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THE EFFECT OF TEMPERATURE, OXYGEN CONCENTRATION AND STORAGE INTERRUPTION ON PHYSIOLOGICAL DISORDERS OF 'MCINTOSH' APPLES

By

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ABSTRACT

THE EFFECT OF TEMPERATURE, OXYGEN CONCENTRATION AND STORAGE INTERRUPTION ON PHYSIOLOGICAL DISORDERS OF 'MCINTOSH' APPLES

By

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'McIntosh' apples are subjected to a physiological disorder termed 'brown core' when stored at 32°F, particularly in controlled atmosphere (CA) low in O_2 and high in CO_2. Accumulation of toxic metabolites is thought to be a contributing factor. Experiments were conducted with 'McIntosh' to determine the effects of hypobaric storage at 32°F and CA storage at 32° and 36°F in 1.5 and 3% O_2 on the development of physiological disorders. Storage treatments were applied continuously or intermittently by returning fruits to an atmosphere of air at the storage temperature or to 68°F.

Brown core was prevalent in fruits stored at 32°F and in 3% O_2 and was not attenuated by storage interruption. It worsened during 7 days at 68°F after 7 months of storage. Low temperature breakdown was observed in only one instance and was not related to storage temperature, O_2 level or storage interruption. The incidence of senescent breakdown increased as the O_2 level increased and was greater in fruits stored at 36° than at 32°F. The same results were found for scald with respect to O_2 level.

CA and hypobaric storage retarded the loss of acidity in 'McIntosh'. Concentration changes in organic acids of

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'Empire' apples were the same during aerobic and anaerobic metabolism at 68°F; malic decreased, citric remained constant, and succinic and fumaric acids increased.

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INTRODUCTION

Physiological disorders are often the primary factor limiting the preservation period of fresh apples during conventional refrigerated or controlled atmosphere storage. The disorders usually appear toward the end of the cold storage period and some become worse after transferring the fruits to warmer temperatures during the marketing period. This increases the magnitude of the problem. 'McIntosh' is particularly susceptible to the brown core disorder which has been responsible for considerable losses of this cultivar during storage.

The causal factors for several of the physiological disorders are unknown but lately the accumulation of some metabolites, namely tricarboxylic acid (TCA) cycle acids, had been implicated as a factor responsible for the appearance of some disorders. When certain of these metabolites accumulate, they are toxic and may eventually kill the tissue which in turn discolors. The incidence of some physiological disorders, especially brown core and low temperature breakdown, can be lessened in some cultivars by briefly returning the fruits to warm air at intervals during the storage period. This interruption treatment is thought to dissipate the accumulated metabolites allowing the fruits to be stored for a longer period of time.

This study was conducted to investigate the incidence of physiological disorders as influenced by temperature and oxygen concentration during storage. Also investigated was the effect of interim warming treatment and aeration at low temperatures at the midpoint during the storage period.

Analysis of TCA cycle acids was made to determine if oxygen deprivation causes changes in these metabolites that may lead to the development of physiological disorders.

REVIEW OF LITERATURE

This review of literature will deal primarily with physiological disorders of the 'McIntosh' cultivar with special reference to brown core since this disorder is the main problem encountered during the storage of this cultivar. Some attention will also be given to low temperature breakdown and to attempts to control both disorders in stored apples. The physiological and biochemical considerations pertinent to the subject will also be reviewed.

The nomenclature adopted for internal physiological disorders of apples is the one suggested by Smock (107) which is an agreement made by investigators from around the world concerning the terminology for those disorders.

A general description of the symptoms of the disorders dealt with in this review can be found in several other reviews (26, 28, 81, 85, 125).

Brown Core of Apples

1. Causal Factors

There is a great deal of controversy in the literature concerning the causal factors of brown core of stored apples, but the causes most frequently reported are: low storage temperature, high carbon dioxide levels and senescence.

However, most workers seem to agree that 'McIntosh' is the most susceptible variety to this disorder.

The factors that may influence the appearance of brown core are listed below.

a. Effects of Pre-harvest Factors

(1) Growing season

Susceptibility to brown core seems to vary from year to year with different climatic conditions during the growing season (13, 28, 106, 109, 110), and there are some indications that it may be worse following growing seasons that have been cloudy and cool (21, 85, 109, 123).

(2) Irrigation

In the cultivar 'Grand Alexander', a direct correlation was found between the frequency of irrigation and the incidence of brown core (98).

(3) Fertilization

Heavy nitrogen fertilization and high manuring are reported to increase the susceptibility of the fruits to brown core (21, 37, 85, 105, 109). Nitrogen exerted some effect other than merely influencing the growth of the fruit, since there was no correlation found between cell size and incidence of brown core (21, 57).

(4) Limb and fruit shading

Smock (106) found that limb shading consistently increased susceptibility to brown core. However, he found little to no effect due to fruit shading on the development of the disorder. The susceptibility to brown core in this

case seems to be more related to the position of the fruit in the tree, since Jackson (57) found more brown core in fruit from "inside" than fruits from "outside" the tree.

b. Effects of Pre-storage Factors

(1) Fruit source

Brown core is known to vary from orchard to orchard (4, 13, 28, 37, 63) and from area to area and from tree to tree in the same orchard (37). While these differences are recognized, no fully satisfactory explanations have been advanced for them.

(2) Fruit maturity

Most of the researchers on fruit storage present data showing that the more mature the fruits are at harvest time, the less brown core they develop during storage (26, 37, 75, 81, 85, 105, 107, 109). However, some researchers obtained opposite results in which the more immature fruits were less susceptible (63, 126), while others had inconsistent results (9, 13, 57). Fidler and North (28) stated that the influence of maturity of the fruit at the beginning of the storage period depends on the variety.

(3) Delayed storage

It has been suggested that a five day delay before storage would greatly decrease the amount of brown core (90). For the 'Starking Delicious' cultivar a 48 hr. period was also reported to reduce the incidence significantly (75). Other studies have shown that delayed storage did reduce the intensity of the problem but that this finding had little

practical application (79, 106, 109). A delay in storage of 4 to 6 days at 38°C has also been reported to eliminate the problem in 'Spartan' and 'Golden Delicious' apples (87).

(4) Applied chemicals

Phorone (2,6 - dimethyl - 2,5 - heptadien - 4 - one) markedly reduced brown core when fruits were exposed to vapors (0.25 to 1 g. per 25 fruit) during storage. The action of phorone in reducing brown core was found to be reasonably specific since a large number of compounds with a similar structure to phorone were found to have no effect (95).

GA_3 had little to no effect in controlling the disorder (95) when applied as a post-harvest dip or injection into the fruit.

B - 995 (N-dimethylaminosuccinamic acid) when sprayed once during the growing season (2,500 ppm B-995) markedly increased brown core in stored fruits (96).

DPA (diphenylamine) applied as a pre-storage dip (1,000 ppm) also reduced the incidence of brown core (71), but no explanations were advanced.

c. Effects of Fruit Factors

(1) Fruit size

Larger fruits have long been recognized as more susceptible to brown core (4). Light crops, which produce bigger fruits, or fruits from trees heavily fertilized with nitrogen, tend to develop more brown core during storage (4, 37, 109). However, Wilkinson (123) stated that the

available evidence suggests that fruit size, as such, is not a factor influencing brown core.

(2) Fruit color

Fisher and Porritt (37) have noted that poorly colored fruits were more likely to have brown core at the end of storage. Smith (105) observed that fruits with the highest percentage of green and lower percentage of yellow as ground color developed more brown core during storage. It seems that poor color is an indication of more immature fruits and therefore more susceptible to develop the problem.

(3) Seeds

The effect of seed number in the fruit on brown core was first noticed by workers in Canada (81). They demonstrated that brown core is related to a particular phase of physiological activity of seeds. Brown core did not develop when the seeds were killed by controlled dosages of irradiation. However, the same type of treatment failed to prevent brown core in New Zealand apples (81). Côme (11), working with apples in France, suggested that the seeds do not exert any effect on the development of brown core; on the contrary, the disorder had a detrimental effect on the seeds, since it rendered the embryos unable to germinate.

d. Effects of Storage Factors

(1) Temperature

The majority of the reports concerning brown core in apples, especially in 'McIntosh' fruits, implicate low temperature of storage as being the main cause and several

investigators suggest that brown core is essentially a low temperature disorder (4, 37, 81, 85, 106, 107, 109). However, inconsistent or even contradictory results can be found in the literature concerning the role of temperature in causing brown core. Meheurick and Porritt (75) found little or no effect due to temperature on brown core and Fidler and North (28) stated that this injury had no correlation with temperature of storage between 0°C (32°F) and 3°C (37.4°F) and also that the cultivars 'Fameuse' and 'Baldwin' had less brown core at 0°C than at 2°C (35.6°F) to 4°C (39.2°F).

(2) Humidity

In an experiment with fruits subjected to different degrees of water loss, Wilkinson (124) observed that the percentage of brown core was greater in apples which had lost the most weight, and so it was suggested that permeability of the fruit skin to gaseous exchange decreases rapidly when evaporation takes place and that the greater effect of early water loss may be due to a physical effect resulting in restricted ventilation of the intercellular air spaces (123). However, Scott and Wills (94) noted that there was less brown core when apples were stored in the presence of compounds which absorbed water and furthermore, while the loss of water reduced brown core, the addition of water to the fruit increased the incidence of the disorder.

Forsyth and Lightfoot (40) also noted that high humidity would increase considerably the amount of brown core in stored 'McIntosh' fruits.

(3) Carbon dioxide and oxygen levels

Fidler and North (28) are among those who strongly support the view of brown core as being caused by CO_2. In fact, they presented evidence from storage trials over a period of ten years in which the incidence of brown core was always more severe in the presence of CO_2 than in its absence, although the incidence varied greatly from year to year and from one orchard to another. This same view is expressed in a number of other reports (26, 27, 75, 94, 125). However, it was recognized by Fidler and North (28) that brown core is a senescence problem since it only occurs toward the end of the storage period.

Some evidence presents brown core as being a consequence of an association of low temperature and high CO_2 atmospheres (over 3%), especially in the presence of high O_2 levels (67, 106, 112).

Eaves *et al.* (19) found a high susceptibility to brown core in fruits from trees with low nitrogen, as determined by leaf analysis, stored in presence of CO_2 at all O_2 concentrations above 2.5%. In contrast, the disorder appeared to be reduced by CO_2 in the high nitrogen fruit.

The benefits of CA storage on controlling this disorder have been widely recognized (81, 85, 106-109, 117) and Pierson *et al.* (85) attribute to this fact the promotion of CA as the principal method of storing this variety.

Controlled atmosphere storage of apples as it is being investigated nowadays, namely low levels of O_2 (2.5-3%)

and almost no CO_2 in the storage environment (49), would seem to have a protective effect in relation to the incidence of brown core, since evidence suggests that apples will develop less injury at low O_2 levels (26-28, 125). However, low O_2 by itself was not sufficient in preventing the appearance of the problem (86). In addition, some inconsistent results were found in which brown core was aggravated, but not necessarily caused, by storage in controlled atmospheres (13,29).

(4) Length of the storage period

The fact that apples show the injury only after a certain period of storage (generally 3 months) led some researchers to state that brown core is a senescence disorder (26, 28, 125). Three interesting hypotheses were developed to try to explain this phenomenon (28). The first accounts for an increase in susceptibility to the injury as fruit ages; the second relates the disorder to the duration of the exposure to the adverse conditions causing it; and the third suggests that the internal concentration of CO_2 increases as the apples age.

There is no available evidence on the question of changing susceptibility. However, Fidler and North (28) stated that the rate of production of CO_2 by 'Cox's Orange Pippin' apples, stored at $3^{\circ}C$ and 5% CO_2 : 16% O_2, rises slowly after about six weeks in storage and that the same happens in CO_2 - free atmospheres, but the rise is progressively delayed as O_2 concentration is reduced. Therefore, if we assume no change in permeability of the fruit, the internal

CO_2 concentration must increase during storage. If CO_2 is, in fact, the adverse factor in causing brown core then this evidence fits perfectly the second and third hypotheses advanced by Fidler and North (28). Furthermore, there is some published evidence (Kidd and West, 1939 cited in 28) that the permeability of the fruit to CO_2 decreases after the climacteric. If this is so, it will contribute to the rise in the internal CO_2 concentration.

(5) Ethylene levels

The effect of atmosphere of storage containing low levels of ethylene on brown core was first noticed by Forsyth et al. (39). They found significantly less disorder in 'McIntosh' fruits after storage for 189 days at 3.3°C in chambers containing $KMnO_4$. Forsyth and Lightfoot (40) confirmed this observation with 'McIntosh' apples.

More recently Scott and Wills (94) found no correlation between brown core and the ethylene level of the storage environment in an experiment in which apples were stored in plastic bags in the presence of $KMnO_4$ at 10°C. It is interesting to notice that this temperature is considered to be safe with respect to brown core incidence (107, 109).

e. Effects of Post-storage Factors

(1) Holding period at elevated temperatures after storage

Brown core has been noticed to increase in severity and amount after the apples are removed from cold storage and placed in warmer temperature (28, 37, 81, 85) and this

feature of the disorder seems to be very consistent since it happened with fruits grown in the United States (85), in New Zealand (81), in British cultivars (28) and also in Canada (37).

2. Association of Brown Core to Other Disorders

The association of brown core with the disorder known as stem-cavity browning has long been noticed, first by Rasmussen (90) in 1937 who described briefly a browning of the skin of the stem cavity of 'McIntosh' apples and referred to it as "stem-end breakdown" and then by Smith (105) in 1942 who reported a similar condition as "stem-end rot". The brief treatment by these authors caused these reports to go largely unnoticed. However, McColloch (73) in 1966 finally demonstrated the association of the two disorders and proposed the possibility of them having a common cause.

Webster et al. (122) studied the stem-cavity browning of 'McIntosh' apples as influenced by fruit size, position in the blossom cluster and shape of the stem-cavity region. They found apples from terminal clusters more susceptible to the disorder than apples from lateral clusters. Larger fruits also developed more stem-cavity browning as well as fruits with shallow stem cavities.

