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Effects of Elevated Carbon Dioxide on Key Mitochondrial Respiratory Enzymes in 'Hass' Avocado Fruit and Fruit Disks

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ABSTRACT . Preclimacteric avocado *[Persea americana* (Mill.) cv. Hass] fruit or fruit disks as well as fruit harvested in either June (midseason) or August (late season) and partially ripened were kept in air (21% O_2 + 78% N_2), 20% CO_2 + 17% O_2 (63% N_2), or 40% CO_2 +13% O_2 (47% N_2) at either 10 or 20 °C. Ethylene production by preclimacteric fruit was completely inhibited during CO_2 exposure, whereas there was only partial inhibition of ethylene production when partially ripened fruit were exposed. Compared to the fruit stored in air, O_2 uptake of fruit stored in 20% CO_2 was decreased by 20%, whereas the fruit stored in 40% CO_2 showed 25% more O_2 uptake than air-stored fruit. Fruit subjected to a storage regime of 40% CO_2 at 10 °C followed by 2 d in air had the best visual quality. In general, climacteric fruit treated with 20% CO_2 at 10°C showed increased pyruvate dehydrogenase (PDH) activity and decreased cytochrome oxidase (CytOx) activity. Fruit stored in 40% CO_2 had reduced CytOx activity compared to air-stored fruit, and PDH activity was variable depending on the harvest season of the fruit. Our results show that the effect of elevated CO_2 on a given enzyme depends on concentration of CO_2, duration of exposure, physiological state of the fruit, and type of tissue exposed.

Stress levels of O_2 (<1%), CO_2 (>15%), or both used for short durations can be effective in retarding ripening, controlling decay causing pathogens, and reducing insect infestation of several horticultural perishables (Ke and Kader, 1992a), including strawberries *(Fragaria xananassa* Duch.) (Aharoni et al., 1979; Li and Kader, 1989), sweet cherries *(Prunus avium* L.) (De Vries-Paterson et al., 1991), and avocados (Spalding and Reeder, 1974; Trüter and Eksteen, 1985). Such modifications of the storage atmosphere might be used to decrease our dependency on applications of fungicides and pesticides to the perishable produce. Wang (1977) reported that high

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levels of CO_2 delayed senescence and reduced decay in sweet peppers *(Capsicum annaum* L.), and Prusky et al. (1991) showed that elevated CO_2 exposure of avocado before storage increased the level of an antifungal diene that makes fruit more resistant to *Colletotrichum gloeosporioides.* However, some undesirable physiological changes also may occur in plant tissues during exposure to stress-inducing levels of O_2 and CO_2 For example, Yahia and Carrillo-López (1993) observed exocarp and mesocarp injury on 'Hass' avocado fruit exposed to 0.1% to 0.4% O_2 and 50% to 75% CO_2 at 20 °C for >1 d before ripening in air.

Much of the research on the effects of reduced O_2, elevated CO_2, or both on horticultural commodities has been directed toward the optimization of long-term storage conditions for each of a broad range of fruits and vegetables. Only a limited number of studies have been directed toward understanding the physiological and biochemical responses of the fruit to these altered atmospheres (Kader, 1986; Smock, 1979). These investigations have dealt with the effects caused by a range of O_2 and CO_2 concentrations that are used for long-term storage. There is a need for more information on the basic biochemical and physiological effects of using stress levels of low O_2, and especially elevated CO_2, during quarantine or storage pretreatments of fruit.

One of the major consequences of elevated CO_2 or reduced O_2 levels can be reduced respiration rates of several climacteric fruit. For example, Kubo et al. (1990) showed that 60% CO_2 reduced the respiration rates of climacteric tomatoes *(Lycopersicon esculentum* Mill.) and bananas *(Musa acuminata* Juss.). Conversely, Perez-Trejo et al. (1981) found that CO_2 levels as low as 3% stimulated an increase in potato *(Solarium tuberosum* L.) tuber respiration. Plant tissues can respond to elevated CO_2 levels by inducing fermentative pathways, accumulating glycolytic and Krebs cycle intermediates (especially succinate), acidifying intracellular compartments, and decreasing ATP levels (Kader, 1995). Ke et al. (1995) investigated the effects of 20% O_2 + 80% CO_2 on fermentative metabolism in 'Hass' avocados, and Kerbel et al. (1988) determined the effects of 10% CO_2 (19% O_2) on glycolysis in 'Bartlett' pears *(Pyrus communis* L.). Carbon dioxide decreased the activity of several respiratory enzymes of the Krebs cycle, particularly succinic dehydrogenase (SDH) (Bendall et al, 1960; Frenkel and Patterson ,1973; Knee, 1973). Ke et al. (1993) found that succinate and alanine accumulate in crisphead lettuce *(Lactuca sativa* L.) under elevated CO_2 concentrations. Nanos et al. (1994) found that 'Bartlett' pears stored at 0.25% O_2 possessed higher SDH activity than air-stored fruit, and this result was thought to be due to the less senescent state of the low O_2-treated fruit.

The effects of elevated CO_2 levels on mitochondrial electron transport chain enzymes of fruits and vegetables are unclear. Cytochrome oxidase (CytOx) is the predominant terminal electron-accepting enzyme of the electron transport chain. Bendall et al. (1958) found that concentrations of 13% to 26% CO_2 stimulated CytOx activity of germinating castor bean *(Ricinus communis L,)* mitochondria, but 61% to 78% CO_2 had an inhibitory effect. To our knowledge, there are no published reports regarding the effects of elevated CO_2 concentrations on CytOx in fruit tissue. Shipway and Bramlage (1973) found that elevated CO_2 had widespread effects on several apple *(Malus domestica* Borkh.) fruit mitochondrial enzymes, including SDH. They concluded that there must be pH or enzyme structural-conformational changes occurring within the mitochondria

during and after exposure to elevated CO_2.

