

# Ethylene biosynthesis and ripening behaviour of ‘Hayward’ kiwifruit subjected to some controlled atmospheres

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## Abstract

The effects of storage in air (AS), controlled atmosphere (CA) or ultra low oxygen (ULO) on ethylene biosynthesis and ripening of ‘Hayward’ kiwifruit during storage at 0 °C and post-storage at 20 °C, were investigated. Fruit were stored for 60, 120 and 180 days at 0 °C in AS, CA (2%O<sub>2</sub> + 5%CO<sub>2</sub>) and ULO (0.7%O<sub>2</sub> + 0.7%CO<sub>2</sub> and 1%O<sub>2</sub> + 1%CO<sub>2</sub>). Freshly harvested fruit and fruit removed from storage were treated with 130 µl/l propylene or propylene-free air for 9 days at 20 °C. Fruit treated with propylene at 20 °C at harvest produced ethylene with a lag period of 3 days, had concomitant 1-aminocyclopropane-1-carboxylic acid (ACC) production, 1-aminocyclopropane-1-carboxylate synthase (ACC synthase) and 1-aminocyclopropane-1-carboxylate oxidase (ACC oxidase) activities, and were ripe after 3–5 days while fruit not treated with propylene were not able to produce ethylene and ripen during the 9 days experiment. AS fruit softened faster during the first 60 days of storage. This effect was reduced in CA and ULO treatments. The soluble solids content (SSC) increased markedly during the first 60 days of storage and remained almost constant thereafter in all treatments. After 9 days shelf life, only AS and CA stored fruit were ripe. Fruit from ULO storage required propylene treatment to ripen fully. When kiwifruit were placed at 20 °C, after 60, 120 or 180 days storage at 0 °C, there was an induction of ethylene production with no lag period in fruit from AS or CA, with or without propylene. There was some ACC content and ACC synthase activity after 60 days storage for all treatments, while ACC oxidase activity increased only upon rewarming of the fruit in AS or CA. Kiwifruit removed from ULO-storage showed drastically reduced capacity to produce ethylene mainly due to low ACC oxidase activity rather than reduced ACC production or ACC synthase activity. Respiration increased upon rewarming of the fruit in all treatments. With storage time, there was a decrease in the capacity of the warmed fruit to produce ethylene and CO<sub>2</sub> as well as in the activities of ACC synthase and ACC oxidase, mostly after 60 days storage. © 2002 Elsevier Science B.V. All rights reserved.

**Keywords:** Kiwifruit; Temperature; Ethylene; Controlled atmosphere; Storage

## 1. Introduction

Kiwifruit can be air-stored (AS) for 4–6 months at 0 °C, although extensive softening will

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occur (Arpaia et al., 1987). Low O<sub>2</sub> (2–3%) with 3–5% CO<sub>2</sub> further delayed the rate of kiwifruit softening and increased storage life up to 3–4 months beyond normal AS life (Arpaia et al., 1994b). Removal of ethylene from storage rooms is important (Arpaia et al., 1994b; Stow et al., 2000).

Although ethylene production continues at a slower rate at low storage temperatures for most climacteric fruit (Metzidakis and Sfakiotakis, 1993), in kiwifruit it is almost zero at temperatures below 11–14.8 °C (Stavroulakis and Sfakiotakis, 1993; Antunes et al., 2000). However, kiwifruit stored under low O<sub>2</sub> (<10%) at 20 °C did not produce ethylene even when treated with propylene due to a reduction in 1-aminocyclopropane-1-carboxylic acid (ACC) concentration in the tissue, and O<sub>2</sub> concentrations of less than 5% delayed propylene-induced fruit ripening (Stavroulakis and Sfakiotakis, 1997).

Chilling temperatures similar to those encountered in storage will induce ethylene production and ripening in kiwifruit upon transference to warm temperatures (Hyodo et al., 1987). Hyodo et al. (1987) found ACC accumulation in kiwifruit after 120 days storage in AS, while 1-aminocyclopropane-1-carboxylate oxidase (ACC oxidase) did not show any activity until fruit were rewarmed. Antunes and Sfakiotakis (2002) reported that 12 days chilling stress in kiwifruit advanced the onset of ethylene production in the fruit at 20 °C by stimulating 1-aminocyclopropane-1-carboxylate synthase (ACC synthase) and ACC oxidase activities. After prolonged chilling, ethylene production was reduced in cucumbers (Wang and Adams, 1982; Andersen, 1986), avocados (Eaks, 1983) and nectarines (Brecht and Kader, 1984). This impairment of the ethylene-synthesising capacity may be responsible for the failure of chilled fruit to ripen normally (Eaks, 1983).

Oxygen plays an important role in ethylene biosynthesis since it is a co-substrate of ACC oxidase (Pech et al., 1994). Bufler and Bangerth (1983) reported that in ULO conditions ACC oxidase activity should be low and an accumulation of ACC should be expected. Under low O<sub>2</sub>

conditions, pears accumulated ACC, which was converted to ethylene during post-storage ripening (Blankenship and Richardson, 1986). Bufler and Bangerth (1983), Lau et al. (1984) showed that CA suppressed internal ethylene and ACC levels of apples during storage. The CA storage may change the post-storage ripening behaviour of fruit. Most studies of CA storage of kiwifruit have been conducted on its effect on fruit flesh firmness. However, other ripening parameters as well as shelf life of the fruit have to be considered. Storage atmospheres containing high CO<sub>2</sub> and low O<sub>2</sub> concentrations may induce abnormal metabolism, which would injure fruit tissue (Harman and McDonald, 1989). Previous studies have shown that kiwifruit removed from ULO storage did not ripen normally (Thomai and Sfakiotakis, 1997). Differences in ethylene biosynthesis and ripening rates due to storage treatment can affect marketing decisions.

A systematic study of the effect of low O<sub>2</sub> and high CO<sub>2</sub> concentrations during storage on ethylene production and ripening during shelf life has not been conducted with 'Hayward' kiwifruit. The objective of the present research was to study the effect of AS, CA or ULO-storage at 0 °C on ACC synthase and ACC oxidase activities, ACC concentration, ethylene and CO<sub>2</sub> production and ripening of 'Hayward' kiwifruit during storage at 0 °C and subsequent shelf life at 20 °C with or without exposure to propylene.

