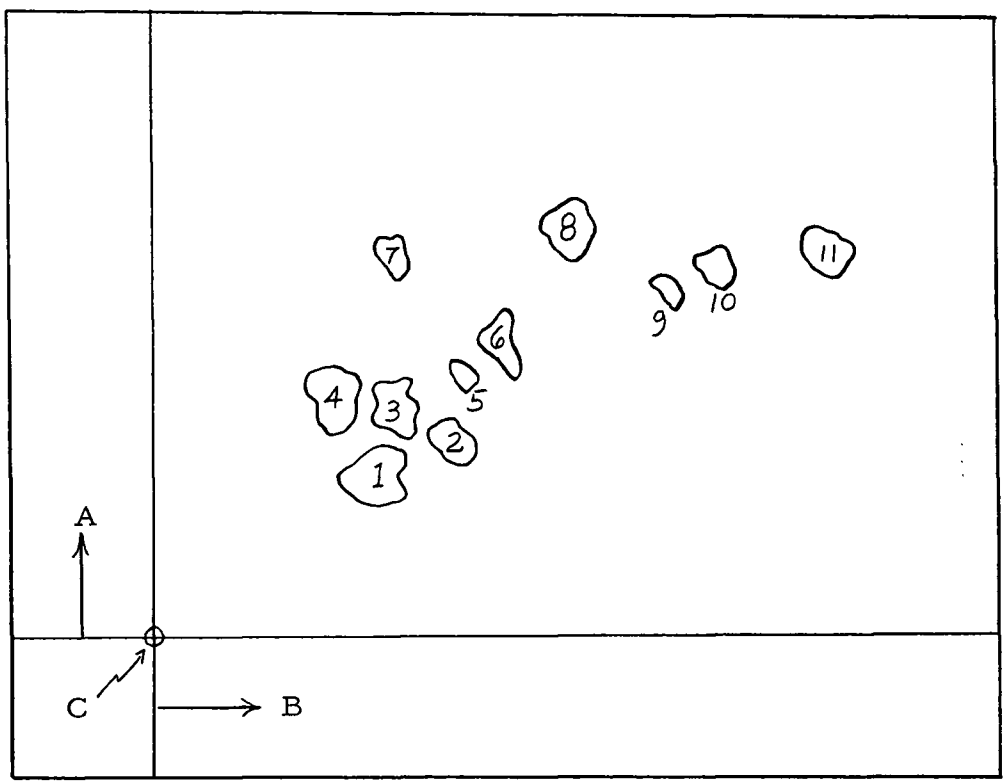



Figure 43. Amino acids in Bartlett pears ripened after 5 months in CAS.

A. phenol:water
B. N-butanol:water:acetic acid
C. original spot

1. aspartic acid
2. glutamic acid
3. serine
4. asparagine
5. threonine
6. alpha-alanine
7. histidine
8. proline
9. alpha-amino-butyric acid
10. valine
11. isoleucine/leucine

Table 17. Changes in free amino acids in Anjou pears during conventional and controlled atmosphere storage, 1960 - 1961.

| Amino acids | Before storage | CS | | | | | | | CAS | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Aspartic acid | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| Glutamic acid | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| Serine | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| Asparagine | x | x | - | - | - | - | - | - | x | - | - | - | - | - | - |
| Threonine | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| Alpha-alanine | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| Histidine | x | - | - | - | - | - | - | - | x | x | x | x | x | x | x |
| Proline | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| Valine | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| Isoleucine/leucine | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| Alpha-amino-butyric acid | - | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| Beta-alanine | - | - | - | - | - | - | - | x | x | x | x | x | x | x | x |
| Cystine | - | - | - | - | - | - | - | - | x | - | - | - | - | - | - |
| Unknown spot | - | - | - | - | - | - | - | - | x | - | - | - | - | - | x |

x = present; - = absent; 1, 2, 3, 4, 5, 6, 7 = months in storage.

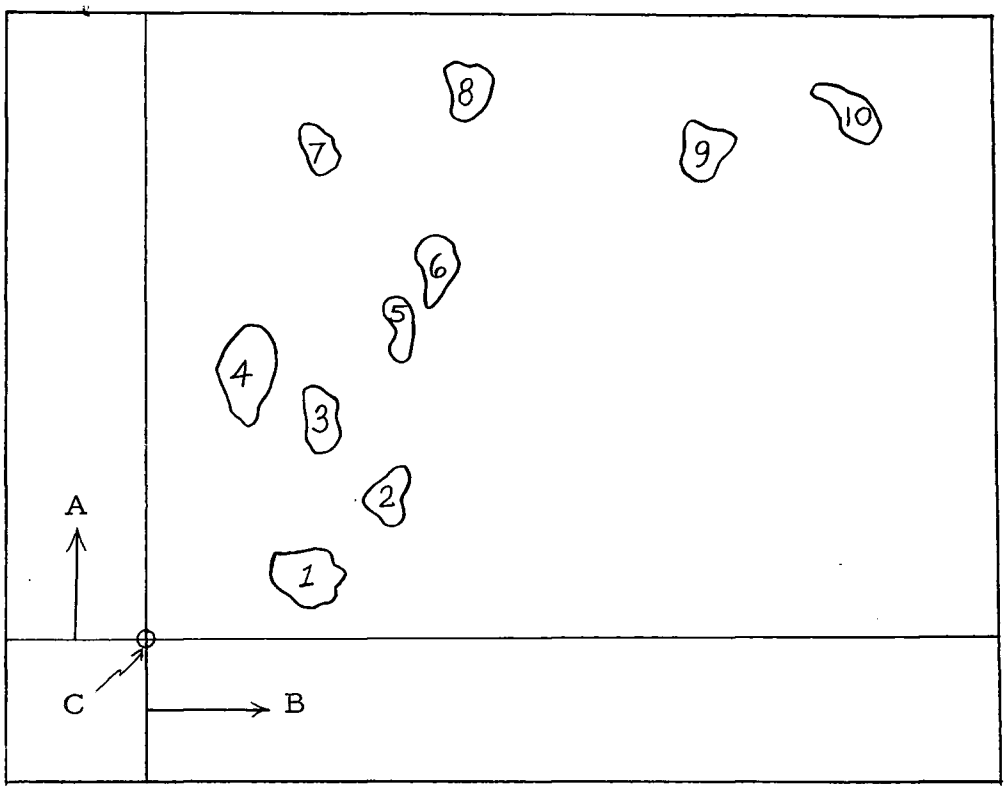



Figure 44. Amino acids in Anjou pears before storage.

