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Chilling Injury During Avocado and Mango Fruit Storage: Development and Prevention

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Abstract:

Shrink-film wraps reduced weight loss and delayed ripening, but had no significant effect on chilling symptoms. Vacuum infiltration of calcium (0.2 M) delayed ripening and reduced chilling symptoms, but had an adverse effect on external quality. Intermittent warming had no significant effect on chilling symptoms and accelerated ripening. Combining shrink-film wraps and intermittent warming did not reduce chilling symptoms significantly. The fatty-acid composition of the lipids showed no significant changes during ripening, chilling or when ripened after chilling. Chilling exposures which result in chilling symptoms reduce the rate of ethylene production and no respiratory climacteric was observed at 20° after severe chilling exposures.

No increase in peroxidase activity was observed in 'Fuerte' avocado fruits which showed chilling injury symptoms as a result of storage at 0 or 2°C. Peroxidase activity in the fruit declined along with fruit softening during the ripening process. It is suggested that peroxidase activity in avocado fruit mesocarp has no role in the development of the chilling injury disorder appearing as dark patches on the skin of the fruit.

Avocado fruit of the 'Pinkerton' cultivar was stored at 5°C for 16 days and then transferred to 20°C for ripening, or kept at 20°C throughout. At 20°C, cellulase and polygalacturonase activity and the reduction in firmness preceded the onset of ethylene production by the fruit. At 5°C, the ethylene-producing apparatus was induced without any significant increase in the activity of these two enzymes.

In both mango and avocado fruits, electrolyte leakage cannot serve as an indication for the development or presence of chilling injury symptoms in the fruit. However, increased leakage of solutes is related to ripening in these fruits.

The activity of peroxidase and cellulase in cold stored (5°C) 'Keitt' mango fruit peel started to increase before any changes in total soluble solids (TSS) and in acid content of the pulp could be observed. It is concluded that the increase in activity of the two enzymes is part of the chilling injury syndrome which develops during storage of mango fruit at cold temperatures.

Objectives of the Research:

As chilling injury in subtropical fruits is caused by metabolic disturbances resulting from low temperatures, above the freezing point of the fruit tissues, it is more difficult to substantially prolong the storage of avocado and mango fruits using low temperature techniques as is done successfully with many deciduous fruits. Therefore, the objectives of the research were:

- a. To study physiological and biochemical processes occurring in avocado and mango fruits stored at low temperatures which may induce chilling injury.
- b. To evaluate various commercial storage practices as well as the handling of these fruits during transport and marketing. The achievement of these objectives will provide better quality fruit and will reduce wastage substantially.

Body of the Report:

Introduction

The increasing demand for high quality avocados and mangos throughout the world requires transport of the fruit long distances and storage for relatively long periods of time after harvest. Low temperatures are required to delay ripening, but subtropical fruits are sensitive to low temperatures which result in loss of quality and failure to ripen due to chilling injury. These investigations were undertaken to gain a better understanding of the fundamental physiological and biochemical processes occurring during low temperature storage which results in chilling injury and loss of fruit quality and to explore practices which could be used commercially to extend the storage life and reduce chilling injury to avocados and mangos.

Effect of Shrink-Wrap Film (Cryovac D-925) on Ripening, Weight Loss and Quality of 'Fuerte' and 'Hass' Avocado Fruits

The effect of shrink-wrap film using Cryovac D-925 film on ripening, weight loss and quality of avocado fruit was evaluated. Experiments were conducted during two seasons with 'Fuerte' and 'Hass' avocado fruit. Control (unwrapped) and wrapped 'Fuerte' fruits were placed directly at 20°C and samples were stored for 2, 4 and 6 weeks at 5°C and then transferred to 20°C. The treatments with 'Hass' avocado fruits were control (unwrapped), heat (unwrapped fruit passed through the heat tunnel used to shrink the film) and wrapped. Fruits were placed directly at 20°C and samples were stored for 2 and 4 weeks at 5°C and then transferred to 20°C. All individual fruits were weighed initially and evaluated for external quality. Six individual fruits were used for respiratory rate and ethylene production determinations at 20°C. Ten fruits of each treatment were placed in open containers at 20°C and ripening and external and internal quality was recorded when ripe. When samples were transferred to 20°C from 5°C for evaluation, each fruit was weighed and the external quality recorded. External and internal quality was rated on a scale of from 1 to 4 as follows: 1 - no damage or discoloration, 2 - slight, 3 - moderate and 4 - severe.

Wrapping individual avocados in shrink-wrap film greatly reduced weight loss during storage at 5°C (Fig. 1). The weight loss after 4 weeks storage at 5°C for unwrapped and wrapped 'Fuerte' avocados was 3.6 and 0.4%, respectively, and for 'Hass' avocados 2.8 and 0.6%, respectively.

Ripening at 20°C was delayed by wrapping for fruit placed directly at 20°C and at 20°C after storage at 5°C (Tables 1 and 2). The internal quality of 'Fuerte' and 'Hass' avocados was not influenced by wrapping. Wrapped 'Fuerte' fruit had better external quality after 2, 4 and 6 weeks storage at 5°C and when ripened at 20° after storage at 5°C than unwrapped fruit (Table 1). All 'Hass' fruit maintained good external and internal quality (Table 2).

The respiratory rate and ethylene evolution of wrapped 'Fuerte' avocados placed directly at 20°C was reduced by about 50% compared with the rates for unwrapped fruit (Fig. 2). The response at 20°C after storage at 5°C was similar to that of the 'Hass' cultivar to be discussed next.

The unwrapped and heated unwrapped 'Hass' fruits had similar climacteric peak heights for respiration and ethylene production when placed directly at 20°C

with the unwrapped heated fruits peaking 1 day earlier than the unwrapped, unheated fruit (Fig. 3). The wrapped fruit had lower peaks (about 33% less) than the unwrapped fruit and peaked 1 to 2 days later.

The respiratory rate and ethylene evolution of 'Hass' avocados at 20°C following 2 and 4 weeks at 5°C for the unwrapped fruit were typical of those previously reported (Figs. 4 and 5). The respiratory rate and ethylene evolution of wrapped fruit was reduced by about 50% and 30%, respectively, compared with unwrapped fruit.

In summary, wrapping individual avocado fruits in shrink-wrap film reduced weight loss, respiratory rate, ethylene evolution and increased the days to ripen at 20°C when placed directly at 20°C and at 20°C after storage at 5°C. The external appearance of 'Fuerte' avocados of wrapped fruit was better than unwrapped fruit. Although the delay in ripening time and improvement in external appearance of 'Fuerte' avocados were not large, film wrapping may provide the advantage necessary to market avocados in export markets.

Effect of Intermittent Warming During Storage at 0° and 5°C on Ripening and Chilling Injury of 'Fuerte', 'Hass' and 'Pinkerton' Avocado Fruits

Intermittent warming during an exposure to chilling temperatures has been reported to reduce chilling injury symptoms of several chilling-sensitive crops. Therefore, the effect of intermittent warming at 15°C for 1 or 2 days every 7 days on ripening, and external and internal quality at 20°C on 'Fuerte' avocados stored at 0° and 5°C was evaluated.

Two experiments were initiated, one on 22 March, 1983 and the other on 26 April, 1983. Randomized samples of 10 fruit were used for each treatment in each experiment. Treatments were: (1) placed directly at 20°C; (2) stored at 0° and 5°C continuously for 4 or 6 weeks (22 March 1983) and 3 or 5 weeks (26 April, 1983) and then transferred to 20°C; (3) stored at 0° and 5°C and after every 7 days transferred to 15°C for 1 or 2 days until the designated holding period and then transferred to 20°C. External and internal evaluation procedures are described in the previous section.

Intermittent warming reduced the time to ripen at 20°C (Tables 3 and 4). Two days warming every 7 days caused a greater reduction in time to ripen at 20°C than 1 day. After the longer storage periods, some intermittent, warmed fruit were beginning to soften and ripened rapidly which would result in commercial marketing difficulties.

In some treatments, intermittent warming reduced internal chilling symptoms, but even in those cases the fruit were still of questionably quality. The external quality of the fruit showed no consistent trends related to treatments.