## 2. Material and methods

### 2.1. Plant material and treatments

Kiwifruit (*Actinidia deliciosa* (A. Chev.) C.F. Liang et A.R. Ferguson cv. 'Hayward') were harvested from an orchard in Pieria-North Greece and immediately transferred to laboratories at the University Farm at Thessaloniki. Fruit of uniform size and free from defects, were placed in 5 l jars through which a continuous, humidified, air stream with or without 130 µl/l propylene was passed at a rate of 100 ml/min. Each set of six jars was kept in a separate water

bath at a constant temperature of 20 °C. Six fruit per replication were removed from the jars at intervals of 0, 3, 5, 7 and 9 days, for measurements of flesh firmness, SSC, ACC content, ACC synthase and ACC oxidase (in vivo) activities. Ethylene production was measured daily. Respiration was measured four times during the first day and twice per day thereafter.

Fruit for storage in AS, CA or ULO at 0 °C were treated with 1-butylcarbomoyl-2-benzimidazole (600 ppm) for 10 s before storage, as fungicide. Atmospheres were attained within 24 h, the desired concentrations of O<sub>2</sub> and CO<sub>2</sub> being monitored by a paramagnetic and infrared gas analyser connected to a computer. The control was air storage. Ethylene scrubbers (K<sub>2</sub>MnO<sub>4</sub>) were placed in all storage chambers and the relative humidity was maintained at 90–95% by humidifiers. At intervals of 60, 120 and 180 days, fruit of each storage treatment were removed, six fruit per replication were analysed and 24 fruits per replication were kept at 20 °C under the same conditions as described above for freshly harvested fruit.

The experimental design was that of a two-factor experiment distributed in a completely randomised design with the atmosphere composition as the first factor, the number of days in storage as the second and the jars as replications. Each treatment consisted of four replications with 60 fruit per replication. Experiments were repeated twice. Statistical analyses were made with a SAS computer program. Two-way analysis of variance (ANOVA), Least Significant Difference and Duncan's Multiple-Range Tests ( $P < 0.05$ ) for comparisons between treatments over shelf life time were conducted.

## 2.2. Firmness and soluble solids content (SSC)

Flesh firmness was recorded by puncture with a Chatillon penetrometer fitted with a flat-8 mm diameter tip. The tip was inserted after skin removal, at the fruit equator, in opposite sides, to a depth of 7 mm. The SSC were measured using a digital Atago refractometer in juice from the equatorial zone of the fruit.

## 2.3. Gas analysis

Ethylene measurements were performed by withdrawing a 1 ml headspace gas sample from the jars with a syringe, and injecting it into a Varian 3300 gas chromatograph, equipped with a stainless steel column filled with Porapak, length 100 cm, diameter 0.32 cm, at 50 °C and a flame-ionisation detector at 120 °C. The carrier gas was N<sub>2</sub> at a flow rate of 20 ml/min. Respiration was calculated by CO<sub>2</sub> production in the gas phase of the jars, measured automatically by an infrared gas analyser connected to a computer.

## 2.4. ACC concentration, ACC synthase and ACC oxidase activities

ACC concentration and ACC synthase activity were assayed as described previously (Antunes and Sfakiotakis, 1997a). One unit of ACC synthase activity was defined as the formation of 1 nmol of ACC per 2 h at 30 °C. ACC oxidase activity was measured in vivo by infiltrating flesh disks with 1 mM ACC under vacuum as described elsewhere (Metzidakis and Sfakiotakis, 1993).

## 3. Results

### 3.1. Flesh firmness

Kiwifruit treated with propylene at harvest softened rapidly in the first 3 days at 20 °C, becoming ripe (< 9.807 N) by day 5 (Table 1). In contrast, fruit not treated with propylene did not soften during 9 days at 20 °C.

Until 3 days shelf life at 20 °C and propylene-treatment, fruit stored for 60 days in CA at 0 °C were the most firm, followed by ULO and AS. By day 5 all propylene-treated fruit were of similar softness (Fig. 1A). Non propylene-treated fruit softened during the shelf life except for AS fruit, which were already soft (Fig. 1D). However by day 9, only CA and AS fruit were ripe. Fruit stored in ULO did not ripen by day 9 without propylene-treatment.

Fruit treated with propylene after 120 or 180 days storage softened similarly to that stored for

60 days, but reached a ripe firmness value by day 3 at 20 °C (Fig. 1B and C). Fruit not treated with propylene after 120 days storage followed the same pattern as after 60 days storage (Fig. 1E). When not treated with propylene, fruit from 180 days storage at 2%O<sub>2</sub> + 5%CO<sub>2</sub> and 1%O<sub>2</sub> + 1%CO<sub>2</sub> had significantly higher firmness than fruit in 0.7%O<sub>2</sub> + 0.7%CO<sub>2</sub> and AS after 3 days at 20 °C, but all treatments had a firmness value of approximately 9.807 N after 7 days (Fig. 1F).

### 3.2. Soluble solids content (SSC)

The SSC of kiwifruit treated with propylene at harvest reached a value of °Brix 14 after 3 days at 20 °C, continuing to increase over 9 days (Table 1). In propylene-free air, kiwifruit only reached 10 °Brix.

The SSC increased in the first 60 days of storage, reaching values of 14 °Brix, and remained almost constant thereafter for all treatments (data not shown). The SSC did not change during the 9 days shelf life at 20 °C regardless of treatment with propylene.

### 3.3. Ethylene production

After 3 days exposure to propylene at 20 °C, freshly harvested fruit had initiated ethylene production reaching 189 µl/kg per h after 9 days

(Table 2). Fruit without propylene-treatment did not produce ethylene over the 9 days.

Ethylene production was negligible in all treatments on removal from 0 °C (Fig. 2). Upon transfer to 20 °C in air with propylene (Fig. 2A, B and C) or propylene-free air (Fig. 2D, E and F), only fruit from CA and AS storage produced ethylene without delay, with no significant differences between treatments. However, maximum ethylene production after 60 days storage was about 10% of that at harvest, 7% after 120 days storage and 3% after 180 days storage. Fruit kept in ULO did not show increased ethylene production during the shelf life period at 20 °C, even when treated with propylene.

