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Effect of 1-Methylcyclopropene and Controlled Atmosphere on Storage of Kiwifruits

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ABSTRACT

'Hayward' kiwifruits were treated with 0.5 µL/L of 1-methylcyclopropene (1-MCP) for 24 hours at 0°C, and then stored either in air or in controlled atmosphere (CA) with 3 kPa O_2 + 5 kPa CO_2 at 0°C. Initially, and after 13, 20, 28, 36, 41, and 55 days of storage, respiration rate, firmness, soluble solids content (SSC), and titratable acidity (TA) were measured in order to assess fruit ripening. Both 1-MCP and controlled atmosphere resulted in effective firmness retention compared to untreated control fruit held in air, although throughout the experiment, fruit treated with 1-MCP presented only 17% higher firmness than untreated fruit, while firmness of kiwifruit stored in CA resulted 40% higher compared to fruit stored in air. Treatments showed no synergistic effect during the first 4 weeks of storage, while fruit pre-treated with 1-MCP and stored in CA presented significantly higher firmness compared to all other treatments starting from the fifth week of storage. Treatment under 1-MCP and CA significantly affected SSC but not TA, and weight loss. Pre-reatment with 1-MCP did not affect respiration rate.

Keywords: 1-MCP, ethylene production, firmness, respiration rate, soluble solids content
Abbreviations: 1-MCP, 1-methylcyclopropene; **CA**, controlled atmosphere; **DMRT**, Duncan's multiple range test; **SSC**, soluble solids content; **TA**, titratable acidity

INTRODUCTION

Kiwifruits (*Actinidia deliciosa* cv. 'Hayward') can be stored for several months at 0°C and 90–95% RH, but they are very sensitive to ethylene, which at concentrations as low as 0.005–0.01 µL/L, may induce premature ripening and flesh softening, thus limiting long-term cold storage (Crisosto *et al.* 2000). The objective of this work was to detect the different effects and possible interaction between 1-methylcyclopropene (1-MCP) treatment and controlled atmosphere (CA) storage on kiwifruit shelf-life at 0°C.

Rapid initial softening is the most important postharvest problem for this commodity: kiwifruits harvested at 70-85 N, soften to around 8-10 N within 10-14 weeks (Harvey and Harris 1986; MacRae *et al.* 1989). Ethylene scrubbing from the cold room is a commercially applied technology adopted to prevent rapid softening. Other strategies include inhibition of ethylene biosynthesis and action.

1-MCP is a synthetic cyclic olefin that blocks access of ethylene to its receptor (Sisler and Serek 1997; Lurie 2007) and, therefore, prevents major undesirable effects on ethylene-sensitive plant tissues. Postharvest treatment with 1-MCP can be used to control both the rate of fruit ripening and premature softening of a wide range of fruits, its usefulness depending on the physiological characteristics of each commodity (Blankenship and Dole 2003; Watkins and Miller 2003).

Kim *et al.* (2001) showed that application of 1-MCP at harvest reduced ethylene production and softening in kiwifruit subsequently exposed to 20°C. When fruit were submitted to a 32 days cold storage period prior to exposure to 10 µL/L 1-MCP, no significant difference in the softening rate was found between 1-MCP-treated and untreated fruits. However, significant differences were observed between fruit treated with 10 µL/L 1-MCP and fruit submitted to 10 µL/L ethylene for 16 hours after cold storage. Crisosto and Garner (2001) showed less softening incidence in kiwifruits treated with µL/L of 1-MCP for 48 hours, during the first four weeks of storage at 1°C, while after this period differences between treated and untreated fruits became smaller. Ethylene production of fresh-cut kiwifruit slices treated with 1-MCP was reduced as well as softening (Colelli and Amodio 2003; Koukounaras and Sfakiotakis 2007). Colelli *et al.* (2004) reported a strong action of 1-MCP on delaying ripening of whole kiwifruits, even when stored with exogenous ethylene, while Boquete *et al.* (2004) reported 1-MCP efficacy not only on control of softening, but also on retarding changes in soluble solids, lightness and chromaticity in the outer cortex.