Experiments were conducted in 1984 using 10°C as the warming temperature in place of the 15°C used the previous year. Two experiments were conducted with 'Fuerte', two with 'Hass' and one with 'Pinkerton' (a new green skin cultivar with a skin texture similar to 'Hass'). Each treatment consisted of 6 fruit for respiratory rate and ethylene evolution determinations, and 10 fruit for ripening and external and internal quality evaluations. Treatments were: (1) placed directly at 20°C; (2) stored at 0° and 5°C continuously for 4 or 6 weeks and then

transferred to 20°C for observation; (3) stored at 0° and 5°C for 4 and 6 weeks which were transferred each week to 10°C for 1 day and then transferred to 20°C for observation; (4) stored at 0° and 5°C for 4 or 6 weeks which were transferred every 2 weeks to 10°C for 1 day. Intermittent warming for 1 day each week or 1 day every 2 weeks had no significant effect on chilling symptoms as determined by external and internal quality compared with the continuous exposures at the respective storage temperatures (Tables 5 and 6). Fruit warmed 1 day each week ripened in a shorter time at 20°C, with the fruit warmed 1 day every 2 weeks ripening next, and the fruit held continuously at the cold temperatures ripened last (Tables 5 and 6). The response of fruit in the other 'Fuerte' and the two 'Hass' experiments were similar to those presented in Tables 5 and 6.

Effect of Shrink-Wrap Film and Intermittent Warming, Individually and Combined, on Chilling Injury, Weight Loss, Ripening and Quality of Avocado Fruits

The effects of shrink-wrap film and intermittent warming, individually and combined, on the responses of avocado fruit during storage and at 20°C after storage, were evaluated. Experiments were conducted on early-, mid- and late-season 'Pinkerton', 'Fuerte' and 'Hass' avocados. The holding conditions for unwrapped and wrapped avocados were: (1) placed directly at 20°C; (2) stored continuously at 0° and 5°C for 2, 4 or 6 weeks and then transferred to 20°C; (3) stored at 0° and 5°C for 2, 4 or 6 weeks with a transfer to 10°C for 1 day each week and then transferred to 20°C, and (4) stored at 0° and 5°C for 4 and 6 weeks with a transfer to 10°C for 1 day every 2 weeks and then transferred to 20°C. Each treatment consisted of 6 fruit for respiratory rate and ethylene evaluation determinations at 20°C, and 10 fruit which, when transferred to 20°C, were held in open containers and evaluated for days-to-ripen and external and internal quality. All fruit were marked and weighed initially, and weighed at time of transfer to 20°C, and when ripe. Data recorded included weight loss, external quality (initially, when transferred to 20°C and when ripe), internal quality when ripe, and respiratory rate, and ethylene evaluation of the fruit placed directly at 20°C, and at 20°C after the various storage treatments.

The responses of avocado fruits to shrink-film wrapping and intermittent warming evaluated separately were similar to those reported previously. Shrink-film wrapped fruit subjected to intermittent warming, ripened at essentially the same time as unwrapped fruit after the same chilling exposure, while intermittent warming of unwrapped fruit resulted in ripening in a much shorter time when transferred to 20°C. The combined treatments of shrink-film wrapping and intermittent warming had no significant effect on the development of chilling injury symptoms compared with the treatments, individually or with unwrapped fruit, held continuously at 0° and 5°C (Tables 7, 8 and 9). Early-season fruit tended to be more susceptible to chilling injury than mid- or late-season fruit; significant chilling symptoms were observed on early-season fruit after 4 weeks storage at 0° and 5°C, while only slight or no symptoms developed on mid- or late-season fruit after 4 weeks storage at 0° or 5°C (Tables 8 and 9). Significant differences in chilling injury symptoms were not detected between avocados held at 0° or 5°C and, in some cases, fruit held at 0°C were slightly better in quality after storage and when ripe than fruit held at 5°C.

Shrink-film wraps significantly reduced weight loss and were particularly important for early-season fruit which tended to show shrivelling without the film wraps. Prolonged chilling exposures at 0° or 5°C reduced and, in cases of

severe chilling injury, eliminated the respiratory climacteric peak and reduced the maximum rate of ethylene production. Wrapping reduced the respiratory rates and rate of ethylene production compared with the same storage treatment which were unwrapped (Figures 2-5). Late season 'Hass' avocados tended to develop off-flavors which have been characterized as rancid, when ripe, whether chilled or not.

The Effect of Calcium on Ripening, Respiratory Rate, Ethylene Evolution and Quality of Avocado Fruits

Postharvest treatments with calcium have been reported to suppress physiological disorders in apples and to delay ripening and reduce chilling in avocados. Therefore, experiments were conducted to further evaluate the responses of 'Fuerte' and 'Hass' avocados to postharvest treatment with calcium. Several experiments compared a range of calcium concentrations between 0.05 and 0.5 M calcium chloride as a dip and vacuum infiltration (50 mm Hg for 1 min) into the fruit with untreated fruit and water treated fruit. The ripening rate, respiratory rate and ethylene evolution was determined at 20°C. Experiments on the effect of ethylene and propylene applied exogenously on the ripening rate, respiratory rate and ethylene evolution of avocados vacuum infiltrated with 0.1 and 0.3 M calcium were conducted at 20°C. Storage experiments were conducted to determine the effect of 0.2 M calcium vacuum infiltrated into the fruit on storability and chilling injury. Control (untreated) fruit and calcium treated samples were placed directly at 20° and other samples were held for 1, 3 and 5 weeks at 0° and 5°C and then transferred to 20°C. At 20°C, 6 fruits were used for respiratory rate and ethylene evolution determinations and 10 fruits were placed in open containers for ripening rate and external and internal evaluations when ripe.

Dipping avocados for 15 minutes in a range of calcium concentrations between 0.05 and 0.5 M had no significant influence on ripening, respiratory rate or ethylene evolution at 20°C compared with untreated fruit or fruit dipped in water.

The respiratory climacteric peaks of mid-season 'Hass' avocados for control (untreated) and vacuum infiltrated with water and 0.05, 0.1, 0.2 and 0.3 M calcium were 220, 220, 200, 170, 135 and 80 ml CO₂/Kg-Hr, respectively (Figure 6). The respective peak rates of ethylene production were 240, 240, 210, 150, 100 and 70 µl ethylene/Kg-Hr (Figure 7). Similar results were obtained with early- and late-season 'Hass' avocados and with 'Fuerte' avocados. Although the climacteric peaks of all treatments occurred on the ninth or tenth day, ripening of the fruit treated with 0.1 and 0.2 M calcium was delayed 1 and 2 days, respectively compared with untreated fruit. Fruit treated with 0.3 M calcium softened somewhat about 4 days after untreated fruit ripened, but did not attain acceptable eating quality. In preliminary experiments, 0.4 and 0.5 M calcium vacuum-infiltrated into the fruit essentially eliminated the climacteric and ethylene production, and the fruit failed to ripen even when exposed to 10 ppm exogenous ethylene for 2 days beginning 1 day after treatment.

Avocados were vacuum-infiltrated with calcium and then exposed to 10 ppm ethylene or 1000 ppm propylene for 2 days. Propylene elicits the same physiological responses as ethylene and permits ethylene production determinations during the treatment. Untreated and 0.1 and 0.3 M calcium (vacuum infiltrated) Hass avocados not exposed to propylene reached climacteric peaks of 200, 140 and 70 ml CO₂/Kg-Hr, respectively (Figure 8). The respective peak respiratory

rates for fruit exposed to 1000 ppm propylene for 2 days beginning immediately after treatment were 225, 170 and 100 ml CO₂/Kg-Hr (Figure 8). The peak rate was attained 2 days after initiation of the experiment for the untreated and 0.1 M calcium-treated fruit. The 0.3 M calcium treated fruit maintained a respiratory rate near 100 ml CO₂/Kg-Hr for 4 days and then slowly declined to a rate of about 50 ml CO₂/Kg-Hr by the tenth day. The respective peak rates of ethylene production of treatments not exposed to propylene were 225, 155 and 145 µl ethylene/Kg-Hr (Figure 9) which were attained 7, 8 and 10 days, respectively, after initiation of the experiment. The peak rates of ethylene production for fruits exposed to propylene were 250, 180 and 40 µl ethylene (Kg-Hr) and were attained after 3, 4 and 5 days. The untreated and 0.1 M calcium-treated fruit not exposed to propylene, ripened after 9 and 10 days, respectively, and the propylene treated fruit of both ripened after 5 days. The 0.3 M calcium-treated fruit, exposed to propylene or not exposed to propylene, softened some after 6 and 12 days, respectively, and both failed to complete normal ripening. Experiments with exogenous ethylene at 10 ppm resulted in similar respiratory and ripening rates as with propylene, but ethylene evolution could not be determined. Also, 'Fuerte' avocados responded in the same manner as Hass.