Few studies have focused on the effects of elevated CO_2 on the pyruvate dehydrogenase (PDH) complex located in the mitochondria, which is the first enzyme that directs the end product of glycolysis (pyruvate) into the Krebs cycle. This enzyme may be an important regulatory point during exposure to stress levels of CO_2. Ke et al. (1995) found that PDH activity was reduced by exposing 'Hass' avocados to 0.25% O_2 and/or 80% CO_2 atmospheres. In this study, we investigated the effects of less severe CO_2 concentrations on PDH.

Avocado fruit is an excellent model system to test the metabolic effects of elevated CO_2 on key mitochondrial enzymes because of its high respiratory activity when ripe and the maintenance of mitochondrial structural integrity during senescence (Biale and Young, 1971; Blanke, 1991). In addition, extracting mitochondria from avocados results in a high yield of viable mitochondria. Eaks (1976) and Lutz and Hardenburg (1968) reported that storage of avocados at 10°C for a few weeks can slow ripening effectively, without causing the development of chilling injury. So, this temperature was chosen for the longer-term avocado storage used in this study.

In our study, the effects of two levels of elevated CO_2 on respiration, ethylene evolution, and extractable activities of certain key mitochondrial respiratory enzymes were determined in 'Hass' avocados, which were harvested in 2 months, held at two storage temperatures, and exposed to elevated CO_2 at two maturity stages.

Materials and Methods

PLANT MATERIAL AND TREATMENTS. Preclimacteric 'Hass' avocado fruit were harvested at commercial maturity (>24% dry mass) from an orchard in Santa Barbara County, Calif.; transported to our laboratory in Davis, Calif.; and held at 20 °C. Within 2 d of harvest, they were stored at 10 °C for <1 week until initiation of the experiments. Fruit that were uniform in size and free of defects were selected. Unripe preclimacteric fruit and partially ripe climacteric fruit were used in separate experiments. Partially ripe fruit were stored at 20 °C for 2 d and were treated with a continuous flow of ethylene at 10 $\mu L\text{-}L^{-1}$ in humidified air at a flow rate of 400 $mL\text{-}min^{-1}$ before the 4-d experimental period.

Experiments were conducted either at 10°C for up to 15 d or at 20°C for up to 5 d. Nine fruit were placed in a 4-L glass jar and were ventilated with humidified air or a specified gas mixture at a continuous flow rate of 100 $mL\text{-}min^{-1}$. The specific gas mixtures were created by mixing 100% CO_2 and air (21% O_2) to produce 20% CO_2 + 17% O_2 and 40% CO_2 + 13% O_2. Avocados were kept at 20°C for 4 d and then transferred to air for 1 d. Similar O_2 uptake rates occurred using air (21% O_2) or 100% O_2 in the gas mixtures, so air was used for ease of experimentation. For air-treated fruit, measurements were taken initially and after 2, 4, and 5 d. For fruit stored under elevated CO_2 atmospheres, measurements were taken 2 and 4 d after storage under elevated CO_2 atmospheres and after 1 d following transfer to air to observe signs of recovery from high CO_2 effects. In addition, avocado fruit were kept at 10°C for 12 d (June 1994) or 7 d (Aug. 1994) and then transferred to air for 3 (June) or 2 d (August). In a separate experiment, 5 x 15-mm avocado mesocarp disks were cut (as described by Starrett and Laties, 1991) from fruit harvested in Mar. 1993 and were ventilated in humidified, plastic, 24-well plates with air or air + CO_2 at a flow rate of 20 $mL\text{-}min^{-1}$ for 2 d at 20 °C as previously described. Each

type of experiment was replicated at least two times.

Individual fruit were selected for each experiment based on screening for uniform C_2H_4 production rates as described by Kerbel et al. (1988). In the case of partially ripened avocados, the fruit were screened after 4 d of ripening. Three fruit were pooled into each of three replicates per treatment for each sampling date. Each 4-L jar contained all of the fruit for one sampling date for each treatment. Flesh firmness measurements were made on both sides of avocado fruit and averaged for the nine fruit within each treatment-sampling date. Oxygen consumption and C_2H_4 production rates also were monitored during the sampling period. Cytochrome oxidase (CytOx) activity was monitored in all experiments. Pyruvate dehydrogenase (PDH) activity was only monitored in the experiments conducted at 10 °C.

QUALITY EVALUATION. Flesh firmness was determined on opposite peeled cheeks with a Univ. of California fruit firmness tester (Western Individual Supply Co., San Francisco) fit with a 6.2-mm plunger (Claypool and Fridley, 1966).

GAS ANALYSIS. The mixtures for elevated CO_2 treatment were tested periodically to determine the compositional accuracy by taking a 10-mL gas sample and analyzing the O_2 and CO_2 concentrations using a gas Chromatograph (model 111; Carle Instruments, Anaheim, Calif.) equipped with a thermal conductivity detector.