Webster and Eaves (121) found that the incidence of brown core and stem-cavity browning increased with increase in fruit size. They confirmed their previous results, namely stem-cavity browning being more severe in terminal apples than in lateral apples and decreasing with increase in stem-cavity depth.

Lougheed et al. (70) recognized several features in common for both disorders: the increase in incidence after removal from storage; the highest incidence in immature fruits; and considerable variation among years and locations. However, they suggested that the physiological relationship is not clearly defined and regarded the association of both disorders as being a coincidence.

Low Temperature Breakdown

Low temperature breakdown (LTB) has been reviewed by Faust et al. (21) and more recently by Wilkinson and Fidler (125) in an excellent review.

It seems obvious for this particular disorder that the cause is a low temperature of storage and the time of exposure of the fruits to that low temperature (21, 26, 107, 125). However, a number of other factors militate to aggravate the injury and will be mentioned here.

The fact that LTB varies greatly from year to year and from location to location indicates that it can be influenced by pre-harvest or orchard factors. Perring (84) observed that LTB is less likely in apples with high levels of K, P and Mg than in apples with low levels of these elements.

An interesting feature of LTB is that the disorder becomes worse if the apples are cooled when they are at a critical physiological state, namely in the climacteric rise (26) and this seems to be the origin of some contradictory reports on the effect of delayed storage on LTB. Delayed storage can either increase (63) or decrease (12) LTB,

depending on the physiological stage of the fruits when subjected to the delaying treatment.

The composition of the storage atmosphere is known from early days to have an effect on the extent and severity of LTB (125). Accumulation of CO_2 to levels above 5% has been reported to be detrimental to the quality and to increase LTB (125). More recently, Knee and Bubb (63) found that levels of 5% CO_2 + 3% O_2 instead of 8-10% CO_2 significantly decreased the incidence of LTB in 'Bramley's Seedling' apples.

A decrease in susceptibility in LTB was found in Tasmanian apples stored at low levels of O_2, but this effect was shown to be subject to seasonal variations in English cultivars (125).

Humidity in the storage environment was also noted as having an effect in LTB. High humidity is known to increase its incidence while low humidity generally decreases the incidence (125).

Alleviation of Brown Core and Low Temperature Breakdown

Most storage trials are conducted at constant temperatures, but it is possible to arrange an interruption of the exposure to the low temperature by introducing one (interim) or more (intermittent) periods at a higher temperature, or to arrange a gradual reduction or a gradual increase of the temperature during storage. The first two methods are perhaps the less feasible in practice but are effective in reducing the incidence of both low temperature breakdown and brown core.

Smith (103) was probably the first to utilize interim warming for storage of fruits. He found that low temperature injury in plums was virtually eliminated by using an interim warming treatment whereas storage at constant low temperature for an equivalent period of time resulted in extensive injury. Subsequently a "dual temperature method" for storage of plums was developed in which fruits were kept at 31°F for 5 days and then the temperature was raised to 45°F to 50°F (101). The theoretical basis for this treatment derived from the assumption that while capacity to ripen is impaired sooner or later by low temperatures, the injury is not immediate, or at least is not, in its earliest stages, irreversible.

More recently, it was found by Smith (104) that an interim warming on the 16th day at 65°F for 2 days resulted in an extension of the period of storage in 'Victoria' plums, regularly stored in 1% O_2 and 34°F, by reducing the amount of low temperature injury.

Following the early work with plums, the interim warming treatment was applied to peaches and nectarines (2) and apples (32, 53, 80, 102, 103, 125).

Interim warming was proved to be an effective method of controlling low temperature breakdown in apples (53, 102, 103, 125).

Intermittent warming treatment (in which the fruit receives more than one warming period) has also been used in controlling brown core in apples (67, 80, 81, 107).

Padfield (80) reported a good control of brown core in 'Granny Smith' apples when fruits stored at 31°F were warmed for three periods after 6, 9 and 13 weeks at 64-65°F for 2 days. Only one warming period (either at 6, 9 or 13 weeks) was not useful in controlling the problem.

Landfald (67) also reported a reduction in brown core when Scandinavian apples stored at 32°F were warmed for periods of 5 days at 59°F.

Hypothesis Advanced to Explain Brown Core and Low Temperature Breakdown

1. Brown Core

Despite the considerable amount of literature on brown core, very few hypotheses have been advanced to explain the appearance of the disorder. This is probably because there is still a great deal of controversy concerning the cause of this injury.

Basically, the disorder has been regarded as due to senescence (Carne, 1958 cited in 28), as a form of low temperature injury (81, 106, 107), or as a form of carbon dioxide injury (27, 28). No hypotheses were presented for the first two possible causes. However, Fidler and North (28) presented their hypothesis on brown core as being caused by CO_2. The evidence in the literature clearly supports this hypothesis in view of the reports on brown core being aggravated by high levels of CO_2, especially at high O_2 levels. Most of the time brown core was more severe in the presence of CO_2 than in CO_2-free atmospheres. Furthermore, it has been

demonstrated that the respiration rate of 'Cox's Orange Pippin' apples at 3°C and 2% O_2 is only approximately 60% of the respiration rate of fruits kept at the same temperature in air (28). Consequently, one would expect less CO_2 accumulation in apples preserved in low O_2 concentrations.

Brown core was also tentatively linked with senescence on the basis that apples will slowly increase their CO_2 production throughout storage (28). Also, if permeability to CO_2 decreases after the climacteric, as suggested by Kidd and West, 1939 (cited in 28), one should expect an enhancement of the internal CO_2 concentration as the fruits age.

2. Low Temperature Breakdown

Although it is generally accepted that LTB is caused by an exposure of the fruits to a low temperature for a certain period of time, there is not an agreement on how the low temperature effect brings about the appearance of the disorder.

The first hypothesis was advanced by Plank in 1941 (cited in 103). He suggested that poisoning of the cells could occur through the accumulation of a volatile product of the metabolism. Several investigators have tried to prove this hypothesis by linking LTB to the accumulation of several volatile substances during the storage period. Wills *et al.* (132) observed that increased rates of water loss, which reduce susceptibility of 'Jonathan' apples to LTB, caused a reduction in the level of acetic acid in the fruit. Increasing the level of acid by the injection of acetic acid into

the fruit increased the incidence of the disorder. They suggested that acetic acid could promote LTB and its removal as acetate esters or free acid would reduce the disorder.

Wills and Scott (131) also found that apples injected with acetate, mevalonate, malonate, formate, butyrate, benzoate, caffeine, amytal, ATP and octanol showed an increase in LTB after storage at -1°C. They suggested that isoprenoid metabolism may be involved in the metabolic events leading to LTB. It was also shown that fruits stored at -1°C retained the greatest amount of acetic acid (129). At 0°C and 2.5°C the amount retained was also high, but at 5°C and 10°C it was very low. The loss of butyl, isopentyl and hexyl acetates was highest at 10°C and decreased markedly with decreasing temperature. It was suggested that LTB does not occur at higher temperatures because the increased loss of acetate as esters results in a reduction of the amount of acetic acid available to produce the disorder (129).

Acetaldehyde has also been shown to accumulate in fruits subjected to low temperatures, however, Smagula and Bramlage (100), in a recent review on the involvement of acetaldehyde accumulation on physiological disorders of fruits, concluded, based on a whole body of evidence, that its accumulation is a result, rather than cause, of tissue disorganization.

Accumulation of non-volatile substances has also been shown to occur in fruits showing LTB. Fidler and North (33-35) have shown an accumulation of sorbitol in fruits injured by low temperature but a causal connection was not established.

LTB also increased when geraniol and a number of inhibitors of isoprenoid synthesis were injected into the fruit after harvest (130).

Probably the most known hypothesis on the mechanism of LTB is the one put forward by Hulme et al. (53). They found a relationship between the accumulation of oxaloacetic acid (OAA) in the tissue and subsequent development of LTB in cold stored apples. A short interim warming treatment reduced both the accumulation of OAA and intensity of LTB. They suggested that LTB is caused by an interference in the operation of the Krebs cycle in the tissue.

However, Fidler and North (31) have more recently demonstrated that the disorder is not entirely dependent on OAA concentration since they found comparatively high levels of OAA in fruits not showing LTB.

Organic Acid Metabolism

Organic acid metabolism, especially that of the intermediates of the tricarboxylic acid (TCA) cycle, may be important in physiological disorders such as brown core and low temperature breakdown. The metabolism of organic acids in fruits was reviewed by Ulrich (116) and in the apple fruit specifically by Hulme and Rhodes (49).

Organic acids are widespread throughout higher plants. Buch (7) listed 79 different nonvolatile, non nitrogen-containing, carboxylic acids present in higher plants from which 18 were present in the apple fruit, either in the pulp or in the peel.

The organic acid composition of apple fruits varies in both amount and type of acids according to variety and location (60).

At early stages of the fruit development, quinic acid accounts for more than 50% of the total acid content of the cortical tissue (45). As the fruit matures the concentration and amount of this acid decreases rapidly (45), and it was suggested that it may be oxidized to citric and malonic acid (47). Malic acid reaches a peak 50-60 days after petal fall and begins to decline (54), and at maturity it predominates, being reported to vary from 80% (66) to 95% (49). Citric acid remains at a low and steady concentration throughout fruit development (54).

Krotkov et al. (66) reported that while malic acid decreased after harvest, "other organic acids" tended to increase. And the same observation was made by Robertson (93) who noted that "unknown organic acids" increased rapidly compared to malic and citric acid through the climacteric rise.

The metabolism of organic acids has been studied in apples subjected to low temperature and CA storage because of the physiological problems that may arise from such preservation methods.

Kollas (64) found a much greater total acid concentration in 'McIntosh' fruits from CA (3% O_2 + 5% CO_2 at 38°F) storage as compared to regular cold storage at 32°F after 6 months of storage. However, three acid peak fractions

were higher in fruits from air storage. We can interpret these results in basically three ways: a) by supposing a difference in the rate of depletion of the acids; b) by supposing a greater production of the acids in CA storage; or c) a combination of both.

Malic acid loss has been demonstrated to be independent of oxygen since it was the same in air and in nitrogen over a period of 80 days at 12°C (24). There is also evidence that 'McIntosh' apples in 5% CO_2 produce malic acid at significant rates by fixation of CO_2 (1, 77). Thus it was suggested that the higher malic acid content in apples stored in CA atmosphere may have resulted from CO_2 fixation (64). Fidler and North (30) advocate that CA storage reduces the rate of loss of acid from the fruit.

Hulme and Wooltorton (55) observed that citric acid in the apple pulp increased from about 6mg/100g fresh tissue to almost 10mg/100g fresh tissue over a period of 100 days at 15°C and that citric from the peel remained the same at about 1.8mg/100g fresh tissue throughout.

An accumulation in both CA and air storage of oxaloacetic acid (OAA), pyruvic and α-oxoglutaric acids was also observed (53). An interim warming treatment after 5 weeks of storage for 5 days at 15°C was sufficient to lower the level of the acids to their original concentration which then started to accumulate again toward the end of storage.

Conversion of succinic acid -^{14}C into fumaric acid -^{14}C was slightly less in apples kept in high CO_2 atmosphere than

those kept in air (77).

It is generally accepted that CA storage and/or low temperature storage can cause an accumulation of some organic acids in apples (43, 53, 77, 99) as well as in other fruits (56, 69, 74, 99, 120, 127). The acids reported to accumulate are OAA (25, 53, 74, 133) and succinic acid (43, 46, 56, 65, 88, 120, 127). However, Wills and McGlasson (128) were not able to find any accumulation of OAA, ∝-keto-glutaric and pyruvic acids during storage of 'Jonathan' apples at -1°C.

Generally, it is assumed that high levels of CO_2 in CA storage would interfere in the activity of succinic dehydrogenase (41, 61) causing succinic acid to accumulate. High levels of CO_2 also interfered with the activity of succinic oxidase (89) and succinic-cytochrome C reductase (6).

The activity of citrate synthase was also inhibited by low temperature and there was an accumulation of ∝-keto acids in banana peel (76).

High levels of succinic acid were found in apples exposed to unusually high CO_2 concentrations (up to 20%), but only when the fruits were kept at low temperatures (37°F) since high CO_2 at 50°F did not cause succinic acid to accumulate (46). It was noticed that the accumulation of succinic acid was accompanied by carbon dioxide injury and it was proposed that succinic acid poisoning was the cause for CO_2 injury (46). However, intentional application of succinate to the peel of apple fruits was not sufficient to

cause CO_2-associated peel injury in 'Golden Delicious' apples (68). Furthermore, it was recently found that in the presence of high CO_2, transfer of fruits from 0°C to 21°C inhibited further induction of CO_2 injury but did not inhibit the accumulation of succinic acid (43). This suggests that CO_2 injury may be a low temperature and CO_2 interaction and rules out succinic acid as a sole causal agent.

Apple fruits decarboxylate malic acid oxidatively (malate effect) to pyruvate (14, 38, 59, 62, 91) and subsequently to acetaldehyde and alcohol (10, 78, 91).

Dilley (14) demonstrated malic enzyme activity in 'McIntosh' fruits in the oxidative decarboxylation of malate and postulated that the malate effect is a reflection of metabolic processes concerned with pyruvic acid utilization and that is perhaps regulated by the availability of oxidized pyridine nucleotide.

Since the demonstration of the operation of the classical Krebs cycle-cytochrome oxidase respiratory system in cut tissue and mitochondria from apple fruits (44), a great number of reports dealing with the metabolism of TCA cycle intermediates in apple tissue have been put forward (20, 48, 50-52, 58, 91, 97).