The effect of 0.2 M calcium vacuum-infiltrated into the fruit on external and internal quality and days to ripen at 20°C after 1, 3 and 5 weeks storage at 0 and 5° was determined with 'Fuerte' and 'Hass' avocado fruit. The use of 0.2 M calcium for the storage experiments was based on the fact that this concentration delayed ripening, respiratory rates and ethylene production without interfering with normal ripening. Calcium treatment delayed ripening of 'Fuerte' and 'Hass' avocados placed directly at 20°C (Tables 10 and 11). The external quality was reduced by the calcium treatment. Calcium-treated fruit displayed lower internal quality indicating a reduction in chilling injury symptoms.

The respiratory rates and ethylene production of the untreated fruit were typical of those reported in the literature for fruit placed directly at 20°C and at 20°C after storage at 0 and 5°C (Figures 10 and 11 data given for 'Fuerte'; 'Hass' avocados were similar). Calcium treatment reduced the respiratory rates and rates of ethylene production of the fruit compared to respective untreated fruit (Figures 10 and 11).

In summary, dipping avocados in calcium solutions up to 0.5 M had no significant effect on ripening rate, respiratory rate or ethylene evolution at 20°C. Vacuum-infiltration of calcium solutions of 0.05 to 0.5 M into avocados reduced the respiratory rate and rate of ethylene evolution at 20°C as the molarity increased. The higher molarities (0.4 and 0.5 M) essentially eliminated the climacteric and ethylene production. The days to ripen at 20° were delayed as the concentration increased to 0.2 M calcium. Avocados vacuum-infiltrated with 0.3 M calcium failed to ripen normally and 0.5 M calcium completely inhibited ripening. Storage experiments for 1, 3 and 5 weeks at 0° and 5°C of avocados vacuum-infiltrated with 0.2 M calcium delayed ripening at 20°C after storage, reduced external quality and reduced internal chilling symptoms when ripe, compared with respective untreated avocados. Commercial application of calcium to avocados to delay ripening and reduce internal chilling symptoms does not appear practical because of the necessity to vacuum-infiltrate the calcium solution into the fruit and the adverse effect on external appearance of the fruit.

The Effect of Chilling Storage Temperatures on the Fatty Acid Composition of the Lipids in Avocado Fruits

The ratio of the saturated fatty acids to unsaturated fatty acids has been implicated in the sensitivity of plant materials to chilling injury. Chilling sensitive plant materials have been reported to have a higher mole percent of saturated fatty acids than plant materials not sensitive to chilling injury. Changes in the fatty acid composition have also been reported for chilling sensitive crops, such as tomato, during and following exposure to chilling temperatures and it has been suggested that these changes may be related to chilling sensitivity. Therefore, the fatty acid composition of avocado lipids were determined initially, after exposure to various chilling and non-chilling temperatures and when ripe at 20°C following the chilling exposures.

Five experiments were conducted, two with 'Fuerte' and two with 'Hass' and one with 'Pinkerton' avocados. The fatty acid composition of the avocado lipids were determined initially, after ripe at 20°C, after storage at 0° and 5°C for 2, 4 and 6 weeks and after 2 and 4 weeks at 10°C and when ripe following transfer to 20°C after the respective storage periods. Also, one experiment with 'Hass' intermittently warmed for one day at 10°C once each week and every two weeks was conducted.

The fatty acid composition of avocado lipids is composed of myristic acid [(14-0); length of carbon chain - number of double bonds]; palmitic acid (16-0), palmitoleic acid (16-1), stearic acid (18-0), oleic acid (18-1), linoleic acid (18-2), and linolenic acid (18-3)]. Oleic acid is the most abundant acid accounting for between 70-80% of the total fatty acids depending upon variety and maturity. Palmitic and linoleic acids each account for from 10 to 15% of the total fatty acids. Palmitoleic acid accounts for about 2% of the total fatty acids. Myristic, stearic and linolenic acids were present in trace amounts (less than 1%).

The fatty acid composition of the lipids from the avocado fruit in all experiments did not change significantly at the end of the storage treatments or after ripening at 20°C. Data are shown for 'Hass' avocado fruit in Figures 12 and 13. Changes in the fatty acid composition of the lipids of other chilling-sensitive fruits have been reported during the chilling exposure and after ripening at a non-chilling temperature following the chilling exposure and these changes have been implicated in chilling susceptibility. The lack of significant changes in the fatty acid composition of the lipids in avocados indicates that fatty acids may not be involved in the mechanism of chilling injury in avocados.

Table 1

Effect of Film Wrap on External and Internal Quality and Ripening of
Fuerte Avocado Fruit at 20°C after 2, 4, and 6 Weeks Storage at 5°C.

Storage Weeks at 5°C	Treatment	External			Internal Ripe	Days to Ripen at 20°C
		Initial	Transfer	Ripe		
0	CK	2.0	-	2.0	1.0	4.0
0	Wrap	2.0	-	2.5	1.0	6.6
2	CK	2.0	2.0	2.4	1.0	2.9
2	Wrap	2.0	2.1	2.2	1.0	4.0
4	CK	2.0	3.0	3.6	1.8	1.8
4	Wrap	2.0	2.0	2.9	1.7	2.9
6	CK	2.0	3.7	3.8	2.4	1.0
6	Wrap	2.0	2.0	2.9	2.4	2.4

Table 2

Effect of Heat Tunnel (Heat) and Film Wrap on External and Internal Quality and Ripening of Hass Avocado Fruit at 20°C after 2 and 4 Weeks Storage at 5°C.

Storage Weeks at 5°C	Treatment	External			Internal Ripe	Days to Ripen at 20°C
		Initial	Transfer	Ripe		
0	CK	1.1	-	1.1	1.0	5.1
0	Heat*	1.0	-	1.0	1.0	5.9
0	Wrap	1.0	-	1.0	1.0	8.1
2	CK	1.0	1.0	1.0	1.0	3.1
2	Heat*	1.0	1.0	1.0	1.0	3.2
2	Wrap	1.0	1.0	1.0	1.0	3.6
4	CK	1.0	1.0	1.0	1.0	2.1
4	Heat*	1.0	1.0	1.0	1.0	2.2
4	Wrap	1.0	1.0	1.0	1.1	2.6

* Run through heated tunnel used to shrink film wrap.

Table 3

Effect of Intermittent Warming for 1 or 2 Days at 15°C every 7 Days on External and Internal Quality and Ripening at 20°C of Fuerte Avocado Fruit Stored for 4 and 6 Weeks at 0° and 5°C.

Treatment		Days at 15°	External			Internal Ripe	Days to Ripen
Storage Period Weeks	Storage Temp. °C		Initial	Transfer	Ripe		
0	-	0	1.4	-	1.4	1.0	5.6
4	5	0	1.5	1.6	1.8	1.8	4.0
4	0	0	1.7	1.7	2.0	2.0	4.3
4	5	1	1.5	1.7	2.3	1.9	2.7
4	0	1	1.3	1.4	2.3	1.9	2.4
4	5	2	1.2	1.9	2.7	1.9	1.4
4	0	2	1.5	2.0	2.9	2.1	1.6
6	5	0	1.6	2.3	3.1	2.8	2.6
6	0	0	1.5	1.7	3.5	3.3	3.0
6	5	1	1.7	2.3	3.3	2.3	1.3
6	0	1	1.6	2.0	3.2	2.6	1.5
6	5	2	1.8	2.0	2.9	2.0	0.5
6	0	2	1.3	2.1	3.0	2.3	1.1

Table 4

Effect of Intermittent Warming for 1 or 2 days at 15°C every 7 Days on External and Internal Quality and Ripening at 20°C of Fuerte Avocados Stored for 3 and 5 Weeks at 0° and 5°C.

