

Prestorage Low-oxygen Atmosphere Treatment Reduces Chilling Injury Symptoms in 'Fuerte' Avocado Fruit

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Abstract. Prestorage treatment of avocado fruit (*Persea americana* Mill. cv. Fuerte) with a low-O₂ atmosphere (3% O₂ + 97% N₂) for 24 hours at 17C, significantly reduced chilling injury (CI) symptoms after storage at 2C for 3 weeks. Fruit softening was also delayed by this treatment. The treated fruit had lower respiration and ethylene production rates during storage at 2C and subsequently at 17C. Electrolyte leakage was significantly lower in peel disks from treated fruit. Reducing power, expressed as total sulfhydryl groups, was higher in the peel and pulp of low-O₂-treated fruit. The amount of peel chlorophyll was inversely correlated with the severity of CI symptoms.

The avocado is a subtropical fruit sensitive to chilling injury (CI) when exposed to low but nonfreezing temperatures. The main symptoms of CI are black spots on the peel and a gray or dark-brown discoloration of the mesocarp (Chaplin et al., 1982; Zauberman et al., 1985). 'Fuerte' avocados exhibited CI symptoms after only 10 days of storage at 2C (Zauberman et al., 1985). Lyons (1973) suggested that electrolyte leakage and disrupted ion balance resulting from ultrastructural changes in membranes lead to development of CI symptoms. Increased ion leakage is characteristic of many plant tissues that show CI symptoms (Hariyadi and Parkin, 1991; Lafuente et al., 1991; McCollum and McDonald, 1991).

Pretreatments with N₂ at ≥97% (low O₂) atmospheres or with air enriched with 30% to 90% CO₂ delay ripening in various fruit and vegetables. Exposing preclimacteric bananas (*Musa* sp.) to 1% O₂ + 99% N₂ for 3 days, followed by storage in air, extended the time required for the fruit to ripen (Wills et al., 1982, 1990). Exposure to anaerobic conditions using either CO₂ or N₂ removed astringency of persimmon fruit by inducing the production of acetaldehyde (AA) and ethanol and led to retention of fruit firmness (Pesis and Ben-Arie, 1984). Treatments with high N₂ or CO₂ for 24 to 48 h, which resulted in increased endogenous AA and ethanol, inhibited ripen-

ing in tomato (*Lycopersicon esculentum* Mill.) (Kelly and Saltveit, 1988; Pesis and Marinansky, 1993). In peaches and nectarines [*Prunus persica* (L.) Batsch], the insoluble pectin content remained higher and polygalacturonase activity showed a slower increase in fruit treated with AA, CO₂, or N₂ (Lurie and Pesis, 1992).

Prestorage treatments of citrus fruit with 10% to 40% CO₂ in air reduced CI symptoms during cold storage (Bertolini et al., 1991; Hatton et al., 1975). Exposing 'Hass' avocado to 20% CO₂ three times during 21 days at 4C reduced CI (Marcellin and Chaves, 1983). Storing avocado in polyethylene bags also reduced CI symptoms (Scott and Chaplin, 1978). Treating 'Fuerte' avocado with 25% CO₂ for 3 days before 28 days of storage at 5C reduced incidence of disorders and lowered the total phenols level (Bower et al., 1989).

Oxidative stress, an early response of cucumber (*Cucumis sativus* L.) fruit to chilling at 4C, caused a major reduction in glutathione and α-tocopherol levels within 2 days of chilling (Hariyadi and Parkin, 1991). An association between enhanced cold hardiness of plants and increasing sulfhydryl (SH) content was demonstrated earlier (Levitt et al., 1962). Endogenous SH groups increase during fruit ripening, and a role of detoxificant agents was suggested for them (Fuchs et al., 1981; Tabachnik-Ma'ayan and Fuchs, 1982).

In an attempt to extend storage life of avocado fruit, we stored them at 2C and examined the effect of low-O₂ atmosphere pretreatments on maintaining their quality and preventing CI development at this otherwise injurious temperature.