Worthy of mention is the "in vivo" inhibitory action of OAA to all TCA cycle oxidations found in mitochondria of potato tubers and mung bean hypocotyls (115) and the action of CO_2 in several reactions of the cycle (118, 119). CO_2 at 18% stimulated malate oxidation about 10%; suppressed

α-ketoglutarate, citrate and NADH oxidations about 10% and suppressed fumarate, pyruvate and succinate oxidations about 32% in 'Richared Delicious' apple mitochondria (97). This experiment suggests that CO_2 can have a strong controlling effect on apple mitochondrial activity. The suppressed oxidations of succinate and citrate could explain the reported accumulations of these acids under high CO_2. The effect of CO_2 on mitochondria was clearly not due to an effect on a single enzyme, such as succinic dehydrogenase. Many enzyme systems must be sensitive to CO_2 and to different degrees.

Behavior of Apple Fruits Under Low Oxygen and Anaerobic Conditions

Apples will shift their respiration from aerobic to anaerobic (alcoholic fermentation or zymasis) when subjected to very low levels or complete absence of oxygen leading to an accumulation of considerable amounts of ethyl alcohol and smaller amounts of acetaldehyde (22).

The extinction point for anaerobic respiration in many tissues is below 2% oxygen (88). Early in the storage season, in 'Newton Wonder' and 'Bramley's Seedling' apples, the extinction point lies between 1 and 3% oxygen, but it was noticed that the extinction point shifts to higher concentrations of oxygen later in the storage season, and eventually in senescent apples alcohol may accumulate even at 100% oxygen (113).

Fidler (23) observed that the capacity for anaerobic respiration of apples would change over the storage period

and would follow the same seasonal course as aerobic respiration. McLean et al. (72) observed a similar seasonal variation in the capacity for anaerobic respiration, but they concluded that the loss in the capacity for anaerobic respiration was due to the successive cycles of anaerobiosis experimentally imposed previously on the fruits.

The carbon dioxide output in apples kept in anaerobiosis is lower than those kept in air (18, 24, 35). The ratio of anaerobic to aerobic CO_2 production was shown to be lower in fruits from CA storage than that of fruits from air storage, indicating a conserving effect of the modified atmosphere on the mechanism of aerobic CO_2 production (18). The ratio of anaerobic to aerobic CO_2 production by 'McIntosh' fruits increased during ripening at 20°C immediately following harvest (18).

Fidler (24) observed that the presence of oxygen has a conserving effect on the loss of carbohydrate (Pasteur effect) and that the loss of carbon is greater in nitrogen than in oxygen. He found that anaerobic respiration in apples is identical, as far as the nature and quantity of the end products are concerned, with the alcohol fermentation of yeast. It was also observed that the amount of carbon dioxide produced by apples in nitrogen is equivalent to that which would be produced by oxidation of malic acid.

Loss of CO_2 plus alcohol was equivalent to the loss of carbohydrate plus acid (35).

The "localization" theory of the Pasteur effect, which assumes the localization of the glycolytic enzymes and substrates in certain points of the cell, was demonstrated in apples (5), but Barker and Khan (5) were not able to consistently demonstrate the "activation/inactivation" theory of the Pasteur effect, i.e., that hexokinase and phosphofructokinase are activated in anoxia and inactivated in air.

Hulme (45) showed that oxaloacetic, pyruvic and ∝-ketoglutaric acids would decrease from their initial levels to very low levels when apple fruits were kept at 15°C under nitrogen atmosphere during five days with, presumably, an accumulation in their precursors since, on readmission of air, there was a rapid and temporary burst in their rate of production.

The effects of periods of anaerobiosis on the storage of apples can be detrimental in other respects, apart from the accumulation of alcohol. Anaerobiosis can increase the incidence of brown core and breakdown (36). Apple scald was also found to be greatly induced by deliberate anaerobiosis (17, 72).

MATERIALS AND METHODS

Fruit Material

Fruits of the 'McIntosh' cultivar (*Malus domestica* cv. McIntosh) used in the first experiment were obtained from four different locations comprising four different lots. Lots 1 and 2 were obtained from two different orchards at the Graham Experiment Station in Grand Rapids, lot 3 from a commercial orchard at Laingsburg and lot 4 from another commercial orcahrd at Leslie, Michigan. Fruits from all lots were pre-climacteric having less than 0.1 ppm internal ethylene. For the second experiment only fruits from lot 1 were used.

The fruits were harvested and transported to the Horticulture Research Center on the same day where they were randomized and placed in refrigerated storage (36°F) while waiting for the other fruit lots. Harvest dates were as follows:

Lot 1 - September 21, 1978

Lot 2 - September 26, 1978

Lot 3 - September 29, 1978

Lot 4 - September 29, 1978

For the third experiment, the 'Empire' cultivar (a cross between 'McIntosh' and 'Red Delicious'), harvested on October 3, 1979 from Graham Station and kept in hypobaric

storage at 40 mmHg and 31.5°F for 34 days, was used.

Treatments

1. First Experiment

The first experiment was carried out in a commercial CA (Controlled Atmosphere) storage facility. The one chosen was Bull's Storage located at Casnovia. The cold storage control treatments for this experiment were kept in refrigerated storage rooms at the Horticulture Research Center, Michigan State University (MSU) at 32°F and 36°F in air. The temperatures and controlled atmospheres available at Bull's Storage were 32°F and 36°F with 3% O_2 + 3% CO_2. The fruits were transferred to the definitive storage temperatures and atmospheres on October 18 and the treatments were as follows:

(1) 32°F, 3% O_2 + 3% CO_2 continuously
(2) 32°F, 3% O_2 + 3% CO_2 w/interruption at 3 months for 2 days at 68°F
(3) 32°F, 3% O_2 + 3% CO_2 w/interruption at 3 months for 14 days at 32°F
(4) 36°F, 3% O_2 + 3% CO_2 continuously
(5) 36°F, 3% O_2 + 3% CO_2 w/interruption at 3 months for 2 days at 68°F
(6) 36°F, 3% O_2 + CO_2 w/interruption at 3 months for 14 days at 36°F
(7) 32°F in air continuously
(8) 32°F in air w/interruption at 3 months for 2 days at 68°F

(9) 36°F in air continuously

(10) 36°F in air w/interruption at 3 months for 2 days at 68°F

One box containing 150 fruits, approximately, was used for each of the four lots comprising a total of approximately 600 fruits per treatment.

Fruits from the interim warming treatments were removed from storage after 3 months and placed at room temperature (68°F) for 2 days and then returned to their original storage conditions. Some of the treatments were only interrupted at 3 months with respect to the storage atmosphere but remained at the same storage temperature for 14 days and then were returned to the original atmospheres. The CA control treatments were kept continuously at 3% O_2 + 3% CO_2 at 32°F and 36°F.

Fruits of all treatments remained in storage for 7 months.

2. Second Experiment

For the second experiment only fruits from the lot 1 were used. A box containing approximately 150 fruits was used per treatment and all the fruits were stored at the Horticulture Research Center at MSU.

For the CA treatments, gas tight aluminum drums with a volumetric capacity of approximately 1.7m^3 were used and the desired atmospheres were achieved by flushing initially or upon atmosphere re-establishment with nitrogen until the

desired gas levels were reached. The oxygen and carbon dioxide levels were monitored daily with an Orsat gas analyzer. Fresh air was admitted whenever the oxygen dropped below the desired levels. Atmospheres containing 1.5 and 3% $\pm$ 0.2% of O_2 were used in the CA treatments. The carbon dioxide levels were kept below 1% with the aid of dry lime placed in the interior of the CA chambers. The chambers were kept at 32°F and 36°F.

The hypobaric storage treatments were maintained in a cylindrical tank with a volumetric capacity of approximately 1.16m^3 placed inside of a storage room at 32°F and hence were "jacket" cooled (Fig.1). The storage pressure (50 mmHg) was achieved by withdrawing the air continuously with a Kinney rotary oil seal vacuum pump located outside the storage room and at the same time admitting ethylene-free air saturated with water vapor through a humidifier maintained at the reduced pressure to avoid dehydration of the stored fruits (15, 16). The coming air was regulated to the desired pressure by a Fisher type 98 LD vacuum regulator and the flow of the air through the system was throttled by an exhaust valve leading to the vacuum pump. The humidifier was kept outside the storage room and had a constant water level regulated by a Sensall Model 501 ultrasonic liquid level switch which activated a solenoid valve to admit filtered and de-ionized water whenever necessary (Fig.1).

The fruits were placed under the treatment conditions on September 25. The interim warming treatments were made

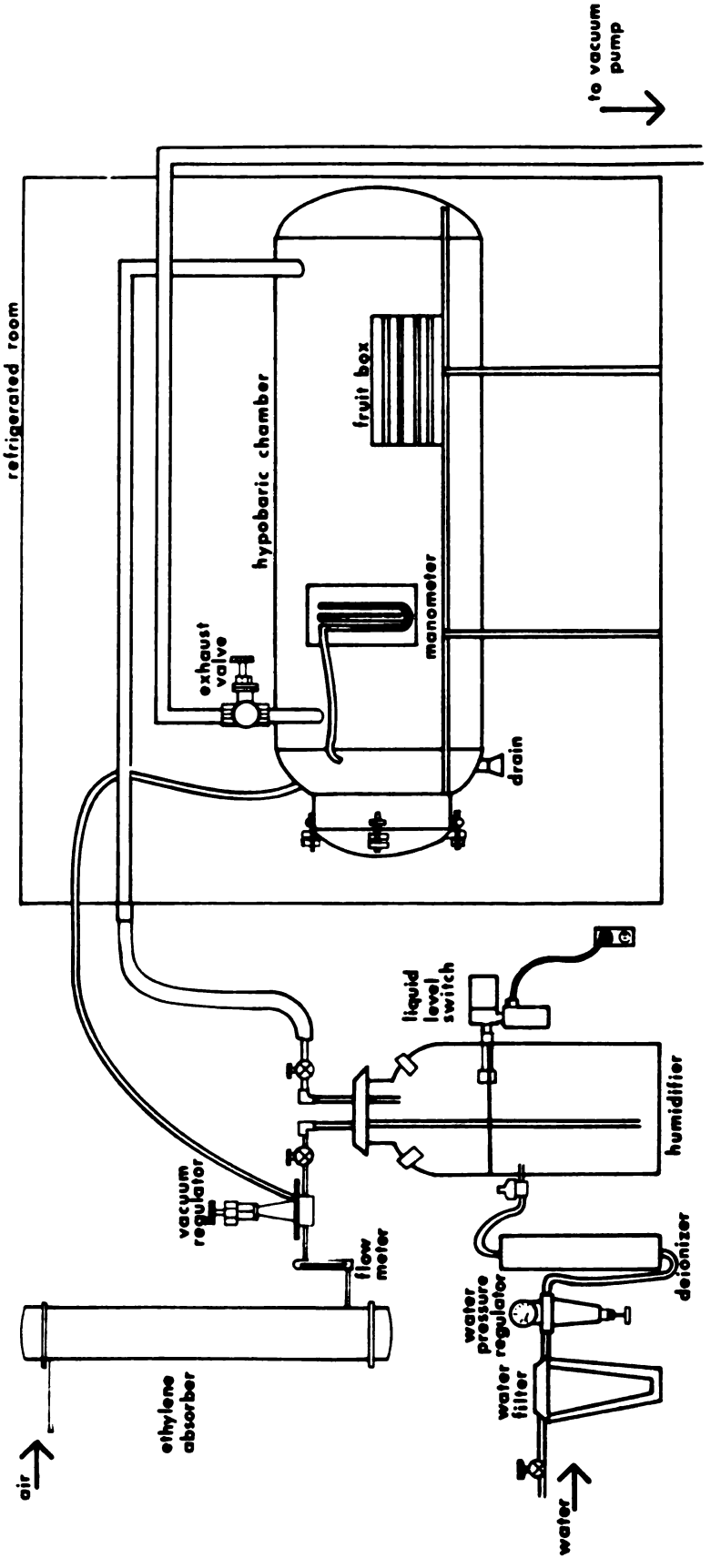

Figure 1. Schematic representation of the hypobaric storage system used in the second experiment.

by interrupting storage after 3 months and transferring the fruits to air for 2 days at 68°F and then returning the fruits to their original storage conditions. A period of approximately 5 hours was necessary to warm the fruits to 68°F. Some of the treatments consisted of interrupting the storage atmosphere but maintaining fruits at the same storage temperature for 14 days after which they were returned to their original storage conditions. There were CA, hypobaric and common refrigeration control fruits which were kept at 32°F and 36°F continuously. The treatments were the following.

(1) 32°F, 3% O_2 continously

(2) 32°F, 1.5% O_2 continuously

(3) 36°F, 1.5% O_2 continuously

(4) 36°F, 3% O_2 continuously

(5) 32°F, 3% O_2 w/interruption at 3 months for 2 days at 68°F

(6) 32°F, 1.5% O_2 w/interruption at 3 months for 2 days at 68°F

(7) 36°F, 1.5% O_2 w/interruption at 3 months for 2 days at 68°F

(8) 36°F, 3% O_2 w/interruption at 3 months for 2 days at 68°F

(9) 32°F, 3% O_2 w/interruption at 3 months for 14 days at 32°F

(10) 32°F, 1.5% O_2 w/interruption at 3 months for 14 days at 32°F

(11) 36°F, 1.5% O_2 w/interruption at 3 months for 14 days at 36°F

(12) 36°F, 3% O_2 w/interruption at 3 months for 14 days at 36°F

(13) 32°F, 50mmHg continuously

(14) 32°F, 50 mmHg w/interruption at 3 months for 2 days at 68°F

(15) 32°F, 50 mmHg w/interruption at 3 months for 14 days at 32°F

(16) 32°F in air continuously

(17) 32°F in air w/interruption at 3 months for 2 days at 68°F

(18) 36°F in air continuously

(19) 36°F in air w/interruption at 3 months for 2 days at 68°F

All the treatments remained in storage for 7 months. In the hypobaric chamber, a malfunction of the humidifier caused the fruits to remain submerged in water for a few days prior to the end of the experiment.

3. Third Experiment

Thirty fruits of the 'Empire' variety ranging from 111 to 188 grams were divided into two lots of fifteen fruits and each lot subjected to a different atmosphere treatment. Each fruit was placed in individual respiration chambers with a volume of 18 ml and a flow rate in the range from 18 to 20 ml/min. The temperature of the room was kept at 20°C throughout the experiment. One lot received ethylene-free air and the other received pure nitrogen.