MITOCHONDRIAL ISOLATION AND PARTIAL PURIFICATION. Avocado fruit mitochondria were isolated using the method of Moreau and Romani (1982b). All extraction procedures were performed over ice. Homogenization of the fruit tissue from the 10°C experiments was conducted in a 10°C storage room. In experiments involving the use of fruit disks and fruit at 20°C, 24 disks (25 g) or 50 g of mesocarp tissue, respectively, were homogenized in isolation medium (3 mL-g_{-1} fresh mass) using a Polytron homogenizer (Brinkmann Instruments, New York) at low speed. In the 10°C experiments, 100 g of tissue were homogenized in 300 mL isolation medium by maceration through a fine wire-mesh screen submersed in medium as described by Romani et al. (1969). The isolation medium consisted of 0.25 M sucrose, 50 mM potassium phosphate (pH = 7.2), 5 mM EDTA, 5 mM ß-mercaptoethanol, 0.2% (w/v) soluble (40,000 MW) PVP, and 0.1% BSA. In all experiments, the homogenate was filtered through four layers of cheesecloth and centrifuged at 2000x *g* for 10 min. The supernatant was filtered through four layers of cheesecloth and centrifuged at 10,000x *g* for 15 min. This supernatant was discarded, and the pellet was resuspended in 1 (disk and 20°C experiments) or 3 mL (10°C experiments) wash medium and was homogenized in the presence of 3 (disk and 20°C experiments) or 1 mL (10°C experiments) additional wash medium. The wash medium consisted of 0.25 M sucrose, 50 mM potassium phosphate (pH = 7.2), 5 mM ß-mercaptoethanol, and 0.1% BSA. The homogenate was recentrifuged at 2000x *g* for 5 min. In the short-term and disk experiments, the supernatant was recentrifuged at 5000x *g* for 10 min, and then, the pellet was resuspended in 0.5 mL wash medium and was used as the mitochondrial preparation. Protein content of the crude mitochondrial preparation (20°C experiments) was determined by the standard Bradford (1976) method.

In the 10°C experiments, the 5000x *g* centrifugation step was substituted with a 20,000x *g* centrifugation step through 20 mL of 25% Percoll (Sigma Chemical Co., St. Louis)

"pad" for 10 min [as described in Day and Hanson (1977) and modified by Romani, personal communication]. This partial purification was necessary to eliminate most of the nonmitochondrial organelles and their corresponding enzymes that would interfere with the enzyme assays. A full Percoll purification system was not used (Moreau and Romani, 1982a) because a more rapid cleanup was necessary. The pad medium consisted of 25% Percoll, 0.25 M sucrose, and 50 mM potassium phosphate (pH = 7.2). The resulting mitochondrial pellet was resuspended in wash medium and centrifuged at 2000x g for 5 min several times to remove Percoll and was resuspended in 0.3 to 0.5 mL wash medium. Between addition of wash medium and centrifugation steps, the supernatant was removed by suction and discarded. Protein content of the purified mitochondrial preparation (10°C experiments) was determined by the standard Bradford (1976) method using BSA as the standard.

CYTOCHROME C OXIDASE (CYTOX) ASSAY. The CytOx activity was evaluated using the method of Hoekstra and van Roekel (1983) with modifications. The reaction mixture contained 50 mM reduced cytochrome C in 10 mM potassium phosphate (pH = 7.0). Reduction of cytochrome C occurred by adding 100 µL of 1.4 mM ß-mercaptoethanol into 10 mL of 50 mM cytochrome C (reduced and oxidized forms). One hundred microliters of the original 50 mM cytochrome C was added to the reduced substrate to oxidize any of the unused ß-mercaptoethanol since reduced mercaptoethanol will interfere with the CytOx oxidative assay. Diluted (1:10 in cold deionized water) mitochondrial (100 µL) preparation were added to 0.9 mL of the previously described reduced preparation for initiating the reaction (Bendall et al., 1958). The decrease in absorbance at 550 nm was followed at 25 °C, which was linear for at least 3 min. The activity was reported as micromoles of cytochrome C oxidized per minute per milligram of protein.

PYRUVATE DEHYDROGENASE COMPLEX (PDH) ASSAY. The overall activity of the PDH complex was assayed using the method of Budde and Randall (1987) with some modification. The reaction mixture was obtained by mixing 0.55 mL of 200 mM potassium phosphate buffer (pH 8.0), 0.05 mL of 4% (v/v) Triton X-100, 0.05 mL of 50 mM $MgCl_2$, 0.05 mL of 40 mM NAD^+, 0.05 mL of 5 mM TPP (thiamin pyrophosphate), 0.05 mL of 2.5 mM CoA (coenzyme A), 0.05 mL of 20 mM cysteine, 0.05 mL of 20 mM Napyruvate, and 0.1 mL of mitochondrial preparation (diluted in some cases). Formation of NADH was measured by recording the increase in absorbance at 340 nm using a spectrophotometer. PDH activity was expressed as nanomoles of NADH formed per minute per milligrams of protein.

Results and Discussion

Avocado fruit kept in CO_2-enriched atmospheres or in air softened similarly during storage for 5 d at 20°C or 9 to 15 d at 10°C (data not shown). Storage for 5 d at 20°C was not long enough to observe softening of preclimacteric avocado fruit in any of the treatments. Experiments using fruit stored at 10°C used fruit that had been partially ripened with ethylene before storage at 10°C for 9 to 15 d. These fruit were of comparable firmness at the initiation of 10°C storage. Exposure to ethylene stimulated softening in the fruit and CO_2 treatments were not effective in maintaining firmness once the softening had been initiated.

