

## **The Role of Ethylene in Browning of Avocado Pulp during cold storage**

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### **Summary**

Application of exogenous ethylene, irrespective of the method of application, caused intensification of avocado fruit pulp browning during cold storage in all cultivars tested. Avocado fruit (cv. Ettinger) was treated with Ethrel (2-chloroethyl phosphonic acid) prior to packing and storage. The Ethrel-treated fruits developed severe pulp browning after 3 weeks storage at 5C.

Treating avocado cv. Fuerte with ethylene gas (100 ppm) for 24 h at 20C prior to storage at 5C for 3 weeks caused pulp browning, which increased dramatically during ripening.

Treating avocado cv. Hass with 50 ppm ethylene, for 12, 24, and 48 h at 5C, prior to storage at 3C, caused a gradual increase in pulp browning after 3 weeks at 3C followed by ripening. The 48h ethylene-treated fruit exhibited the most severe browning.

### **Introduction**

It is well established that ethylene, the ripening hormone, accelerates ripening in all climacteric fruit (Biale, 1960). Avocado is one of the fruits in which internal levels of ethylene accumulate dramatically during ripening to high levels of around 70 ppm (Burg and Burg, 1962).

Discoloration of the avocado mesocarp (pulp browning) during cold storage is one of the symptoms of chilling injury (CI) expressed in avocado (Chaplin et al., 1982; Bower et al., 1989; Cutting et al., 1990). Browning of the pulp causes high percent of losses every year in the export of avocado (Van Lelyveld and Bower, 1984). The mesocarp discoloration in avocado has been attributed to oxidation of phenols by the enzyme polyphenol oxidase (Kahn, 1977) and the amount of phenols in the pulp (Van Lelyveld and Bower, 1984; Bower et al., 1989).

In order to reduce CI symptoms in avocado, expressed as peel injury and pulp browning, several ways have been examined including: controlled atmosphere (CA), modified atmosphere (MA), anaerobiosis and heat treatments. Hatton and Reeder (1972) showed that removal of ethylene from avocado cv. Lula stored in CA at 10C increased the percentage of acceptable fruit at the end of the storage period. Storage in CA (2% O<sub>2</sub> 10% CO<sub>2</sub>) at 5.5C for 28 days reduced mesocarp discoloration of cv. Fuerte (Bower et al., 1989). Application of anaerobiosis for 24h prior cold storage at 2C, reduced ethylene production and CI symptoms in avocado cv. Fuerte (Pesis et al., 1994). It was shown that acetaldehyde, which is the intermediate metabolite in anaerobic condition inhibits ethylene formation in avocado and inhibits ACC oxidase activity (Pesis et al., 1998). Heat treatments at 38C for 48h reduced CI symptoms in avocado cv. Hass at 1C (Woolf et al., 1995). Chan (1986) demon-

strated that heat treatment in water at 49C for 20 to 60 minutes reduced the conversion of ACC to ethylene in papaya fruit disks by ACC oxidase. Also in apples heat treatments inhibit ripening and ethylene production (Lurie and Klein, 1990).

Continuous ethylene application to avocado at 6C cold storage accelerated ripening. However, application of ethylene to avocado for 24 h before cold storage did not cause acceleration in fruit ripening after removal to shelf life (Zauberman and Fuchs, 1973). It is well known that application of ethylene prior to marketing avocado enhances the ripening process without causing injury to the fruit (Zauberman et al., 1988). However, the influence of applied exogenous ethylene on peel injury and pulp browning in avocado stored at cold temperature has not been reported.

In this work we studied the effect of application of ethylene, before or during cold storage or removal of ethylene during cold storage, on avocado peel injury and pulp browning.

### **Material and Methods**

**Fruit, material and treatments.** Avocado fruit (cv. Ettinger) was dipped in Ethrel (2-chloroethyl phosphonic acid) solution (concentration 500 ppm) at the packinghouse prior to packing and storage. Ethrel is an ethylene-generating compound and is used for color development in fruits and to accelerate the ripening process (DeWilde, 1971).

The Ethrel-treated fruit and nontreated fruit were stored at 3 temperatures, 4, 6 and 8C, for 3 weeks. In addition Ethrel-treated and nontreated fruit were ripened at 20C without cold storage. Following cold storage the firmness was determined and the fruit removed to 20C ripening. The fruit were held until the fruit was soft before pulp browning was examined.

Avocado cv. Fuerte fruit were treated with ethylene gas in ethylene-applied room (100 ppm, 24 h at 20C). The control fruit were held for 24 h at 20C in a ethylene-free room. All fruit were held for 3 weeks at 5C and then removed to 20C for ripening.

Avocado cv. Hass fruit were treated with ethylene gas in ethylene-applied room (40 ppm, 24h at 5C) and after ethylene exposure the fruit were removed and stored at either 1C, 3C, or 5C). Control fruit was placed immediately at the 3 different temperatures without ethylene treatment.

In a second experiment cv. Hass fruit were treated with ethylene gas in ethylene-applied room (50 ppm at 5C) for various durations, 12, 24 and 48h. After treatment the fruit were stored at 3C for 2 to 3 weeks. Control fruit were placed immediately at 3C. Firmness was checked upon removal from cold storage after 2 and 3 weeks. Pulp browning was evaluated following ripening at 20C.