At given intervals (0, 8, 32 and 128 hours) samples were drawn consisting of three fruits from each lot and subsequently analyzed for organic acids. Samples of the gas atmosphere inside the respiration chambers were also taken at periodic intervals for CO_2 analysis.

Fruit Maturity at Harvest

The maturity of the fruit lots was determined following harvest by measuring the internal concentration of ethylene of 10 fruits from each lot. One ml gas samples were taken from apples submerged in water and injected into a Varian aerograph series 1700 gas chromatograph which used nitrogen at 60°C as the carrier gas and was equipped with a 2mm x 1m column of activated alumina and a flame ionization detector.

Fruit Firmness

Fruit firmness was determined with an Effe-gi fruit tester equipped with a probe of 11.1mm (7/16 inch) diameter. Determinations were made at the beginning of the storage, after 3 months of storage, at the end of the interruption periods, at the end of the storage period, and after 7 days at 20°C following storage. At the beginning of the storage period, 50 fruits per treatment were used and two measurements were made on opposite sides of the fruits. After 3 months of storage and the end of the interruption periods, 10 fruits per treatment were used and 4 measurements were made per fruit. At the end of the storage period and after 7 days following storage, 20 fruits per treatment were used and 2 measurements were made on opposite sides of the fruits. In the case of 2 measurements per fruit, one of the measurements was taken in the blushed side of the fruit.

Internal and External Disorders

Fruits were examined for disorders after 3 months of storage and after the interruption periods using 10 fruits

per treatment and at the end of the storage period and after 7 days following removal from storage using 30 to 90 fruits per treatment. A cut was made longitudinally in each fruit and both surfaces were visually examined. Fruits were then classified into different categories.

1. Brown Core

Brown core was characterized by a browning of the cortical parenchyma in the intervascular region. This is generally a very characteristic arrow-shaped discoloration in the core region. The browning sometimes extended beyond the core region. Four arbitrary categories were chosen as follows:

None - Fruits completely sound.

Slight - Brown core hardly discernible. Only identified by a trained person.

Medium - Brown core in a level as to be easily identified by a non-trained person, but not in a level as to render the fruit unmarketable.

Severe - Brown core in a level as to render the fruit unmarketable.

2. Low Temperature Breakdown

Low temperature breakdown was characterized by an evenly distributed discoloration of the whole cut surface of the fruit. Sometimes a clear halo of unaffected tissue was noticed beneath the skin. The same four categories as above were used to classify this disorder.

3. Senescent Breakdown

This disorder, also known as internal breakdown, was characterized by the softening and crumbling of the flesh and by the eventual browning of the affected area. The disorder was classified in four categories as described above.

4. Scald

Superficial scald was identified by the characteristic browning of the fruit peel which became worse following removal from storage. In all cases, only the peel of the fruits was affected, the underlying tissues remaining sound. The fruits were classified according to the degree of incidence of the disorder using the same four categories as above.

Titratable Acidity

Titratable acidity was determined prior to storage, after 3 months of storage, after the storage interruption periods and at the end of the storage period.

For the initial determinations (prior to storage) 12 fruits per lot were used and for the subsequent determinations 5 fruits per treatment were used. Apples were peeled, cored, and sliced and the pieces were randomized. Fifty grams of tissue were weighed and placed in a Waring blendor with 100ml of distilled water. The fruit tissue was blended for one minute at full speed and filtered on a Buchner filter flask using two layers of milk filter discs. An aliquot of 25ml was titrated against a standardized solution of 0.1N NaOH. The titratable acidity was calculated as percentage of malic acid.

Organic Acids

1. Organic Acids Extraction

The method used for organic acids extraction was especially developed for the purpose of the present study and is herein described.

A 50 gram sample of apple pulp tissue was blended with 100ml of boiling absolute ethanol in a Waring blendor for 5 minutes at full speed. The slurry was filtered through two layers of milk filter and two layers of Whatman's filter paper no. 2 in a Buchner filter flask to remove cell wall material and the coagulated pectins. The ethanol was then eliminated in a rotary evaporator (Rotavapor, Buchi) at 20°C and the remaining aqueous extract was made basic to pH 8.5 with concentrated NH_4OH and passed over a column of 1.5 x 30cm packed with 18ml of a precycled (12) strong anion exchange resin (Amberlite IRA-400 grade 1-X8) in the acetate form to avoid degradation of the sugars (8).

The flow rate was maintained in the range from 0.5 to 1ml/minute. The resin with the absorbed organic acids was washed with 10 bed volumes of distilled water to remove all the remaining sugars and then was eluted with 40ml of 1N HCl. The eluate was then concentrated in a rotary evaporator at 35°C to an appropriate volume to be filtered and injected in the chromatographic system. Using this method of extraction, recoveries very close to 100% were obtained when samples were spiked with known amounts of standard acids.

Hydrochloric acid present in the eluate made the final concentration process difficult, but addition of concentrated NaOH was found to facilitate the process and therefore the acids were analyzed in their salt form.

2. Organic Acids Analysis

For the analysis of the organic acids present in the samples a modification of the method suggested by Waters Associates, Inc. was used.

The High Performance Liquid Chromatograph (HPLC) system consisted of a Waters Associates solvent delivery pump (model 6000A), a Waters Associates Universal liquid chromatograph injector (model U6K), a Waters Associates Differential Refractometer detector (model R401) and two μ BONDAPAK C_{18} columns (Waters Associates, Milford, Mass.).

The mobile phase was a buffer solution of 0.1M $NH_4H_2PO_4$ with pH adjusted to 2.5 with H_3PO_4 and the flow rate maintained at 1.4ml/min. with an operational pressure in the pump of 3000psi. This procedure gave a better separation of the acids, a better response in terms of peak height and was faster than the procedure suggested by the manufacturer of the equipment.

With this procedure, all of the organic acids of interest present in the samples could be analyzed in 9 minutes.

The standards used were acids of high purity obtained from Sigma Chemical Co. and a typical chromatogram of the standards can be seen in Figure 2.

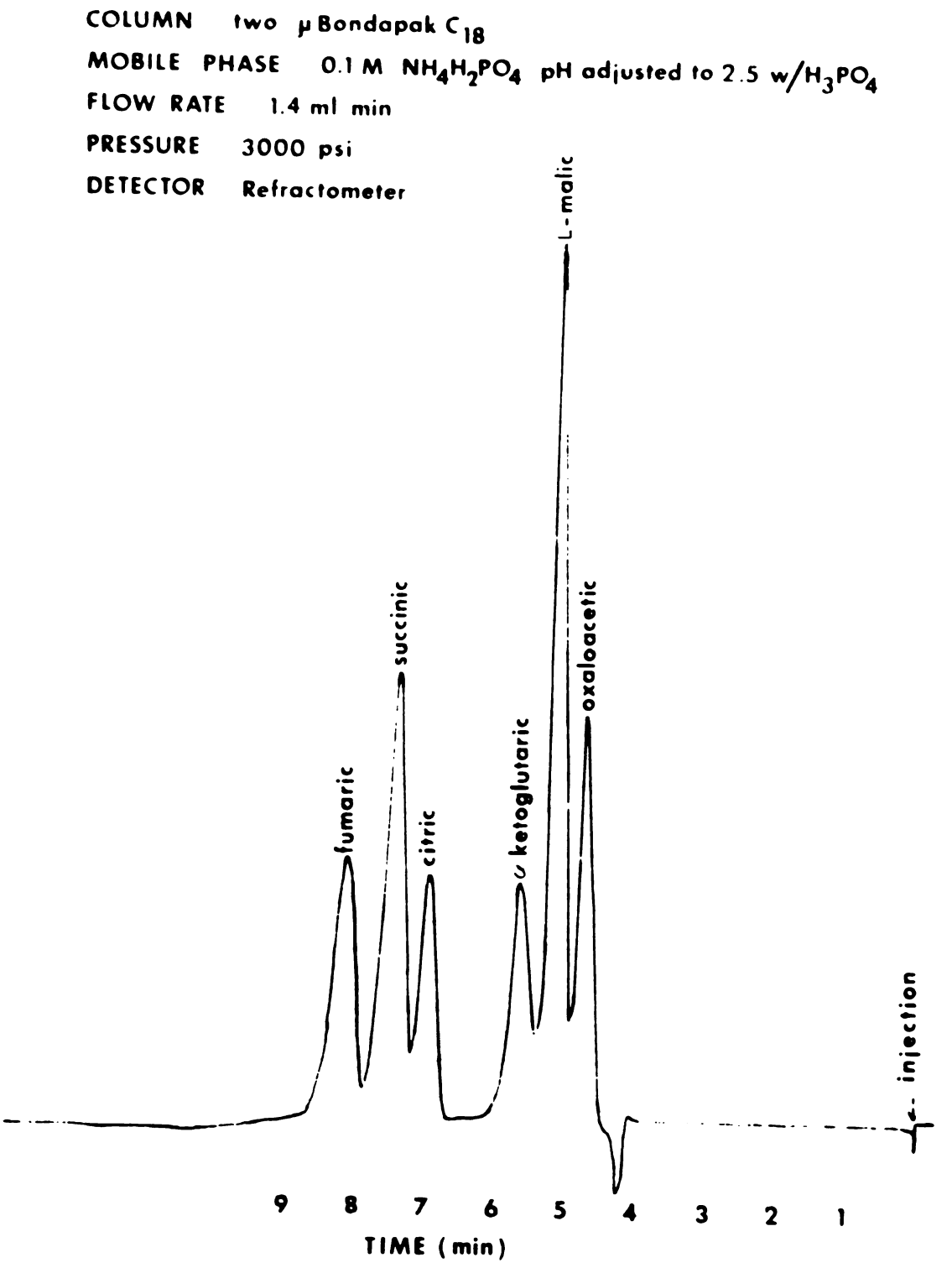

Figure 2. Separation of the tricarboxylic acid cycle acids by HPLC.

The peaks appearing in the chromatograms of the samples were measured for retention time and compared with the retention time of the peaks from the chromatograms of the standards and also samples were mixed with authentic organic acids to additionally confirm the identity of the acids.

This system provided qualitative and quantitative analysis for malic, citric, succinic and fumaric acid in the apple extracts. Oxalacetic acid could not be quantified because of the appearance in the chromatograms of the samples of an unidentified peak which exceeded by far the normal concentration of this acid in the fruit. There was an overlap of the acetate peak (from the anion exchange column) with the peak of ∝-Ketoglutaric acid and therefore it also could not be quantified.

Carbon Dioxide Determinations

Gas samples were taken from the individual respiration chambers, previously described, with a 2cc syringe through septa located at the lid of the chambers and analyzed in a gas chromatograph equipped with a thermal conductivity detector (Carle Instruments, Inc. GC 8700) using Helium as carrier gas.

Statistical Analysis

Statistical analysis of data was by analysis of variance procedure. Means comparison was by Duncan's multiple range test.

To analyze the effect of the treatments on the physiological disorders a procedure was developed as follows:

For the category "none" an arbitrary value of one was assigned, for "slight" a value of three, for "medium" a value of 6 and for "severe" a value of 10. The number of fruits going into each category was multiplied by the category value and the results were summed and divided by the total number of fruits used for the observation. This procedure generated a single value for each physiological disorder at each observation period which was then used for statistical analysis.

RESULTS

First Experiment

The first experiment tested the incidence of physiological disorders under commercial CA storage conditions and included fruits from 4 orchards.

The results for firmness and titratable acidity expressed as percentage of malic acid of the first experiment can be observed in Table 1.

After 7 months of storage, firmness was retained better in CA storage even when a temperature of 36°F was employed as compared to air storage. Interruption treatments didn't significantly affect firmness and the air stored fruits which received a 2 days at 68°F interruption had, in fact, a higher firmness after 7 months of storage, but this effect was not noticed in CA stored fruits. After the holding period, the same trend was observed; namely, CA stored fruits were firmer than air fruits. No statistical significance was found for temperature and interruption effects or for the temperature/interruption interaction.

The total acidity content of CA stored fruits was significantly higher after 7 months of storage than that of air stored fruits. The acidity of the fruits from the storage interruption treatments was generally higher than the acidity

Table 1. Firmness and titratable acidity of 'McIntosh' apples stored for 7 months under different temperature, atmosphere composition and storage interruption regimes.

Treatments[w]	Firmness (lbs) After storage[y]	Firmness (lbs) Plus 7 days at 20°C	Titratable Acidity (% malic acid) after storage[y]
32°F CA[x] continuously	10.5 ab[z]	9.1 ab	0.259 a[z]
32°F CA (2 days at 68°F)	10.3 ab	9.6 a	0.274 a
32°F CA (14 days at 32°F)	10.5 ab	9.3 ab	0.244 a
36°F CA continuously	10.8 a	9.4 ab	0.252 a
36°F CA (2 days at 68°F)	10.1 abc	9.4 ab	0.274 a
36°F CA (14 days at 36°F)	10.3 ab	9.0 abc	0.268 a
32°F Air continuously	9.3 de	8.3 cd	0.177 b
32°F Air (2 days at 68°F)	9.9 bcd	8.7 bcd	0.201 b
36°F Air continuously	9.1 e	8.3 cd	0.160 b
36°F Air (2 days at 68°F)	9.4 cde	8.2 d	0.190 b

[w] All storage interruption treatments were made after 3 months of storage for the periods and conditions indicated in the parentheses.

[x] CA = 3% CO_2 + 3% O_2

[y] Firmness at harvest was 14.1 lbs. and the titratable acidity was 0.66% malic acid.

[z] All means in a column followed by the same letter are not significantly different by the Duncan's multiple range test at 5% level.

of the fruits from continuous storage and this effect was found significant at the 2.5% level for the air stored fruits but not for the CA stored fruits.