Materials and Methods

Mature 'Fuerte' avocado fruit were harvested from the central plain of Israel and treated on the day of harvest. The fruit were enclosed in three 30-liter plastic containers

(30 fruit in each one) and exposed to near-anaerobic conditions for 24 h at 17C. Nitrogen from a N₂ generator (Rittal, Enfield, U.K.) was flushed for 1 h at 400 ml·min⁻¹ via a water flask into the containers. After reaching 3% O₂ + 97% N₂, the containers were sealed for 24 h, then opened, and the fruit were transferred to fiberboard boxes. The control fruit had remained in the treatment room at 17C [90% relative humidity (RH)]. After treatment, all fruit were stored in air at 2C (90% RH) for 3 weeks and then transferred to 17C.

At the end of the treatment, head space samples were taken from the jars, and the concentrations of AA, ethanol, CO₂, N₂, and O₂ were determined by gas chromatography using either a flame ionization detector (AA, ethanol) or a thermal conductivity detector (CO₂, O₂, N₂) as described by Pesis and Ben-Arie (1984).

CI severity based on external damage was scored on 60 fruit per treatment using a subjective scale: 0 = no damage, 1 = very light damage, 2 = light damage, 3 = medium damage, 4 = severe damage, 5 = very severe damage. The CI index was calculated according to the following formula:

$$\text{Injury index} = \frac{\sum (\text{injury level}) \times (\text{number of fruits at this level})}{\text{total number of fruits}}$$

Chlorophyll concentration in the peel was also used as a damage index. Six peel disks, 12 mm in diameter (total weight 0.6 g), were put in 10 ml of boiling 85% ethanol. The flasks were shaken at 70C for 2 h, and chlorophyll a and b were measured at 665 and 649 nm. The amount of chlorophyll was calculated according to Wintermans and De Mots (1965). The results were expressed as the ratio between the chlorophyll concentrations of low-O₂-treated fruit and controls.

Carbon dioxide and ethylene production were determined using individual fruit held in 2-liter jars at 2C for 3 weeks before transfer to 17C for 8 days. Carbon dioxide and ethylene in the headspace were detected by gas chromatography as described previously (Lurie and Pesis, 1992; Pesis and Ben-Arie, 1984). Data are means of five measurements for each treatment at each temperature.

Samples of five fruit from each treatment were removed once a week during the 2C storage and every 2 days during 8 days at 17C for further examination. Fruit firmness was measured on two pared sides of each fruit (10 measurements per treatment) using an electronic penetrometer (Chatillon, New York) with a 6.5-mm conical tip.

Six 12-mm-diameter disks were prepared from each avocado peel (total weight 0.6 g) and incubated in 10 ml 0.3 M mannitol (five samples per treatment). Electrolyte leakage was measured after 3 h of incubation in a shaking water bath at 25C. The electrical conductivity was measured at room temperature with a microprocessor conductivity meter (WTW, Weilheim, Germany). The tissue and the mannitol solution then were frozen at -20C, thawed, and boiled for 10 min. Total

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conductivity was then measured. Rates of electrolyte leakage are expressed as changes in the percentage of the total conductivity per hour (Lafuente et al., 1991).

Total SH content was determined by boiling disks (12 mm in diameter; 0.6 g) of peel or pulp in 5 ml of 0.02 M phosphate buffer (pH 5.7; five samples per treatment) for 10 min. After centrifugation at 10,000× g, the water-soluble SH compounds were determined in the supernatant using 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) reagent. The DTNB-reactive compounds were determined spectrophotometrically at 412 nm according to the method of Ellman (1959).

All measurements were done on five fruit per treatment, except for the CI measurements, which were measured on 60 fruit per treatment after 3 weeks at 2C. All data were subjected to analysis of variance. The experiments were done in two subsequent years several times. Results of two representative experiments are presented.

