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Avocado (*Persea americana* Mill.) quality changes in response to low-temperature storage

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Abstract

Avocado (*Persea americana* Mill. cv. Hass) fruit were stored at 2, 5, and 8°C for four weeks to determine maximum cold-storage life. Skin colour, fruit firmness, mesocarp appearance, pectinmethylesterase (PME), polygalacturonase (PG), and cellulase (CX) activity were determined weekly during cold storage, and three times a week during subsequent transfer to 22°C. Fruit held at 2°C remained hard green during four weeks, and ripened normally upon removal to 22°C. At the two higher temperatures fruit ripening started during storage. At 5°C, ripening commenced during the fourth week of storage, and minor mesocarp discolouration was observed at full ripeness at 22°C. Fruit held at 8°C ripened after two weeks in storage, resulting in more intense mesocarp discolouration and vascular browning at full ripeness, upon transfer to 22°C. For current Australian commercial storage of avocados cv. Hass, 7°C is too warm, and may be reduced to 2°C for preclimacteric fruit. Results indicate that fruit held at 2°C can be stored for at least four weeks, and possibly five weeks without injury.

Keywords: Avocado; *Persea americana*; Cold storage; Cellulase; Polygalacturonase; Pectinmethylesterase

1. Introduction

The critical temperature for cold storage of unripe avocados has been reported to be 8°C (Lyons, 1973), and in the range 5–8°C for cv. Hass (Snowdon, 1990; Dopico et al., 1993).

Avocado fruit response to cold storage has been studied by Bleinroth et al. (1976); Zauberman et al. (1977); and Berger et al. (1982). Based on their results,

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Australian avocados are currently stored at 7°C (Debney et al., 1980). Although maximum storage life at this temperature is not documented for Australian avocados, it is thought to be too short for commercial sea-freight to European markets. If storage life of avocados can be extended through a reduction in transit storage temperature, sea transport to European markets may be feasible.

This study aimed to define the maximum storage life of cv. Hass at 8°C, and the potential of reducing storage temperature to 5 or 2°C to further extend storage life. Results were confirmed by using fruit from three harvests over the commercial season. Data for only one harvest are presented.

2. Materials and methods

Fruit and treatments

Avocado fruit (cv. Hass) were harvested from a commercial orchard near Nambour (26° S, 153° E), south-east Queensland. Trials were conducted monthly from June to August to obtain a range of fruit maturities.

Fruit were transported to the laboratory within 2 h, dipped in Prochloraz^R (1 min, 0.55 ml l^{-1}, at ambient temperature), and air dried. Fruit were packed in cartons and stored at either 2, 5, or 8°C in well-ventilated rooms. At weekly intervals, 15 to 20 fruit were removed from each temperature and ripened at 22°C. Controls were stored continuously at 22°C and assessed daily. Determination of fruit maturity at harvest was based on dry matter (Horwitz, 1970).

Internal discolouration and fruit firmness

Skin and mesocarp colour were assessed following removal from 22°C. Fruit were assessed visually for discolouration on a 1–4 scale (1 = nil and 4 = severe) using a five-member panel. Fruit firmness was determined using an Instron Universal Testing Machine 1122 fitted with a round-tipped probe of 12.5 mm diameter. The probe was driven towards the equatorial region of the intact fruit with a speed of 20 mm min^{-1}. The peak force, measured in Newtons (N) at 2 mm compression, was assessed twice on each fruit (opposite sides). Resistance to hand pressure was conducted in parallel to qualitatively determine ripening. A comparison of the two methods was established with >25 N = hard, and 8–5 N = eating ripe.

Enzyme activity determination

Pectinmethylesterase (PME; EC 3.1.1.11), polygalacturonase (PG; EC 3.2.1.15) and cellulase (CX; EC 3.2.1.4) were determined weekly during cold storage, and three times a week during ripening at 22°C. Mesocarp (25 g) was homogenised using a hand blender, and solubilised in 75 ml 1 M NaCl for 2 min. The solution was centrifuged at 4300 rpm for 20 min, filtered and the solute used as the crude extract. PME activity was assayed based on a method by Rouse and Atkins (1955). A 10 ml aliquot of the crude extract and 100 ml 1% pectin in 0.2 M NaCl (substrate) were reacted at 30°C. Solution pH was monitored, and the reaction mixture titrated with 0.05 *N* NaOH to maintain pH 7.5. Activity was calculated based on the volume of NaOH required to maintain constant pH for 30 min. PG activity was assayed