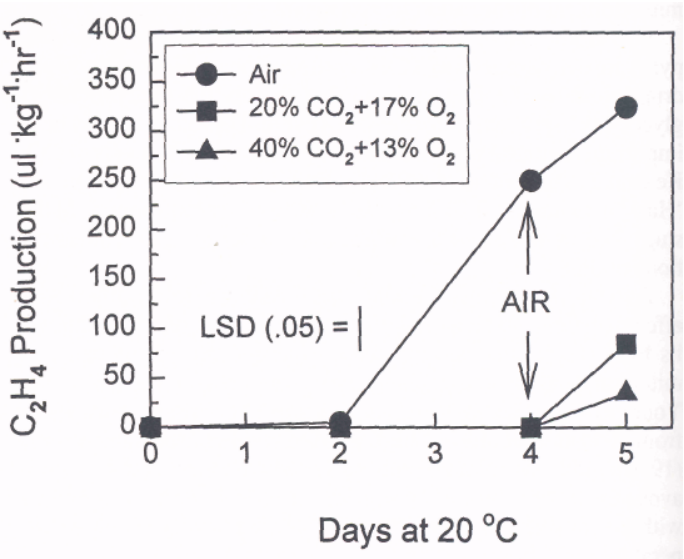

Fig. 1. Changes in C_2H_4 production of July-harvested, preclimacteric 'Hass' avocados kept at 20 °C in air (21% O_2), 20% CO_2 + 17% O_2, or 40% CO_2 + 13% O_2 for 4 d and subsequently transferred to air for 1 d.

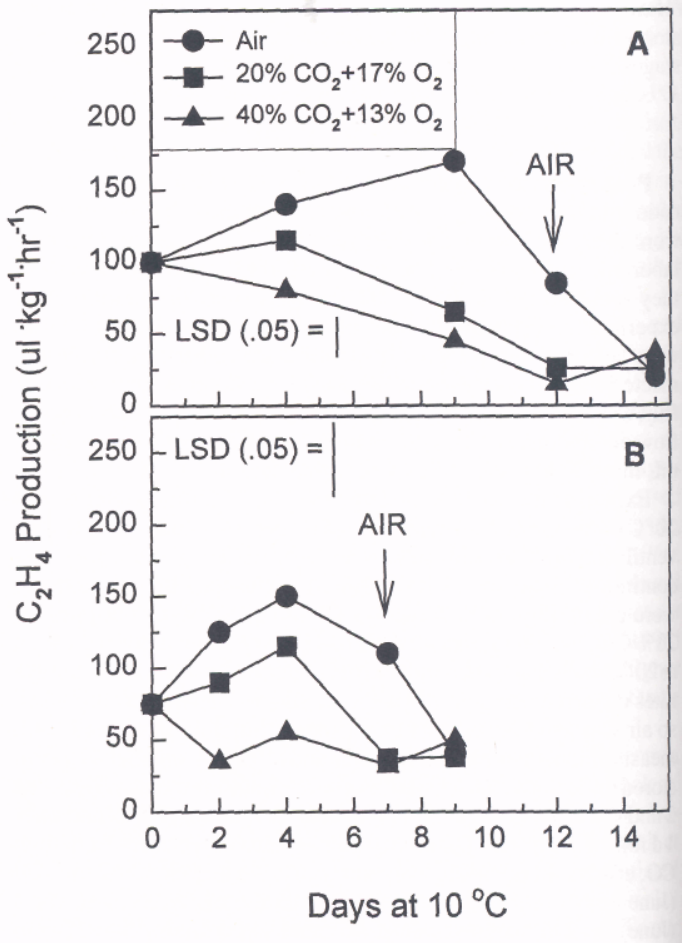

Fig. 2. Changes in C_2H_4 production of (**A**) June-harvested and (**B**) August-harvested partially ripe 'Hass' avocados kept at 10 °C in air (21% O_2), 20% CO_2 + 17% O_2, or 40% CO_2 + 13% O_2 and subsequently transferred to air.

Ethylene production of preclimacteric avocados was very low in all treatments until after day 2 (Fig. 1). Air-stored fruit produced 250 to 350 [µL-kg^{-1}-h^{-1} by days 4 and 5, while the CO_2-treated fruit did not produce measurable ethylene until after transfer to air on day 5. Ethylene production was completely inhibited by the elevated CO_2 treatments, but only while under high CO_2 atmospheres similar to what has been reported for other fruits (Yang, 1985).

Avocados that had been partially ripened at 20°C and placed at 10°C exhibited a moderate level of ethylene production initially (Fig. 2 A). Avocado fruit harvested in June and stored in air evolved about twice the ethylene on day 9 of storage when compared to the initial levels (Fig. 2 A). This phase was followed by a decrease in production to 25% of the initial rate. The CO_2-stored fruit had similar patterns of ethylene production throughout storage. On day 9 the CO_2-stored fruit produced only 30% of the ethylene that air stored fruit produced on the same day. Fruit harvested in August and stored in air at 10°C had double the ethylene production rate on day 4 when compared to day 0, followed by a decrease to 30% of the initial (Fig. 2B). Again the CO_2-stored fruit exhibited significantly lower ethylene production rates than the air-stored fruit; although in this test, the 40% CO_2-stored fruit had less than half of the production of the 20% CO_2-stored fruit until day 7. Partially ripened 'Hass' avocado fruit are inhibited partly by these CO_2 treatments in contrast to the complete inhibition observed with the preclimacteric fruit. Once ethylene biosynthesis was