Results and Discussion

During the 24 h that the fruit were exposed to low O₂, there was an accumulation of the anaerobic metabolites AA, ethanol, and CO₂ (Table 1). The CO₂ produced was mainly due to anaerobic respiration. The change in O₂ concentration during the 24 h was only 1.7%, while that of CO₂ was 7.1%, which resulted in a respiratory quotient of 4.2. The ethanol levels produced were >30 times higher than the AA level, probably because ethanol is an end-product metabolite in anaerobic respiration while AA is an intermediate. In other fruit, such as persimmon (*Diospyros kaki* L.F.), peach, and tomato, the accumulation of ethanol under anaerobic conditions is also much higher than that of AA (Lurie and Pesis, 1992; Pesis and Ben-Arie, 1984; Pesis and Marinansky, 1993).

Pretreatment with low O₂ for 24 h before storage at 2C reduced CI symptoms significantly (Fig. 1). After 3 weeks at 2C, some of the treated fruit exhibited only slight CI, while most of the control fruit showed severe CI on >50% of the peel surface, resulting in a CI index 3 times higher than in the treated fruit (Fig. 1). During holding at 17C, the control fruit showed more intense CI symptoms and they had started to decay, while the treated fruit maintained a better appearance with milder CI symptoms. CI in 'Fuerte' avocado is a common occurrence in storage at 2C (Zauberman et al., 1985). The reduction in CI attributable to low-O₂ pretreatment is similar to the effects of pretreatment with high CO₂ (Bertolini et al., 1991; Hatton et al., 1975; Marcellin and Chaves, 1983) and by temperature conditioning (Hariyadi and Parkin, 1991;

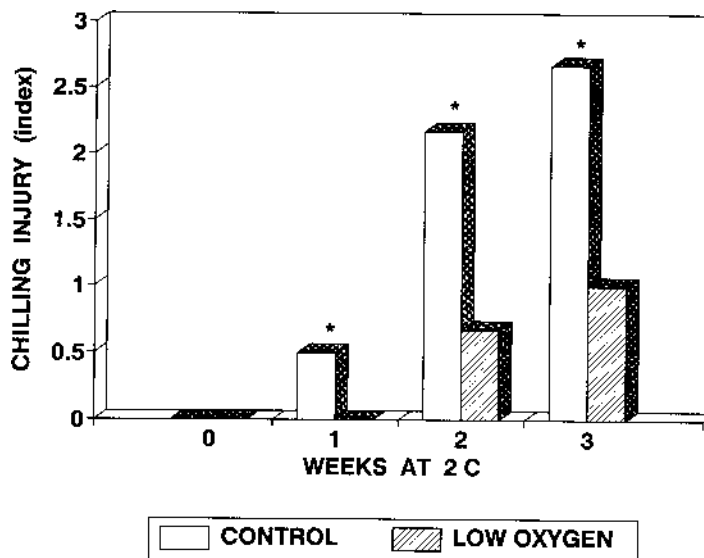


Fig. 1. Effect of a 24-h low-O₂ pretreatment at 17C on chilling injury (CI) in avocado during 3 weeks at 2C. CI index was calculated from 60 fruit. CI index: 0 = none, 5 = severe. *Significant differences between treatments, $P \leq 0.05$.