according to the method by Kertesz (1951), while CX activity according to that by Pesis et al. (1978). Both methods were based on a reduction in viscosity of a mixture of the enzymes and their respective substrates (1% pectin, which is demethoxylated by PME present in avocado extracts, in 0.1 M acetate buffer pH 5 for PG; 1% carboxymethylcellulose in 0.05 M acetate buffer pH 5 for CX) after 30 min at 30°C. The change between the initial and final drainage time of the mixture in the viscosimeter was used as a measure of loss of viscosity.

3. Results and discussion

Fruit dry matter (DM) increased during the season (23.3% in June, 27.9% in July, and 35.1% in August) indicating that maturity increased (Kader, 1992). As maturity increased, the time for unstored (control) fruit to ripen at 22°C decreased (Table 1). Similar results have also been demonstrated by Eaks (1980), Fuchs and Zauberman (1987), and Cutting and Wolstenholme (1992). Although fruit maturity seems then to play an important role in optimal storage life of non-stored fruit, in the present study we did not detect any obvious differences in fruit response at the three storage temperatures for fruit from the three different harvests (data not presented). The ripening time decreased, also, following cold storage (Table 2). At either 8 or 5°C for one week, ripening was reduced to five days upon removal to 22°C. Storage at 2°C negligibly increased this period (Table 2). A small decrease in ripening time seemed also to occur following two-week storage (Table 2) at all cold temperatures. The ripening time at removal to 22°C following storage beyond two weeks was independent of the storage time (Table 2). Firmness decreased rapidly in control fruit during ripening at 22°C, while CX and PG activities increased (PG at a slower rate) (Fig. 1), as has been shown previously (Zauberman and Schiffmann-Nadel, 1972; Pesis et al., 1978; Awad and Young, 1979; Christoffersen et al., 1984), and PME activity remained constant (Fig. 1). This pattern was maintained in the ripening at 22°C following cold storage of fruit at 2, 5, and 8°C (Fig. 1), but occurred over a shorter period (about five days). A reduction of the ripening period following cold storage, was also observed by Cutting and Wolstenholme (1992) who found that fruit (cv. Fuerte) transferred to 21°C after storage at 5.5°C ripened faster than ambient-stored fruit. Pears (Wilson et al., 1990) and apples (Jobling et al., 1991; Larrigaudière and Vendrell, 1993) also exhibit loss of storage life following cold storage. In these fruits low temperatures induced changes in

Table 1
The effect of maturity as determined by percent pulp DM on days to ripen for avocado (cv. Hass). Fruit were stored continuously at 22°C until firmness was 8 N

Harvest date	Maturity (% DM)	Time to ripen (days)
June	23.3	13
July	27.9	8
August	35.1	6

Table 2
The effect of cold storage temperature on days to ripen for avocado (cv. Hass). Fruit were obtained from a single harvest, DM = 27.9%. Full ripeness was at 8 N

Storage temperature	Time (weeks)	Days to ripen
8°C	1	5
	2	4
	3	4
	4	4
5°C	1	5
	2	4
	3	4
	4	4
2°C	1	6
	2	5
	3	5
	4	5