Storage Period Weeks	Treatment		External			Internal	Days to
	Storage Temp. °C	Days Days at 15°	Initial	Transfer	Ripe	Ripe	Ripen
0	-	0	2.0	-	2.0	1.0	5.5
3	5	0	2.1	2.1	3.0	1.5	4.3
3	0	0	1.9	2.0	3.0	1.9	4.5
3	5	1	2.1	2.3	2.8	1.7	3.3
3	0	1	2.1	2.1	2.7	1.8	3.1
3	5	2	2.0	2.0	2.8	1.9	2.6
3	0	2	1.8	1.9	2.9	1.8	3.0
5	5	0	1.7	2.1	3.0	2.8	3.2
5	0	0	2.0	2.2	3.3	3.0	3.6
5	5	1	2.1	3.0	3.4	2.3	0.5
5	0	1	2.1	2.7	3.2	2.7	0.8
5	5	2	2.0	3.5	3.8	3.0	0.3
5	0	2	2.0	3.4	3.6	2.5	0.4

Table 5

Effect of Intermittant Warming for 1 Day at 10° Every Week and Every 2 Weeks on External and Internal Quality and Days to Ripen at 20°C, After Storage Treatments of Fuerte Avocado Fruit Stored for 4 and 6 Weeks at 0° and 5°C.

Storage period (weeks)	TREATMENT		Initial	EXTERNAL		INTERNAL Ripe	Days to ripen at 20°C
	Storage temp. °C	Warmed* at 10° weeks		Transfer to 20°C	Ripe		
0	-	-	1	-	1.1	1	6.4
4	5	-	1	1	1.6	1	4.4
4	5	1	1	1	1.6	1	3.2
4	5	2	1	1	2.1	1	4.1
4	0	-	1	1	1.9	1	5.7
4	0	1	1	1	1.8	1	4.7
4	0	2	1	1	1.7	1	4.8
6	5	0	1	2.1	3.1	2.5	2.5
6	5	1	1	2.0	2.6	2.0	2.0
6	5	2	1	2.3	2.9	1.7	1.7
6	0	-	1	1	2.5	2.2	3.9
6	0	1	1	1.6	3.1	2.3	2.8
6	0	2	1	1.6	3.0	2.4	2.8

* - Indicates continuous at storage temperature.
 1 Indicates transfer to 10°C for 1 day each week.
 2 Indicates transfer to 10°C for 1 day every 2 weeks.

Table 6

Effect of Intermittant Warming for 1 Day at 10° Every Week and Every 2 Weeks on External and Internal Quality and Days to Ripen at 20°C, After Storage Treatments of Pinkerton Avocado Fruit Stored for 4 and 6 Weeks at 0° and 5°C.

Storage period (weeks)	TREATMENT		Initial	EXTERNAL		INTERNAL Ripe	Days to ripen at 20°C
	Storage temp. °C	Warmed* at 10° weeks		Transfer to 20°C	Ripe		
0	-	-	1	-	1	1	9.0
4	5	-	1	1	1.1	2.0	5.4
4	5	1	1	1	1	1.5	4.9
4	5	2	1	1	1.4	3.1	6.4
4	0	-	1	1	1	1.7	6.3
4	0	1	1	1.1	1.3	2.0	5.4
4	0	2	1	1	1.2	1.6	5.1
6	5	-	1	1.3	1.7	3.7	3.3
6	5	1	1	1.2	1.6	3.2	2.9
6	5	2	1	1.1	1.3	3.4	3.7
6	0	-	1	1	1.3	2.4	5.9
6	0	1	1.1	1.1	1.3	2.9	3.3
6	0	2	1	1.1	1.1	2.9	4.2

* - Indicates continuous at storage temperature.

1 Indicates transfer to 10°C for 1 day each week.

2 Indicates transfer to 10°C for 1 day every 2 weeks.

Table 7

Effect of Intermittant Warming for 1 Day Each Week at 10°C and Shrink-film Wrapping on the External and Internal Quality and Days to Ripen at 20°C After the Storage Treatment of Fuerte Avocado Fruit Stored for 2, 4 and 6 Weeks at 0° and 5°C.

Storage temp. °C	TREATMENT			Wrap	EXTERNAL			INTERNAL Ripe	Days to ripen
	Storage period (weeks)	Warmed 1 day/wk at 10°C			Initial	Transfer	Ripe		
Initial	-	-	No		1.9	-	1.9	1	5.8
Initial	-	-	Yes		1.3	-	1.3	1	9.0
5	2	No	No		2	2	2	1	3.5
5	2	No	Yes		1.5	1.5	2	1	5.8
5	2	Yes	No		1.5	1.5	1.6	1	4.1
5	2	Yes	Yes		2	2	2	1	5.8
0	2	No	No		1.5	1.5	1.9	1	4.4
0	2	No	Yes		1.5	1.5	1.9	1	7.3
0	2	Yes	No		1.1	1.2	1.7	1	3.5
0	2	Yes	yes		1	1	2.6	1	6.5
5	4	No	No		2	2	2	1	2.9
5	4	No	Yes		1.7	1.7	2	1	5.0
5	4	Yes	No		1.8	1.8	2.2	1	2.7
5	4	Yes	Yes		1.9	1.9	2	1	5.1
0	4	No	No		1.7	1.7	1.8	1	4.9
0	4	No	Yes		1.2	1.2	2.4	1.4	8.2
0	4	Yes	No		1.9	1.9	1.9	1	4.0
0	4	Yes	Yes		1.8	1.8	2.1	1	5.2
5	6	No	No		2	2.2	3.0	1.9	2.3
5	6	No	Yes		1.9	1	2.1	2.3	3.9
5	6	Yes	No		1.9	2.7	3.1	2.1	1.4
5	6	Yes	Yes		1.2	1.6	1.9	2.1	2.8
0	6	No	No		1.7	1.7	2.5	2.5	6.0
0	6	No	Yes		1	1	2	2	8.1
0	6	Yes	No		1.7	1.7	2.7	2.6	3.7
0	6	Yes	Yes		1.4	1.4	1.9	2.7	4.0

Table 8

Effect of Intermittant Warming for 1 Day Each Week at 10°C and Shrink-film Wrapping on the External and Internal Quality and Days to Ripen at 20°C After the Storage Treatment of Early Season Hass Avocado Fruit Stored for 2, 4 and 6 Weeks at 0° and 5°C.

Storage temp. °C	TREATMENT			EXTERNAL			INTERNAL Ripe	Days to ripen
	Storage period (weeks)	Warmed 1 day/wk at 10°C	Wrap	Initial	Transfer	Ripe		
Initial	-	-	No	1	1	1.0	1.0	12.1
Initial	-	-	Yes	1	1	1.0	1.0	15.3
5	2	No	No	1	1	1.0	1.0	11.2
5	2	No	Yes	1	1	1.0	1.0	14.1
5	2	Yes	No	1	1	1.0	1.0	8.2
5	2	Yes	Yes	1	1	1.0	1.0-	12.3
0	2	No	No	1	1	1.0	1.0	11.8
0	2	No	Yes	1	1	1.0	1.0	14.7
0	2	Yes	No	1	1	1.0	1.0	8.5
0	2	Yes	Yes	1	1	1.0	1.0	13.5
5	4	No	No	1	1	1.3	2.2	7.1
5	4	No	Yes	1	1	1.2	2.1	10.4
5	4	Yes	No	1	1	1.1	2.3	6.3
5	4	Yes	Yes	1	1	1.3	2.1	9.8
0	4	No	No	1	1	1.2	2.3	7.2
0	4	No	Yes	1	1	1.1	2.0	11.3
0	4	Yes	No	1	1	1.3	2.3	6.5
0	4	Yes	Yes	1	1	1.2	2.1	10.6
5	6	No	No	1	1	1.5	3.6	5.7
5	6	No	Yes	1	1	1.6	3.4	8.4
5	6	Yes	No	1	1	1.4	3.7	4.9
5	6	Yes	Yes	1	1	1.6	3.3	8.5
0	6	No	No	1	1	1.5	3.0	6.1
0	6	No	Yes	1	1	1.4	3.1	9.2
0	6	Yes	No	1	1	1.6	2.9	5.4
0	6	Yes	Yes	1	1	1.4	3.0	8.1

Table 9

Effect of Intermittant Warming for 1 Day Each Week at 10°C and Shrink-film Wrapping on the External and Internal Quality and Days to Ripen at 20°C After the Storage Treatment of Late Season Hass Avocado Fruit Stored for 2, 4 and 6 Weeks at 0° and 5°C.