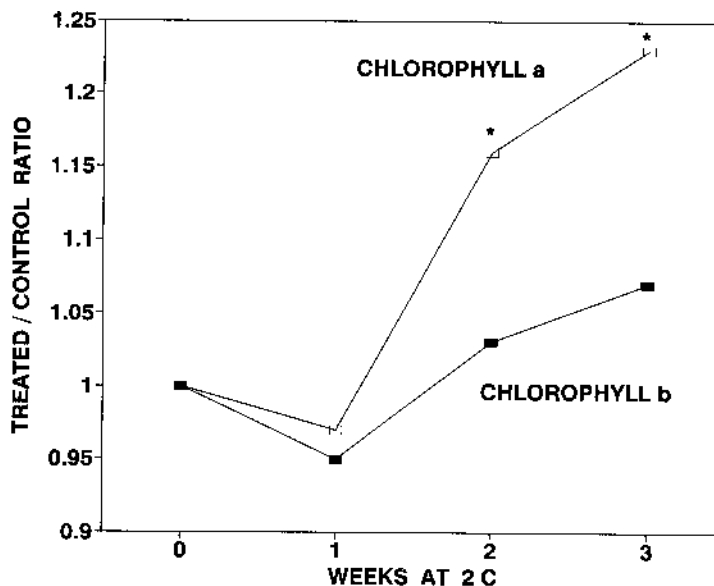


Fig. 2. Effect of a 24-h low-O₂ pretreatment at 17C on chlorophyll a and chlorophyll b as expressed by the ratio between low-O₂-treated and control fruit of avocado during 3 weeks at 2C. Results are the means of five measurements. *Difference significant from ratio = 1.0, $P \leq 0.05$.

Lafuente et al., 1991; Lurie and Klein, 1991).

Avocado peel is rich in chlorophyll, and the amount of chlorophyll extracted from the peel was found to be inversely correlated with the severity of the CI ($r = 0.821$). The higher the CI index, the less chlorophyll extracted, so that in low-O₂-treated fruit more chlorophyll was extracted compared to the chilled control fruit (Fig. 2). The reduction in chlorophyll in damaged peel compared with nondamaged peel was mainly in chlorophyll a and not in chlorophyll b (Fig. 2). Measurement of chlo-

rophyll as an index for CI is a different method from the one suggested by Chaplin et al. (1982), who measured CI as the absorbance of extracts from the discolored areas of CI-affected fruit.

Upon removal of the fruit from 2C storage, there was no difference in fruit firmness between treated and nontreated fruit (Fig. 3). However, during subsequent holding at 17C, the low-O₂-treated fruit remained slightly firmer than those not treated. In control and treated fruit, there was a dramatic decrease in firmness between 4 and 6 days, but the difference between treatments was significant only at 8 and 10 days. Only after 11 days at 17C did the difference in firmness between treated and nontreated fruit disappear (Fig. 3).

Nontreated avocado fruit increased in ethylene production during the 19 days of storage at 2C, while the low-O₂-treated fruit had consistently low ethylene production (Fig. 4A).

Table 1. Headspace concentration of various volatiles in containers containing 30 'Fuerte' fruit after 1 h and 24 h of sealing. Data are means of three jars \pm SE.

Time (h)	CO ₂ (%)	O ₂ (%)	N ₂ (%)	Acetaldehyde (ppm)	Ethanol (ppm)
1	1.4 \pm 0.08	3.2 \pm 0.16	95.8 \pm 0.69	0.0	3.2 \pm 0.12
24	8.5 \pm 0.70	1.5 \pm 0.23	89.9 \pm 0.46	1.8 \pm 0.43	59.4 \pm 8.69

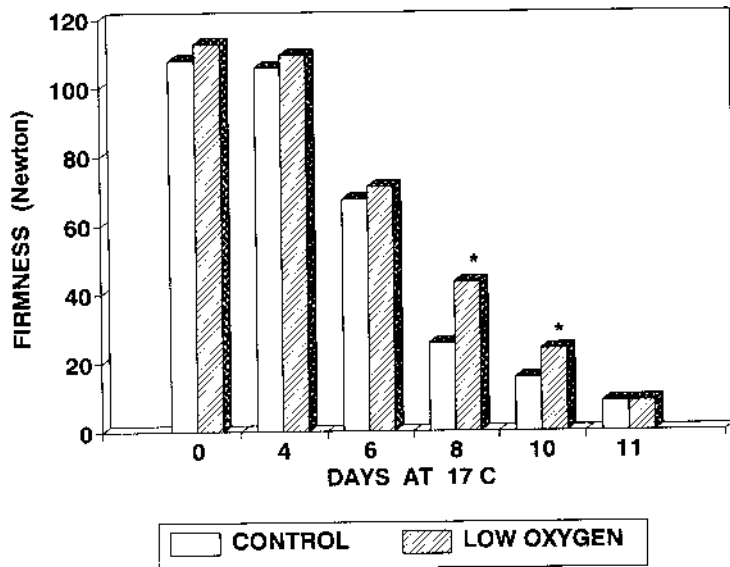


Fig. 3. Effect of a 24-h low-O₂ pretreatment at 17C on firmness of avocado stored at 2C for 3 weeks and then held at 17C for periods indicated. Results are the means of 10 measurements (two measurements per fruit). *Significant differences between treatments, $P \leq 0.05$.