The incidence of brown core was higher in air stored fruits than in CA stored fruits after 7 months of storage (Table 2). Brown core was slightly higher in both CA and air stored fruits at 32°F than at 36°F. No statistical significance was found for this effect in CA stored fruits, but in air stored fruits a significance at the 2.5% level was observed for the temperature effect. Interruption of the storage conditions had no effect on brown core development. Significant differences (at 0.1% level), however, were observed in the amount of brown core among the different lots of fruits after 7 months of storage. After holding the fruit in air at 20°C, brown core generally increased in all treatments, and the same trend was observed with air storage having more incidence of brown core than CA stored fruits and the disorder being worse at 32°F than at 36°F. Interruption of the storage environment had no effect on the final amount of the disorder. Significant differences were also found in the amount of the disorder among the different lots of fruits (at 0.1% level).

The effect of the different treatments on the amount of superficial scald was not found to be significant after 7 months of storage. For CA stored fruits, interruption of the storage environment tended to increase scald (significant at 1% level). This effect was not observed in air stored

Table 2. Physiological disorders[w] of 'McIntosh' apples stored for 7 months under different temperature, atmosphere composition and storage interruption regimes.

Treatments[x]	Brown Core		Scald		Senescent Breakdown	
	After storage	Plus 7 days at 20°C	After storage	Plus 7 days at 20°C	After storage	Plus 7 days at 20°C
32°F CA[y] continuously	1.42 c[z]	1.85 c	2.66n.s.	4.48 bcd	1.00	1.39 bc
32°F CA (2 days at 68°F)	1.40 c	1.52 c	2.87n.s.	4.04 d	1.00	1.20 c
32°F CA (14 days at 32°F)	1.43 c	1.81 c	3.01n.s.	5.03 bcd	1.00	1.47 bc
36°F CA continuously	1.35 c	1.27 c	2.69n.s.	4.19 cd	1.00	1.07 c
36°F CA (2 days at 68°F)	1.26 c	1.19 c	3.06n.s.	4.22 cd	1.00	1.08 c
36°F CA (14 days at 36°F)	1.23 c	1.26 c	3.43n.s.	4.96 bcd	1.00	1.10 c
32°F Air continuously	4.56 a	6.68 a	3.62n.s.	6.22 ab	1.00	2.74 a
32°F Air (2 days at 68°F)	4.05 ab	6.92 a	3.61n.s.	6.98 a	1.00	2.12 ab
36°F Air continuously	3.14 b	3.90 b	3.78n.s.	5.68 abcd	1.00	1.43 bc
36°F Air (2 days at 68°F)	3.09 b	4.25 b	3.21n.s.	5.87 abc	1.00	1.69 bc

[w] Weighed physiological disorder = (None x 1) + (Slight x 3) + (Medium x 6) + (Severe x 10)/ number of fruits examined; ranging from 1 = no disorder to 10 = all fruits severely affected by disorder.

[x] All storage interruption treatments were made after 3 months of storage for the periods and conditions indicated in the parentheses.

[y] CA = 3% CO_2 + 3% O_2

[z] All means in a column followed by the same letter are not significantly different by the Duncan's multiple range test at 5% level.

fruits which showed lower amounts of scald when previously subjected to the storage interruption treatment. Different sources of fruits had significantly different levels of scald (at 0.1% level).

During the 7 day post-storage holding period scald increased in all treatments. No marked benefit was evident from the storage interruption treatments on the final amount of scald. In fact, CA stored fruits which were interrupted showed increased scald on holding after storage. Air stored, which previously showed less scald when interrupted, showed more scald after the holding period. Air stored fruits had a significantly higher level of scald when kept at 32°F (at 5% level), but this effect was not observed in CA stored fruits. The final amount of scald showed to be significantly different (at 0.1% level) among the different lots of fruit.

Senescent breakdown was absent in all the treatments following 7 months of storage. But after the holding period, fruits stored at 32°F had more breakdown than those kept at 36°F (significant at 5% level) in both CA and air stored fruits. Senescent breakdown was slightly more severe in fruits kept in air at 32°F. No clear-cut benefit was observed due to the storage interruption treatments and breakdown was significantly different among the different fruit lots (5% level) in CA stored fruits.

Second Experiment

The second experiment was conducted in order to gain additional information on the factors involved in the

physiological disorders, especially brown core and low temperature breakdown using the more precise and reliable research facilities available at the Horticulture Research Center at MSU. Hypobaric environment, apart from its excellent prospects as a storage method, is a very good research tool whenever one wants to study the involvement of ethylene or other volatiles in a given physiological process, and therefore, was included in the experiment.

Due to physical constraints we were unable to replicate each treatment, which reduces considerably the scope of inference of the experiment, and this should be considered in interpreting the results. Statistical analysis was conducted by analysis of variance for the first 12 treatments (CA treatments) which are arranged in a factorial experiment and the significance was tested by the F test. The main effects analyzed were temperature, O_2 concentration and interruption treatments. The air storage treatments were analyzed separately but, perhaps due to the lack of replications and to the small number of treatments, no significant differences were found.

The fruits used in this experiment were in the preclimacteric stage at the beginning since their internal ethylene concentration averaged 0.1ppm (average of ten fruits).

The results of firmness and titratable acidity expressed in percentage of malic acid are shown in Table 3. After 3 months of storage firmness was higher in the fruits stored at $32^{o}F$ and 1.5% O_2 (statistically significant at 0.1% level). The interaction between temperature and oxygen level was also

Table 3. Effect of temperature, oxygen concentration, atmospheric pressure and storage interruption on firmness and titratable acidity of 'McIntosh' apples.

Treatments[x]	Firmness (lbs)[y]			Titratable Acidity (% malic acid)	
	3 months	7 months	Plus 7 days[z]	3 months	7 months
32°F 3% O_2 continuously	15.0	14.2	13.2	0.651	0.435
32°F 1.5% O_2 continuously	15.3	16.8	15.3	0.622	0.473
36°F 1.5% O_2 continuously	14.8	16.1	15.5	0.552	0.388
36°F 3% O_2 continuously	12.7	11.3	10.6	0.599	0.266
32°F 3% O_2 (2 days at 68°F)	14.7	14.4	14.4	0.585	0.424
32°F 1.5% O_2 (2 days at 68°F)	15.2	16.2	16.3	0.645	0.480
36°F 1.5% O_2 (2 days at 68°F)	14.8	14.7	15.1	0.611	0.336
36°F 3% O_2 (2 days at 68°F)	12.5	11.8	11.0	0.547	0.325
32°F 3% O_2 (14 days at 32°F)	14.6	14.9	12.1	0.594	0.405
32°F 1.5% O_2 (14 days at 32°F)	15.3	17.3	15.9	0.610	0.506
36°F 1.5% O_2 (14 days at 36°F)	14.7	14.6	14.8	0.630	0.420
36°F 3% O_2 (14 days at 36°F)	12.4	12.6	12.2	0.686	0.421
32°F Hypobaric continuously	15.2	16.1	13.5	0.583	0.386
32°F Hypobaric (2 days at 68°F)	15.5	15.4	14.4	0.547	0.368
32°F Hypobaric (14 days at 32°F)	15.2	15.2	13.5	0.630	0.355

Table 3 (cont'd.)

Treatments[x]	Firmness (lbs)[y]			Titratable Acidity (% malic acid)	
	3 months	7 months	Plus 7 days[z]	3 months	7 months
32° Air continuously	12.1	12.9	9.7	0.517	0.290
32° Air (2 days at 68°F)	12.1	12.1	11.3	0.580	0.294
36° Air continuously	11.3	11.5	9.4	0.504	0.262
36° Air (2 days at 68°F)	10.8	11.4	10.1	0.445	0.204

[x] All storage interruption treatments were made after 3 months of storage for the periods and conditions indicated in the parentheses.

[y] The firmness at harvest was 15.8 lbs. and the titratable acidity was 0.833% malic acid.

[z] Holding period consisted of an additional week at 68°F after the 7 months of storage.

significant at the 0.1% level suggesting a possible interdependence of both factors up to that time in storage.

After 7 months of storage, oxygen was more efficient than temperature in maintaining the firmness, since fruits kept at 1.5% O_2 had higher firmness values (significant at 2.5% level) regardless of the storage temperature. However, the temperature effect was also significant at the 5% level. The interruption treatments didn't affect the firmness enough to be significant. After the additional holding period of 7 days at 68°F, however, only the fruits from the treatments which had 1.5% O_2 showed high firmness values (significant at 5% level) and no effect due to temperature was observed.

No significant differences in acidity were detected after 7 months of storage and also after the additional holding period of 7 days at 68°F. This was probably because of the limited number of observations taken with the experimental set-up used.

Firmness of the hypobarically stored apples was consistently high up to 7 months in storage as compared to firmness of CA stored and air stored fruits. However, near the end of the storage period a failure of the humidifier caused the hypobaric chamber to be filled with water and this unexpected problem, though promptly corrected, resulted in a very unfavorable environment for the fruits for a short period of time, and this is probably the origin of the lower levels of firmness and acidity of hypobaric fruits, as compared to some of the CA storage treatments. Fruits kept at 32°F and 1.5% O_2 had the highest firmness throughout the experiment.

The results for physiological disorders can be seen in Table 4.

No significant differences were found in brown core at the end of the storage period for CA stored fruits. Brown core was absent in hypobaric storage and slightly higher in air stored fruits after 7 months of storage. However, after the holding period at a high temperature, in which brown core is enhanced, brown core was found worse in air stored fruits and significantly higher in 3% O_2 fruits as compared to 1.5% O_2 fruits (significant at 2.5% level). Interruption of the atmosphere in low O_2 treatments yielded no clear-cut benefit. Interruption for 14 days at low temperature had the least brown core in low O_2 treatments after the holding period. Fruits from continuous hypobaric storage treatment had more brown core than those fruits interrupted for 14 days at 32°F. And interruption for 2 days at 68°F had the least brown core. For the fruits kept in air the trend was similar in that fruits that had been interrupted for 2 days at 68°F showed less brown core after the holding period of 7 days at 68°F than if held continuously in storage at 32°F.

Scald showed no significant difference among the treatments for CA stored fruits after 7 months of storage. A trend, however, was observed in which fruits kept at 3% O_2 showed consistently more scald than those kept at 1.5% O_2.

For hypobaric storage a browning of the peel resembling scald was observed in the fruits after 7 months of storage and was, therefore, rated as scald. This may be related to

Table 4. Effect of temperature, oxygen concentration, atmospheric pressure and storage interruption on physiological disorders[x] of 'McIntosh' apples after 7 months in storage and after 7 days at 20°C.

Treatments[y]	Brown Core		Scald		Senescent Breakdown		Low Temperature Breakdown	
	7 months	Plus 7 days	7 months	Plus 7 days	7 months	Plus 7 days	7 months	Plus 7 days
32°F 3% O_2 continuously	1.03	1.12	2.26	3.17	1.00	1.11	1.14	2.94
32°F 1.5% O_2 continuously	1.03	1.15	1.41	2.05	1.00	1.00	1.26	1.33
36°F 1.5% O_2 continuously	1.00	1.05	1.45	1.19	1.00	1.03	1.03	1.09
36°F 3% O_2 continuously	1.10	1.11	2.36	4.84	1.00	1.78	1.73	2.01
32°F 3% O_2 (2 days at 68°F)	1.07	1.15	2.46	4.07	1.00	1.00	1.07	1.10
32°F 1.5% O_2 (2 days at 68°F)	1.03	1.03	1.83	2.16	1.00	1.00	1.03	1.11
36°F 1.5% O_2 (2 days at 68°F)	1.03	1.07	1.95	1.49	1.00	1.00	1.13	1.17
36°F 3% O_2 (2 days at 68°F)	1.00	1.23	3.04	8.44	1.00	2.39	1.08	1.85
32°F 3% O_2 (14 days at 32°F)	1.03	1.00	1.29	3.27	1.00	1.21	1.03	3.45
32°F 1.5% O_2 (14 days at 32°F)	1.12	1.00	1.88	3.13	1.00	1.03	1.16	1.48
36°F 1.5% O_2 (14 days at 36°F)	1.03	1.02	1.65	2.59	1.00	1.36	1.07	1.48
36°F 3% O_2 (14 days at 36°F)	1.00	1.07	2.55	4.58	1.00	2.13	1.20	2.20
32°F Hypobaric continuously	1.00	1.28	2.75*	2.08*	1.16	2.31	1.29	4.01
32°F Hypobaric (2 days at 68°F)	1.00	1.15	3.36*	1.68*	1.72	1.75	1.32	1.76
32°F Hypobaric (14 days at 32°F)	1.00	1.24	3.52*	2.42*	1.45	2.58	1.45	3.78

Table 4 (cont'd.)

Treatments[y]	Brown Core		Scald		Senescent Breakdown		Low Temperature Breakdown	
	7 months	Plus 7 days	7 months	Plus 7 days	7 months	Plus 7 days	7 months	Plus 7 days
32°F Air continuously	1.43	6.23	3.33	7.62	1.14	2.07	1.09	1.74
32°F Air (2 days at 68°F)	1.46	4.72	3.21	9.54	1.30	2.43	1.03	1.37
36°F Air continuously	1.28	5.75	3.78	9.23	1.00	3.57	1.20	1.38
36°F Air (2 days at 68°F)	1.31	3.28	4.91	9.09	1.38	2.34	1.23	1.51

[x] Weighed physiological disorders = (None x 1) + (Slight x 3) + (Medium x 6) + (Severe x 10)/ number of fruits observed; ranging from 1 = no disorder to 10 = all fruits severely affected by disorder.

[y] All storage interruption treatments were made after 3 months of storage for the periods and conditions indicated in the parentheses.

* Browning of the peel resembling scald by visual evaluation (see text for explanation).

the flooding mishap near the end of the storage period. This injury decreased after the holding period.

Air stored fruits showed a higher amount of scald as compared to CA stored fruits after 7 months of storage.

After the holding period at room temperature, a greater amount of scald was found in fruits kept at 3% O_2 as compared to 1.5% O_2 (significant at 2.5% level) confirming the trend observed after 7 months of storage. Scald appeared worse in fruits kept at 36°F than at 32°F, however, this difference was not found statistically significant.