A. phenol:water
B. N-butanol:water:acetic acid
C. original spot

1. aspartic acid
2. glutamic acid
3. serine
4. asparagine
5. threonine
6. alpha-alanine
7. histidine
8. proline
9. valine
10. isoleucine/leucine

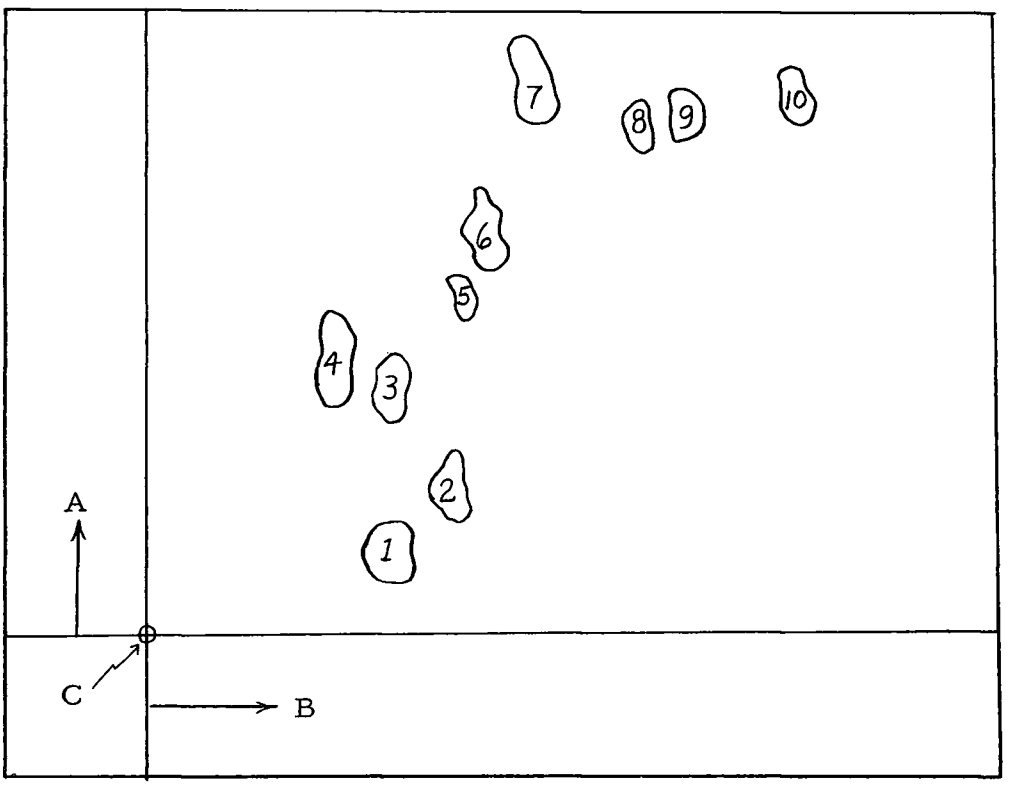



Figure 45. Amino acids in Anjou pears after 1 month in CS.

A. phenol:water

B. N-butanol:water:acetic acid

1. aspartic acid
2. glutamic acid
3. serine
4. asparagine
5. threonine
6. alpha-alanine
7. proline
8. alpha-amino-butyric acid
9. valine
10. isoleucine/leucine

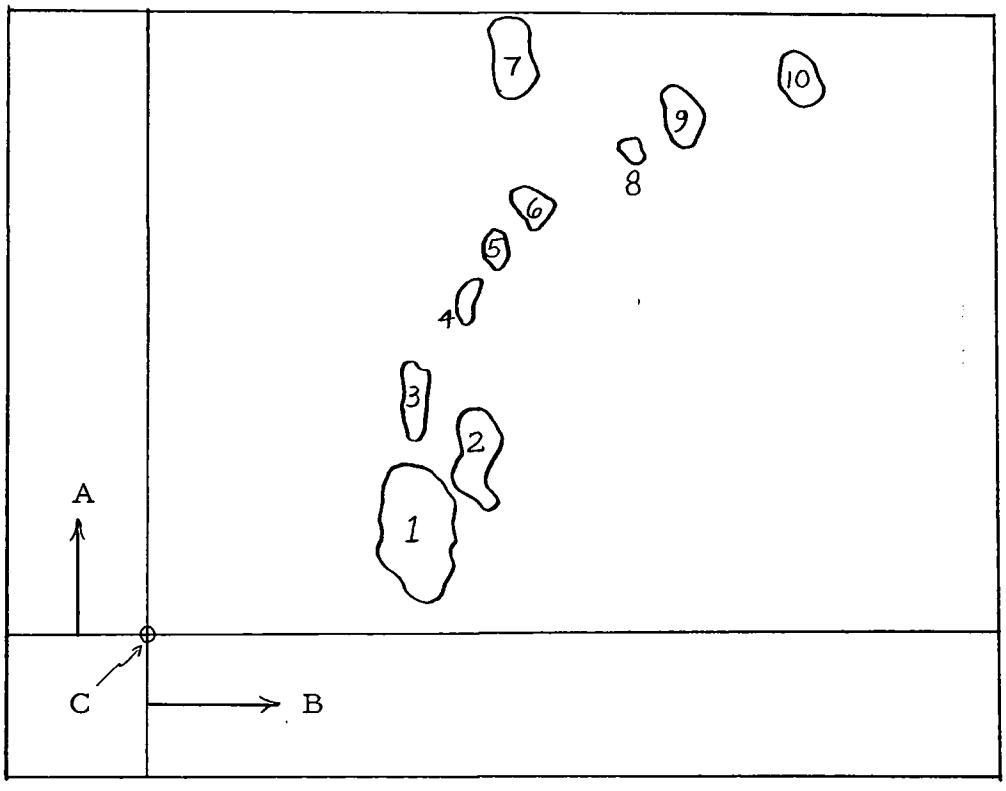



Figure 46. Amino acids in Anjou pears after 7 months in CS.