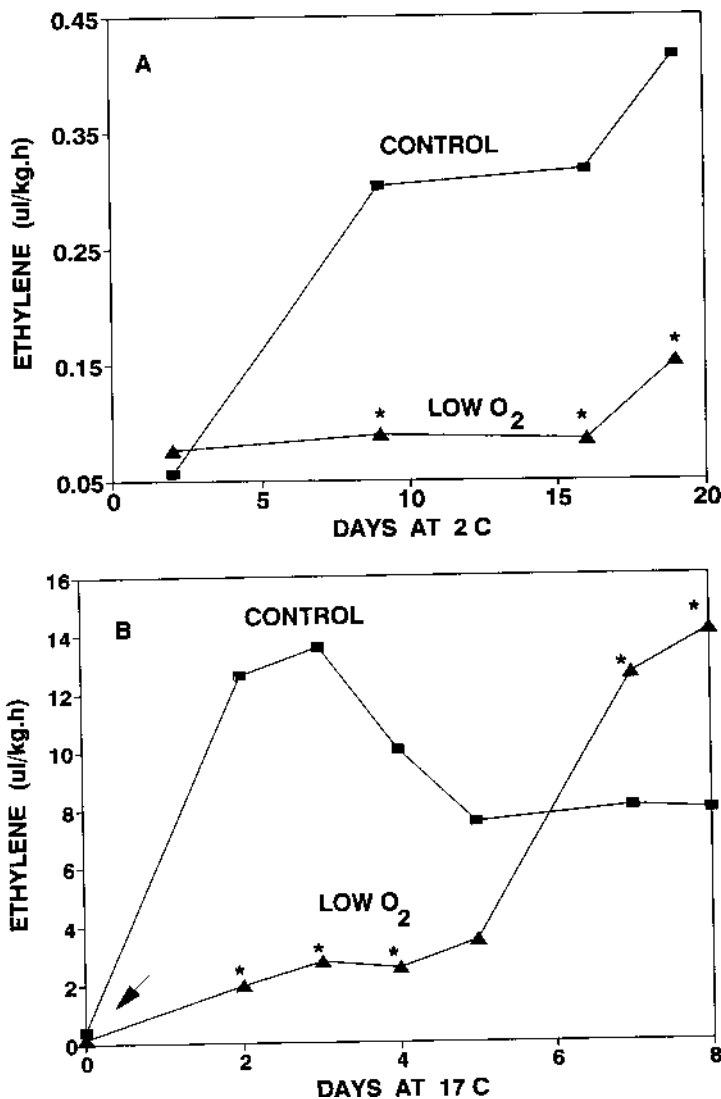


Fig. 4. Effect of a 24-h low-O₂ pretreatment at 17C on ethylene production in avocado during storage at (A) 2C followed by storage at (B) 17C. Results are the means of five measurements. Arrow in B indicates time of transfer from 2 to 17C. *Significant differences between treatments, $P \leq 0.05$.

After removal of the control fruit to 17C for ripening, the climacteric peak of ethylene production occurred within 2 days, while in the low-O₂-treated fruit, the climacteric increase was delayed 4 days (Fig. 4B). This result is similar to data presented by Marcelline and Chaves (1983), who showed a reduction in ethylene production at 4C in avocados pretreated with 20% CO₂. Anaerobic metabolites produced during exposure to low O₂ and/or elevated CO₂ concentrations may affect ethylene production. Saltveit and Mencarelli (1988) suggested that ethanol vapors inhibit 1-aminocyclopropane-1-carboxylic acid (ACC) conversion to ethylene in tomato disks. In grape (*Vitis vinifera* L.) halves treated with ACC, ethylene production was inhibited by AA but not by ethanol (Pesis and Marinansky, 1992).