### 3.4. ACC concentration

Fruit treated with propylene at 20 °C after harvest produced ACC by 3 days, increasing more rapidly from day 5 to 9 (Table 1). Fruit not treated with propylene did not show increased ACC concentration.

By 60 days of storage, ACC had accumulated in fruit from all treatments. ACC concentration increased slightly during 9 days in air with or without propylene at 20 °C (Fig. 3A and D). However, maximum concentrations were about 42% of those of fruit ripened with propylene at harvest.

After 120 days storage, fruit from CA or AS had higher ACC concentrations than fruit stored

Table 1

Flesh firmness, SSC, ACC, ACC synthase (ACCS) and ACC oxidase (ACCO) activities of harvested 'Hayward' kiwifruit kept at 20 °C under a continuous, humidified, air stream with or without 130 µl/l propylene

Days	Firmness (N)		SSC (%)		ACC (nmol/g)		ACCS (U/mg)		ACCO (nl/g per h)	
	Prop	Air	Prop	Air	Prop	Air	Prop	Air	Prop	Air
0	70	70	7.2	7.2	0	0	0	0	0	0
3	13	66	13.3	9.6	0	0	1.6	0	3.2	0
5	9	67	14.8	9.7	0.1	0	8.9	0.8	11.5	0.3
7	5	61	15.6	10.6	0.5	0	30.6	0	15.3	0.4
9	4	66	16.9	10.2	1.2	0.1	76.6	1.2	21.4	0.4
LSD ( <i>P</i> <0.05)	4.32	7.75	1.11	0.91	0.05	n.s.	4.3	n.s.	2.36	n.s.

n.s., not significant at *P*<0.05. 1 U/mg = 1 pmol ACC/mg protein per 2 h. Prop, 130 µl/l propylene. Air, Propylene-free air.

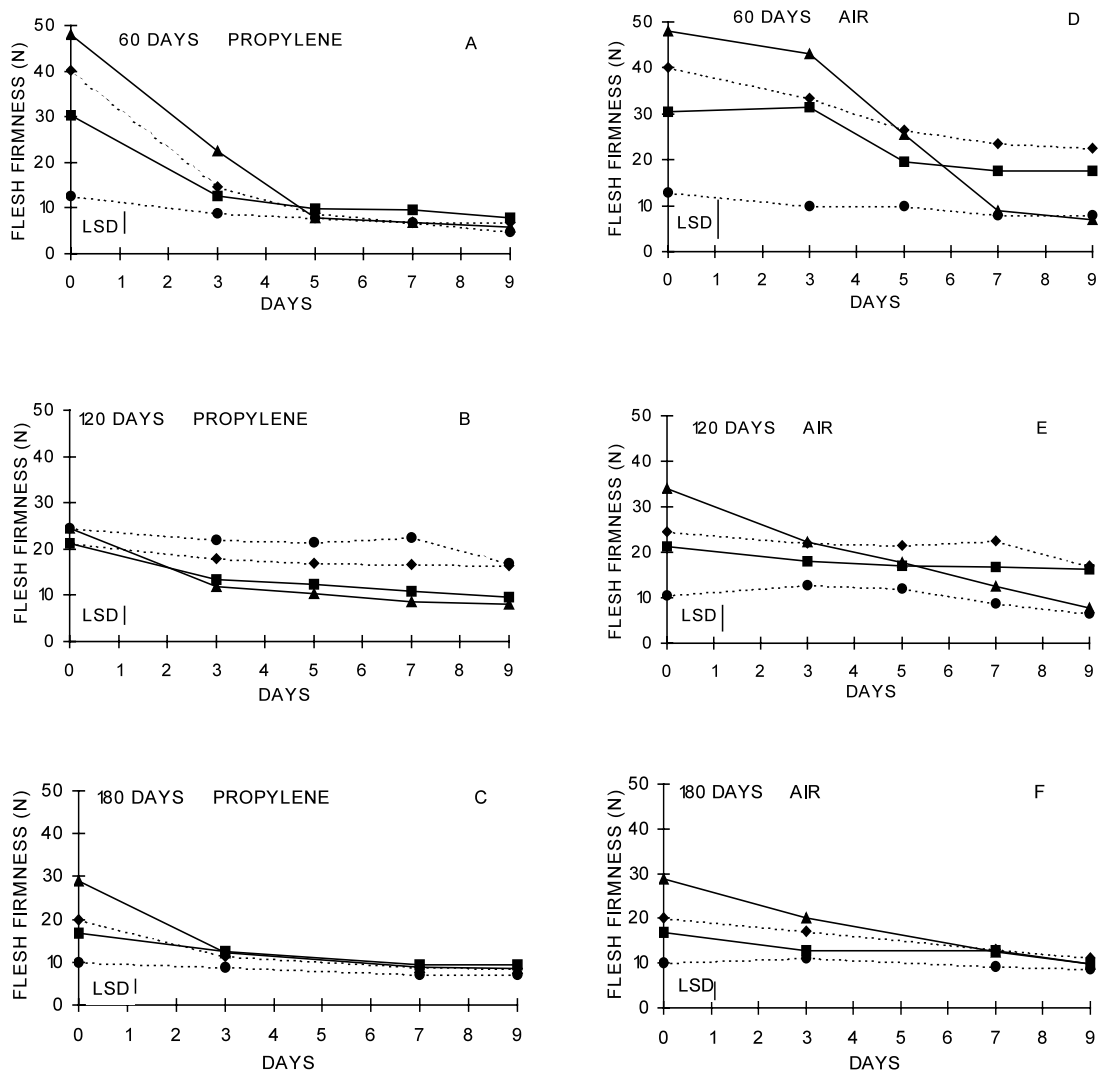


Fig. 1. Flesh firmness of 'Hayward' kiwifruit placed at 20 °C under a continuous, humidified, air stream with 130  $\mu$ l/l propylene (A, B and C) or propylene-free air (D, E and F), after 60, 120 and 180 days storage at 0 °C in 0.7%O<sub>2</sub> + 0.7%CO<sub>2</sub> (—■—), 1%O<sub>2</sub> + 1%CO<sub>2</sub> (---◆---), 2%O<sub>2</sub> + 5%CO<sub>2</sub> (—▲—) or air (---●---). LSD at  $P < 0.05$ .

in ULO (Fig. 3B and E) The ACC concentration increased slightly in fruit from AS or CA during the shelf life in air with or without propylene, while it remained almost constant in fruit from ULO storage. After 180 days storage, and during shelf life in air with or without propylene, ACC concentrations of fruit followed the same patterns as those after 120 days storage for all treatments, but ACC values were 40–50% lower (Fig. 3C and F).