A. phenol:water
B. N-butanol:water:acetic acid
C. original spot

1. aspartic acid
2. glutamic acid
3. serine
4. threonine
5. alpha-alanine
6. beta-alanine
7. proline
8. alpha-amino-butyric acid
9. valine
10. isoleucine/leucine

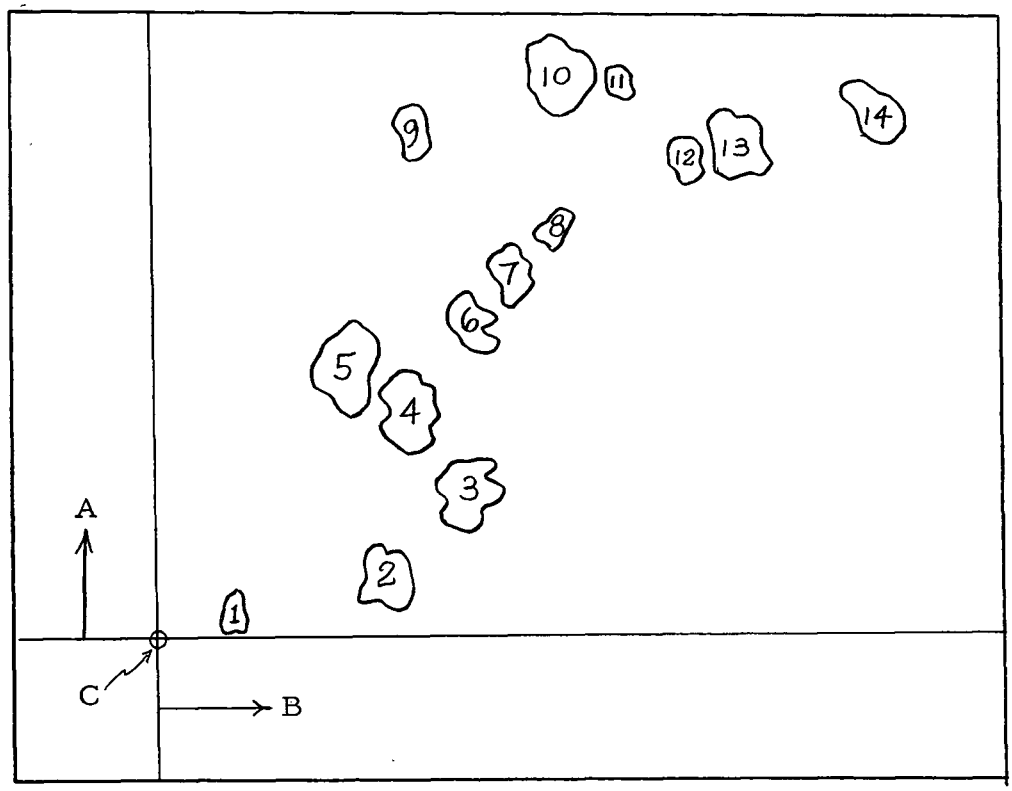



Figure 47. Amino acids in Anjou pears after 1 month in CAS.

A. phenol:water
B. N-butanol:water:acetic acid
C. original spot

1. cystine
2. aspartic acid
3. glutamic acid
4. serine
5. asparagine
6. threonine
7. alpha-alanine
8. beta-alanine
9. histidine
10. proline
11. unknown spot
12. alpha-amino-butyric acid
13. valine
14. isoleucine/leucine

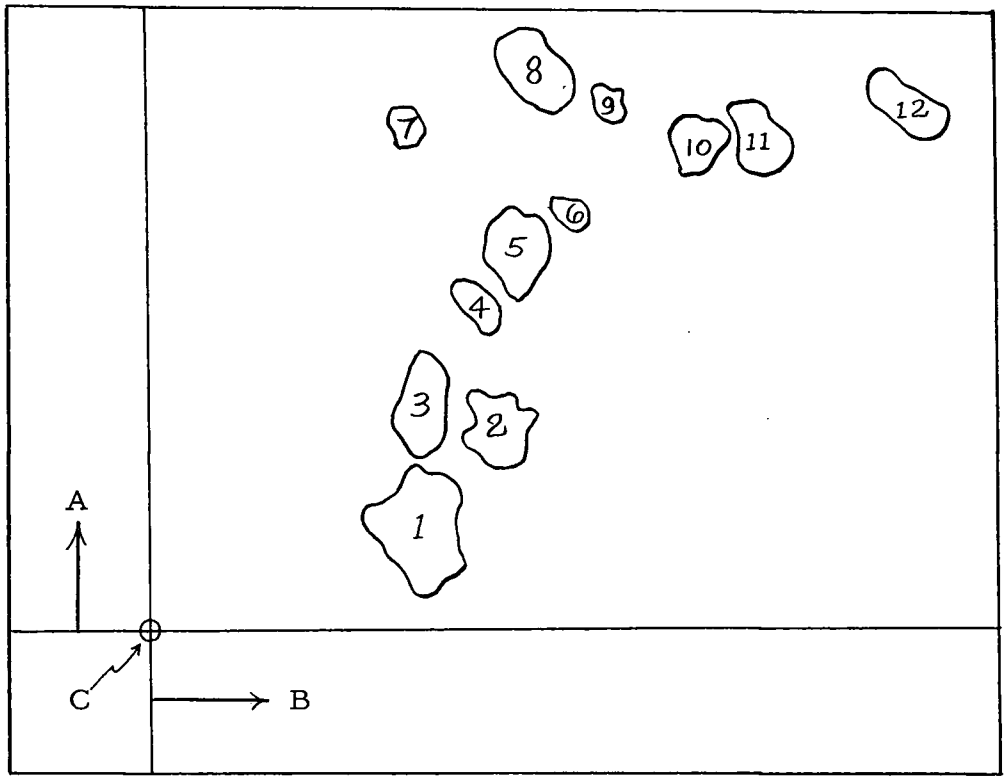



Figure 48. Amino acids in Anjou pears after 7 months in CAS.

A. phenol:water
B. N-butanol:water:acetic acid
C. original spot

1. aspartic acid
2. glutamic acid
3. serine
4. threonine
5. alpha-alanine
6. beta-alanine
7. histidine
8. proline
9. unknown spot
10. alpha-amino-butyric acid
11. valine
12. isoleucine/leucine

Table 18. Free amino acids in Anjou pears ripened after conventional and controlled atmosphere storage, 1960 - 1961.

| Amino acids | Before storage | CS | | | | | | | CAS | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Aspartic acid | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| Glutamic acid | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| Serine | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| Asparagine | x | x | x | x | x | - | - | - | x | x | x | x | x | x | x |
| Threonine | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| Alpha-alanine | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| Histidine | x | - | - | - | - | - | - | - | x | x | - | - | - | x | x |
| Proline | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| Valine | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| Isoleucine/leucine | x | x | x | x | x | x | x | x | x | x | x | x | x | x | x |
| Alpha-amino-butyric acid | - | - | - | - | x | x | x | x | - | x | x | x | x | x | x |
| Beta-alanine | - | - | - | - | - | - | - | x | - | - | - | - | - | x | x |

x = present; - = absent; 1, 2, 3, 4, 5, 6, 7 = months in storage.