Carbon dioxide production was higher in nontreated fruit than in treated fruit during 2C storage (Fig. 5A). However, upon removal to 17C, a dramatic increase in respiration occurred in both types of fruit, which might have been a result of the increase in temperature. Still, in the first days after removal, fruit formerly under low O₂ had a lower respiration rate than the nontreated fruit (Fig. 5B). Grapefruit (*Citrus paradisi* Macfad.) with CI had higher rates of CO₂ and ethylene evolution (McCollum and McDonald, 1991). Delay in the climacteric peak of CO₂ and ethylene production after low-O₂ pretreatment was shown also in banana and peach stored at 20C (Lurie and Pesis, 1992; Wills et al., 1982).

During 24 days of storage at 2C, there was a gradual increase in electrolyte leakage in the peel of treated and nontreated fruit, although the rate of increase mostly was lower in fruit treated with low O₂ (Fig. 6). During ripening at 17C, there was a further substantial increase in electrolyte leakage, but differences between treated and nontreated fruit, although nonsignificant at $P \leq 0.05$, remained (Fig. 6). Ion leakage is considered a qualitative indicator of CI (McCollum and McDonald, 1991). Temperature conditioning for a short period before cold storage significantly reduced the electrolyte leakage and CI in cucumber cotyledons and tomato fruit (Lafuente et al., 1991; Lurie and Klein, 1991). However, Fuchs et al. (1989) stated that in 'Hass' avocado and 'Haden' mango (*Mangifera indica* L.), electrolyte leakage from fruit pulp is related to ripening and cannot serve as an indication for the development or presence of CI symptoms in the fruit. The lower leakage reported here was found to be significant only after 2 and 3 weeks at 2C and can be related to the CI because there were only minor differences in firmness at this stage between the treated and nontreated fruit.

The fruit pretreated with low O₂ had higher concentrations of total free SH groups in pulp (Fig. 7A) and peel (Fig. 7B) than the control. The difference in the pulp was significant at 17 and 24 days of storage at 2C, while in the peel, it was significant from day 9 to 24 at 2C and also later, after removal to 17C (day 27 and 32). In the pulp, the levels of SH groups were 3 times higher than those in the peel. However, the relative difference in the SH concentration

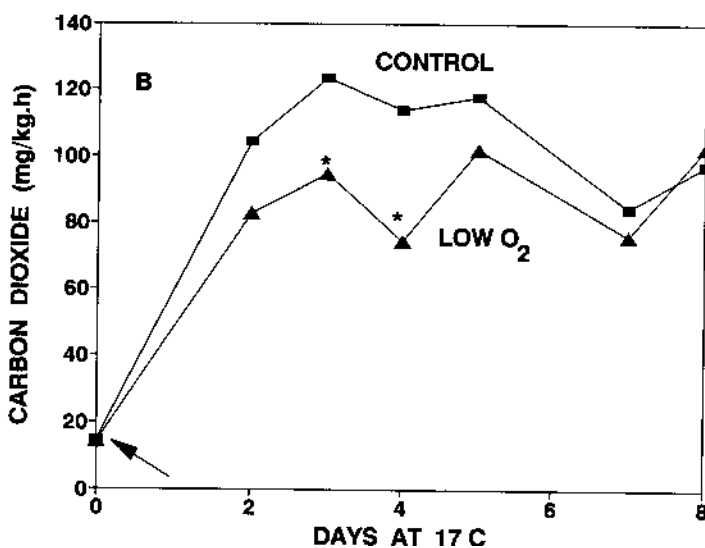
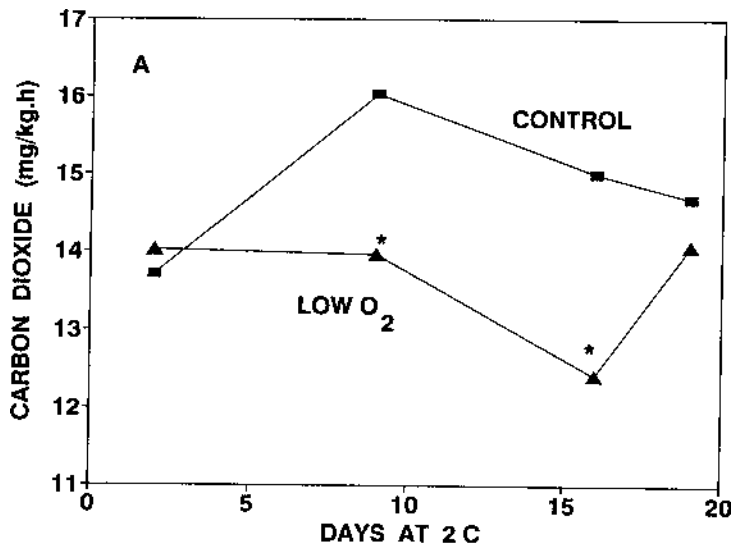