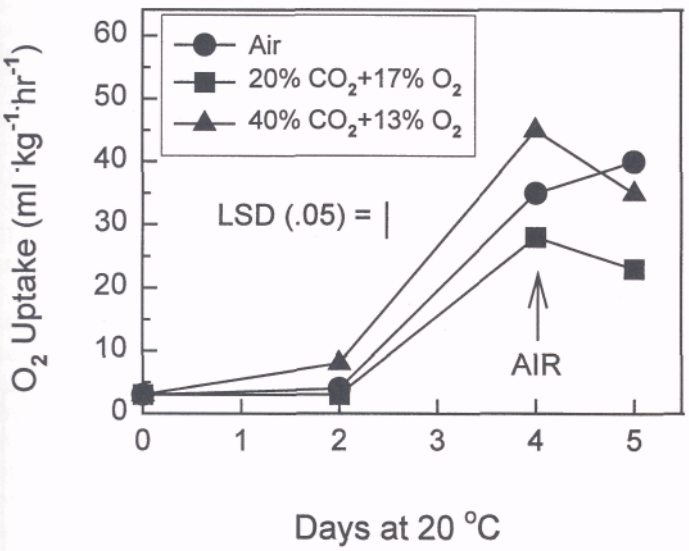

Fig. 3. Changes in O_2 uptake of July-harvested, preclimacteric 'Hass' avocados kept at 20 °C in air (21% O_2), 20% CO_2 + 17% O_2, or 40% CO_2 + 13% O_2 for 4 d and subsequently transferred to air for 1 d.

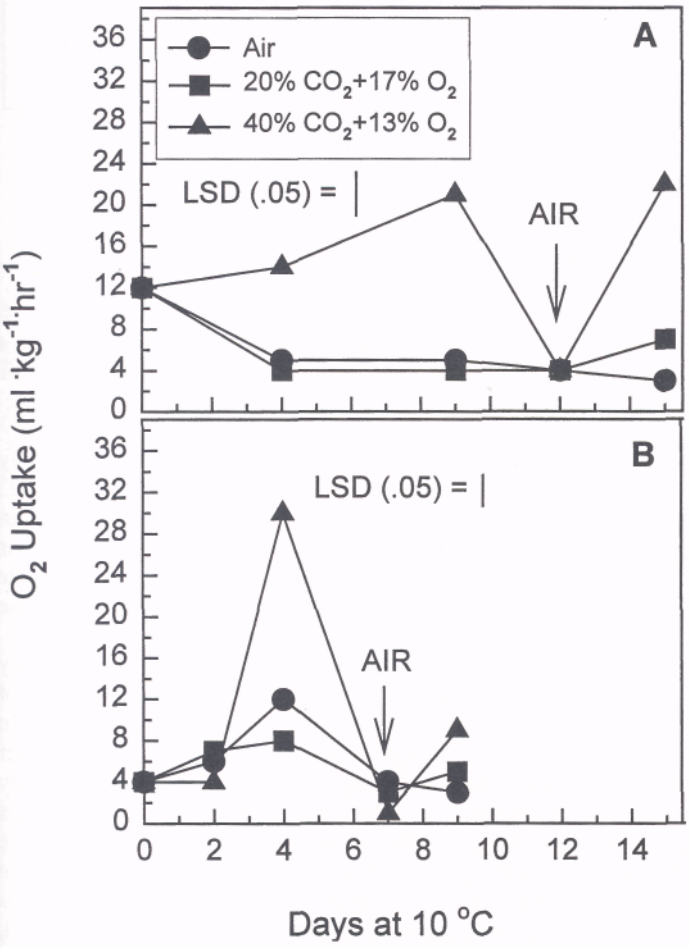

Fig. 4. Changes in O_2 uptake of (**A**) June-harvested and (**B**) August-harvested, partially ripe 'Hass' avocados kept at 10 °C in air (21% O_2), 20% CO_2 + 17% O_2, or 40% CO_2 + 13% O_2 and subsequently transferred to air.

stimulated by ethylene exposure, as with the partially ripened fruit, CO_2 was only partially effective in decreasing ethylene evolution.

Preclimacteric fruit stored at 20°C had a low rate of O_2 uptake until after day 2 (Fig. 3). On day 4 the order of O_2 uptake rates was 40% CO_2, air, and 20% CO_2. The observed increase in respiration rate of fruit stored in 40% CO_2 was probably due to a stress respiration response. Twenty percent CO_2 partially inhibited O_2 uptake of these preclimacteric fruit, and this inhibition remained 1 d after transfer to air.

Partially ripened fruit harvested in June had a moderate level of O_2 uptake on day 0 (Fig. 4A). During storage, the respiration rate of fruit stored in 40% CO_2 slowly increased and almost doubled by day 9 of storage. This increase was followed by a drastic decrease to below initial levels on day 12. Three days after transfer to air, the O_2 uptake of the 40% CO_2-stored fruit increased to a rate that was comparable to the peak in respiration observed on day 9, possibly signifying residual stress respiration. Fruit harvested in August (Fig. 4B) behaved quite differently in respiratory responses when compared to the June-harvested fruit (Fig. 4A). The O_2 uptake of the August fruit was at a low level on day 0. Again the 40% CO_2-stored fruit had a higher uptake rate than the air-stored fruit, but only on day 4 (during treatment) and 9 (after 2 d of air transfer). On day 4, the O_2 uptake of 40% CO_2-stored fruit was ~4-fold when compared to that of the other

treated fruit. Storage at 40% CO_2 appeared to result in a transient increase in stress respiration. The air and 20% CO_2-stored fruit exhibited similar respiratory patterns, although the 20% CO_2-stored fruit had significantly less O_2 uptake on day 4 than the fruit stored in air. Young et al. (1962) found a similar partial inhibition of avocado fruit respiration using 5% or 10% CO_2.