Storage temp. °C	TREATMENT		Wrap	EXTERNAL			INTERNAL Ripe	Days to ripen
	Storage period (weeks)	Warmed 1 day/wk at 10°C		Initial	Transfer	Ripe		
Initial	-	-	No	1	-	1	1	6.1
Initial	-	-	Yes	1	-	1	1	8.9
5	2	No	No	1	1	1	1	4.4
5	2	No	Yes	1	1	1	1	6.6
5	2	Yes	No	1	1	1	1	4.1
5	2	Yes	Yes	1	1	1	1	6.0
0	2	No	No	1	1	1	1	4.5
0	2	No	Yes	1	1	1	1	7.8
0	2	Yes	No	1	1	1	1	4.1
0	2	Yes	yes	1	1	1	1	7.6
5	4	No	No	1	1	1	1.4	3.6
5	4	No	Yes	1	1	1	1.3	5.2
5	4	Yes	No	1	1	1	1.3	3.1
5	4	Yes	Yes	1	1	1	1.9	4.1
0	4	No	No	1	1	1.1	1.7	4.1
0	4	No	Yes	1	1	1	1.3	5.2
0	4	Yes	No	1	1	1	1	3.0
0	4	Yes	Yes	1	1	1	1.4	4.5
5	6	No	No	1	1	1	1.7	1.1
5	6	No	Yes	1	1	1	1.6	2.5
5	6	Yes	No	1	1	1	1.4	0.5
5	6	Yes	Yes	1	1	1	1.7	1.1
0	6	No	No	1	1	1	2.4	2.9
0	6	No	Yes	1	1	1	1.5	4.0
0	6	Yes	No	1	1	1	1.8	1.2
0	6	Yes	Yes	1	1	1	1.5	2.7

Table 10

Effect of Calcium (0.2 M CaCl_2 Vacuum Infiltrated) on External and Internal Quality and Days to Ripen at 20°C after 3 and 5 Weeks Storage at 0° and 5°C on Fuerte Avocado Fruit

Storage Period Weeks	Treatment		External*			Internal*	Days to Ripen
	Storage Temp. °C	Calcium	Initial	Transfer	Ripe	Ripe	
0	-	No	2.0	-	2.0	1.0	5.7
0	-	Yes	2.0	-	2.5	1.0	6.9
3	5	No	2.0	2.0	2.6	2.0	5.1
3	5	Yes	2.0	2.0	2.9	1.7	6.0
3	0	No	2.0	2.0	3.1	2.7	5.2
3	0	Yes	2.0	2.0	3.3	2.2	6.1
5	5	No	2.0	2.0	3.3	2.9	4.2
5	5	Yes	2.0	2.0	3.8	2.3	5.6
5	0	No	2.0	2.3	3.6	3.4	5.2
5	0	Yes	2.0	2.8	4.0	2.6	7.0

* External and internal quality rated as follows: 1 = None; 2 = Slight;
3 = Moderate and 4 = Severe damage or discoloration.

Table 11

Effect of Calcium (0.2 M CaCl_2 Vacuum Infiltrated) on External and Internal Quality and Days to Ripen at 20°C after 1, 3 and 5 Weeks at 0° and 5°C on Hass Avocado Fruit

Storage Period Weeks	Treatment		External*			Internal*	Days to Ripen
	Storage Temp. °C	Calcium	Initial	Transfer	Ripe	Ripe	
0	-	No	1.0	-	1.0	1.0	6.4
0	-	Yes	1.0	-	1.0	1.0	8.1
1	5	No	1.0	1.0	1.0	1.0	6.3
1	5	Yes	1.0	1.0	1.0	1.0	7.0
1	0	No	1.0	1.0	1.0	1.0	6.3
1	0	Yes	1.0	1.0	1.0	1.0	7.1
3	5	No	1.0	2.0	2.5	1.0	4.6
3	5	Yes	1.0	2.9	3.1	1.0	5.1
3	0	No	1.0	1.0	1.7	1.0	4.7
3	0	Yes	1.0	1.6	2.6	1.0	5.2
5	5	No	1.0	2.6	2.7	2.2	5.8
5	5	Yes	1.0	3.0	3.5	1.8	6.2
5	0	No	1.0	2.2	2.5	2.5	5.6
5	0	Yes	1.0	2.5	3.1	2.0	6.4

* External and internal quality rated as follows: 1 = None; 2 = Slight;
3 = Moderate and 4 = Severe damage or discoloration.

Figure 1. Percentage weight loss of control (unwrapped) and film-wrapped Hass avocados after 2 and 4 weeks at 5°C and Fuerte avocados after 2, 4 and 6 weeks at 5°C.

Figure 2. Respiratory rates and ethylene production of control (unwrapped) and film-wrapped Fuerte avocado fruit at 20°C.

Figure 3. Respiratory rates and ethylene production of control (unwrapped), heat-treated (unwrapped fruit exposed to the shrink-film heat treatment) and film-wrapped Hass avocado fruit at 20°C.

Figure 4. Respiratory rates and ethylene production of control (unwrapped) and film-wrapped Hass avocado fruit at 20°C after 2 weeks storage at 5°C.

Figure 5. Respiratory rates and ethylene production of control (unwrapped) and film-wrapped Hass avocado fruit at 20°C after 4 weeks storage at 5°C.

Figure 6. Respiratory rate of Hass avocado fruit vacuum infiltrated with water (H_2O) and 0.05, 0.1, 0.2 and 0.3 M calcium solutions and control (untreated) at 20°C.

Figure 7. Ethylene production of Hass avocado fruit vacuum-infiltrated with water (H₂O) and 0.05, 0.1, 0.2 and 0.3 M calcium solutions and control (untreated) at 20°C.

הספיקה של פליטת
למדע המזון

Figure 8. Respiratory rate of Hass avocado fruit vacuum-infiltrated with 0.1 and 0.3 M calcium solutions and control (untreated) in air and 1000 ppm propylene applied immediately after treatment at 20°C.

Figure 9. Ethylene production of Hass avocado fruit vacuum-infiltrated with 0.1 and 0.3 M calcium solutions and control (untreated) in air and 1000 ppm propylene applied immediately after treatment at 20°C.

Figure 10. Respiratory rate of Fuerte avocado fruit vacuum-infiltrated with 0.2 M calcium and control (untreated) at 20°C after 0, 3 and 5 weeks storage at 5°C.

Figure 11. Ethylene production of Fuerte avocado fruit vacuum-infiltrated with 0.2 M calcium and control (untreated) at 20°C after 0, 3 and 5 weeks storage at 5°C.

Figure 12. Changes in fatty acid composition as percentage of total lipids of Hass avocados during storage for 0, 2, 4 and 6 weeks at 5°C and after subsequent ripening.

Figure 13. Changes in fatty acid composition as percentage of total lipids of Hass avocados during storage for 0, 2, 4 and 6 weeks at 0°C and after subsequent ripening.

Peroxidase Activity in Avocado Fruit Stored at Chilling Temperatures

Introduction

Storage of avocado fruit, at temperatures lower than 10°C but higher than 0°C, for relatively long periods of time always results in the development of chilling injury. This physiological disorder has been described by several workers (Eaks, 1976; Chaplin et al., 1982; Couey, 1982; Van Lelyveld and Bower, 1984; Swarts, 1984). The most common symptoms are darkening of the skin and the appearance of gray or dark-brown discoloration of the mesocarp tissue. Recently, it was reported that an avocado fruit stored at 5.5°C for 31 days and kept under restricted ventilation conditions for 1 or 2 days, the activity of polyphenol oxidase and of peroxidase in the mesocarp increased (Van Lelyveld and Bower, 1984), as well as fruit mesocarp discoloration.

Since chilling may involve oxidation phenomena, it was of interest to study peroxidase activity in 'Fuerte' avocado fruit, at different storage temperatures and during different storage periods as related to chilling injury symptoms development, changes in firmness, respiration and ethylene production.

Materials and Methods

Fully mature (as determined by oil content) 'Fuerte' avocado fruits were harvested and on the same day were stored untreated, in different, well ventilated, refrigerated chambers at 0°, 2°, 5° or 20°C. The fruit was packed in ordinary commercial 4-kg cartons. At each temperature, five fruits were monitored individually for their respiration and ethylene production for 18 consecutive days in the cold storage and then at 20°C for ripening. Carbon dioxide and ethylene evolution were determined by gas chromatography in the headspace of 2-liter jars inside which a single fruit was enclosed for 1 h. Periodically (after 8, 11 and 18 days), five fruits from each temperature were transferred to 20°C for ripening and tested for chilling injury symptoms (dark patches on the skin of the fruit) and peroxidase activity in the fruit mesocarp (McCune, 1961). Fruit firmness was determined with a mechanized "Chatillon" pressure tester, equipped with a conical tip, 6.5 mm in diameter. The pressure which was required to penetrate the fruit through the peel was recorded (Zauberman and Fuchs, 1973). All determinations were carried out in five replicates of a single fruit, and the standard deviation was calculated.