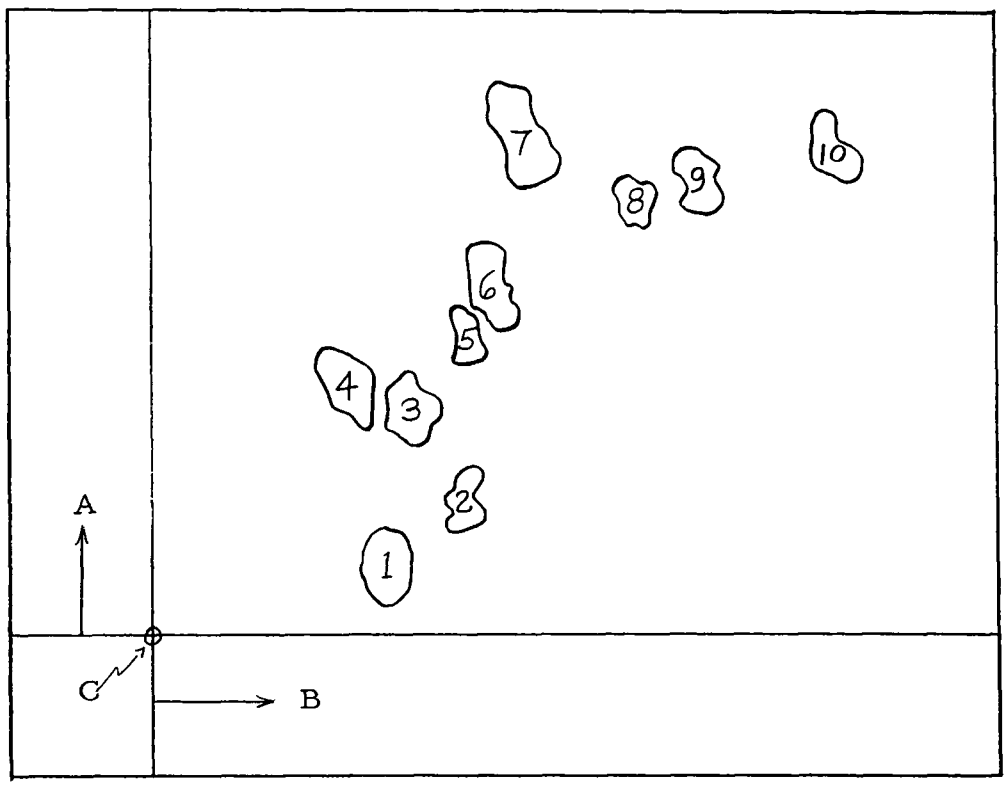



Figure 49. Amino acids in Anjou pears ripened after 4 months in CS.

A. phenol:water
B. N-butanol:water:acetic acid
C. original spot

1. aspartic acid
2. glutamic acid
3. serine
4. asparagine
5. threonine
6. alpha-alanine
7. proline
8. alpha-amino-butyric acid
9. valine
10. isoleucine/leucine

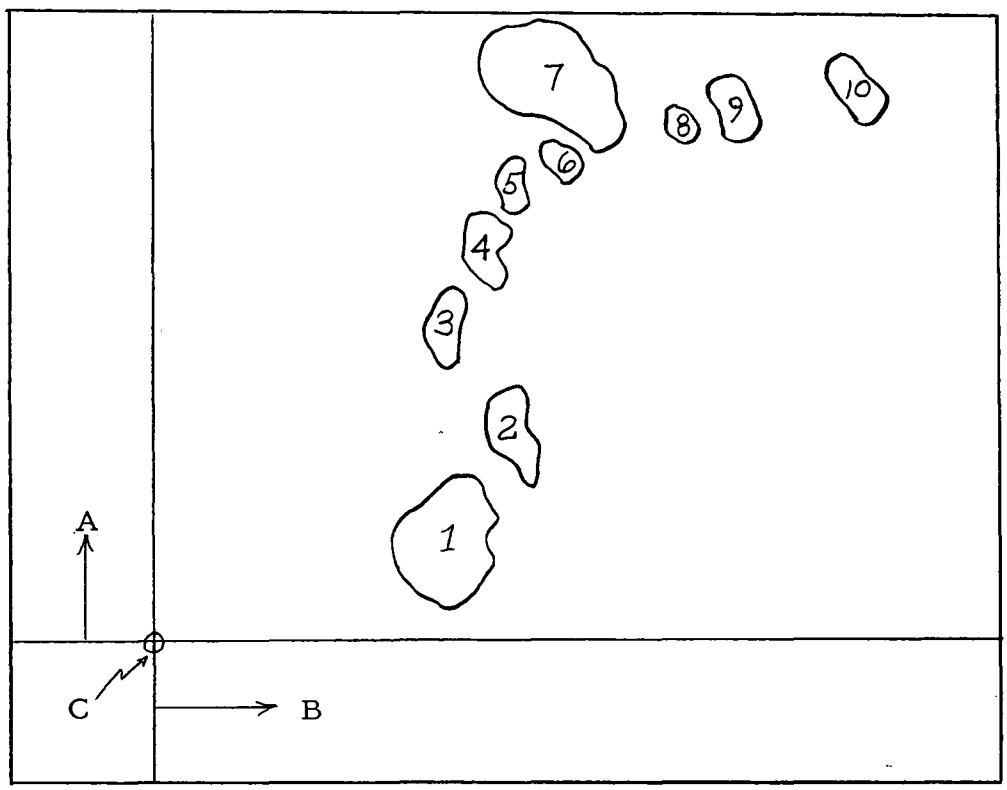



Figure 50. Amino acids in Anjou pears ripened after 7 months in CS.

A. phenol:water
B. N-butanol:water:acetic acid
C. original spot

1. aspartic acid
2. glutamic acid
3. serine
4. threonine.
5. alpha-alanine
6. beta-alanine
7. proline
8. alpha-amino-butyric acid
9. valine
10. isoleucine/ leucine