The visual quality of the 'Hass' avocado fruit stored at 10°C after 7 (June) or 12 d (August), followed by 3 d of air transfer, was variable (data not shown). Air-stored fruit in both tests had stem end decay, vascular decay, and browning after treatment and storage. Fruit harvested in June and stored in 20% CO_2 for 12 d followed by 6 d in air had intermediate visual quality due to the appearance of stem-end decay and slight browning. The 40% CO_2-stored fruit had excellent visual quality without the presence of any decay or browning in both tests. Treatment with 20% CO_2 at 10 °C for 7 d, followed by 2 d in air was effective in the prevention of decay and browning; however, 12 d of 20% CO_2 followed by 6 d in air was not effective. An informal taste panel found that the flavor quality was not adversely affected by elevated CO_2 treatments, and on the contrary, the 40% CO_2-stored fruit had the best retention of flavor quality (data not shown).

Table 1. Cytochrome oxidase (CytOx) activity of preclimacteric and climacteric 'Hass' avocado fruit disks kept in air, air + 20% CO_2, or air + 40% CO_2 at 20 °C for 2 d. Activity was measured at the optimum pH of 7.0.

	Preclimacteric fruit disks		Climacteric fruit disks	
Treatment	CytOx activity protein mass basis ($\mu mols \cdot min^{-1} \cdot mg^{-1}$)	% of initial	CytOx activity protein mass basis ($\mu mols \cdot min^{-1} \cdot mg^{-1}$)	% of initial
Initial	185	100	263	100
Air (21% O_2)	200	108	264	100
20% CO_2 + 17% O_2	288	156	603	229
40% CO_2 + 13% O_2	340	184	117	44
$LSD_{0.05}$	13.7		11.9	

Changes in CytOx activity from avocado fruit disks depended on the CO_2 level and the maturity of the tissue at the initiation of treatment (Table 1). In preclimacteric disks, the increase in CytOx activity was directly proportional to the CO_2 level. After 2 d, the 40% CO_2-treated disks exhibited activity that was 184% of the initial activity, whereas the activity of the disks held exclusively in air only increased to 108% of the initial. The CytOx activity of climacteric fruit disks was greater than that of preclimacteric disks. The disks held in air for 2 d had activity similar to the initial values. CytOx activity from the climacteric disks was highest under the 20% CO_2 treatment (229% of the initial) and was inhibited by 56% under 40% CO_2 (44% of the initial). CytOx activity of climacteric fruit disks was stimulated >20% CO_2 than preclimacteric disks (Table 1), possibly due to the increased sensitivity of the more senescent tissue. However, exposure of climacteric disks to 40% CO_2 resulted in decreased CytOx activity. Perhaps, 40% CO_2 in combination with an advanced stage of senescence resulted in mitochondrial membrane damage that caused the decreased CytOx activity. Similar mixed results of CytOx activity under various levels of CO_2 were reported by Bendall et al. (1958) using germinating castor bean endosperm tissue; 13% to 28% CO_2 stimulated activity and 61% to 78% inhibited the activity. In their work, 43% CO_2 caused just a slight elevation

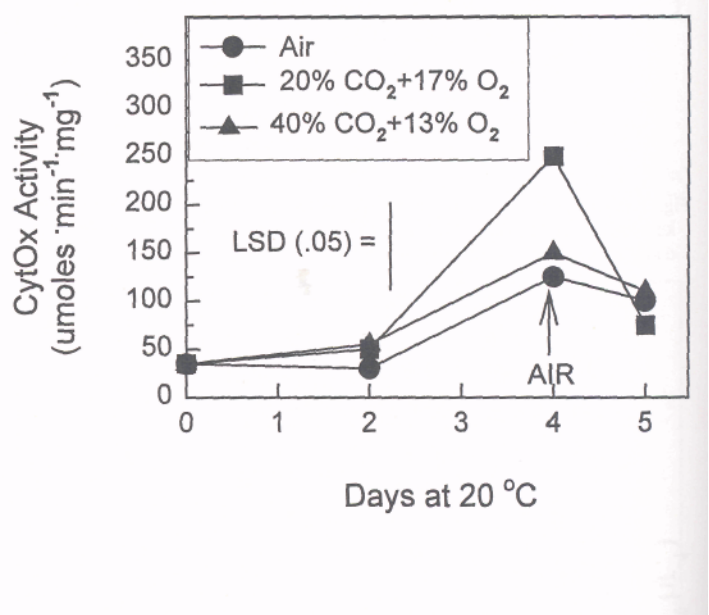

Fig. 5. Changes in cytochrome oxidase (CytOx) activity (protein mass basis) of preclimacteric 'Hass' avocados kept at 20 °C in air (21% O_2), 20% CO_2 + 17% O_2, or 40% CO_2 + 13% O_2 for 4 d and subsequently transferred to air for 1 d.

in activity compared to mitochondria held in air.