Results

When avocado fruits were stored at 0°, 2° or 5°C for 18 days, their peroxidase activity stayed at about the same level as at harvest time (Figure 1). The same was true for the firmness of these fruits (Figure 2). In all cases in which avocado fruits were transferred to 20°C either immediately after harvest or after various periods of storage at 0°, 2° or 5°C, peroxidase activity in the mesocarp declined, as did the firmness of the fruit (Figures 1 and 2).

The first chilling injury symptoms (darkening of the fruit skin) could be observed after 8 days of storage at 0°C or after 10 days of storage at 2°C and the injury became more severe with time, either in the cold storage or subsequently at 20°C (not shown in the figures). No such injuries were observed in fruits stored at 5°C for 18 days or in fruits kept continuously at 20°C from the first day after harvest.

The maximal rates of ethylene production were lower in fruits stored for 18 days at 0°, 2° or 5°C and then transferred to 20°C, than in those which were stored at 20°C immediately after harvest (Table I). Similar trend can be observed for fruit respiration as well.

Discussion

The results (Figures 1 and 2) show that peroxidase activity is related to avocado fruit ripening. When softening of the fruit was prevented, during storage at 0°, 2° or 5°C, no change in the relatively high peroxidase activity had occurred. The lowest activity was exhibited always in the soft, ripe fruit. In mature green tomatoes stored in a modified atmosphere at 12°C, peroxidase activity was reported to remain unchanged during a period of 8 weeks (Goodenough, 1982). On the other hand, peroxidase activity was reported to increase as a result of mechanical injury or viral infection of the avocado tree bark (Da Graca and Van Lelyveld, 1978; Van Lelyveld and Bester, 1979). Also, Van Lelyveld and Bower (1984) reported the enhancement of peroxidase activity in avocado fruit stored at 5.5°C and under restricted ventilation (low oxygen) and associated the phenomenon with initiation of avocado fruit mesocarp discoloration. They suggested that the increased peroxidase activity "may be associated with ethylene evolution".

In our study, no increase in peroxidase activity was detected at any storage temperature in spite of the fact that chilling injuries were observed at 0° and 2°C. However, in our experiments the chilling injury appeared as darkening of the exocarp (skin), similar to the description of Swarts (1984), rather than as dark brown discoloration of the mesocarp as described by Van Lelyveld and Bower (1984). No anaerobic conditions prevailed in our experiments. Our data do not support the suggestion that increased peroxidase activity may be associated with ethylene evolution, as in Table I it can be seen that in cold-stored fruit, the maximal rates of ethylene production (during subsequent ripening at 20°C) were significantly lower than in fruit stored continuously at 20°C. Also, in all cases (Figures 1 and 2), the activity of peroxidase declined during ripening (softening), a stage at which ethylene is produced and evolved at the highest rates in avocado fruit.

Therefore, we conclude that peroxidase activity in avocado fruit mesocarp has no role in the development of the chilling injury disorder appearing as dark patches on the skin of the fruit.

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Figure 1 Peroxidase activity in mesocarp of avocado fruit stored at 0, 2 or 5°C (—). Arrows (∇) indicate the day of transfer of fruit to 20°C for ripening. Changes in peroxidase activity after transfer to 20°C (- - - - -). Vertical bars indicate the standard deviation of five replicates.

Figure 2 Firmness (pressure required to penetrate the mesocarp) of avocado fruit stored at 0, 2 or 5°C (————). Arrows (↓) indicate the day of transfer of fruit to 20°C for ripening. Changes in firmness after transfer to 20°C (-----). Vertical bars indicate the standard deviation of five replicates.

Table I. Maximum rates of respiration and ethylene production of 'Fuerte' avocado fruits stored at different temperatures for 18 days and then transferred to ripen at 20°C. Numbers are average of five replicates $\pm$ their standard deviation.

Storage temperature °C	Maximum rate of respiration mg CO ₂ kg ⁻¹ h ⁻¹	Maximum rate of ethylene production μl kg ⁻¹ h ⁻¹
20 continuously	187 $\pm$ 27	44 $\pm$ 19
5	109 $\pm$ 3	6 $\pm$ 4
2	132 $\pm$ 17	11 $\pm$ 8
0	140 $\pm$ 43	10 $\pm$ 3

Polygalacturonase, Cellulase and Softening, as Related to Ethylene Production in Avocado Fruit Stored at Chilling Temperatures

Introduction

Today, it is common practice to trigger the ripening of climacteric fruits with ethylene. It has been well documented that ethylene treatment enhances the ripening of detached mature avocado fruit (Biale, 1960; Eaks, 1966; Zauberman and Fuchs, 1973a,b). Also, it has been shown (Pesis et al., 1978) that cellulase activity in avocado fruit is enhanced as a result of ethylene treatment. On the other hand, it has been reported (Awad and Young, 1979) that in 'Fuerte' avocado stored at 20°C, the onset of cellulase activity preceded any measurable production of ethylene by the fruit. As polygalacturonase and cellulase activity and fruit softening are related to ripening, we studied the pattern and time of onset of the above parameters as related to ethylene production by the fruit at storage temperatures of 5° and 20°C.

Materials and Methods

Avocado fruit (*Persea americana* Mill.) cv. 'Pinkerton' was stored at 20° and 5°C from the day of harvest. Periodically, ethylene production, fruit firmness and polygalacturonase and cellulase activities were determined. After 16 days, the fruit stored at 5° was transferred to 20°C for ripening.

Ethylene production. Five fruits were sampled periodically and their ethylene production was determined by enclosing each fruit for exactly 1 h in a 2-liter jar. A 1 ml sample of the head space was injected into a gas chromatograph for ethylene determination. The limit of the detection was 20 ppb ethylene/ml. The same fruits were then tested for their firmness and later for their enzymatic activities.

Fruit firmness. Firmness was determined, without removing the peel, by a "Chatillon" pressure tester, using a conical tip 6.5 mm in diameter (Zauberman and Fuchs, 1973a). The beginning of softening could be sensed by hand when the resistance to penetration was approximately 35 Newtons. Five fruits per treatment were tested at two points at each determination, and then sampled and extracted for polygalacturonase and cellulase activities.

Enzyme extraction. Twenty grams of pulp from each of the five fruits per treatment were extracted with 60 ml of 1 M NaCl dissolved in 0.05 M acetate buffer, pH 5.0, with a blender for 3 min. The filtered extract was used as the enzyme solution for both polygalacturonase and cellulase activity determination.

Cellulase assay. Five ml of enzyme solution were incubated with 10 ml of 1% carboxymethylcellulose dissolved in 0.1 M acetate buffer, pH 5.5. The enzyme activity was expressed as percent loss of viscosity determined with Ostwald Viscometer (Volac No. 150) during 30 min incubation at 30°C.

Polygalacturonase assay. The activity of this enzyme was determined by incubating 5 ml of enzyme solution with 10 ml of 1% citrus pectin (Yakhin, Israel) dissolved in 0.05 M acetate buffer, pH 5.0. The enzyme activity was expressed as percent loss of viscosity determined with an Ostwald Viscometer (Volac No. 150) during 30 min incubation at 30°C.

Results

At 20°C, the increase in cellulase and polygalacturonase activities and the decrease in firmness preceded the onset of ethylene production by the fruits (Figure 1). In fruit which was stored at 5°C for 16 days and then transferred to ripen at 20°C, all the above parameters of ripening increased together after the transfer to 20°C. Ethylene was detected one day after the fruit was transferred, and its peak of production preceded the peaks of the enzyme activities and of softening (Figure 1).