Carbon dioxide also inhibits ethylene production in climacteric fruit by inhibiting ACC synthase and ACC oxidase activity (Chavez-Franco and Kader 1993) and probably competing for the ethylene active sites in the cells (Burg and Burg 1967), while low oxygen concentrations reduce the rate of ethylene production and its concentration within the fruit (Stow 1989). Controlled atmospheres of 1–2 kPA O_2 and 3–5 kPa CO_2, with ethylene concentration lower than 0.02 µL/L, can extend the storability of cold-stored kiwifruits (Kader 1997; Crisosto *et al.* 1999). Atmospheres with 2–3 kPa O_2 and 3–5 kPa CO_2 further delayed the rate of kiwifruit softening and increased storage life up to 3–4 months beyond normal life in air flow (Arpaia *et al.* 1994).

Several studies on CA and 1-MCP interaction were conducted on apples (Watkins *et al.* 2000; Zanella 2003; Delong *et al.* 2004; DeEll *et al.* 2006), while very scarce information is available for other fruit. Watkins *et al.* (2000) observed a better effect of 1-MCP on softening of apple, compared to CA, and obtained best results combining both treatments. In persimmons the rates of ethylene production and fruit softening declined with the increase of 1-MCP from 0 to 0.6 µL/L, and this response was enhanced in an 80 kPa CO_2 atmosphere compared with air treatment (Tsviling *et al.* 2003). Klieber *et al.* (2003) reported that low oxygen atmospheres after ripening did not extend the shelf

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life of bananas due to skin injury, while 1-MCP extended the shelf-life by 100%. Positive interaction between CA and 1-MCP was found by Amodio *et al.* (2005) on tomatoes. On kiwifruits the interaction of 1-MCP and modified atmosphere packaging was investigated by Neves *et al.* (2003), that reported the best results in terms of firmness and titratable acidity, for 1-MCP treated fruits held in low density polyethylene, while no information are available on the effect of 1-MCP and CA in cold storage.

The objective of this study was to provide evidence of the possible interaction between 1-MCP pre-treatment and CA on quality of stored kiwifruits.

MATERIALS AND METHODS

'Hayward' kiwifruits, grown in the province of Bari, Italy, were obtained from a commercial packinghouse, where they had been stored for 5 weeks in ethylene-scrubbed air at 0°C, and promptly transported to the postharvest laboratory of the University of Foggia within 2 hours.

Fruits were randomly divided into six 120-fruit lots. Each lot was placed in a polyethylene container (37 L) which was hermetically sealed and kept at 1°C. In three of these, 0.5 µL/L,of 1-MCP were generated from a stock solution of 1-MCP in air (344 µL/L,), diluted in the head space, after injection of 41 mL through a rubber tube inserted in the container lid. 1-MCP was released in the stock solution by adding 1200 mg of 1-MCP powder (SmartFresh™ a.i. 0.14%; Rohm & Hass) to a 5 mL of water in a volume of 2.2 L according to the manufacturer's instructions.

After 24 hours all containers were opened, and fruit of each container divided in 2 60-fruit sub-lots, and then transferred in 12 containers, replicating 3 times the following treatments:

- untreated fruit in humidified flow of air;
- 1-MCP-treated fruit in humidified flow of air;
- untreated fruit in humidified flow of CA (3 kPa O_2 + 5 kPa CO_2);
- 1-MCP-treated fruit in humidified flow of CA (3 kPa O_2 + 5 kPa CO_2).

Initially and after 13, 20, 28, 36, 41 and 55 days of storage at 0°C, the following quality attributes were determined:

- firmness, measured with a firmness tester by measuring force required by an 8-mm probe to penetrate into 5 mm of the peeled surface in two opposite locations;
- soluble solids content (SSC) of the juice at room temperature, from all fruit of each replicate, using a digital refractometer with temperature compensation (Atago, PR-32; Tokyo, Japan);
- titratable acidity (TA) of the juice from all fruit of each replicate titrated with 0.1 N NaOH solution to a pH 8.1 and expressed as per cent of citric acid;
- weight loss as % on initial weight.

On the samples stored in air, initially and at each evaluation time, respiration rate (in mL CO_2/kg*h) was measured using the static head-space system. At each storage duration, 10 fruits for each replicate were transferred in a closed jar (9.4 L). Air within each jar was sampled with an infrared sensor (MultiRAE IR, Copenhagen, Denmark) after a given elapsed time, and measured CO_2 partial pressure was referred to the fruit weight, the elapsed time, and the head-space volume.

A two-way factorial analysis was performed on raw data of all the experiment testing 1-MCP application and atmosphere composition, while at each storage evaluation a one-way ANOVA was performed by treatment; means separation was assessed with the DMRT test.