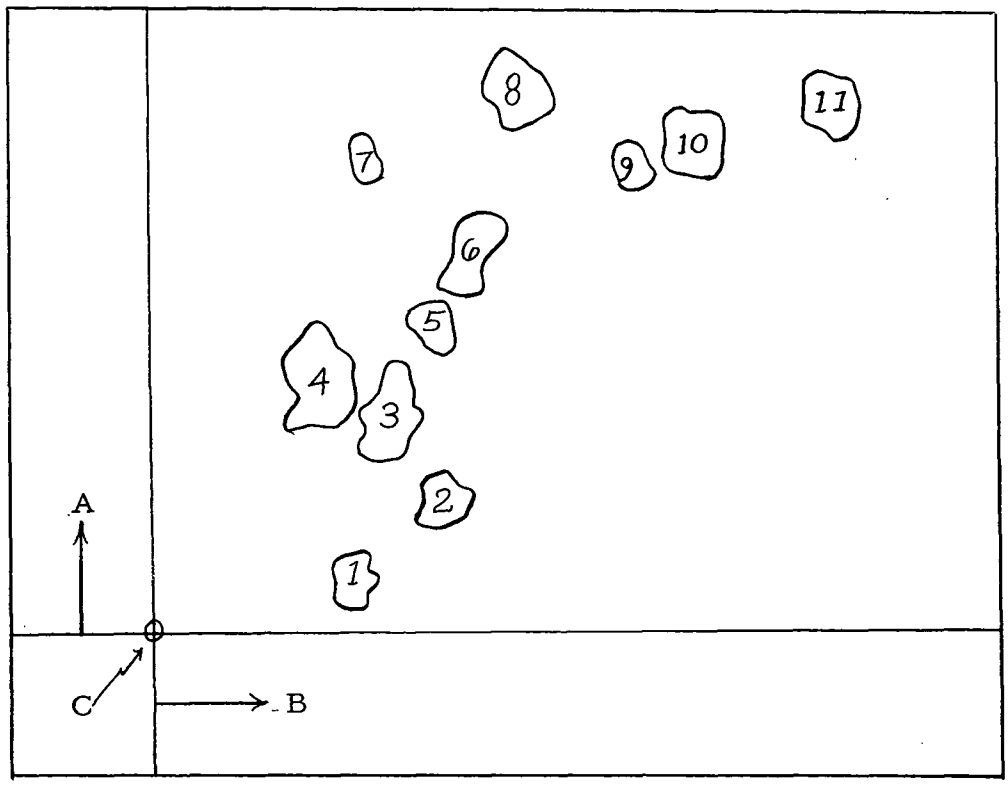



Figure 51. Amino acids in Anjou pears ripened after 2 months in CAS.

A. phenol:water
B. N-butanol:water:acetic acid
C. original spot

1. aspartic acid
2. glutamic acid
3. serine
4. asparagine
5. threonine
6. alpha-alanine
7. histidine
8. proline
9. alpha-amino-butyric acid
10. valine
11. isoleucine/leucine

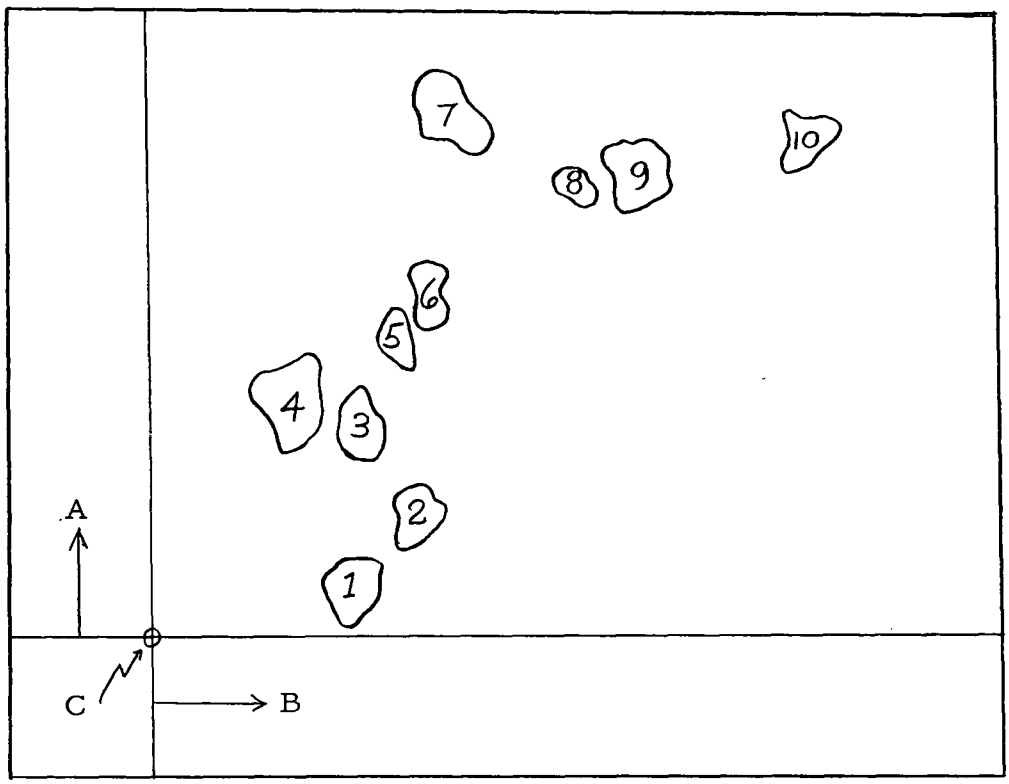



Figure 52. Amino acids in Anjou pears ripened after 3 months in CAS.

A. phenol:water
B. N-butanol:water:acetic acid
C. original spot

1. aspartic acid
2. glutamic acid
3. serine
4. asparagine
5. threonine
6. alpha-alanine
7. proline
8. alpha-amino-butyric acid
9. valine
10. isoleucine/leucine