Senescent breakdown was absent in low O_2 storage fruits after 7 months of storage, showing only after the holding period at high temperature. At that point, fruits which were kept at 36°F showed more senescent breakdown (significant at 5% level) as well as fruit from the 3% O_2 treatments (significant at 5% level). Interruption treatments showed no effect on the level of senescent breakdown. In air stored fruits the same temperature trend was observed, namely, fruits stored at 36°F having more disorder than those kept at 32°F after the holding period. Senescent breakdown was higher in both observation periods in hypobaric storage and air storage as compared to low oxygen storage.

No significant difference was found in low temperature breakdown following 7 months of storage or after the holding period in low oxygen or in air. However, fruits kept at 3% O_2 showed a tendency to have more LTB after the holding period.

Hypobaric storage, however, showed a greater amount of LTB after the holding period when the fruits were kept continuously in storage and decreased when an interruption treatment was used, with 2 days at 68°F being more efficient in reducing the level of LTB than 14 days in air at low temperature. However, as noted earlier, this damage may be related to anoxia from a flooding mishap to the hypobaric chamber.

Third Experiment

1. Organic Acids

Comparison of the results was done by the analysis of variance and the differences evaluated by the F test.

No statistical difference was found in the amount of organic acids between aerobic and anaerobic atmospheres when the two sets of data were compared at the same observation period throughout the experiment.

However, when two sets of data from the same treatment (aerobic or anaerobic) were compared at different observation periods, some statistical significance was found indicating changes in the amount of some acids during the experimental period. The results for individual acids are described below.

a. Malic Acid

The results from the High Performance Liquid Chromatography determinations of malic acid are shown in Figure 3.

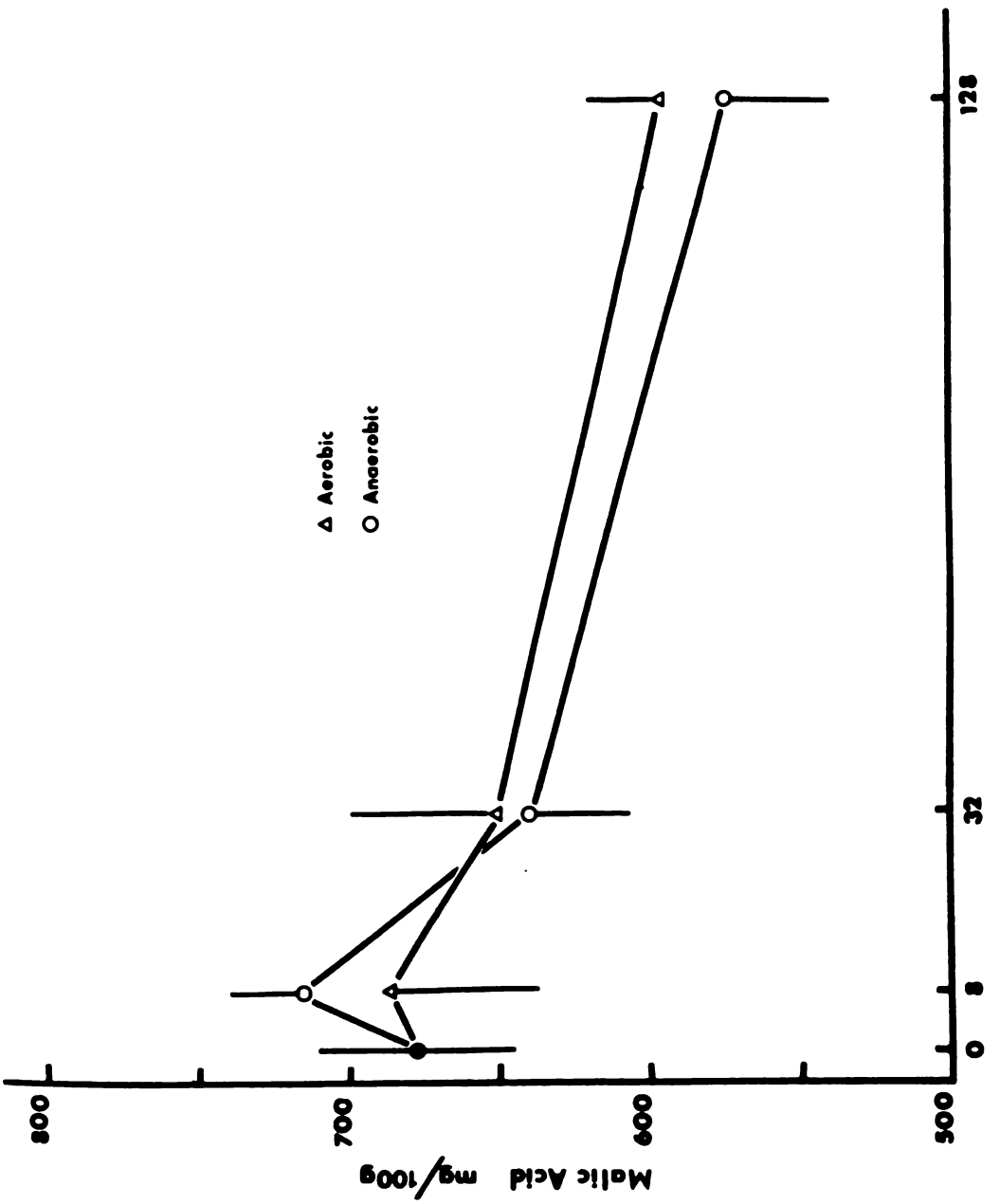

Figure 3. Changes in malic acid content of 'Empire' apples kept in air or nitrogen atmospheres at 20°C. Each data point represents an average of 3 determinations using individual fruits. Standard deviations of the means are shown for each data point.

Aerobic and anaerobic fruits behaved similarly in respect to malic acid content during the experiment period and, in fact, no statistical difference was found between the two treatments.

Although the data suggests a decrease in the amount of malic acid in both aerobic and anaerobic conditions over the period of 128 hours, only anaerobic fruits were found significantly different from the initial concentration at 1% level.

b. Citric Acid

The level of citric acid, in both aerobic and anaerobic stored fruits, remained constant during the experiment period (Figure 4). The data shows the anaerobic fruits with a citric acid content consistently higher than aerobic fruits, but no statistical differences were found between the two treatments in any of the three observation periods and also no statistical differences were found when the same treatment was compared among the different observation periods, which shows that the amount of the acid was not changed by any of the treatments and that it remained constant throughout the experiment period (128 hours).

c. Succinic Acid

The amount of succinic acid in aerobic and anaerobic fruits showed a similar behavior (Figure 5). They initially decreased slightly toward 8 hours and then increased toward 32 hours, aerobic fruits having a more pronounced increase than anaerobic fruits. The level then remained constant toward 128 hours and at the end of the experiment they had

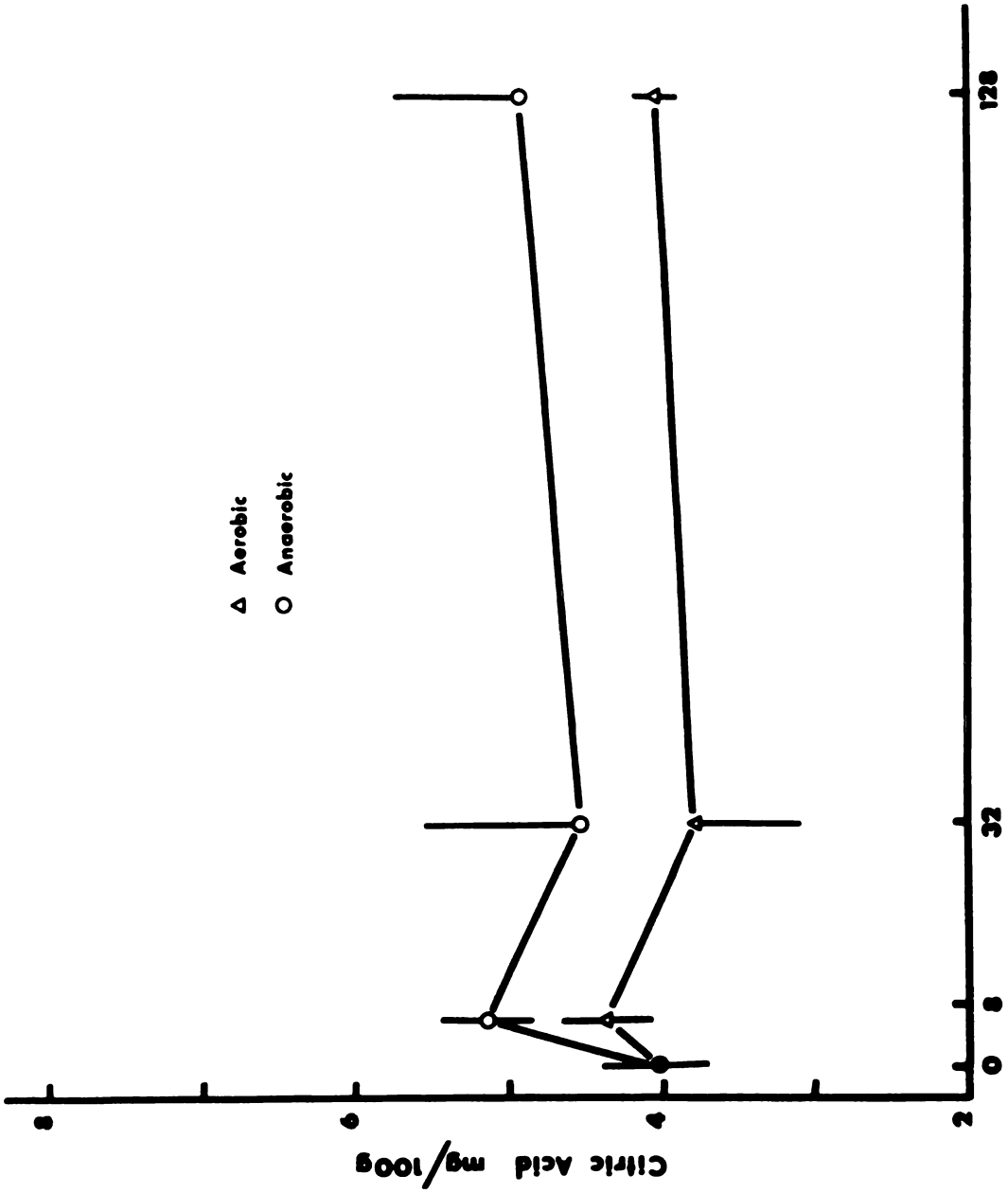

Figure 4. Changes in citric acid content of 'Empire' apples kept in air or nitrogen atmospheres at 20oC. Each data point represents an average of 3 determinations using individual fruits. Standard deviations of the means are shown for each data point.

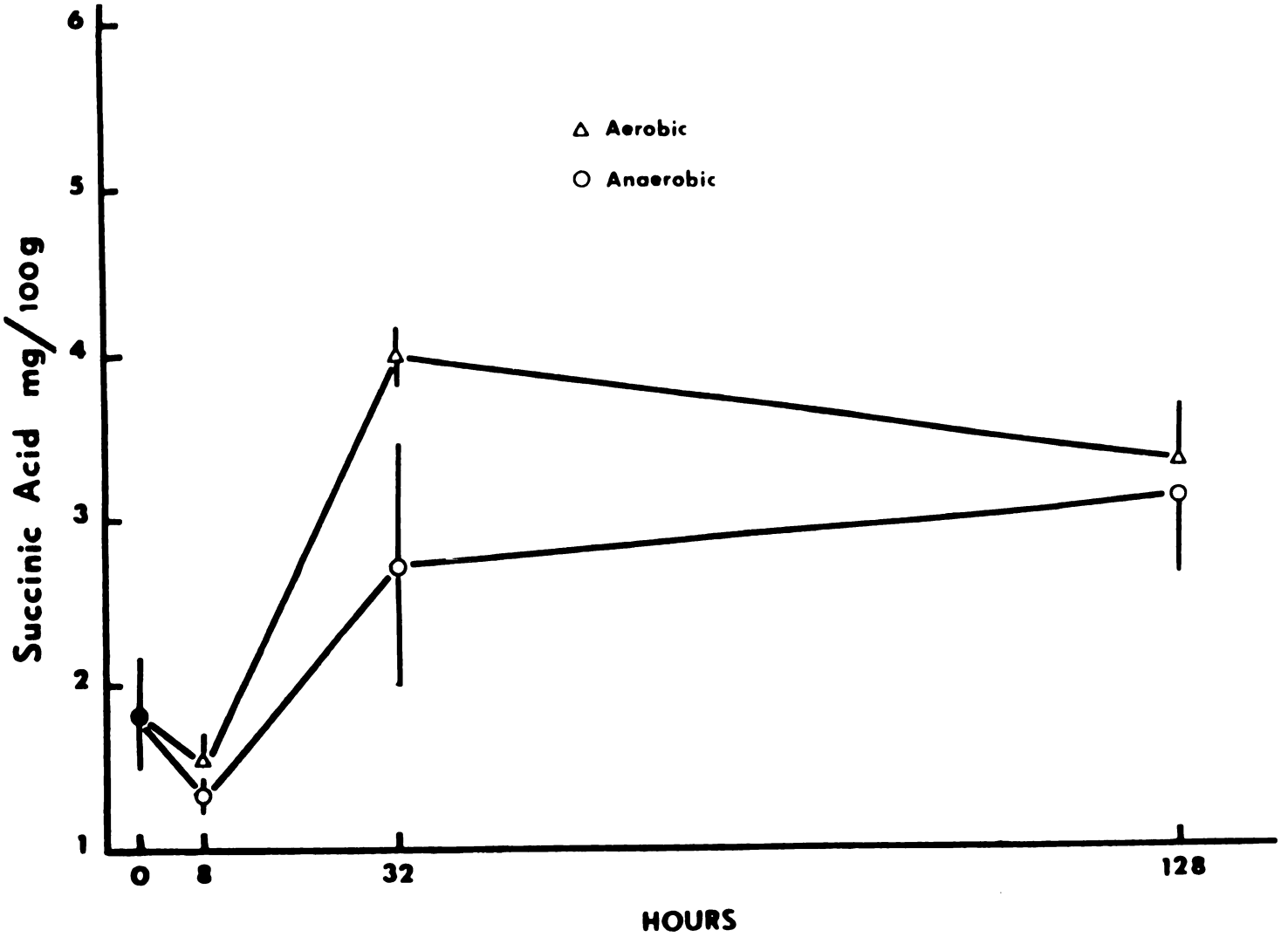

Figure 5. Changes in succinic acid content of 'Empire' apples kept in air or nitrogen atmospheres at 20°C. Each data point represents an average of 3 determinations using individual fruits. Standard deviations of the means are shown for each data point.

almost the same concentration.