RESULTS AND DISCUSSION

Initially, fruit had a SSC of 10.2% (±1.02), TA of 1.40% (±0.08), and firmness of 34.7 N (±10.8). At this stage of maturity, the respiration rate at 0°C was 15 mL CO_2/kg*h.

In **Table 1** the effect of time on all evaluated attributes is shown. As expected, firmness and acidity decreased during storage life of kiwifruits, while SSC increased from 10 to 13% after 55 days at 0°C. Firmness rapidly decreased from 34.7 to 19.1 N in the first 2 weeks, loosing about 45% of initial value, and showing a final value of 13 N after 55 days at 0°C. Similar results are in accord with what observed previously on kiwifruits by Colelli *et al.* (2004).

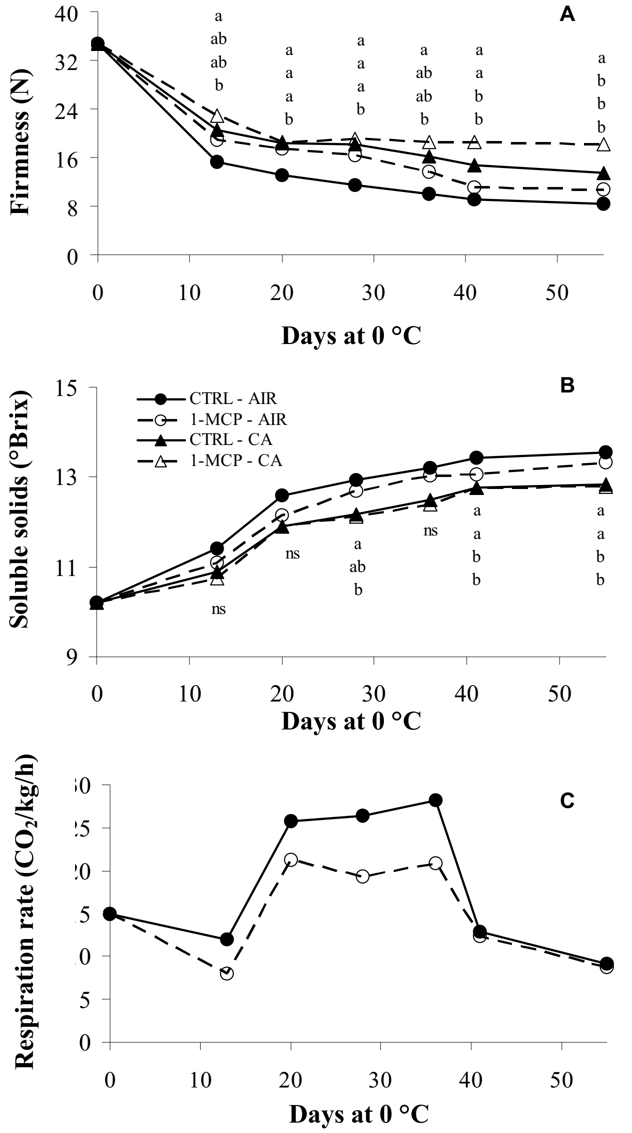

Fig. 1 Effect of 0.5 µL/L 1-MCP for 24 hours (1-MCP) and storage in 3 kPa O_2 + 5 kPa CO_2 (CA) on firmness (**A**), soluble solids contents (**B**) and respiration rate (**C**) of 'Hayward' kiwifruit during cold storage at 0°C. Fruit used as control (CTRL) were no pre-treated with 1-MCP and stored in air conditions (AIR). Within each storage time, different letters indicate significant difference for P<0.05. Where no letters are shown, no significant differences were found among treatments.

Respiration rate showed a maximum for both treatments ranging from 22 to 28 mL CO_2/kg*h between 20 and 37 days of storage, probably because most of the fruits were in the climacteric stage, and then decreased (**Fig. 1**).

Both 1-MCP and CA affected firmness and SSC, as resulted from the two-way ANOVA (**Table 2**), but no interaction was found, for any of the measured attributes.

No other attribute presented significant differences due to 1-MCP or CA.

In **Tables 3** and **4** the effects of 1-MCP and of CA are respectively shown (mean values for the entire storage period).

Flesh firmness is probably the best predictor of kiwifruit shelf life (Boquete *et al.* 2004) and both treatments were effective in delaying softening. Fruit treated with 0.5 µL/L of 1-MCP and fruit stored in CA showed higher firmness values than untreated fruit and fruit stored in air respectively.