### 3.5. ACC synthase activity

ACC synthase activity followed the same patterns as those of ACC concentration both at harvest and during shelf life, after 60 days storage, and in fruit treated or not with propylene (Table 1 and Fig. 4A and D). The maximum ACC synthase activity during shelf life, in fruit after 60 days storage, was about 50% lower than at harvest and decreased slightly with storage time.

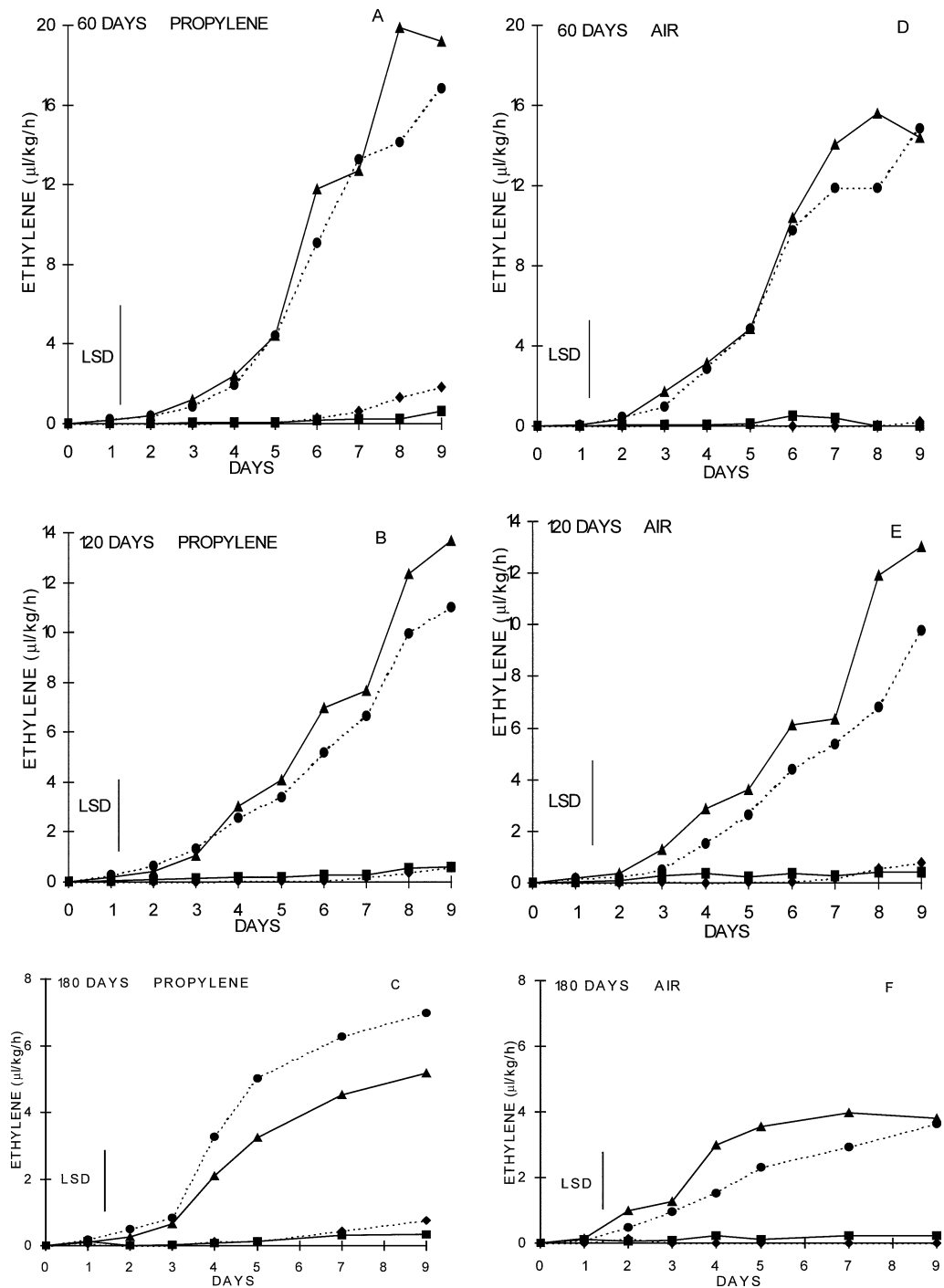


Fig. 2. Ethylene production of 'Hayward' kiwifruit placed at 20 °C under a continuous, humidified, air stream with 130 µl/l propylene (A, B and C) or propylene-free air (D, E and F), after 60, 120 and 180 days storage at 0 °C in 0.7%O<sub>2</sub> + 0.7%CO<sub>2</sub> (—■—), 1%O<sub>2</sub> + 1%CO<sub>2</sub> (---◆---), 2%O<sub>2</sub> + 5%CO<sub>2</sub> (—▲—) or air (---●---). LSD at *P* < 0.05.

After 120 days storage, ACC synthase activities were similar in all treatments increasing after 5 days at 20 °C in fruit from CA treated or not with propylene, and in AS fruit treated with propylene, but remained almost constant in fruit from ULO treatments (Fig. 4B and E). After 180 days storage, fruit from CA or AS had slightly higher ACC synthase activities than in fruit from ULO during the shelf life period in air with or without propylene (Fig. 4C and F).

### 3.6. ACC oxidase activity

ACC oxidase activity of kiwifruit was negligible at harvest but increased upon exposure to propylene (Table 1). ACC oxidase activity did not increase in propylene-free air.