The 40% CO_2 treatment increased the in vitro CytOx activity of preclimacteric disk extracts (Table 1) but had no effect on the CytOx activity extracted from whole preclimacteric fruit (Fig. 5). Possibly the wounding of the disks during preparation in combination with the CO_2 stress caused a stimulation in CytOx activity. On day 4, the 20% CO_2-treated fruit had twice the CytOx activity of fruit stored in air, but similar activities were exhibited 1 d after transfer to air. The 40% CO_2-treated fruit tended to have slightly higher extractable CytOx activity than the air-held fruit, but the difference was not significant. The effect of CO_2 shown in our study is indirect. Perhaps elevated O_2 increased the extractability or synthesis of CytOx. The pattern of an increase in CytOx activity in all treatments during storage at 20 °C (Fig. 5) resembled the increase in O_2 uptake (Fig. 3).

CytOx activity of the partially ripened avocado fruit harvested in either June or August and stored at 10°C initially was at a similar moderate rate (Fig. 6), although the patterns of CytOx activity during storage in the CO_2 treatments were much different between the two tests. In the fruit harvested earlier, the only significant differences in CytOx activity occurred on day 9 of storage, with air-stored fruit having the highest activity, followed by 20% CO_2, then 40% CO_2 (Fig. 6A). CytOx activity of air-stored fruit followed a pattern of an increase in activity from day 4 to 9 followed by a decline to initial levels of activity on day 15. The 20% CO_2-stored fruit had similar activity throughout storage, while the 40% CO_2-stored fruit had a decline in activity at day 9 with a return to initial levels by day 12. The later season fruit only had different CytOx activities on day 4 of a 7-d storage period followed by 2 d in air (Fig. 6B). On day 4, the air-stored fruit had CytOx activities that were 4-fold higher than that of the initial activity, while the 40% CO_2-stored fruit tripled in activity over that same period. The 20% CO_2-stored fruit merely doubled in activity during this period. By day 7, the CytOx activity in all of the treatments fell below the initial level. In other studies, CytOx was partially inhibited by bicarbonate, which was in equilibrium with high levels of CO_2 (Miller and Evans, 1956; Palet etal., 1991). The changes in CytOx activities of climacteric fruit disks and intact fruit in response to 20% CO_2 differed (Table 1, Fig. 6); in disks, CytOx was stimulated relative to the air control, and in fruit, CytOx was lower. Wounding that occurred during preparation of the disks in combination with exposure to 20% CO_2 may have increased extractable CytOx activity.

Some of these changes in CytOx activity in avocado fruit may be due to a stress response of the tissue to CO_2 (Mathooko et al., 1995). The CytOx activity also may have

decreased in some cases due to the accumulation of fermentative metabolites, such as acetaldehyde, in the tissue, which has been reported to accumulate under elevated CO_2 (Saltveit and Ballinger, 1983) and to decrease CytOx activity (Cedarbaum et al., 1974).

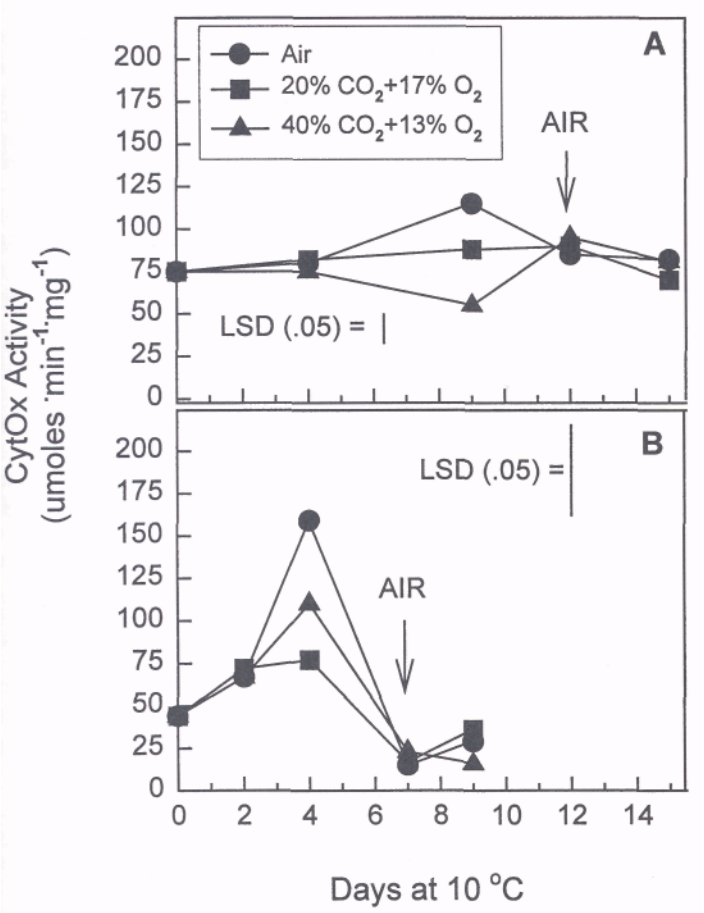

Fig. 6. Changes in cytochrome (CytOx) activity (protein mass basis) of (**A**) June-harvested and (**B**) August-harvested partially ripe 'Hass' avocados kept at 10 °C in air (21% O_2), 20% CO_2 + 17% O_2, or 40% CO_2 + 13% O_2 and subsequently transferred to air.

The PDH activity was only slightly and transiently affected by elevated CO_2 concentrations (Fig. 7). The initial PDH activity of June-harvested avocado fruit was relatively high (Fig. 7A). In general, PDH activity declined over the storage period, no matter what the treatment. Fruit stored in 20% CO_2had the highest activity on day 9. The air and CO_2-stored fruit had similar PDH activities throughout storage until after 3 d of air transfer, at which time the 40% CO_2-stored fruit had the lowest activity (50% of fruit held in air). In fruit harvested in August (Fig. 7B), PDH activity was initially low and under all treatments remained steady or increased. Two days after transfer to air (day 9), PDH activity of fruit stored in 20% CO_2 rose to a level that was 7-fold higher than the initial PDH activity level. With these late-harvested fruit, the PDH activity of 40% CO_2-stored fruit was higher than the air-stored fruit on day 9, 2 d after transfer to air.