**Fruit ripening and injury indices.** Fruit firmness was determined by destructive and non-destructive methods. In the destructive method fruit firmness, expressed as Newtons (N), was measured on two pared sides of each fruit, using an electronic penetrometer (Chatillon, New York, NY) with a 6.5-mm conical tip.

Non-destructive measurement of firmness was accomplished by grading the fruit by hand touch using a descending 10 to 1 point scale: Firm fruit = 10, elastic fruit = 5, soft fruit = 1.

The Firmness Index was calculated by the following formula:

$$\text{Firmness Index} = \frac{\sum (\text{Index level}) * (\text{no. of fruit in this level})}{\text{Total no. of fruit}}$$

Chilling injury (CI) of the peel was represented by peel darkening and pitting. The peel injury index was evaluated using a 0 - 10 point scale: 0 = no injury, 1 = low injury, 10% of the fruit with injury; 5 = medium damage, 30% of the fruit with injury; 10 = high injury level, 50% and above of the fruit peel with injury. The index was calculated according the following formula:

$$\text{Peel Injury Index} = \frac{\sum (\text{Index level}) * (\text{no. of fruit in this level})}{\text{Total no. of fruit}}$$

Pulp browning was checked on completely soft fruit (ready to eat) after ripening at 20C. The fruit was cut into 2 halves longitudinally for examination of the browning. The internal damage is expressed as the percentage of the fruit with internal browning out of total number of fruits.

For all experiments each treatment was done with 5 export quality boxes of 4kg fruit/box for a total of around 80 fruit per treatment. All fruits were taken from commercial packing-houses on the day of packing. In cvs. Ettinger and Fuerte the 4kg/box contain 14 - 16 fruit/box. In cv. Hass the 4kg/box contain 16 - 18 fruit/box.

The results are averages  $\pm$  SD.

### **Results and Discussion**

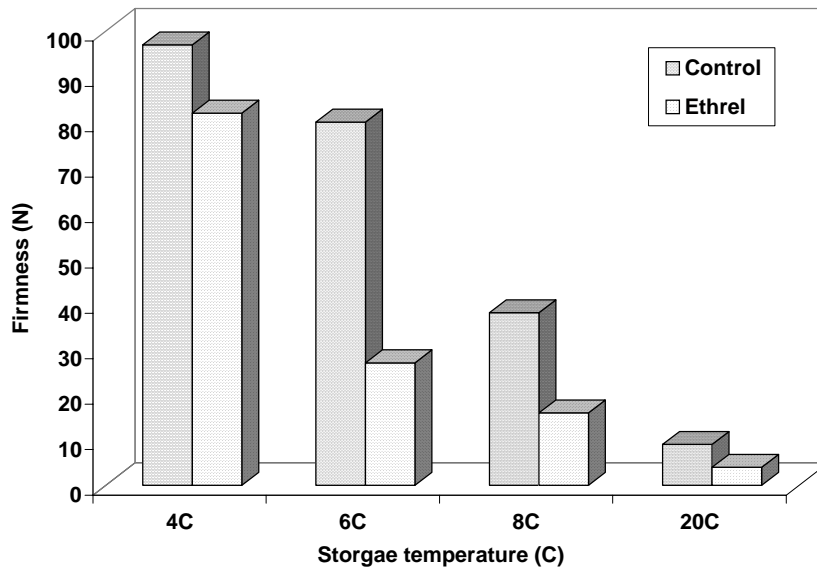
The effect of Ethrel dipping prior to storage on 'Ettinger' fruit softening and pulp browning was very significant. After 3 weeks of cold storage, all fruit softened much quicker when removed from the higher storage temperature (8C) compared to 4 and 6C (Fig. 1). The nontreated and Ethrel-treated fruit showed the same trend of softening although the Ethrel-treated fruit had lower fruit firmness upon removal from cold storage. The influence of Ethrel on the acceleration of fruit softening can be attributed to the effect of ethylene on acceleration of the activity of the enzyme cellulase, which is one of the main cell wall degrading enzymes responsible for avocado softening (Pesis et al., 1978).

The Ethrel-treated fruits developed severe pulp browning after 3 weeks storage at all temperatures. On the other hand, in the fruit stored at 20C no pulp browning was observed (Fig. 2). The CI symptoms expressed as pulp browning is probably a result of combined conditions of ethylene existence in the tissue and an environment of low temperature.

Storage of avocado cv. Fuerte at 5C after application of ethylene gas (100 ppm, 24h at 20C) caused severe CI symptoms shown as peel injury and pulp browning (Table 1). It is interesting that the difference in firmness between the ethylene-treated and the control fruit disappeared during 6 days of shelf life. This result is in agreement with results shown by Zauberman and Fuchs (1973) who showed that only continuous applied ethylene in cold storage caused acceleration of softening and ripening, while application for 24h was not enough for acceleration of softening. Application of ethylene prior to cold storage to cv.