The effect of 1-MCP pre-treatment on firmness retention of kiwifruits is reported by other authors for both

Table 1 Effect of cold storage time (days) on quality parameters of '*Hayward*' kiwifruits.

Quality attributes	Days at 0°C						
	0	13	20	28	36	41	55
Firmness (N)	34.7 a	19.1 b	17.3 bc	16.6 c	14.8 cd	13.9 d	13.0 d
Soluble solids content (°Brix)	10.2 f	11.0 e	12.1 d	12.4 c	12.8 b	12.9 ab	13.1 a
Respiration (mL CO_2/mg*h)	15.0 bc	10.0 bc	23.4 a	18.8 ab	24.6 a	12.6 bc	9.0 c
Acidity (% citric acid)	1.45 b	1.30 c	1.39 bc	1.55 a	1.16 d	1.37 bc	1.28 c
Weight loss (%)	0	0.4 d	0.5 c	0.5 c	0.6 c	0.8 b	0.9 a

Within the same row, values followed by the same letter are not significantly different, P < 0.05.

Table 2 Two-way ANOVA for the effect of 0.5 µL/L 1-MCP for 24 hours (1-MCP) and Controlled Atmosphere with 3 kPa O_2 + 5 kPa CO_2 (CA) treatments on quality parameters of 'Hayward' kiwifruits stored at 0°C.

Source	Sum of Square	df	Mean Square	F	p
Firmness (N)					
1-MCP	1031.9	1	1031.9	32.9	****
CA	3885.7	1	3885.7	124.0	****
1-MCP*CA	39.3	1	39.3	1.3	ns
Soluble solids content (°Brix)					
1-MCP	6.8	1	6.8	5.4	*
CA	69.9	1	69.9	55.5	****
1-MCP*CA	0.4	1	0.4	0.3	ns
Acidity (% citric acid)					
1-MCP	0.03	1	0.03		
CA	0.04	1	0.04		
1-MCP*CA	0.01	1	0.01		
Weight loss (%)					
1-MCP	0.025	1	0.025	0.5	ns
CA	0.031	1	0.031	0.7	ns
1-MCP*CA	0.007	1	0.007	0.1	ns

*: statistically significant for 0.5≥P≤0.1
**: statistically significant for 0.1≥P≤0.01
***: statistically significant for 0.01≥P≤0.001
****: statistically significant for P≤0.001
ns: not significant

Table 3 Effect of pre-treatment with 0.5 µL/L 1-MCP for 24 hours (1-MCP, mean values of all evaluation times) on quality parameters of 'Hayward' kiwifruits stored for 55 days at 0°C.

Quality attributes	Treatment effect	
	CTRL	1-MCP
Firmness (N)	14.4 b	16.9 a
Soluble solids content (°Brix)	12.5 a	12.3 b
Respiration (mLCO_2/mg*h)	18.7 ns	13.7 ns
Acidity (% citric acid)	1.3 ns	1.4 ns
Weight loss (%)	0.6 ns	0.6 ns

Within the same row, values followed by the same letter are not significantly different, P < 0.05. ns: not significant.

Table 4 Effect of Controlled Atmosphere with 3 kPa O_2 + 5 kPa CO_2 (CA, mean values of all evaluation times) on quality parameters of 'Hayward' kiwifruits stored for 55 days at 0°C.

Quality attributes	Atmosphere effect	
	AIR	CA
Firmness (N)	12.9 b	18.0 a
Soluble solids content (°Brix)	12.7 a	12.1 b
Acidity (% of citric acid)	1.4 ns	1.3 ns
Weight loss (%)	0.6 ns	0.6 ns

Within the same row, values followed by the same letter are not significantly different, P < 0.05. ns: not significant.

whole (Koukounaras and Sfakiotakis 2007) and fresh-cut kiwifruits (Colelli and Amodio 2003; Vilas-Boas and Kader 2007). Colelli *et al.* (2004) reported a 70%-shelf life extension of fruit treated with 1 µL/L of 1-MCP for 24 hours compared to untreated fruits; in addition 1-MCP-treated fruit better retained initial firmness also when stored in exogenous ethylene (1 µL/L).

Kim *et al.* (2001) reported a dose-dependent delay in softening for treated fruits kept at 20°C and a less marked effect when fruits were kept at 0°C. These authors also observed an effect of 1-MCP on fruit stored in presence of exogenous ethylene.