The ACC oxidase activity was very low and similar in all treatments on removal from 60 days storage and increased during 9 days in air with or without propylene at 20 °C in fruit from AS or CA treatments (Fig. 5A and D). ACC oxidase activity increased slightly in fruit from the ULO treatments during the shelf life period when treated with propylene, but not in propylene-free air. The maximum ACC oxidase activity was

about 40% lower than at harvest and decreased slightly thereafter with storage time.

After 120 days storage, ACC oxidase activity followed the same pattern as for fruit stored for 60 days, except that activity did not increase in fruit from ULO treatments during shelf life regardless of propylene-treatment (Fig. 5B and E). During shelf life at 20 °C, ACC oxidase activity in fruit removed from storage after 180 days followed the same pattern as in fruit removed after 120 days, except that fruit from AS had higher values than CA stored fruit after 7 and 9 days shelf life in fruit treated or not with propylene (Fig. 5C and F).

### 3.7. Carbon dioxide production

Kiwifruit treated with propylene at harvest showed increased respiration rates, reaching a peak after 1 day and decreased until day 6, after which it remained constant (Table 2). Respiration of fruit not treated with propylene increased from 3 to 6 days and then remained almost constant. Rates were always significantly lower than fruit treated with propylene.

When fruit were removed from 60, 120 or 180 days storage and placed at 20 °C in air with or without propylene, there was a significant increase in respiration within 1 day for all treatments, and then no significant changes occurred during the remaining 8 days of shelf life (Fig. 6). After 60 days storage, the maximum CO<sub>2</sub> production during shelf life at 20 °C was about 65% of that at harvest and decreased slightly thereafter with storage time.

Table 2

Ethylene and CO<sub>2</sub> production of harvested 'Hayward' kiwifruit kept at 20 °C under a continuous, humidified, air stream with or without 130 µl/l propylene

Days	Ethylene (µl/kg per h)		CO <sub>2</sub> (µmol/kg per h)	
	Prop	Air	Prop	Air
	0.0	0.0	0.36	0.47
1	0.0	0.0	2.01	0.56
2	0.0	0.0	2.02	0.64
3	0.1	0	1.91	0.50
4	2.4	0.0	1.91	0.66
5	16.3	0.0	1.56	0.70
6	65.5	0.0	1.33	0.91
7	127.4	0.0	1.31	0.84
8	155.4	0.1	1.51	0.89
9	189.0	0.0	1.37	0.84
LSD ( <i>P</i> <0.05)	28.2	n.s.	0.25	0.18

n.s., not significant at *P*<0.05. Prop, 130 µl/l propylene. Air, propylene-free air.

## 4. Discussion

The 2%O<sub>2</sub> + 5%CO<sub>2</sub> and 1%O<sub>2</sub> + 1%CO<sub>2</sub> treatments were effective in prolonging storage life of kiwifruit with few changes in quality factors as reported by Arpaia et al. (1994b), Thomai and Sfakiotakis (1997). As observed by Arpaia et al. (1994b), the 2%O<sub>2</sub> + 5%CO<sub>2</sub> stored fruit became ripe during a shelf life period, while 1%O<sub>2</sub> + 1%CO<sub>2</sub> did not, unless treated with propylene (Thomai and Sfakiotakis, 1997). In the present study, ripening of fruit from AS and CA