Fuerte caused tremendous peel injury shown as pitting and darkening on the green peel which intensified during ripening at 20C. In cv. Fuerte, which is a green-peel cultivar, the peel damage is very obvious. In this experiment the peel injury and the pulp discoloration was not associated with softening, but with applied ethylene. The percentage of pulp browning in the ethylene-treated fruit was very high (61.5%) compared to no browning in the control fruit (Table 1).

**Figure 1. Effect of Ethrel dip (500 ppm) before storage on fruit firmness (N) of cv. Ettinger upon removal from 3 weeks storage at 4, 6 and 8C or immediately held at 20C for 8 days.**



Exposure of avocado cv. Hass for various durations of ethylene (12, 24 and 48h) caused only slight amount of fruit softening upon removal from cold storage after 3 weeks in the 24 and 48h ethylene-treated fruit (Table 2). However, the most pronounced result was, the longer the ethylene exposure the higher the level of pulp browning after the fruit softened (Table 2). It was shown in the past that the longer the duration of ethylene exposure (24 h vs. 18 h) the faster is the softening process, while 1, 6 and 12 h did not trigger softening (Zauberman et al., 1988). Also in our experiment a 12 h exposure to ethylene did not trigger softening, but increased significantly mesocarp browning.

**Table 1. Effect of ethylene application (100 ppm, 24h at 20C) on fruit firmness (index 10 - 1), peel injury (index 0 - 10) and pulp browning (%) of cv. Fuerte after 2 and 6 days at 20C following 3 weeks at 5C.**

Treatment	After 2 days at 20C		After 6 days at 20C		
	Firmness (index)	Peel Injury (index)	Firmness (index)	Peel Injury (index)	Pulp browning (%)
Control	8.60±1.17	0.14±0.14	4.10±0.83	0.71±0.07	0.0
C <sub>2</sub> H <sub>4</sub>	6.87±0.33	2.57±0.49	5.56±0.28	4.27±0.65	61.5

Figure 2. Effect of Ethrel dip (500 ppm) before storage on pulp browning of cv. Ettinger following 3 weeks storage at 4, 6 and 8C and removal to shelf life at 20C or immediately stored at 20C for 8 days. Pulp browning (% of fruit) was determined after the fruit softened completely.

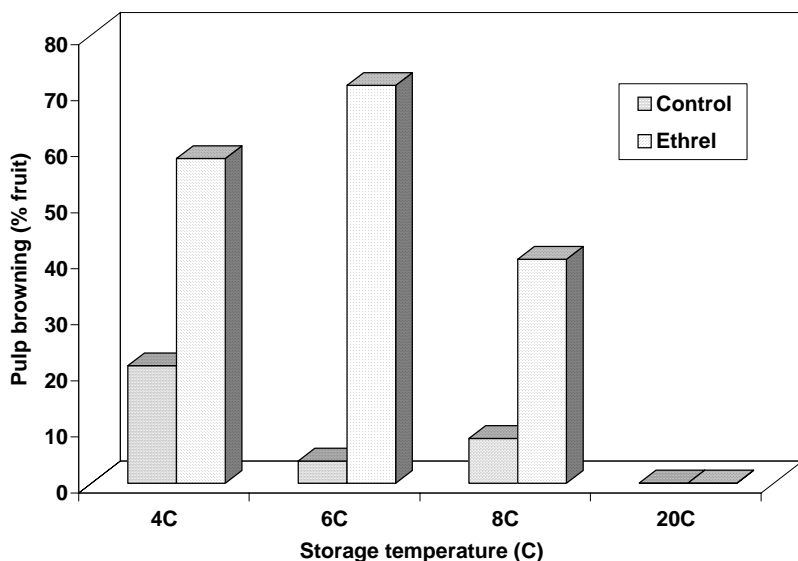


Table 2. Effect of application of ethylene on cv. Hass (50 ppm at 5C) for different durations (12 h, 24 h and 48 h) on fruit firmness (Newtons) and pulp browning (% Fruit). The firmness was checked at the removal from cold storage after 2 and 3 weeks at cold storage in 3C. Pulp browning was determined at 20C in soft fruit, following storage.

Cold wks.	Exposure ethylene (hours)			Control
	12	24	48	
<b>Pulp Browning (%)</b>				
2 wk	2.6 ± 4.4	20.9 ± 6.9	43.8 ± 10.8	0.5 ± 0.0
3 wk	52.7 ± 14	85.1 ± 2.8	78.2 ± 19.2	5.6 ± 5.1
<b>Firmness (N)</b>				
2 wk	139 ± 8.7	129 ± 16.4	134 ± 14	139 ± 10.7
3 wk	129 ± 10.0	91.3 ± 34.5	114 ± 26.2	130 ± 9.6

### Conclusions

Application of exogenous ethylene, irrespective of the method of application, caused intensified chilling injury symptoms, after cold storage, in three avocado cultivars: 'Ettinger', 'Fuerte' and 'Hass'.

Ethylene is the main agent combined with the low temperature that accelerates peel injury and pulp browning even after 2 weeks of cold storage. Control fruit suffered from much lower chilling injury symptoms after 2 - 3 weeks storage.

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