Fig. 5. Effect of a 24-h low-O₂ pretreatment at 17°C on CO₂ production in avocado during storage at (A) 2°C followed by storage at (B) 17°C. Results are the means of five measurements. Arrow in B indicates time of transfer from 2 to 17°C. *Significant differences between treatments, $P \leq 0.05$.

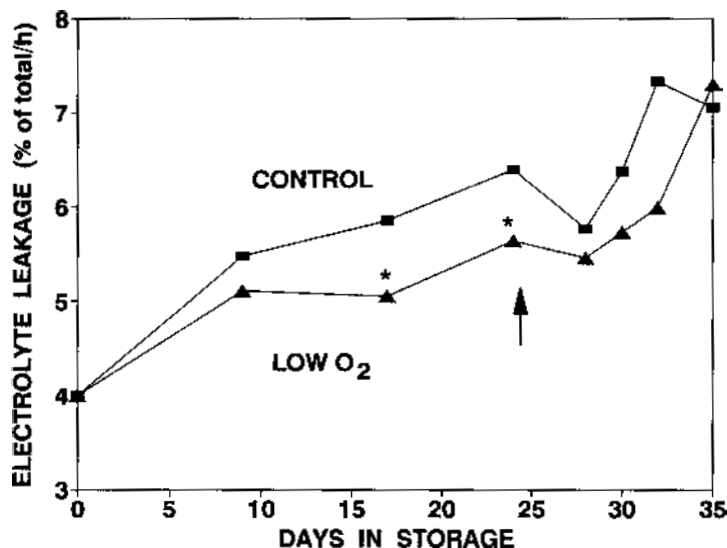


Fig. 6. Effect of a 24-h low-O₂ pretreatment at 17°C on ion leakage in the peel of avocado during storage at 2°C followed by storage at 17°C. Results are the means of five measurements. Arrow indicates time of transfer from 2 to 17°C. *Significant differences between treatments, $P \leq 0.05$.

between the low-O₂-treated fruit and the nontreated fruit was much greater in the peel (Fig. 7B) than that in the pulp (Fig. 7A). In avocado, the symptoms of CI begin in the peel (Zauberman et al., 1985). During ripening of climacteric fruit, such as tomato and mango, there is a gradual increase in free SH groups (mainly cysteine and glutathione) that may serve as natural detoxification agents (Fuchs et al., 1981; Tabachnik-Ma'ayan and Fuchs, 1982). Levitt et al. (1962) suggested that an increase in SHs enhanced cold hardness. Glutathione also helps protect plant tissues from peroxides that are generated during oxidative stress (Foster and Hess, 1980). Lower levels of reduced glutathione were found in chilled than in nonchilled fruit (Hariyadi and Parkin, 1991).

Higher levels of reducing (SH) groups in low-O₂-pretreated fruit might indicate a more active antioxidant defense mechanism, which in turn can explain the milder CI symptoms. The external application of antioxidants reduced the severity of CI in chilled cucumber and bell pepper (*Capsicum annuum* L.) fruit (Wang and Baker, 1979). Low-O₂ pretreatment of avocado possibly results in a higher level of reducing power (SH) and greater membrane integrity, which together increase the fruit resistance to CI.