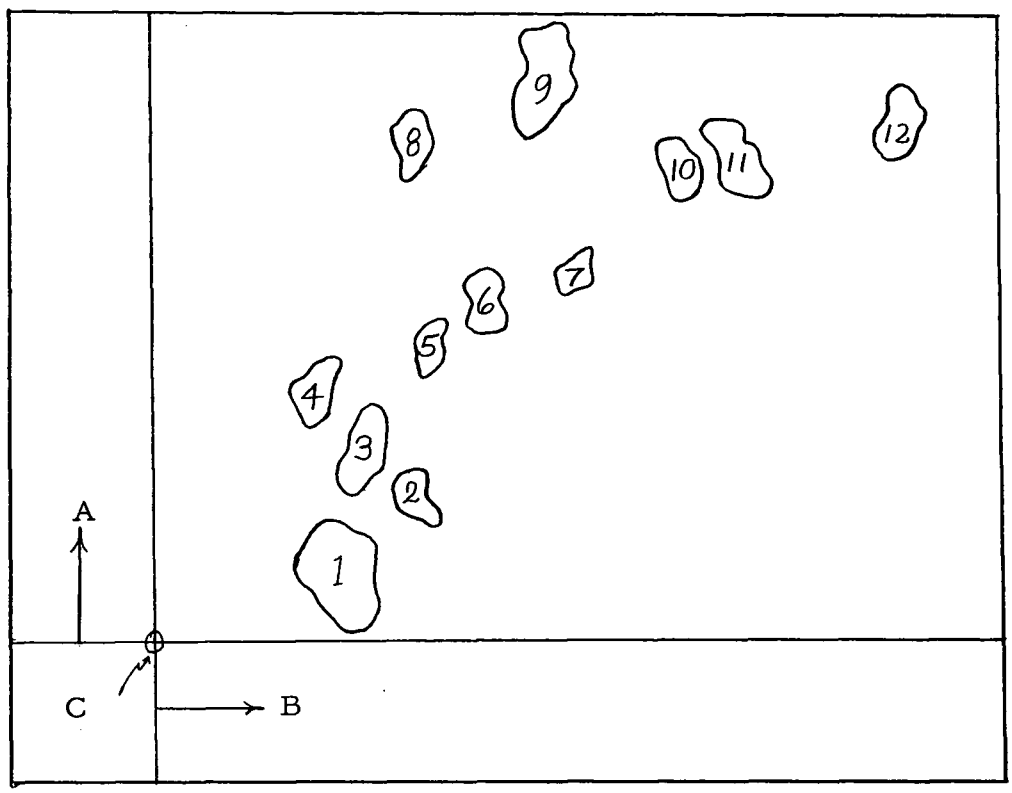



Figure 53. Amino acids in Anjou pears ripened after 6 months in CAS.

A. phenol:water
B. N-butanol:water:acetic acid
C. original spot

1. aspartic acid
2. glutamic acid
3. serine
4. asparagine
5. threonine
6. alpha-alanine
7. beta-alanine
8. histidine
9. proline
10. alpha-amino-butyric acid
11. valine
12. isoleucine/leucine

# DISCUSSION

In considering the data obtained in this investigation, three features evolve which appear to be of primary significance: (1) that while the main metabolic pathways involved in the carbohydrate, organic acid and nitrogen metabolism of pears appear to conform to the general pattern found in various other plant tissues, there are certain specific differences between the two varieties; (2) that modification in the storage atmosphere results in specific alterations in metabolism, and (3) that certain of these changes induced appear to be reflected in improved storage and fruit quality.

The differences in metabolism of Bartlett and Anjou pears during storage as indicated by the data are shown diagramatically in Figures 54 A and B.

The participation of the pentose cycle is suggested in both varieties but appears to be more active in Bartlett pears, especially during the early storage period. This is indicated by a large increase in shikimic acid which was not observed in Anjou pears. So far as known, shikimic as well as quinic acids are formed from 4 and 7 carbon phosphate sugars, both of which are intermediates of the pentose cycle, reacting with phosphoenolpyruvate. The occurrence of these acids is of special interest, since they are

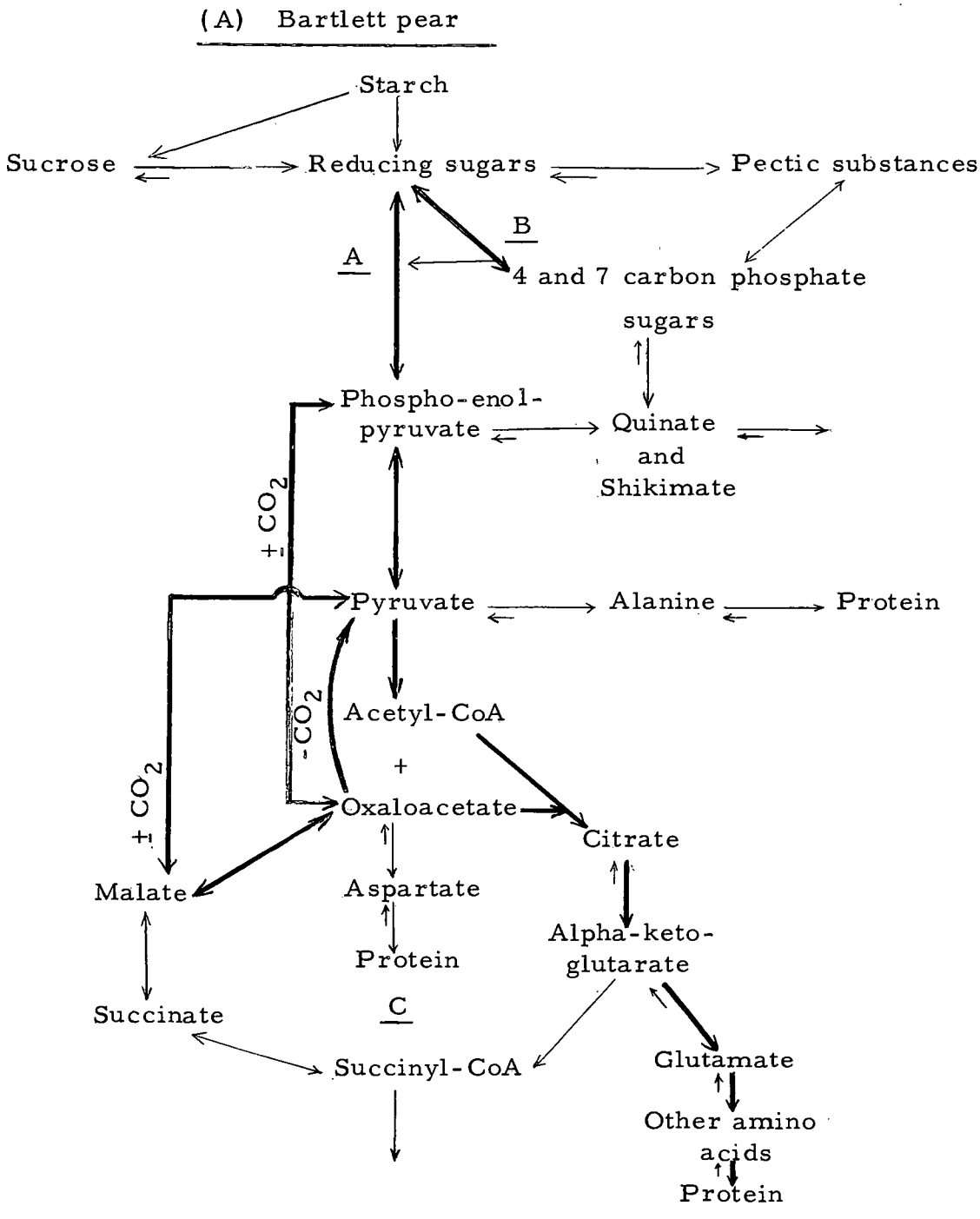
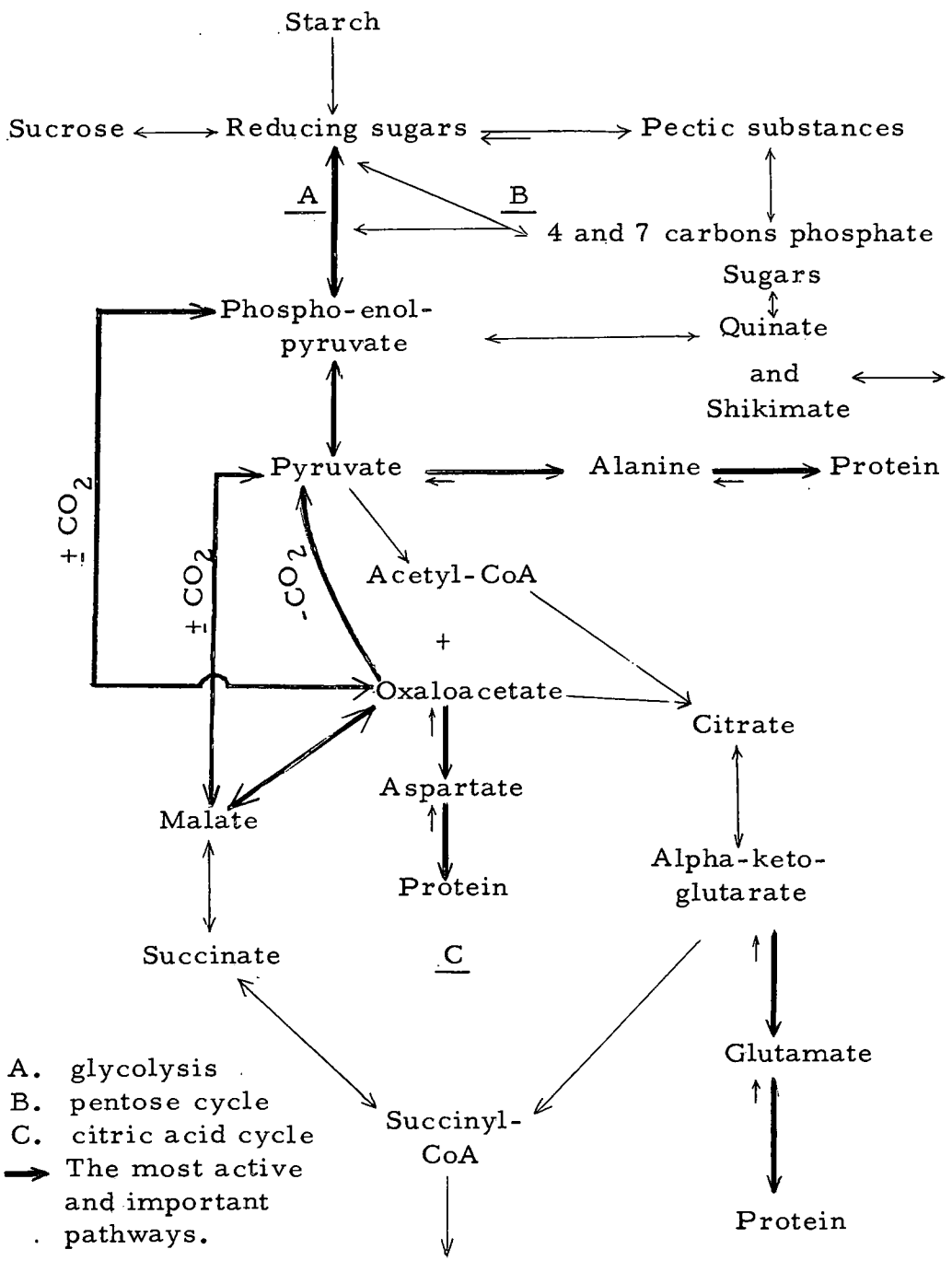