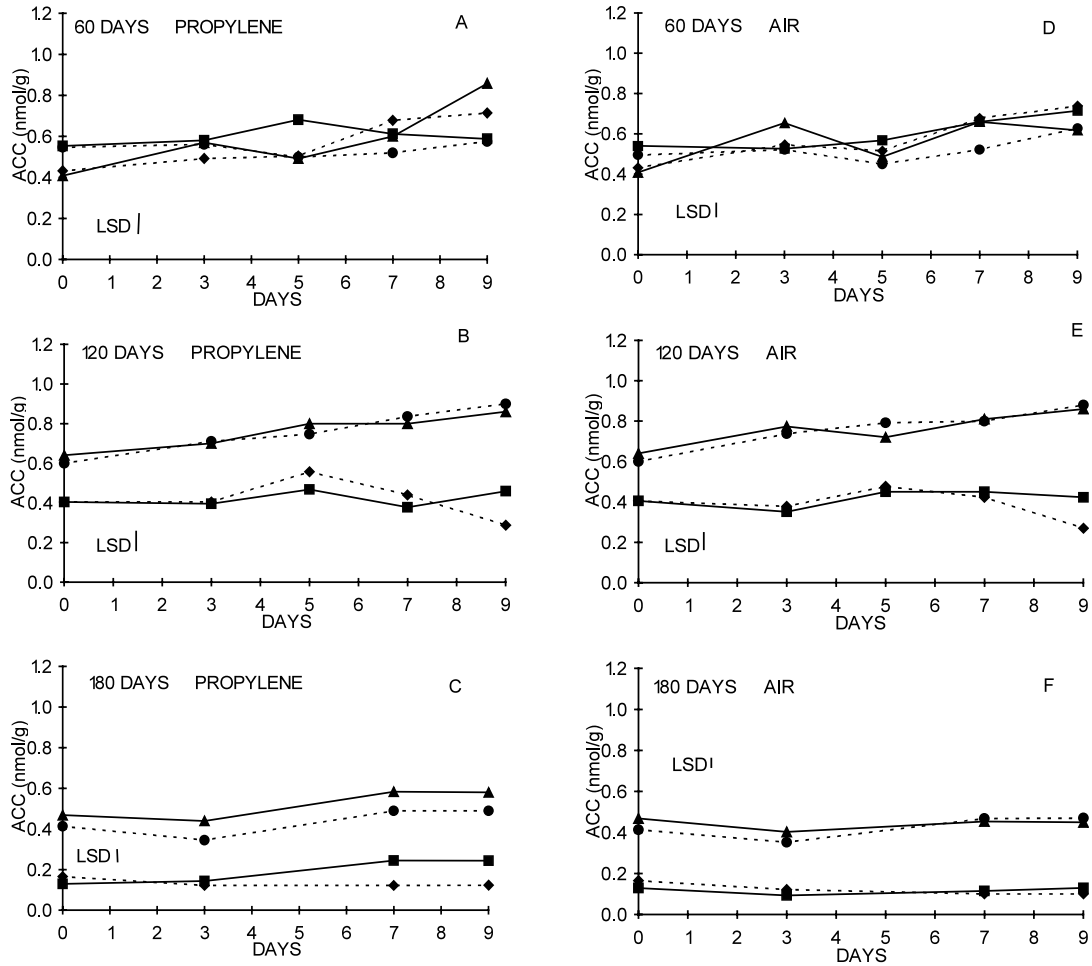


Fig. 3. ACC concentration of 'Hayward' kiwifruit placed at 20 °C under a continuous, humidified, air stream with 130  $\mu$ l/l propylene (A, B and C) or propylene-free air (D, E and F), after 60, 120 and 180 days storage at 0 °C in 0.7%O<sub>2</sub> + 0.7%CO<sub>2</sub> (—■—), 1%O<sub>2</sub> + 1%CO<sub>2</sub> (---◆---), 2%O<sub>2</sub> + 5%CO<sub>2</sub> (—▲—) or air (---●---). LSD at  $P < 0.05$ .

storage was associated with ethylene production in agreement with Arpaia et al. (1994b). In contrast, fruit from ULO did not ripen during 9 days at 20 °C and did not produce ethylene.

Kiwifruit storage in 0.7%O<sub>2</sub> + 0.7%CO<sub>2</sub> for more than 120 days resulted in development of flesh breakdown, making this ULO treatment not suitable for long kiwifruit storage (Antunes and Sfakiotakis, 1997b; Thomai and Sfakiotakis, 1997). It appears that the pattern of SSC evolution is not greatly influenced by CA (Harman and McDonald, 1989; Arpaia et al., 1994b) or ULO treatments.

At harvest, kiwifruit kept at 20 °C ripened in 3 days and started ethylene autocatalysis thereafter when treated with propylene and did not increase ethylene production during the 9 days in propylene-free air (Antunes and Sfakiotakis, 2002).

Antunes and Sfakiotakis (2002) showed that AS for at least 12 days at 0 °C induces ethylene production and ripening immediately upon transfer to 20 °C, due to induction of ACC synthase and ACC oxidase activities. Chilling induces ethylene production upon rewarming, by stimulating ACC synthesis in cucumber and pears (Knee, 1987) or ACC oxidase activity in apples

(Gaudierre and Vendrell, 1993). It was observed in this study that fruit from 60 to 180 days AS or CA started to produce ethylene without delay upon rewarming due to ACC accumulation and ACC synthase activity in storage and induction of ACC oxidase activity upon rewarming. However, as for cucumbers (Andersen, 1986), avocados (Eaks, 1983) and nectarines (Brecht and Kader, 1984) stored in AS, the capacity of kiwifruit to produce ethylene during shelf life decreased, mostly in the first 60 days. Andersen (1986) reported that prolonged chilling could reduce ethyl-

ene production upon rewarming by damaging ACC oxidase. The results of the present study suggest that the loss of the capacity to produce ethylene with storage time was related to decreased ACC synthase and ACC oxidase activities, in comparison with freshly harvested fruit ripened with propylene at 20 °C.

Interestingly, fruit previously stored in ULO were not able to produce ethylene for up to 9 days at 20 °C even if treated with propylene. It was previously reported that ACC accumulates in pears stored in low O<sub>2</sub> and that it was converted