In spite of the consistently higher amounts of succinic acid under aerobic conditions, no difference was found between the two treatments in any of the observation periods, although the data suggests a more sharp increase in succinic acid in aerobic fruits from 8 to 32 hours.

However, a significant difference was found at the 1% level between the initial and the final concentrations under aerobic conditions which shows an increase in the amount of the acid over the period of 128 hours. A similar significance was not found under anaerobic conditions. But if we compare the concentration of succinic acid from 8 hours (minimal inflection point of the curve) with the final concentration (128 hours), then both the aerobic and anaerobic treatment show a significant increase.

d. Fumaric Acid

The behavior of fumaric acid under aerobic and anaerobic conditions was quite similar to the behavior of succinic acid described above (Figure 6).

The inflection point of the two curves at 8 hours was more pronounced than that of succinic acid and the level of fumaric acid increased thereafter under both conditions toward 128 hours. Again, the aerobic condition consistently showed higher levels of the acid but no statistical differences were found in any of the observation points.

The increase in the amount of fumaric acid from 8 to 128 hours was more pronounced in the aerobic atmosphere than

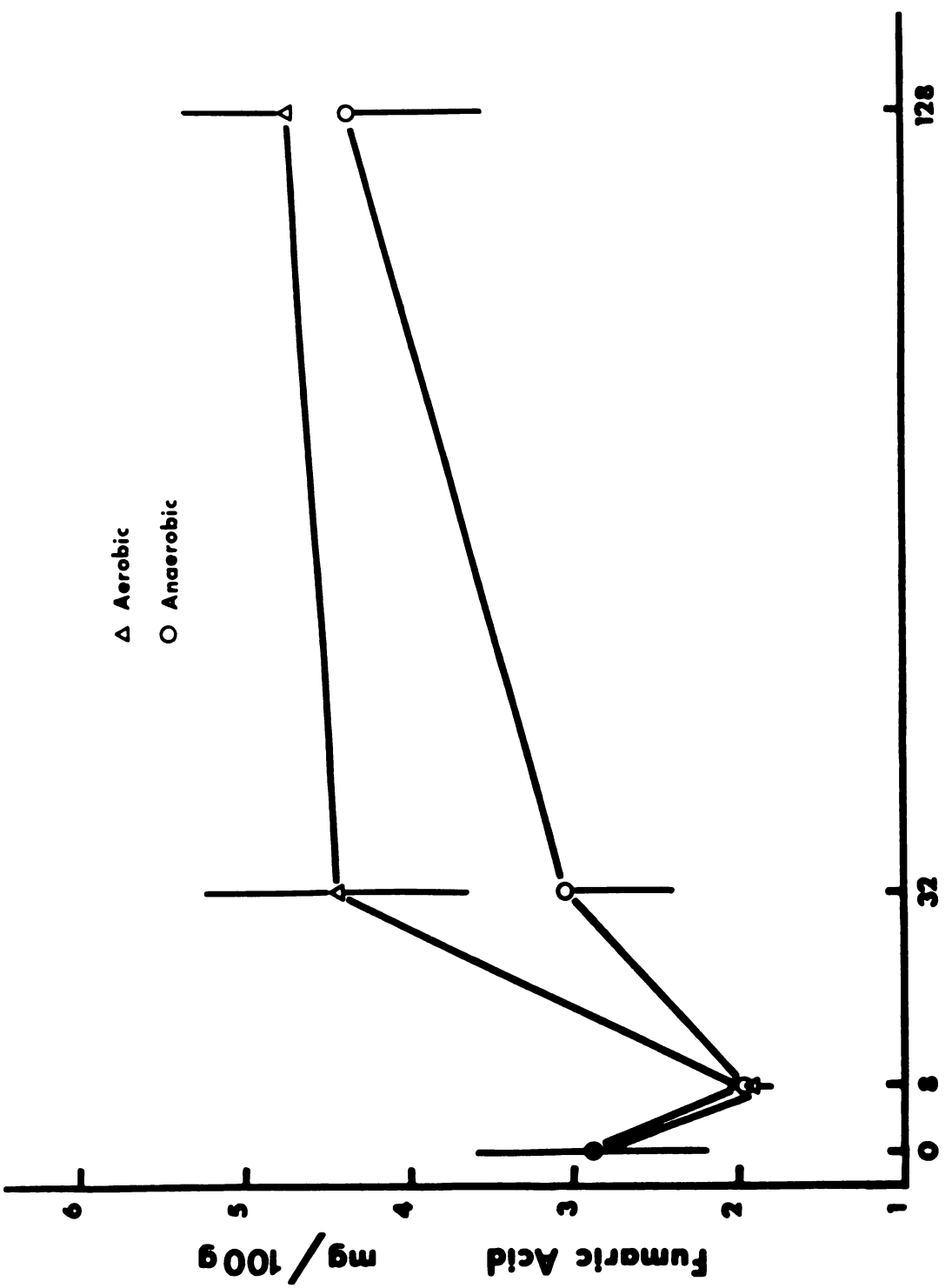

Figure 6. Changes in fumaric acid content of 'Empire' apples kept in air or nitrogen atmospheres at 20°C. Each data point represents an average of 3 determinations using individual fruits. Standard deviations of the means are shown for each data point.

in the anaerobic atmosphere, but the increase was significant in both cases.

e. Carbon Dioxide Production

Carbon dioxide production of 'Empire' fruits kept in air or nitrogen at 20°C is shown in Figure 7. Each data point represents the average of CO_2 production of eight individual fruits.

Data gathered eight hours after the fruits had reached equilibrium in the respirometers shows a marked reduction in CO_2 evolution of the fruits kept in nitrogen as compared to the CO_2 evolution of the fruits kept in air. The anaerobic CO_2 output decreased even more toward 128 hours while aerobic respiration increased until 56 hours and then held fairly steady through 128 hours.

The ratio of anaerobic to aerobic CO_2 evolution decreased from 0.61 at 8 hours to 0.17 at 128 hours.

No visible symptoms of low O_2 injury were observed in the fruits kept under nitrogen up to 128 hours.

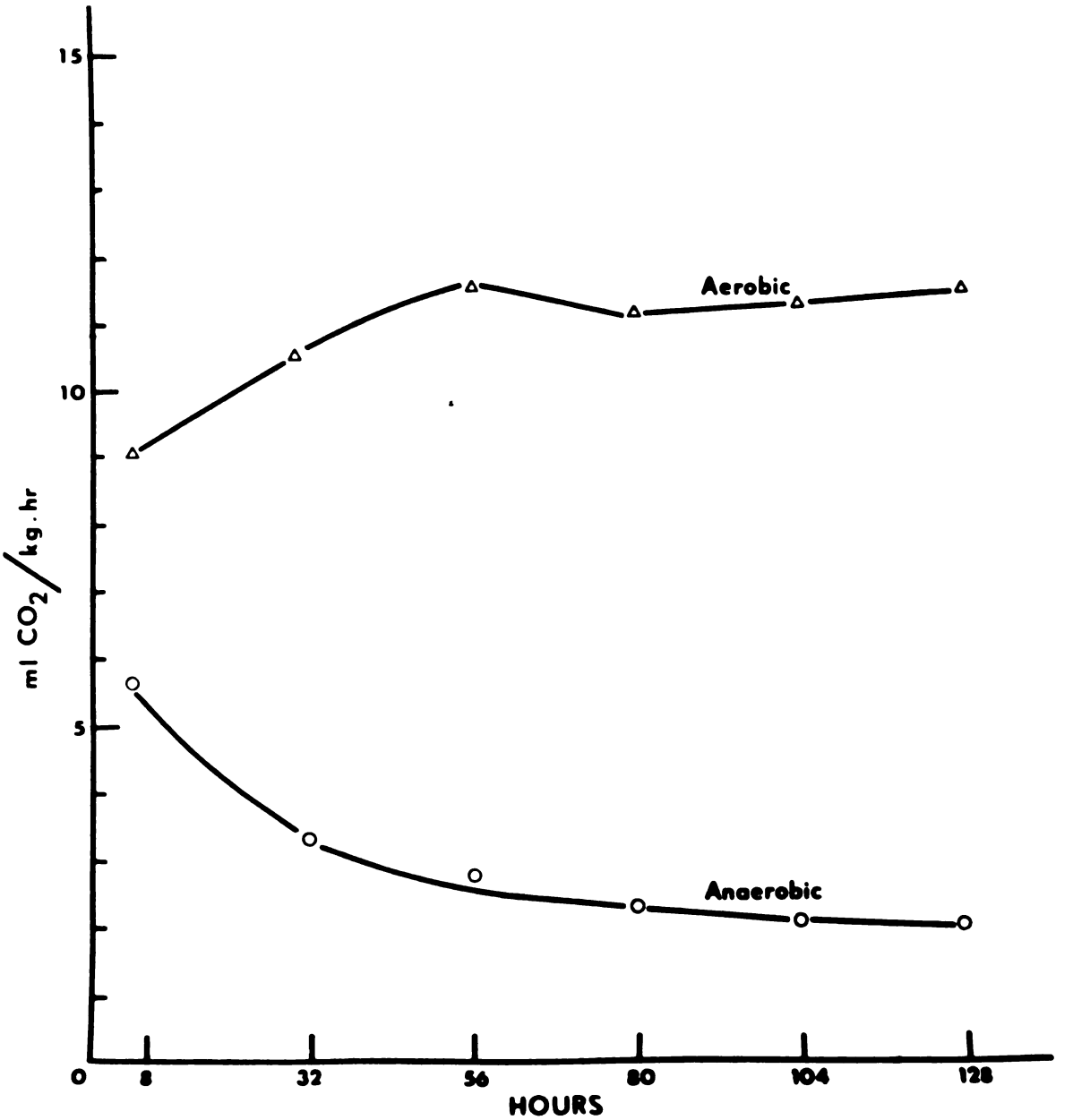

Figure 7. CO_2 evolution of 'Empire' apples kept in air or nitrogen at 20°C. Each data point represents an average of 8 determinations using individual fruits.

DISCUSSION

From a review of the existing literature, it becomes clear that the task of interpreting the results of experiments on physiological disorders of apples, such as the disorders included here, is not an easy one. Smock (107) recognized that there is considerable confusion in the literature on the terminology of internal storage disorders of apples. They presented as an example "internal breakdown", which is a term widely used to cover all such problems. Many types of flesh breakdown can only be described visually, and they are therefore difficult to define and are sometimes given different names in different parts of the world.

The terms "low temperature breakdown", "senescent breakdown", "brown core", and "CO_2 injury" are all used to describe brown or dead cortical tissue of pome fruits. The descriptive nature of these terms implies that the cause of the disorder has been established, but this is usually not so. Faust *et al.* (21) observed that it is not feasible to differentiate consistently on symptoms alone and that critical metabolic studies appear to be necessary for identification of the cause of breakdown.

It is not surprising, therefore, that conflicting results may very often be found since there is always the possibility

of different investigators using the same term to make reference to different problems having different physiological origins.

First Experiment

The results of flesh firmness data obtained from CA stored fruits after 7 months of storage show the remarkable effect of low oxygen in the maintenance of the physical integrity of the fruits up to that time. Firmness was higher in CA fruits kept at 36°F than in air stored fruits at 32°F, indicating their younger physiological stage at the end of storage time. This effect was also noticed after the holding period at 68°F. There are some evidences that fruits kept under CA conditions have a younger physiological age at the end of storage when compared with fruit of the same chronological age kept under air storage (18).

The total acidity content of CA stored fruits was significantly higher than air stored fruits after 7 months of storage. This behavior of CA stored fruits was also observed by other authors (1, 31, 64) and it has been explained as due to a greater production of organic acids (mainly malic) (1, 64), due to a slower depletion of the total acidity (31), and due to a combination of both aspects (64). Although it has been shown that 'McIntosh' fruits will fix CO_2 into malic acid at considerably high rates (1), there seems to be no grounds to explain the noticed effect with this hypothesis alone since it was observed in the second experiment (which is going to be discussed next) that there was a higher total

acidity at the end of the storage when low levels of CO_2 were used (below 1%) associated with low levels of oxygen throughout the storage. The data gathered in this research (both first and second experiment) indicates that CA storage has an effect in retaining the total acidity or lowering the depletion rate of the total acidity and, therefore, the higher levels of acidity observed in CA stored fruits at the end of the storage period may be a result of higher CO_2 fixation into malic acid and lower consumption of malic acid during the storage period.

Controlled atmosphere storage decreased the incidence of brown core and this agrees with several other reports (81, 85, 106-109, 117). The incidence of brown core was significantly higher at the lower storage temperature in CA stored fruits which adds more evidence for the hypothesis that brown core is a low temperature-related disorder. It has also been suggested that brown core is a form of carbon dioxide injury induced after continuous exposure of the fruit to high internal CO_2 concentrations and this worsens after the climacteric rise when permeability to CO_2 decreases (28).

Since brown core incidence was significantly higher in air stored than in CA stored fruits, it is tempting to rule out CO_2 as the causal agent. Even though internal CO_2 concentration was not evaluated, it is obvious that CA stored fruits have higher internal CO_2 concentrations throughout storage than air stored fruits. However, it is also possible

that air stored fruits, because of their more advanced physiological stage, would have gone through the climacteric in storage much earlier than CA stored fruits and, consequently, would have been exposed to critically higher internal CO_2 concentrations for a longer period of time than CA stored fruits.