Figure 54. The suggested metabolic pathways in pears in conventional and controlled atmosphere storage.

(B) Anjou pear


Starch
Sucrose
Reducing sugars
Pectic substances
A
B
4 and 7 carbons phosphate Sugars
Phospho-enol-pyruvate
Quinate and Shikimate
Pyruvate
Alanine
Protein
+ CO2
+ CO2
- CO2
Acetyl-CoA
+
Oxaloacetate
Citrate
Aspartate
Malate
Protein
Alpha-keto-glutarate
Succinate
C
Glutamate
Succinyl-CoA
Protein
A. glycolysis
B. pentose cycle
C. citric acid cycle
The most active and important pathways.

regarded as being formed by reactions which are separate and distinct from those concerned with the aliphatic acids of the Krebs' cycle. While the reactions concerned with the synthesis of shikimic and quinic acids have been studied mainly in micro-organisms (49), the enzymes and pathways involved probably occur also in higher plants, according to Davis (10), Higuchi (20) and Srere (48). Tager (50) found the pentose cycle to be operative in the respiration of pre-climacteric bananas, but during the climacteric rise, there appeared to be a shift to the EMP pathway. Hulme isolated quinic acid (23) and shikimic acid (22) from apple fruits, and suggested that the former may be oxidized to citrate. The occurrence of these aromatic acids in apples and pears is of special interest from the standpoint of precursors in the formation of certain polyphenols which appear to be involved in tissue "browning" reactions, superficial scald and similar physiological disorders.

A major difference found between Bartlett and Anjou pears was in the concentration of citric acid in the tissues. In Bartlett pears both citric and malic acids occurred in high amounts in comparison to the other organic acids present. In Anjou pears malic was the single major acid, and citric acid was found in only small amounts. The reason for this difference is not clear from the

data obtained; however, there appear to be several possible explanations. In Anjou pears (Figure 54 B) pyruvate formed in glycolysis may be carboxylated directly to malic acid, thus by-passing the condensing reaction between pyruvate and oxaloacetate catalyzed by acetyl-conenzymeA. Conversely, in Bartlett pears this latter reaction may be very active (Figure 54 A). Another possibility is that the dehydrogenases involved in citric to succinic reactions are likewise very active in Anjou pears and prevent an accumulation of substrate. Malic also may be converted to citric acid in Bartlett pears via the glyoxylate cycle. Whatever the explanation, the carboxylation reactions between phosphoenolpyruvate and oxaloacetate, oxaloacetate and pyruvate as well as the dehydrogenations between malate and pyruvate and malate and oxaloacetate appear to play an important role in the metabolism of both varieties.