The induction of AA and ethanol formation (Table 1) during low-O₂ treatment may play a role in inhibiting ripening and reducing CI. AA is an active molecule in plant tissues because it can form covalent bonds with protein-containing NH₂ groups by forming a Schiff base (Perata, 1992). Frenkel and Erez (1992) suggested that ethanol vapors reduce CI symptoms in cucumber seedlings as a result of biophysical changes. In addition, anaerobic treatment of plant tissues induced the synthesis of anaerobic stress proteins and the disruption of normal protein synthesis (Nover, 1989).

We have shown that a 24-h treatment with 3% O₂ + 97% N₂ resulted in increased production of AA, ethanol, and free SH groups, which led to delayed softening, inhibition of ethylene production, and reduced CI damage of 'Fuerte' avocado during storage at 2°C and upon return to ambient conditions. The mechanisms directly responsible for these phenomena bear further study.

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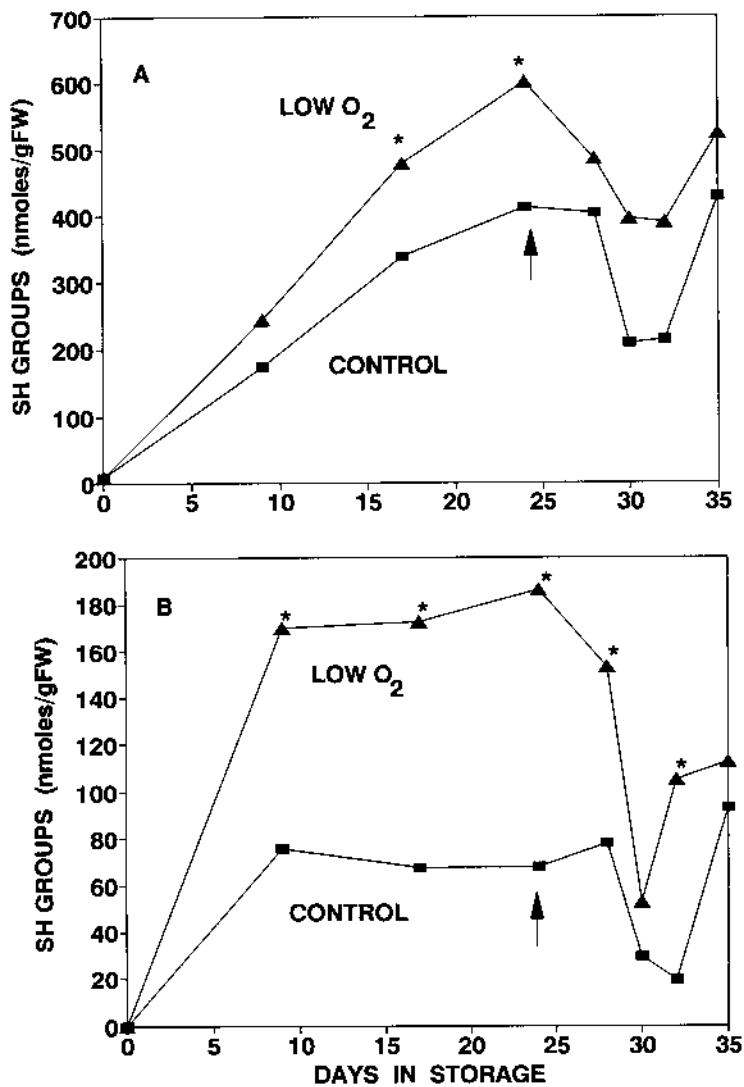


Fig. 7. Effect of a 24-h low-O₂ pretreatment at 17C on the concentration of SH groups content in the (A) pulp or (B) peel of avocado during storage at 2C followed by storage at 17C. Results are the means of five measurements. Arrows indicate time of transfer from 2 to 17C. *Significant differences between treatments, $P \leq 0.05$. Note that ordinate scales for parts A and B differ by a factor of ≈ 3.5 .

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