Discussion

Based on the above data (Figure 1) and those of Awad and Young (1979) with the 'Fuerte' cultivar, it appears that at 20°C softening processes such as cellulase and polygalacturonase activities and the decrease in firmness are not triggered by ethylene but rather precede the onset of ethylene production by the fruit. However, after storage at low temperature (5°C), the onset of ethylene production occurred soon after transfer to 20°C and the peak of production preceded those of polygalacturonase and cellulase activities and pulp softening (Figure 1). Treatment with exogenous ethylene was reported (Pesis et al., 1978) to accelerate the onset of cellulase activity in the 'Fuerte' cultivar but this does not mean necessarily that naturally, at ambient temperatures (approximately 20°C), this is the sequence of events. It has been shown recently (Mauch et al., 1984) for other enzymes, chitinase and β -1,3-glucanase, that exogenous ethylene application may induce their activity in plant tissues; however, under fungal infection conditions there are factors (elicitors) other than ethylene which induce the onset of enzyme activity in the tissues. Also, it has been shown (Anderson et al., 1982) that cellulysin (a commercial cellulase) acts as an inducer of ethylene production in tobacco leaves.

It appears that in avocado fruit the action of cellulase and polygalacturonase on the cell walls is to release or induce the ethylene production processes at 20°C. However, during storage at 5°C, the release or induction of these processes occurs without any significant increase in the activity of either enzyme.

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Fig. 1. The time course of ethylene production, of cellulase and polygalacturonase activities, and of reduction in firmness in ripening avocado fruit stored at 20°C, and in fruit stored at 5°C for 16 days and then transferred to ripen at 20°C.

Chilling Injury and Electrolyte Leakage in Cold-Stored Mango and Avocado Fruits

Introduction

Tropical and subtropical fruits are susceptible to chilling injury when exposed to low but non-freezing temperatures in the range of 0 to 12°C. It has been suggested that solute leakage and disrupted ion balance resulting from ultrastructural changes in membranes and cellular permeability, are events leading to development of chilling injury symptoms in sensitive plant tissues (Lyons, 1973). Increased leakage of solutes related to chilling injury was observed in sweet potato tubers (Lieberman et al., 1958), corn seedlings (Creencia and Bramlage, 1971), cotton seedlings (Guinn, 1971), cucumber leaves (Wright and Simon, 1973), mature-green tomato fruit (Lewis and Workman, 1964; King and Ludford, 1983), and cucumber fruit (Murata and Tatsumi, 1979). However, in other studies with fruit tissues, such as peach (Furmanski and Buescher, 1979), bell pepper and eggplants (Murata and Tatsumi, 1979), and tomato (Lewis, 1956), electrolyte leakage was reported to be unaffected by chilling. King and Ludford (1983) have suggested that electrolyte leakage in tomato fruit seems to be a function of fruit type rather than of chilling sensitivity.

Avocado and mango fruit are known to be susceptible to chilling injury (Couey, 1982). The purpose of this study was to determine whether electrolyte leakage and ripening parameters in these fruits are affected during the development of chilling injury during storage at low temperatures and subsequent ripening at 20°C.

Materials and Methods

Mango. Mature green, preclimacteric 'Haden' mango fruit (*Mangifera indica* L.) was harvested and stored on the following day at 2°, 5° and 20°C. Fruit samples, five from each storage temperature, were taken periodically for determination of electrolyte leakage, total soluble solids, titratable acidity and chilling injury. Additional fruit was transferred at the same time from 2° and 5°C to 20°C for ripening, sampled twice, and the parameters mentioned above were determined in the fruit.

Electrolyte leakage. From each of the five randomly selected fruit in each treatment, ten pulp discs (12 mm in diameter and 3 mm thick) were taken from the equatorial region. The discs were weighed, rinsed three times in deionized water, and placed in 25 ml of 0.4 M mannitol solution (in 50 ml Erlenmeyer flasks) in a shaking water bath (100 strokes per min) at 23 ± 1°C. Electrical conductivity readings of the solution were taken after 1 and 3 h as a measure of electrolyte leakage from the discs, using a TH27 conductometer (El-Hama Instruments, El-Hama, Israel). After 3 h of incubation, the conductivity was, in general, 10% higher than after 1 h in all samples, and therefore data of only 3 h are presented. After 3 h the flasks were frozen at -18°C, thawed, autoclaved for 30 min, cooled to 23°C, and a final conductivity reading was taken for total electrolytes. All leakage data were expressed as a percentage of total electrolyte readings (King and Ludford, 1983).

Total soluble solids. Juice from each of the sampled fruits was determined for its soluble solids using an Atago, automatic temperature compensation, hand refractometer.

Titrateable acidity. The same juice as above was titrated with 0.1 N sodium hydroxide using phenolphthalein as an indicator. The acidity was calculated as percent citric acid in the juice.

Chilling injury. Pitting of the fruit skin and disorders in its color development were the most obvious symptoms of chilling injury.

Avocado. Mature 'Hass' avocado fruit (*Persea americana* Mill.) was harvested and stored on the following day at 0°, 5° and 20°C. Five fruit samples from each storage temperature were taken periodically for determination of electrolyte leakage, firmness and chilling injury. Additional fruit was transferred at the same time from 0° and 5°C to 20°C for ripening and was sampled for the determination of the above mentioned parameters.

Electrolyte leakage. This parameter in avocado was determined in the same way as for mango (described above).

Fruit firmness. The same five fruit samples which were used for electrolyte leakage determinations were first measured for their firmness, as a measure of ripeness, using a mechanized Chatillon pressure testor, equipped with a 6.5 mm diameter conical tip (Zauberman and Fuchs, 1973).

Chilling injury. Pitting of the avocado fruit skin was the most obvious symptom of chilling injury observed in this study.

Results and Discussion

Although there was some increase in electrolyte leakage 14 and 20 days after harvest in mango stored at 2° and 5°C (Figure 1), it was not significantly different from the leakage measured 1, 4 7 and 10 days after harvest (Duncan's multiple range test, 5% level). However, it is obvious that with the advance in ripening at 20°C, there was a significant increase in electrolyte leakage in mango fruit. No significant changes in total soluble solids or in titrateable acidity were observed during 19 days of storage at 2° and 5°C, but these parameters also changed significantly during ripening at 20°C (Figure 1). Chilling injury in the mango fruit was observed for the first time only 20 days after harvest and were more severe in fruit stored at 2° (10% of the peel area) than at 5°C (5% of the peel area). After 7 days at 20°C, the pitted peel area reached 60 and 50% of the peel area for fruit stored at 2° and 5°C, respectively. As data for mango fruit at both temperatures are quite similar, only those of fruit stored at 2°C are presented (Figure 1).

In avocado, no increase in electrolyte leakage occurred during 32 days storage at 0° and 5°C, although chilling injury symptoms (pitting of about 5% of the peel area) were observed after 22 days of storage but only at 0°C (Figure 2). The pitted peel area grew with the time up to 15 and 25% at 0° and after transfer to 20°C, respectively. No injuries were observed in avocado fruit stored at 5°C. Both leakage and firmness change significantly (Duncan's multiple range test, 5% level) during ripening of avocado at 20°C (Figure 2).

Electrolyte leakage has been reported to be unaffected by chilling in some other fruits such as peach (Furmanski and Buescher, 1979), bell pepper and eggplant (Murata and Tatsumi, 1970) and tomato (Lewis, 1956). However, in vegetative tissues (Lieberman et al., 1958; Creecia and Bramlage, 1971; Guinn,

1971; Wright and Simon, 1973), in cucumber fruit (Murata and Tatsumi, 1979) and in mature, green tomato fruit (Lewis and Workman, 1964), electrolyte leakage increased in relation to chilling injury. Our data suggest that in chilling injury-sensitive subtropical fruit, such as mango and avocado, electrolyte leakage cannot serve as an indication for the development or presence of chilling injury symptoms in the fruit. Therefore, it appears that in these fruits, changes in cellular permeability are not the events leading to chilling development. It is obvious, however, that increased leakage of solutes is related to ripening in these fruits.

References

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Fig. 1. Electrolyte leakage, total soluble solids, and titratable acidity in mango fruit stored at 2°C ($\triangle$). Fruit was transferred to 20°C ($\square$) for ripening at 7 and 20 days after harvest. Fruit stored directly after harvest at 20°C ($\square$). Arrow indicates the day at which obvious chilling injury symptoms were observed on the fruit for the first time.

Fig. 2. Electrolyte leakage and firmness of avocado fruit stored at 0°C ($\triangle$) and 5°C ($\circ$). Fruit was transferred to 20°C ($\square$) for ripening at 12, 23 and 33 days after harvest. Fruit stored directly after harvest at 20°C ($\square$).