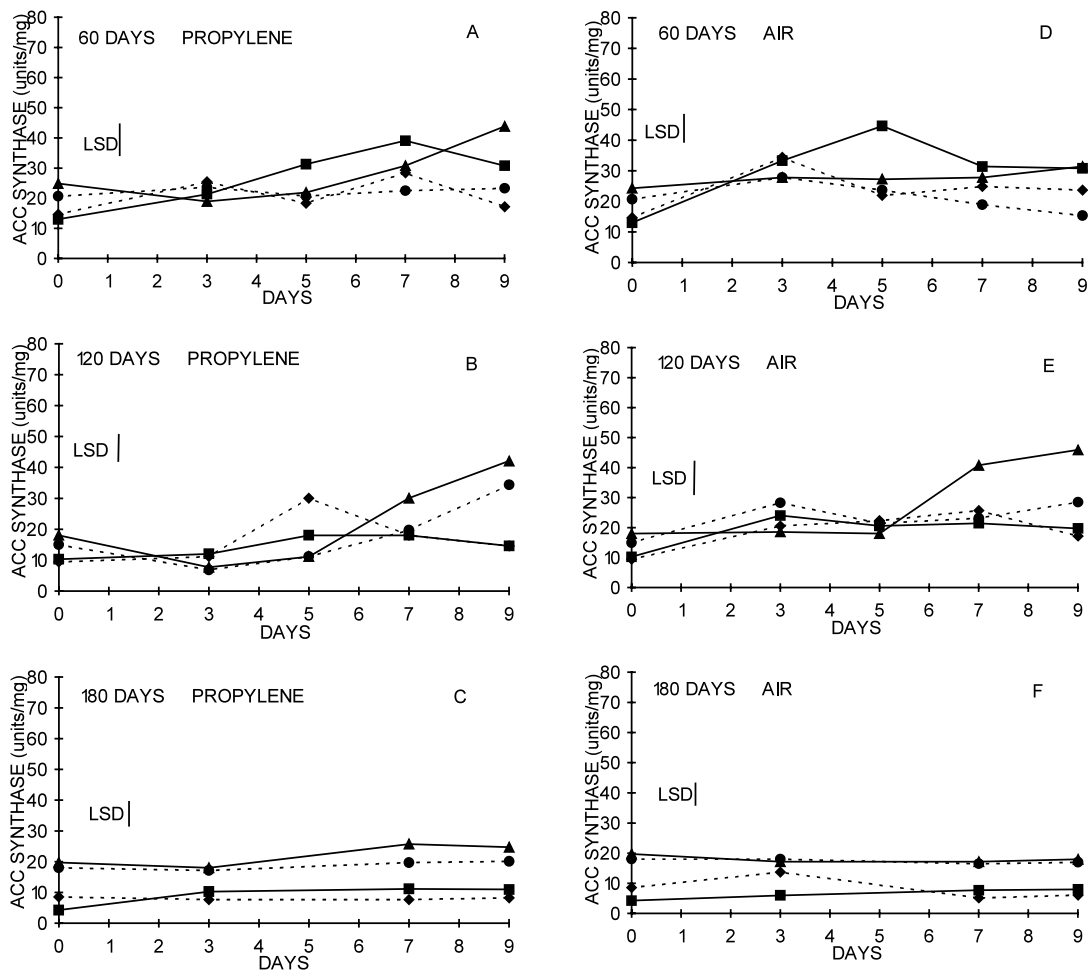


Fig. 4. ACC synthase activity of ‘Hayward’ kiwifruit placed at 20 °C under a continuous, humidified, air stream with 130µl/l propylene (A, B and C) or propylene-free air (D, E and F), after 60, 120 and 180 days storage at 0 °C in 0.7%O<sub>2</sub> + 0.7%CO<sub>2</sub> (—■—), 1%O<sub>2</sub> + 1%CO<sub>2</sub> (---◆---), 2%O<sub>2</sub> + 5%CO<sub>2</sub> (—▲—) or air (---●---). 1 U/mg = 1 pmol ACC/mg protein per 2 h. LSD at *P* < 0.05.

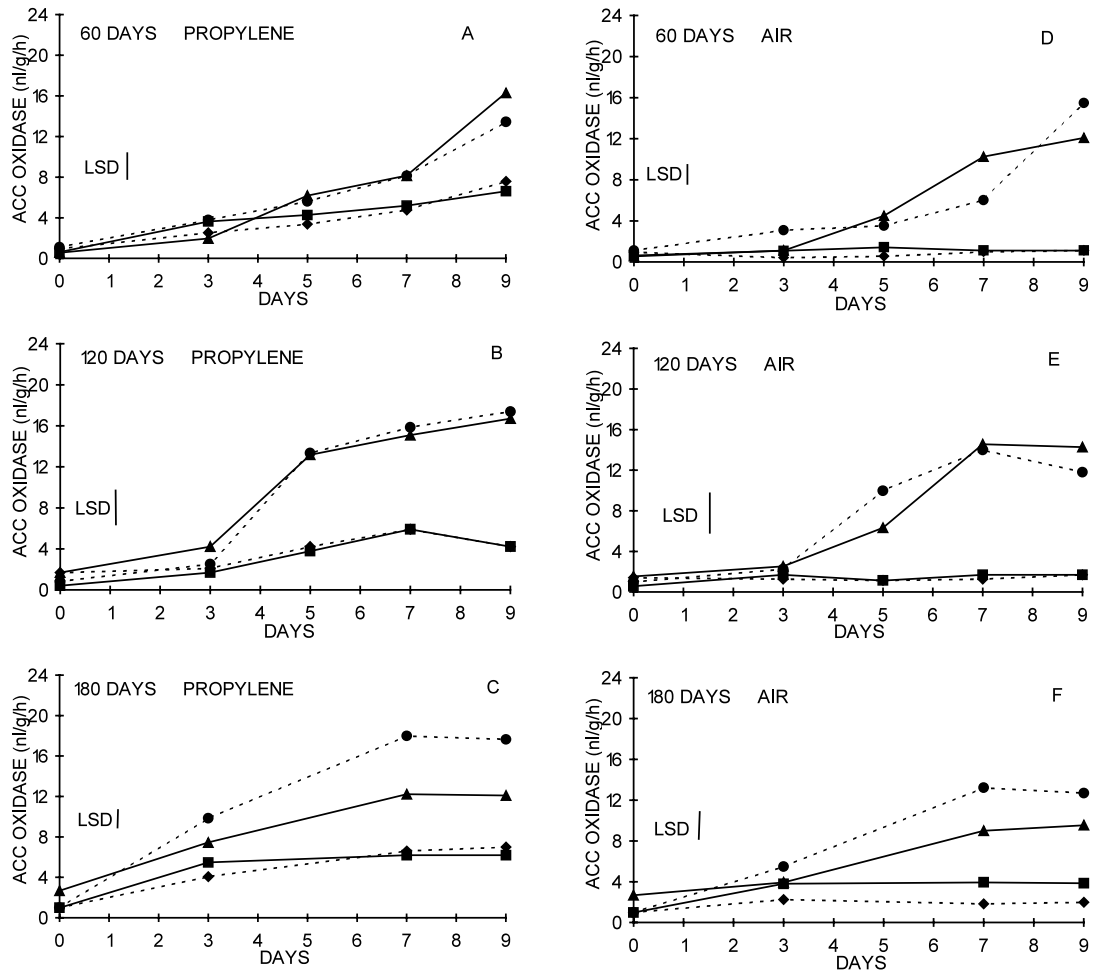


Fig. 5. ACC oxidase activity of 'Hayward' kiwifruit placed at 20 °C under a continuous, humidified, air stream with 130 µl/l propylene (A, B and C) or propylene-free air (D, E and F), after 60, 120 and 180 days storage at 0 °C in 0.7%O<sub>2</sub>+0.7%CO<sub>2</sub> (—■—), 1%O<sub>2</sub>+1%CO<sub>2</sub> (---◆---), 2%O<sub>2</sub>+5%CO<sub>2</sub> (—▲—) or air (---●---). LSD at *P* < 0.05.

to ethylene upon transfer to air at 20 °C (Blankenship and Richardson, 1986). In this study, we observed a small ACC accumulation and corresponding ACC synthase activity in all treatments on removal from 60 days storage. This indicates that lack of ACC accumulation in storage was not the main factor limiting ethylene production as reported for apples by Jobling et al. (1991), and Gaudierre and Vendrell (1993). Hyodo et al. (1987) found levels of 0.6 nmol/g of ACC in kiwifruit after 120 days storage in AS, while ACC oxidase did not show any activity until fruit were rewarmed, in agreement with our studies. During

shelf life at 20 °C, the ACC synthase activity and ACC levels remained constant in fruit from ULO and slightly increased in CA or AS fruit, indicating a small induction of ACC synthase only in fruit stored above 2% O<sub>2</sub>. In this work, it was observed that low ACC oxidase activity was the main factor for the lack of ability of ULO-stored fruit to produce ethylene upon rewarmed. Buefler and Bangerth (1983) suggested that a ripening promoter might be induced before the autocatalytic ethylene production, which depends on O<sub>2</sub>. It appears that kiwifruit exposure to less than 1% O<sub>2</sub> for 60 days at 0 °C damages the receptor of the

stimulus that induces ACC oxidase. Nevertheless, at 120 and 180 days storage, the lower levels of ACC in ULO-stored fruit may also be a cause for the loss of capacity to produce ethylene by those treatments.