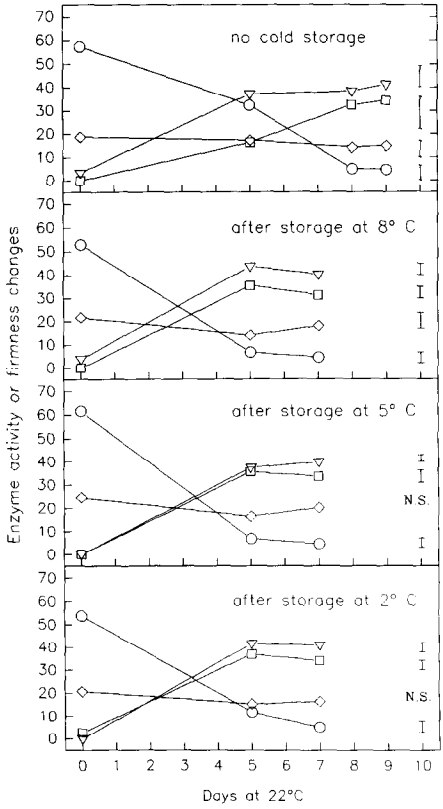

Fig. 1. Changes in fruit firmness (Newtons) (○), PME activity (PME units × 10^4 g^{-1} fresh wt) (◇), PG activity (% loss of viscosity) (□), and CX activity (% loss of viscosity) (▽) of avocado (cv. Hass) ripened at 22°C for controls (no cold storage) and following one week cold storage at 2, 5, and 8°C. [All data represent means of three fruit; July harvest. Vertical bars represent LSD ($P < 0.05$)].

mRNA composition and protein synthesis, that resulted in accumulation of the ethylene precursor, 1-aminocyclopropane-1-carboxylic acid (ACC) (Knee, 1987; Larrigaudière and Vendrell, 1993), believed to be due to activation of ACC synthase as a stress response, or to inhibition of Ethylene Forming Enzyme (EFE) activity. In tomato, Watkins et al. (1990) showed that although EFE mRNA is induced at low temperature, ethylene synthesis is not activated. It seems that this situation also exists in avocado (Dopico et al., 1993).

The higher the storage temperature, the earlier the fruit started to ripen during storage. Fruit stored at 8°C started to ripen after two weeks cold storage. Darkening of the skin was observed at three weeks, concurrent with appearance of PG activity (Fig. 2). CX activity increased progressively with fruit storage. Fruit firmness decreased throughout, with firmness at three weeks half that at harvest (Fig. 2). Fruit stored for up to two weeks at 8°C ripened normally upon removal to 22°C. Although evidence of injury was not expressed during storage, extended cold storage resulted in mesocarp discolouration at full ripeness after transfer to 22°C. Injury was observed either as a darkening of the mesocarp, or localised vascular

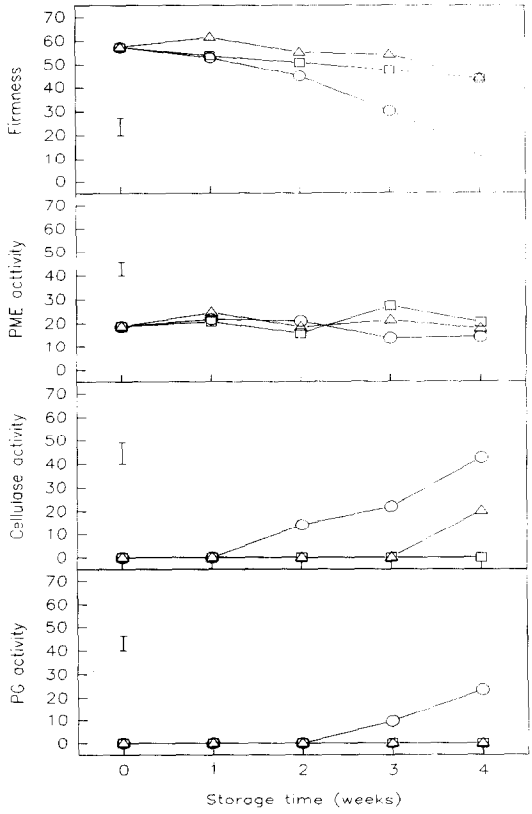

Fig. 2. Changes in firmness (Newtons) and enzyme activity (PME: PME units × 10^4 g^{-1} fresh wt; PG and CX: % loss of viscosity) of avocado (cv. Hass), during cold storage at 2°C (□), 5°C (△), and 8°C (○). [All data represent means of three fruit; July harvest. Vertical bars represent LSD ($P < 0.05$)].

browning possibly associated with polyphenol oxidase activity (Kahn, 1975, 1976). This indicates that maximum storage life at 8°C is between two and three weeks. Reducing the temperature to 5°C prolonged storage life, with ripening initiated after three weeks. Although fruit firmness (measured with the Instron) decreased during storage (Fig. 2), no significant difference in firmness was detected manually. CX activity, indicating that ripening had been initiated, was observed only at four weeks (Fig. 2). At the end of four weeks storage fruit were hard green, and PG activity could not be detected in the tissue. No discolouration of the mesocarp was observed during cold storage. However, following ripening at 22°C, minor mesocarp discolouration and vascular browning was observed at fruit full ripeness. Damage occurred irregularly, and was less intense than in fruit that started to ripen during storage at 8°C.