Chilling Injury, Peroxidase and Cellulase Activity in the Peel of Mango Fruit Stored at Low Temperature

Introduction

The mango, a tropical fruit, is highly sensitive to low storage temperature (Couey, 1982). Mattoo and Modi (1969) showed a decrease in amylase activity and an increase in invertase activity as a result of storage at chilling temperatures. Some of the biochemical changes occurring during cold storage of mango fruit were summarized in two reports (Chhatpar et al., 1971; Mattoo et al., 1975). As chilling injury in mango is characterized also by dark discoloration and pitting of the peel, and by uneven softening, it was of interest to study the peroxidase and cellulase activities of fruit at different stages of chilling injury development.

Materials and Methods

Mature green cv. 'Keitt' mango fruit (*Mangifera indica* L.) was stored at 5° and 20°C. The fruit which was stored at 5°C was transferred after 21 days to 20°C for ripening. Periodically, five fruits of each group were sampled at random and peeled. The peel was extracted with acetone (at -20°C) to yield a bright colored powder which was stored at -20°C until extracted with 20 mM phosphate buffer at pH 6.7 for the enzymatic determinations of peroxidase and cellulase. The pulp of the same fruits was analyzed for titratable acidity calculated as citric acid and for total soluble solids (TSS) using a refractometer as a measure of the ripening state of the fruits. Peroxidase activity was determined according to the method described by Van Lelyveld (1978) for mango fruit. Cellulase activity was assayed by a viscosimetric method as described by Pesis et al. (1978) for avocado fruit. Chilling injury symptoms on the fruit peel were estimated visually.

Results

After 14 days in storage, there were clear symptoms of chilling injury on the peel of fruits stored at 5°C. These consisted of brown discoloration spots on the green peel of the Keitt variety (Table 1). These symptoms were similar in other aspects to chilling injury in mango fruit which were described elsewhere (Couey, 1982).

At 20°C, with the advancement of ripening, there was some increase in peroxidase activity (Figure 1). At 5°C, peroxidase activity remained low for 14 days but, parallel to the appearance of the chilling injury symptoms, there was an increase in this activity although the fruit remained at the low temperature. Considering decrease in acidity and increase in TSS as ripening parameters, the fruit remained unripe (Figures 1, 2). After transferring the fruit to 20°C, peroxidase activity reached much higher levels in the peel of chilling injured fruits than of fruits ripened normally at 20°C (Figure 1).

A similar pattern was observed with the activity of cellulase in the peel (Figure 2) except for the fact that during normal ripening at 20°C, there was only a slight increase in the activity of this enzyme.

Discussion

It is quite clear from our data (Table 1; Figures 1, 2) that the increase in both peroxidase and cellulase activity is part of the chilling injury syndrome, which develops during storage of mango fruit at low (5°C) temperatures (Couey, 1982). This increase in activity is much greater than that which occurs during normal ripening, and it started before any changes in TSS and acid content were observed in the fruit. Upon transfer to higher temperature (20°C), the activity of both enzymes (Figures 1, 2) continued to increase, and chilling injury symptoms (Table 1) continued to develop. The involvement of peroxidase in the development of chilling injury symptoms may lead to the conclusion that suitable antioxidants may possibly alleviate or even prevent at least part of the chilling injury syndrome in mango fruit.

References

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Table 1. Development of chilling damage on mango fruit of the Keitt variety during storage at 5°C and subsequent ripening at 20°C.

Days in storage	Storage temperature (°C)	Chilling injury (% of peel area)
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7	5	0
14	5	15
21 (transfer to 20°C)	5	30
22	20	45
27	20	80
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Fig. 1. Changes in peroxidase activity and acid content in mango fruit peel and pulp, respectively, during storage at 5° and 20°C. The vertical bars represent the standard deviation of five replicates.

Fig. 2. Changes in cellulase activity and total soluble solids (TSS) in mango fruit peel and pulp, respectively, during storage at 5° and 20°C. The vertical bars represent the standard deviation of five replicates.

Description of Cooperation

Dr. Eaks visited the laboratory in Israel in February 1983. He became acquainted with the problems and methods of work in Israel. During his visit, discussions were conducted and experiments were planned. In this way, no repetitions occurred and many aspects of postharvest physiology were covered. Dr. Yoram Fuchs visited the laboratory of Dr. I. L. Eaks at the University of California, Riverside, in July 1984. During his visit he learned the methods of work being used in California and became acquainted with the problems of the California avocado industry. Cold storage and transportation facilities were visited and observed. During his stay, many discussions took place in which results were evaluated and plans for further experimentation were outlined.

Main Achievements of the Research

Vacuum infiltration of calcium solutions into avocados delayed ripening and reduced internal symptoms of chilling, but had an adverse effect on external quality. Dipping avocados in calcium solutions had no effect on fruit response. Therefore, commercial application of calcium to avocados to delay ripening and reduce internal chilling injury symptoms, does not appear practical because of the necessity to vacuum-infiltrate the calcium solution into fruit and the adverse effect on external appearance of the fruit. The individual shrink-film wrapping of avocados had no significant effect on external or internal symptoms of chilling injury where low temperature exposure resulted in chilling injury, but did increase the time-to-ripen and reduce weight loss. The weight loss reduction is important with early-season fruit which tend to lose weight rapidly and may shrivel before ripening. Intermittent warming for 1 day at 10°C each week or every 2 weeks, or at 15°C, had no significant effect on chilling symptoms compared with fruit continuously exposed at the respective storage temperatures. Intermittent warming during the chilling exposure did reduce the ripening time after transfer to 20°C compared to fruit held continuously at the chilling temperatures. Combining shrink-film wrapping and intermittent warming did not reduce chilling symptoms or appear to be of commercial value. The fatty acid composition of the lipids in avocados showed no significant changes during chilling exposures or when ripened, which indicates that fatty acids may not be involved in the mechanism of chilling injury in avocados. Also, changes were not detected in the fatty acid composition of late-season Hass avocados which developed off-flavors characterized as rancid. Chilling exposures, which may or may not result in chilling symptoms, reduced the maximum rate of ethylene production at 20°C after chilling and, in cases of severe chilling, no respiratory climacteric peak was observed, and ethylene production was markedly reduced.

In attempts to learn about the processes involved in chilling injury development, we found that peroxidase activity in avocado fruit pulp has no role in the development of the injury. On the other hand, in mango fruit peel, peroxidase activity have increased at the initial stages of the disorder development. Also, the activity of cellulase in mango fruit has increased before any visible symptoms of the injury were obvious. It seems that in avocado fruit pulp, cellulase induction precedes ethylene evolution at ambient and at cold temperatures. These studies enable a better characterization of the chilling injury syndrome.

During the course of this investigation, several observations were made which can be used as guide lines for the storage and transit of California avocados primarily for export where longer holding periods are necessary. Mid-season fruit should be used for export, since early-season fruit are more susceptible to chilling injury and tend to shrivel, due to more rapid water loss, and late-season fruit may develop off-flavors. The total period between harvest and delivery should not exceed four weeks which, even for export shipments, can be managed with expedient handling at the packinghouse. Holding temperatures should be 5°C in the packinghouse and during transit, although lower temperatures could be used since the results indicated that, in some cases, fruit stored at 0°C were in slightly better condition than fruit held at 5°C. Shrink-film wrapping could be used to reduce weight loss and delay ripening. The respiratory rate and rate of ethylene evolution at 20°C following a chilling exposure, can be used to evaluate the severity of the chilling exposure.

List of Publications

- Eaks, I. L., 1985. Effect of calcium on ripening, respiratory rate, ethylene production, and quality of avocado fruit. J. Amer. Soc. Hort. Sci. 110 (2): 145-148.
- Zauberman, G., Fuchs, Y. and Ackerman, M., 1985. Peroxidase activity in avocado fruit stored at chilling temperatures. Scientia Hort. 26:261-265.
- Eaks, I.L. Changes in the fatty acids of avocado fruit during development, storage and ripening. J. Amer. Soc. Hort. Sci., to be submitted.
- Eaks, I. L. Effect of shrink-film wraps on weight loss, respiratory rate, ethylene production and quantity of avocado fruit, in preparation.
- Eaks, I. L. Effect of intermittent warming during chilling exposures on ripening and quality of avocado fruit. J. Amer. Soc. Hort. Sci., to be submitted.