No significant effect on the amount of brown core following storage was found due to the storage interruptions. If we accept that the effect of interim warming in attenuating physiological disorders is due to the dissipation of some accumulated metabolite(s) toxic to the tissue, then the increase in the general metabolism brought about by both storage interruptions was not sufficient to metabolize the toxic material(s). Padfield (80), working with New Zealand 'Granny Smith' apples, was able to reduce brown core to a low level after storage only when 3 interruptions at 64-65°F for 2 days were used. Landfald (67) also had to apply several warming periods of 15°C for 5 days to control the disorder. Multiple interruptions of the storage period would make this process infeasible on a practical scale. It would have deleterious effects by aging the fruits causing softening, shortening of the storage life and senescence disorders.

The significant difference found in the amount of brown core among the fruits from different orchards used in the experiment suggests that the disorder probably has a pre-storage origin. This fits the hypothesis of organic acids accumulation since differences in the amount and type of

organic acids according to variety and location have been reported in apples following harvest (60).

The level of superficial scald following 7 months of storage was found to be independent of the storage temperature and atmosphere composition since no significant difference was found at that time. Scald increased during a warm post-storage period and a greater incidence was found in air stored fruits as compared to CA stored fruits. The higher amount of scald in air stored fruits presents further evidence of scald being an oxidative process (125). The storage interruption treatments which were found to reduce the incidence of scald (81), afforded no effect and, in fact, they significantly increased scald in CA stored fruits after 7 months of storage and after the holding period. Fruits subjected to the storage interruption treatments had a higher level of exposure to oxygen than those kept continuously in storage and consequently were more likely to exhibit higher levels of scald after storage.

After the holding period, a significantly higher level of scald was observed in air stored fruits kept at 32°F and this agrees with previous reports that scald becomes worse the lower the temperature (81, 125).

The fact that lots of fruits from different orchards had significantly different levels of scald in both observation periods suggests that this disorder is an intrinsic characteristic of the fruits and that it may also have its origin in pre-harvest factors.

Wilkinson and Fidler (125) stated that senescent breakdown would develop further at high temperatures after the fruit has been removed from storage. This characteristic of senescent breakdown was also observed in this experiment since senescent breakdown was absent after 7 months of storage and developed further after a period of 7 days at 68°F. Any treatment that delays ripening would likely reduce the amount of senescent breakdown (125). Therefore, it is logical that CA storage would decrease the amount of senescent breakdown and indeed there was less breakdown in CA stored fruits as compared to air stored fruits. However, senescent breakdown was significantly higher at the lower temperature in both CA and air stored fruits. Normally, more breakdown would be expected at the higher temperature (125), since higher temperatures would tend to age the fruits more, rendering them more susceptible to senescent breakdown.

The significantly different amounts of senescent breakdown among the different lots of fruits observed in CA stored fruits indicate the different inherent ability of the fruits to develop the disorder during storage and clearly links it with pre-storage factors.

Second Experiment

After 3 months of storage, firmness was significantly higher for fruits stored at 32°F and 1.5% O_2, indicating the younger physiological stage of those fruits. Flesh firmness was reduced as the temperature and oxygen levels were increased simultaneously.

After 7 months, however, oxygen level was observed to be more efficient than temperature in maintaining firmness, since fruits kept at 1.5% O_2 had higher values regardless of the storage temperature. After the holding period, only oxygen level showed a significant effect in maintaining firmness. A period of 7 days at 68°F was sufficient to erase the temperature effect but not sufficient to erase the lower oxygen level effect.

Fruits kept under low oxygen levels, either CA or hypobaric storage, had higher levels of total acidity after 7 months of storage and that confirms the results observed in the first experiment. Fidler and North (31) stated that the rate of loss of acid is reduced by reduction of the concentration of oxygen, as long as the respiration remains aerobic, and that it is also reduced by increasing the concentration of carbon dioxide. The results observed confirm this hypothesis.

After 7 months of storage, brown core was only absent in hypobarically stored fruits. Fruits from low oxygen storage had some brown core and air stored fruits had a slightly higher incidence. At that period, 1.5% O_2 level in CA was found to have no significance to the amount of brown core, and since the pressure used (50mmHg) in hypobaric storage is equivalent to approximately 1.4% O_2 relative to air at 1 atmosphere, we tend to attribute the absence of brown core in hypobarically stored fruits to the low pressure in which the fruits were stored. Ethylene (40) and CO_2 (28) have

been implicated in the incidence of brown core and it is possible that the observed effect was due to the removal of both gases from inside the fruits in low pressure storage.

After the post-storage holding period, however, brown core was worse in fruits kept under air storage and a comparison of the low oxygen storage treatments alone revealed a significantly higher level of brown core at 3% O_2 as compared to 1.5% O_2. This result clearly shows the involvement of oxygen in the incidence of brown core.

No conclusive results were obtained for scald after 7 months of storage in the low oxygen treatments since no significant difference was found at that period. Air stored fruits showed higher amounts of scald as compared to low oxygen treatments, confirming the results obtained in the first experiment. In hypobaric storage, the browning of the peel, resembling scald, was also considerably higher than the scald present in the low O_2 treatments; however, two evidences suggest that the disorder observed in hypobaric storage was not, in fact, scald. First, scald is known not to be a problem in hypobaric storage (15), and secondly, in the first experiment, in all the treatments, and in the second experiment, in most of the treatments, scald increased after the holding period at the higher temperature and, in fact, this is a known characteristic of the disorder (85). The peel injury observed in hypobaric fruit decreased in all the three treatments which would be a very unusual behavior of superficial scald. The injury observed under low pressure may be

related to the inadvertent flooding that occurred near the end of the storage period as a result of the humidifier system failure.

After the holding period, scald increased and some air storage treatments had almost all fruits affected by the disorder. Fruits kept at 3% O_2 had a significantly higher scald level when compared to 1.5% O_2. Those results confirm the observations made in the first experiment and clearly link scald incidence to oxygen level. This also adds evidence to the hypothesis of scald being an oxidative process (125).

Senescent breakdown was absent after 7 months of storage in fruits kept at low oxygen levels and this result is in very good agreement with the younger physiological stage of those fruits at that time. In hypobaric storage, however, the observed levels of senescent breakdown are unusual and again this may be due to the unexpected, unfavorable conditions to which the fruits were subjected prior to termination of the storage period. The higher level of senescent breakdown in air stored fruits as compared to low oxygen stored fruits confirms the observed results in the first experiment.

Fruits kept at 36°F in 3% O_2 showed significantly more breakdown after the holding period than those kept at 32°F. This may be expected since at the higher temperature fruits would be more advanced physiologically and consequently more likely to be affected. Interruption treatments, with only a few exceptions, increased the amount of senescent

breakdown and again, interruption treatments would tend to age the fruits.

Air stored fruits showed considerably higher levels of senescent breakdown in both observation periods as compared to low oxygen treatments. Air storage allows fruits to ripen more rapidly than in CA and hence would be least effective in opposing the aging process.

No clear-cut results were obtained concerning the incidence of low temperature breakdown (LTB) after 7 months of storage and after the holding period for low oxygen and air stored fruits. Fruits kept at 3% O_2 tended to have more LTB after the holding period. Interruption was without consistent effect in low oxygen and air stored fruits because it sometimes increased the problem in some instances while decreasing it in others. In hypobaric storage after the holding period, the interim warming did reduce the incidence of LTB. And, it was more efficient in doing so than just interrupting the storage atmosphere while fruits remained at low temperature, indicating that a more drastic increase in the metabolism is needed to metabolize the toxic substances that may have accumulated.

Third Experiment

1. Organic Acids Extraction and Analysis

As observed by Palmer and List (83), the "official" chemical methods for analysis of organic acids in foods are simply too time-consuming for most purposes and, in any case, "official" methods are only available for a few acids (3).

A number of chromatographic methods have been developed for determining organic acids in biological samples. The ion exchange chromatography of organic acids from tobacco plants using gradient elution as described by Palmer (82), proved to be very useful when adapted with minor modifications to the analysis of organic acids of apples (55), and this method was widely used till recently. However, in those methods complete separation of all the acids of interest is not achieved and quantification is achieved through the laborious collection of numerous fractions and the subsequent titrimetric analysis of each fraction with dilute alkali to an indicator end point. More recently, several methods using liquid chromatography (LC) or high performance liquid chromatography (HPLC) for the determination of organic acids have been developed (42, 83, 92, 111, 114). Those methods proved to be very efficient and greatly decreased the analysis time. However, when analysis is needed on a large number of samples, even those methods can be time-consuming, since the fastest method described in the literature takes approximately 25 minutes to separate all the acids of interest investigated in the present work.

The separation of organic acids by the HPLC method developed during this work proved to be quite fast (all the TCA cycle acids of interest were separated in ten minutes) and extremely reproducible and was, therefore, a very useful research tool in this experiment and it is likely that the extraction method, developed in this work for organic acids

of apples, could be used for other biological material.

The purpose of this experiment was to observe the behavior of some of the TCA cycle intermediates in apples when subjected to anoxia since some information gathered so far suggests that apples would accumulate some of those intermediates under low oxygen conditions and at low temperatures. Anaerobic conditions were employed at 20°C to accelerate the process. It is recognized that anaerobiosis at low temperatures may cause different effects.

2. Organic Acids

a. Malic Acid

Malic acid was depleted at the same rate in both conditions, aerobic and anaerobic, at 20°C over a period of 128 hours. Fidler (24) observed that the presence or absence of oxygen is without effect on the rate of loss of acid in apple fruits. The results obtained here confirm the results of Fidler (24) and show specifically that malic acid decreases at the same rate in apple fruits under aerobic and anaerobic conditions. More recently (31) it was observed that the rate of loss of acid is reduced by reduction of concentration of oxygen, as long as the respiration remained aerobic.

b. Citric Acid

The level of citric acid remained constant over a period of 128 hours at 20°C in both aerobic and anaerobic conditions. Fruits under anaerobic conditions were consistently higher in citric acid but the difference was not statistically significant. The concentration of citric acid found and the

stability of the level agree with previous observations of citric acid content in the pulp of apples kept at 15°C in air (55), and additionally suggest that anaerobic conditions do not change the normal behavior of this acid at least over a period of 128 hours.

c. Succinic and Fumaric Acid

The behavior of both acids under aerobic and anaerobic conditions was found to be very similar. Initially, both acids decreased in air or nitrogen over the first 8 hours and then increased toward 128 hours with the increase being more pronounced from 8 to 32 hours. Levels of succinic and fumaric acid in air were consistently higher than those observed under anaerobic conditions, but the difference was not significant. These results agree with previous observations by Handwerker (43) that oxygen concentrations between 3 and 21% did not affect the accumulation of succinic and fumaric acid in apple fruits, and suggests that oxygen is without effect in the accumulation of both acids during the ripening process over a period of 128 hours at 20°C.

d. Carbon Dioxide Production

CO_2 evolution showed to be highly affected by anaerobiosis even after 8 hours of exposure of the fruits to the nitrogen atmosphere, since a marked reduction in CO_2 output could be observed at that time. After 8 hours the CO_2 evolution by fruits in nitrogen continued to decrease gradually and by 128 hours the ratio of anaerobic to aerobic CO_2 evolution was 0.17.

The ratio of anaerobic CO_2 evolution also decreased in 'Sturmer Pippin' apples kept under the same conditions of the present experiment over a period of 35 days (24) and a ratio of 0.6 was observed by Dilley et al. (18) in 'McIntosh' apples following harvest. The pattern of both aerobic and anaerobic CO_2 evolution observed in the present experiment is similar to the one observed by Dilley et al. (18) for fruits following CA storage.

The results gathered in this experiment concerning organic acids suggest that O_2 is without effect on the behavior of malic, citric, succinic and fumaric acid, and consequently accumulation of succinic and fumaric acid would be likely to occur regardless of O_2 concentration. This conclusion is supported by the recent observation that oxygen concentration between 3 and 21% did not affect the accumulation of both acids in apples kept at $0^{o}C$ (43).

However, the accumulation of succinic acid in CA storage as to cause physiological disorders through the inhibition of succinic dehydrogenase by high CO_2 levels (41, 61, 77), is by far greater than the one observed, since apples were reported to accumulate as much as 21mg of succinic acid/100g of fresh tissue (46).

It is more likely that apples would accumulate more toxic organic acids, such as succinic (46), under high CO_2 concentration than under low O_2 concentrations. However, more metabolic studies are necessary to further clarify and establish a cause and effect relationship between organic

acids accumulation and the incidence of physiological disorders in stored apples.

SUMMARY

The effect of temperature, oxygen levels and storage interruption on the incidence of physiological disorders and on the acidity content of 'McIntosh' apples was investigated.

A study of the metabolism of organic acids and respiration of 'Empire' apples in aerobic and anaerobic atmospheres was undertaken and during that study a method of extraction and analysis using High Performance Liquid Chromatography was developed for the quantification of the TCA cycle intermediates.

The incidence of brown core was more pronounced at the lower temperature and higher O_2 concentrations and increased after a holding period of 7 days at $68^{o}F$. A single storage interruption was found not sufficient to attenuate the disorder.

Superficial scald was found to be somewhat influenced by oxygen level, being worse in air and decreasing with decrease in oxygen concentration. It increased after the holding period and storage interruptions tended to increase the incidence of scald.

Senescent breakdown was increased by treatments that increased the aging process of the fruit, such as high O_2 concentration and higher temperatures. However, in one

experiment, low temperature of storage was found to increase senescent breakdown.

Low temperature breakdown, which occurred in only one of the experiments, was found not to be influenced by the factors investigated.

Oxygen was found to be without effect on the amount of malic, citric, succinic and fumaric acid present during ripening at 20°C. Malic acid decreased, citric acid remained constant, and succinic and fumaric acid increased over a period of 128 hours at 20°C in both aerobic and anaerobic atmospheres.

Data are presented which show that the accumulation of organic acids to toxic levels that takes place under CA storage, and to which a role in physiological disorders has been ascribed, may be due to the high CO_2 levels present under those conditions rather than O_2 levels.

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