Fruit stored at 2°C remained hard and green, with no detectable increase in either PG or CX at four weeks (Fig. 2), suggesting that ripening was delayed and fruit were still preclimacteric (Pesis et al., 1978; Awad and Young, 1979). These results agree with the work of Dopico et al. (1993) who did not detect mRNAs for these enzymes in fruit stored 25 days at 3°C. Upon transfer to 22°C fruit ripened normally, both PG and CX increased (Fig. 1), and ripening was complete after five days. No evidence of mesocarp discolouration was observed, indicating that mesocarp damage may be a consequence of ripening during cold storage. The degree of mesocarp injury appears to be closely related to the period of ripening under cold storage. At 2°C (Fig. 2), since ripening was delayed, consequent mesocarp injury was not observed. As storage temperature was increased to 5 or 8°C, fruit started to ripen during storage. The severity of discolouration was therefore more pronounced in the 8°C storage as ripening was initiated earlier, and the remaining time the fruit were subjected to the reduced temperature was longest. We believe that mesocarp injury occurs as a result of ripening during cold storage, where the degree of tissue damage is a function of subsequent storage time. Increased chilling sensitivity of avocados during the climacteric rise was reported by Kosiyachinda and Young (1976). Chilling sensitivity, however, appears to be restricted to the climacteric rise and peak, with Kosiyachinda and Young (1976) suggesting that postclimacteric avocados were less susceptible to chilling injury.

Storage at 2°C was repeated to investigate whether a further extension in storage life beyond four weeks (Fig. 2) was possible. Initial results indicate that fruit remained hard and green at five weeks and ripened normally upon removal to 22°C (Fig. 3), whereas those stored at higher temperatures became brown and discoloured. Although these data need to be confirmed, Jessup (1991) found no evidence of chilling injury in fruit stored for 30 days at 1°C. Further extension of storage life based solely on reduced storage temperature below 1°C, however, is unlikely as Zauberman et al. (1977) and Fuchs and Zauberman (1987), showed that avocados were susceptible to chilling injury at 0°C.

Cold storage of fruit at 2°C resulted in a significant extension of storage life to at least four weeks, and possibly five weeks. Although this is still insufficient to commercially access European markets from Australia by ship, combining cold storage with current advances in controlled-atmosphere technology may be successful.

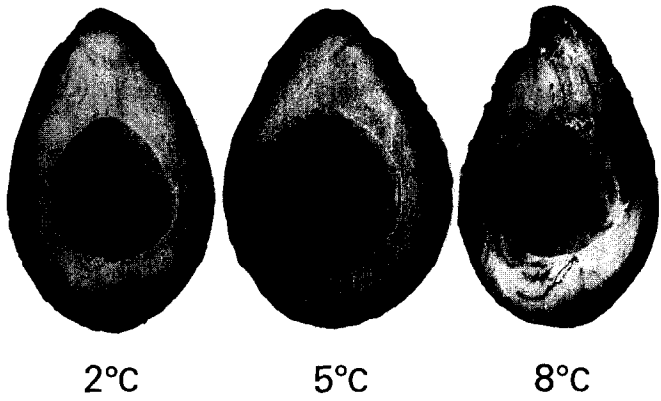

Fig. 3. Pulp discolouration and vascular browning of avocado (cv. Hass) ripened at 22°C following cold storage at either 2, 5, or 8°C for five weeks.

Kanellis et al. (1989) and Metzidakis and Sfakiotakis (1993) have demonstrated that atmospheres of 2.5% O_2 or lower delayed the softening of fruit (cv. Hass), and prevented the rise in EFE, CX and PG activities at 20°C. Preliminary trials in our laboratory (Jordan and Smith, 1993), combining low temperature (7°C) with low O_2 (2%) and high CO_2 (>4%), extended storage to nine weeks. By reducing the temperature to 2°C, as found suitable in this paper, sea-freight of cv. Hass avocados to Europe should become possible.

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