Bartlett and Anjou pears differed also in the changes which occurred in certain of the amino acids during storage and ripening. In Anjou pears proline was present in the fruit at time of storage. In Bartlett pears, proline was not found in CAS samples at any time during the storage period, but appeared in CS fruit after 4 months and in both CAS and CS pears during ripening. It thus appears that appearance of this particular acid in the free state occurs in Bartlett pears only at an advanced stage of senescence or during

ripening. Beta-alanine was detected in Anjou but not in Bartlett pears. It appeared in CAS samples during early storage but could not be detected in the CS fruit until the end of storage period. Beta-alanine could not be identified in ripe fruit of either treatment except in those ripened after 7 months' storage.

Alterations in the $CO_2$ and $O_2$ composition of the atmosphere were reflected in very marked changes in the organic acid and protein metabolism of both varieties. Total organic acid content of CAS pears tended to decrease at a much slower rate in storage and at the termination of the experimental period was 64 and 59 per cent higher, respectively, in CAS Bartlett and Anjou varieties. Malic acid showed the greatest effect from treatment, although tartaric acid also tended to be higher in the CAS fruits. Three possible explanations for the lower rate of acid loss in the CAS pears are suggested. First, rate of respiration has been found to be reduced by as much as 50-66 per cent in apples stored in modified atmospheres (54). Since loss of acid occurs through decarboxylations in the Krebs' cycle, rate of loss would be slower with reduced respiratory activity. This could account for the major difference in total acid concentration between the two treatments. A second possibility is that $CO_2$ fixation occurred in the CAS fruit as a consequence of the elevated $CO_2$ concentration. Goddard (16) found

that $CO_2$ fixation increased as the $CO_2$ concentration of the atmosphere was raised. This reaction has been shown to occur in pears (58). Allentoff, et al. (2) found that mature McIntosh apples were able to incorporate $C^{14}$ into malic acid and in aspartic and glutamic acids, alanine and serine. Malic acid formed by fixation of $CO_2$ was equivalent to about one-half of the daily loss. The contribution of this reaction to the production of amino acids and protein was also quantitatively significant. The fixation rose to a high level at time of harvest and continued to be high throughout the storage period. The uptake of $CO_2$ increased with rising concentration of $CO_2$ in the external atmosphere. Malic enzyme (41), which catalyzes the reaction, pyruvate + $CO_2$ + $TPNH_2$ $\longrightarrow$ malic acid + TPN is widely distributed in plants, according to Vennesland (57). Phosphoenolpyruvic carboxylase which is involved in the fixation of $CO_2$ by phosphoenol pyurvic acid to form oxaloacetic acid also is widely distributed in green and non-green plants (28, 38). These two reactions coupled with a third reaction involving oxaloacetic acid, $DPNH_2$ and malic dehydrogenase would lead to the synthesis of malic acid from phosphoenolpyruvate. Isocitric dehydrogenase studied by Moyle and Dixon (39), Ochoa (40) and Vennesland (56), which is also widely distributed in plants (60) could also bring about $CO_2$ fixation by catalyzing the reaction, alpha-keto-glutaric acid + $CO_2$ + $TPNH_2$ $\longrightarrow$ isocitrate + TPN. A third possible

reason for lower organic acid content of CS pears, could be a more active malic enzyme or malic dehydrogenase whereby malic acid could be converted to pyruvate or oxaloacetate. Alanine and aspartic acid could then be formed by transaminations, resulting in a reduction in malic acid and a net increase in amino acids.

The data obtained from the analyses of the various nitrogen fractions showed that protein synthesis not only to be an important phase of the metabolic processes in mature pears during storage and ripening, but also to differ significantly between the two treatments. That pears actually synthesize protein during storage and ripening is indicated by the changes which occurred in the various nitrogen fractions determined. Protein nitrogen tended to be higher in CS than in CAS Bartlett and Anjou pears during storage, while the concentrations of alcohol soluble and total amino nitrogen were lower. Fruit from both treatments showed net gains in protein nitrogen when ripened after removal from storage early in the season. In CS pears ripened at later dates, however, the magnitude of these increases declined until at the end of the storage, very little difference was found in protein nitrogen before and after ripening. In CAS pears, however, the capacity for protein synthesis during the ripening process was retained throughout storage periods of 5 and 7 months for Bartlett and Anjou pears,

respectively.

Since all experimental evidence indicates that the proteins of mature plant organs are in a state of flux and undergo continuous breakdown and resynthesis (59), the protein levels maintained represent a balance as determined by the relative rates of the two processes. That this situation exists in pears is suggested by the actual appearance and disappearance of specific amino acids as observed during storage and ripening. Conceivably, the failure of the CS pears to show a net gain in protein nitrogen when ripened late in the season could have been due to a high rate of breakdown rather than to a decrease in rate of synthesis. If this were true, significant increases in the alcohol soluble and total amino nitrogen fractions would be anticipated. Actually, increases of this nature did not occur, indicating that the differences found were due to a decrease in rate of protein synthesis.

A characteristic behavior of pears often occurring in commercial storage and observed in these experiments is the failure to ripen normally after prolonged periods in storage. No explanation for this behavior has been apparent in the past. The data obtained in this investigation suggest that this loss in ripening capacity conceivably could be a direct consequence of the observed inability of the fruit to synthesize protein when removed from

storage and held under conditions favorable for ripening. Previous investigations on other fruits indicate that the ripening processes actually are dependent upon the synthesis of protein. Thus, Hulme (24) found that there is a direct relation between the rate of respiration of apples and the content of protein. He also reported (25) that there is a net increase in protein during the climacteric rise in respiration of mature apples. This was observed to occur even in immature fruits in which the climacteric was induced by ethylene treatment (26). Conversely, it was found that when the respiration climacteric was delayed by storing apples in 10 per cent $CO_2$, the rise in protein content was also delayed (27). Pearson and Robertson (43) studied changes in protein and respiration of Granny Smith apples during development on the tree. Their data show a large increase in soluble nitrogen just after the beginning of the climacteric, while a net increase in protein synthesis did not occur until approximately 20 days later. These data would appear to be contradictory to those of Hulme. However, the latter's analyses were made on mature fruits during storage, while the former's were made on apples left on the tree until picked for chemical analyses and respiration determinations. Translocation of amino acids and other metabolites from senescent leaves to the fruit conceivably could occur prior to picking so that the changes in

protein metabolism observed during ripening on and off the tree would not necessarily be comparable. It is a common observation that pears do not soften and ripen normally if left on the tree beyond normal harvest maturity. Marks et al. (36) found the changes associated with ripening of tomatoes to be dependent upon a continual supply of energy. By inhibiting oxidative phosphorylation with dinitrophenol, the fruit failed to ripen, indicating that energy was a necessary requirement for the normal ripening processes. This relationship between ripening and protein synthesis becomes understandable if the protein synthesized consists at least in part of the specific enzymes required for the ripening processes.

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