The moderate stress levels of CO_2 used in these studies had a different effect on PDH than the severe stress level of 80% CO_2 that Ke et al. (1995) used in their studies. They found that 80% CO_2 (20% O_2) at 20 °C for 3 d decreased the PDH activity of preclimacteric 'Hass' avocado fruit to half the activity of the air stored fruit. In our study, 40% CO_2 at 10°C had a similar effect but only after 12 d of treatment and 3 d of air storage and only when using avocado fruit harvested in June (Fig. 7A). This inhibition may be due to an alteration in mitochondrial membranes enzymes. Of course, this effect would not be the only determinant in the level of PDH activity. The decrease in extractable PDH activity by elevated CO_2 appears to be CO_2-level dependent and varies with storage temperature and physiological maturity of the fruit.

The effects of CO_2 on respiratory metabolism varied in these studies depending on the harvest date of the fruit. The peaks and subsequent declines in respiration and ethylene production rates occurred earlier at 10°C when later season (harvested in August) fruit were used relative to the June-harvested fruit (Figs. 2 and 4). Although avocados do not

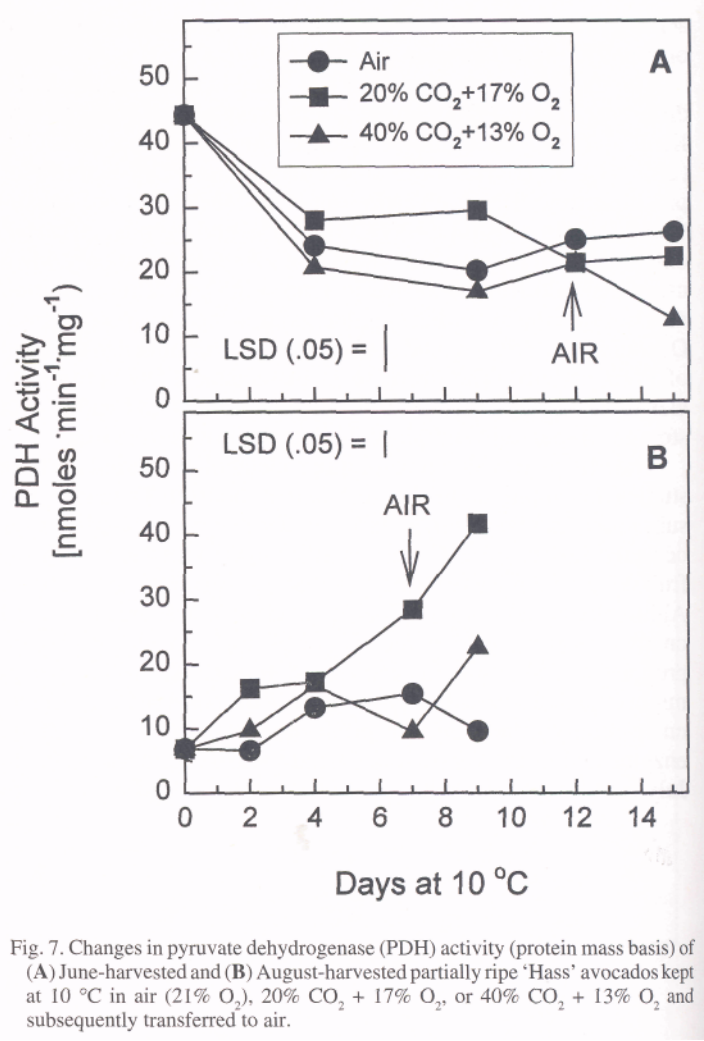

Fig. 7. Changes in pyruvate dehydrogenase (PDH) activity (protein mass basis) of (**A**) June-harvested and (**B**) August-harvested partially ripe 'Hass' avocados kept at 10 °C in air (21% O_2), 20% CO_2 + 17% O_2, or 40% CO_2 + 13% O_2 and subsequently transferred to air.

ripen on the tree (commercially stored on the tree for up to 6 months), ripening-related changes do progress at a slow rate during tree storage (Eaks, 1990), which might explain harvest date differences in the observed CO_2 effects on respiratory and ethylene production patterns as well as on enzyme activities (Figs. 6 and 7). Late-season fruit (harvested in July or August) were reported to have more discoloration due to chilling injury when stored in controlled atmospheres (Faubion, 1992). Possibly, late-season fruit lose their membrane integrity faster during storage due to prolonged periods on the tree, which may lead to physiological disorders or loss of metabolic activity. Lipids could have become more saturated in response to comparatively warmer weather in August, and hence, subsequent chilling injury may have occurred during storage.

Elevated CO_2 levels may have diverse effects on respiratory enzymes in fruit as demonstrated in our study. Those differences depend on the physiological state of the tissue, the harvest season of the fruit, the temperature of treatment, or the dose of CO_2 (Ke and Kader, 1992b). Just as Bendall et al. reported in 1958, we found that the activity of an enzyme (e.g., CytOx or PDH) can be either stimulated or inhibited by CO_2 just by manipulating the CO_2 concentration.

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