Effect of CA on firmness of kiwifruits was stronger than effect of 1-MCP. In fact, while mean firmness value for all storage duration for 1-MCP-treated fruit resulted 17% higher than for untreated fruit (**Table 3**), for fruit stored in CA it resulted about 40% higher than for fruit kept in air (**Table 4**). When firmness evolution during storage is considered (**Fig. 1**), it can be observed that untreated fruit kept in air showed significantly lower values compared to all other treatments for all storage periods, while there were no statistical differences among the other 3 treatments for the first 4 weeks of storage. After 41 days at 0°C the effect of CA was higher than the effect of 1-MCP, with significantly higher firmness values for the two treatments kept in 3 kPa O_2+5 kPa CO_2, compared to the two treatments kept in air. At the last evaluation (55 days at 0°C), only fruit treated with 0.5 µL/L of 1-MCP and held in CA showed a significantly higher firmness value, compared to all other treatments, while the effects of 1-MCP and CA alone vanished resulting in no statistical differences among these treatments. After 4 weeks of storage, the effect of 1-MCP started to decrease as also reported by Crisosto and Gardner (2001), who suggested to repeat the treatment in order to extend the shelf-life of fruit. Many authors suggested that 1-MCP binds permanently to receptors present at the time of treatment and any return of ethylene sensitivity is due to the appearance of new binding sites, with different ability depending on plant tissue (Blankeship and Dole 2003), cultivar, fruit maturity, or growing conditions (Jiang *et al.* 1999; Harris *et al.* 2000; Watkins and Miller 2003).

It is possible that in kiwifruit new binding sites for ethylene are formed in approximately 4 weeks in air, while this time can be longer in CA conditions as in the case of this experiment. On the other hand, Colelli *et al.* (2004) reported an effect of 1 µL/L of 1-MCP after 8 weeks of storage, even in presence of exogenous ethylene, which can be attributed to the different treatment concentration and to the possible different physiological state of the fruit.

At the end of the experiment untreated fruit kept in air retained 25% of the initial firmness, while firmness retention resulted 35% for treated fruit stored in air, 43% for untreated fruit stored in 3 kPa O_2+5 kPa CO_2, and 60% for fruit treated with 1-MCP and stored in CA (**Fig. 1**).

Respiration rate was only measured for samples kept in air and no differences were detected according to 1-MCP treatment, even if the rate in untreated fruit was 36% higher than in treated fruit (**Table 3**, **Fig. 1**). It has been reported a significant reduction of CO_2 production for 1-MCP treated kiwifruits, both intact and sliced (Colelli and Amodio 2003; Mao *et al.* 2007), and for whole fruit also in presence of exogenous ethylene (Colelli *et al.* 2004), although in these works a higher 1-MCP concentration was used.

CA storage and 1-MCP pre-treatment also affected solid soluble content, as reported in **Tables 3** and **4**. Treatment with µL/L ppm of 1-MCP resulted in a significant lower SSC mean value (12.3 compared to 12.5 °Brix); on the other hand the influence of CA storage was more pronounced with a lower SSC mean value of 12.1 °Brix for fruit in 3 kPa O_2+5 kPa CO_2, compared to 12.7 °Brix for fruit stored in air.

The evolution of SSC throughout the experiment for all treatments is shown in **Fig. 1**. Both treatments held in CA always presented lower SSC values, which resulted statistically different after 28, 36, and 55 days at 0°C. Most likely CA conditions affected a wide range of processes related to ripening, including starch degradation (Kader 1986), and its effect was stronger than that of 1-MCP.

Finally neither 1-MCP nor storage in CA affected TA and weight loss on kiwifruits. This is also previously reported (Colelli and Amodio 2003; Colelli *et al.* 2004) for kiwifruit treated with 1 μL/L of 1-MCP.

CONCLUSIONS

Results point to a beneficial effect of 1-MCP pre-treatment and CA on ripening delay of kiwifruits during cold storage. Both treatments allowed the delay of softening, a major problem which limits the shelf-life of these fruit. CA with 3 kPa O_2+5 kPa CO_2 was more effective than 1-MCP alone in delaying softening and solid soluble increase. CA and 1-MCP interaction was not significant when data related to all the experiment duration were considered, but after 4 weeks of storage at 0°C fruit pre-treated with 0.5 μL/L of 1-MCP and stored in CA (3 kPa O_2+5 kPa CO_2) showed the highest firmness retention, indicating a synergistic effect of these treatments, more evident when the effect of 1-MCP treatment started to decrease.

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