Antunes et al. (2000) observed that 8 days storage at low temperature (10 °C) were insufficient to induce transcription of ACC synthase or

ACC oxidase of kiwifruit that were not treated with propylene. In addition, during 17 days at temperatures from 0 to 15 °C, activities of ACC synthase or ACC oxidase were not detected (Antunes and Sfakiotakis, 2002). Our present results suggest that as the chilling period increases, ACC synthase is activated. Wang and Adams (1982) reported that chilling readily stimulates ACC syn-

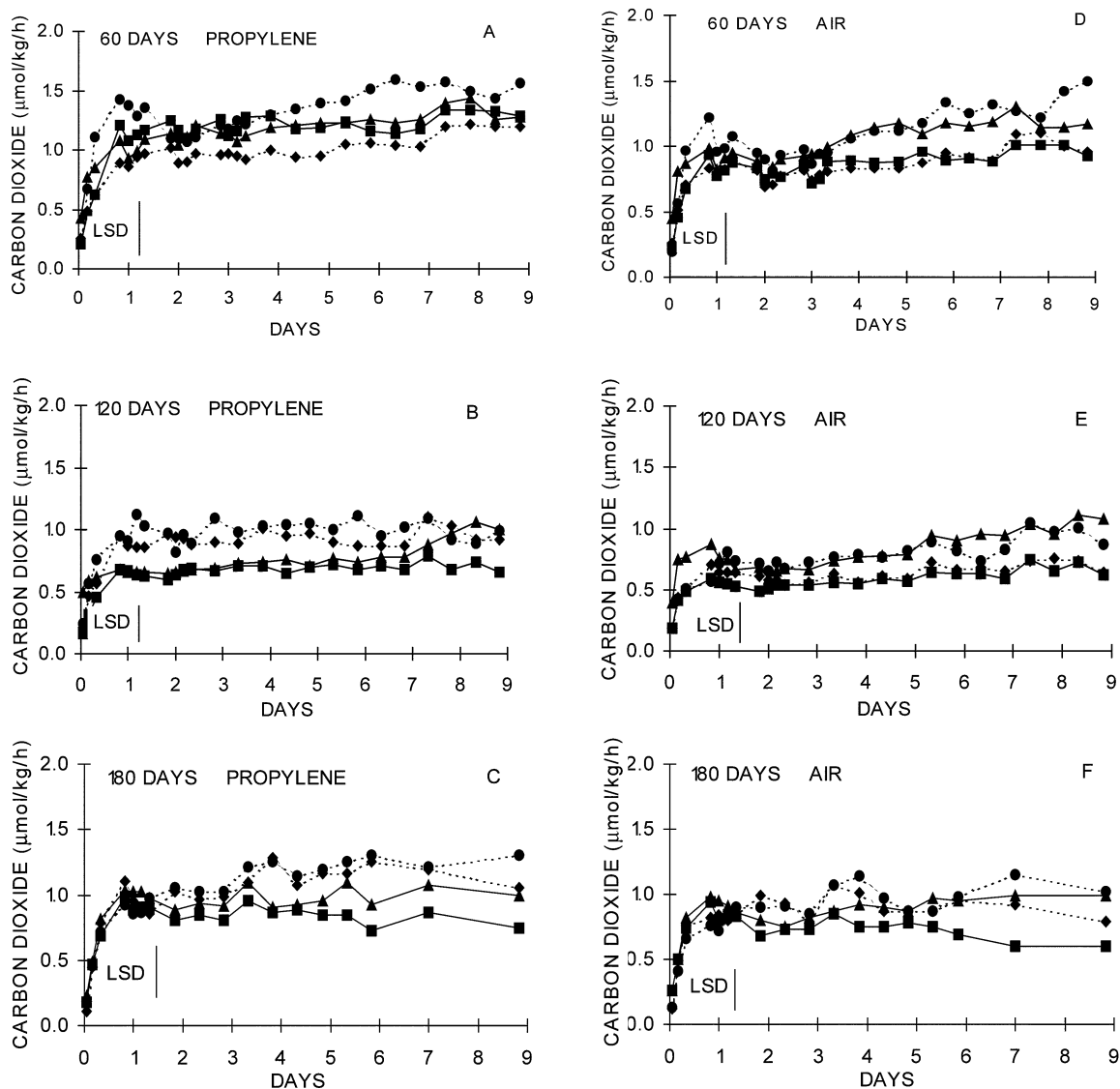


Fig. 6. Carbon dioxide production of 'Hayward' kiwifruit placed at 20 °C under a continuous, humidified, air stream with 130 µl/l propylene (A, B and C) or propylene-free air (D, E and F), after 60, 120 and 180 days storage at 0°C in 0.7%O<sub>2</sub> + 0.7%CO<sub>2</sub> (—■—), 1%O<sub>2</sub> + 1%CO<sub>2</sub> (—◆—), 2%O<sub>2</sub> + 5%CO<sub>2</sub> (—▲—) or air (—●—). LSD at *P* < 0.05.

thesis, whereas ACC oxidase activity was vulnerable to chilling injury. This may be an explanation why ACC oxidase activity is not present in kiwifruit kept at low temperature.

For all treatments, fruit respiration was low in storage and increased upon rewarming of the fruit because the rate of respiration is mostly temperature dependent (Blanke, 1991). The tendency of the AS and CA stored fruit to show higher respiration may be due to ethylene production by these treatments, since respiration increases with ethylene production (Arpaia et al., 1994a; Antunes and Sfakiotakis, 2002). We also observed a decrease in the respiration rate of the rewarmed fruit with storage time. This decrease was lower than the decrease in ethylene production and may be associated mainly with the advance in the natural senescence process.

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