

RESEARCH ARTICLE

Effect of 1-methylcyclopropene (1-MCP) treatment on postharvest quality and antifungal activity of avocado cv. 'pollock' under tropical storage conditions

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ABSTRACT

1-MCP is a competitive, gaseous ethylene inhibitor, which binds irreversibly to ethylene receptors and thereby blocks effects of ethylene in plants. The potential for 1-MCP to delay ripening in avocado cv. 'Pollock' fruit was examined under tropical ambient storage conditions. Fruits harvested at commercial maturity were exposed to 300 nll⁻¹ 1-MCP gas for 20 h under 27±2 °C and then stored under ambient conditions (27±2 °C and relative humidity 65 ± 2%). Fruits were assessed daily for fresh weight, firmness, skin colour, fungal rot development and glossiness. The antifungal activity in diethylether extracts of fruit peel was assessed through thin layer chromatography (TLC) coupled with *Cladosporium* bioassay. Exposure to 1-MCP extended the shelf life up to 9 days of fruit by 2.2 fold as compared to the shelf life of 4 days of the controls. Stem end rot caused by *Botryodiplodia theobromae* was found to be the shelf-life determining factor of the fruits. 1-MCP treatment resulted in a significant (p < 0.05) delay in change in peel colour, softening and glossiness of fruit as compared to the controls. Bioassays using *Cladosporium* fungus revealed the presence of antifungal activity at Rf 0.75 in both 1-MCP treated and control fruits. 1-MCP treatment was effective in delaying the decline of antifungal activity in fruit apparently along with the delay of other ripening-related changes and minimizing losses encountered during storage under warmer ambient conditions.

Keywords: 1-methylcyclopropene, disease, shelf life, storage.

INTRODUCTION

Avocado (*Persea americana* Mill.) is a fruit that is used as a dessert in many parts of the world and is reported to have many health promoting properties (Ding *et al.*, 2007, Dreher and Davenport, 2013). Avocado is a climacteric fruit characterized by an increase in ethylene production at the beginning of climacteric ripening (Wu *et al.*, 2011). Physical properties such as fresh weight, firmness, skin colour, and glossiness are key determinants of aesthetic appeal of avocado fruits (Lee *et al.*, 1983). Harvested fruit undergoes many physiological changes, affecting the quality. The chemical properties of avocado such as total soluble solids (Liu *et al.*, 1999), pH, total titratable acids (Maftoonazad and Ramaswamy, 2008), oils (Hofman *et al.*, 2002), phenolics (Donetti and Manuela, 2011) and antifungal compounds (Adikaram *et al.*, 1993) change upon ripening.

Anthraxnose caused by *Colletotrichum gloeosporioides* and stem-end rot caused by a range of pathogens; i.e. *Dothiorella* spp., *Botryodiplodia theobromae*, *Fusarium* spp., *Phoma* spp., and *Phomopsis* spp. (Kotze, 1978; Johnson and Kotze, 1994) are the most common diseases that cause

postharvest losses in avocado (Crane *et al.*, 2001). However, most of these postharvest pathogens remain quiescent on the fruit surface until the requirements for continuation of the infection process are fulfilled (Prusky and Lichter, 2008).

The presence of constitutive antifungal compounds in the peel of avocado fruit is one of the major attributes for the quiescence of *C. gloeosporioides* (Prusky *et al.*, 1982; Sivanathan and Adikaram, 1989). There is a gradual increase in antifungal compounds with maturity, reaching a maximum level by harvesting maturity stage and then dramatically decreases upon fruit ripening enabling fungal activity in the fruit (Adikaram *et al.*, 1993). Infections, caused by *C. gloeosporioides*, create rounded, dark-colored, sunken lesions that expand rapidly on the fruit skin and into the pulp, whereas the stem-end rot of the fruit initiates around the stem end scar resulting the darkening of the affected tissue externally and a discoloured, brown rotting flesh internally (Snowdon, 1990).

Being a climacteric fruit, a dramatic rise in ethylene production during climacteric period is associated with rapid physico-chemical changes during ripening in avocado (Bower and Cutting, 1988). The

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pathogens remain inactive/quiescent on the fruit become active parallel to the physiological changes occur in the fruit surface during ripening (Prusky, 1996). Therefore, reducing the levels of ethylene in harvested avocado fruit delays ripening and indirectly helps control of decay by pathogens (Jeong *et al.*, 2002).

Compound 1-methylcyclopropene (1-MCP) is a competitive, gaseous ethylene inhibitor, which binds irreversibly to ethylene receptors in plants (Hofman *et al.*, 2001) thus extending postharvest longevity in many fruits and vegetables. Treating banana cv 'Williams' with 500 nll⁻¹ 1-MCP for 24 h at 20 °C effectively inhibited fruit degreening (Harris *et al.*, 2000) whereas tomato cv. 'Clarion' with 5 µll⁻¹ 1-MCP for 1 h at 20 °C increased days to ripen by 70% (Wills and Ku, 2002). 1-MCP also affects the rates of ethylene production in avocado fruits. When avocado (cv. Hass) fruits were treated with 25 ppm 1-MCP for 14 hour at 20 °C, ripening was delayed by 4.4 days with compared to untreated fruits (Hofman *et al.*, 2001). Treated fruits are firmer, softening process and changing skin colour is slow (Adkins *et al.*, 2005). Jeong *et al.* (2002) treated West Indian-type avocado (cv. 'Simmonds') fruits with two different concentrations (0.09 and 0.45 µl l⁻¹) of 1-MCP for three exposure times (6, 12, and 24 h) at 20 °C and found that 1-MCP treatment at 0.45 µll⁻¹ for 24 h at 20 °C delayed the ripening of avocado fruit by 4 days at 20 °C along with significantly less weight loss and retention of green colour than untreated fruit. It has been concluded that avocado ripening is influenced by 1-MCP concentration, duration of exposure and the treatment temperature (Jeong *et al.*, 2002).

In Sri Lanka avocado is mostly targeted for the local market and only small quantities are exported by airfreight mainly to the European and the Middle East countries (Mankotte, 1996). There is no organized marketing process or postharvest handling system for avocado and the postharvest losses mostly during transport and storage, account for about 30 % of the total production in the country (Anon, 2011). The Department of Agriculture (DOA), Sri Lanka, recommended the cultivars 'Booth 7', 'Purple Red', 'Pollock' and 'Tower II' for the local market and cultivars 'Hass' and 'Fuerte' for the export market (Anon, 2013). At present, cv. 'Pollock' dominates the local market, as there is high consumer demand due to its relatively larger size, attractive oval shape and smooth fruit surface (Personal Communications, 2014). However, higher susceptibility to stem-end rot and anthracnose is a major problem associated with this cultivar (Rupasinghe and Peiris, 1992).

Although many avocado cultivars such as 'Ettinger', 'Hass', 'Pinkerton', 'Simmonds' and 'Beta', have been tested for the effect of 1-MCP on

the ripening-related changes in fruit (Feng *et al.*, 2000; Hofman *et al.*, 2001; Jeong *et al.*, 2002; Hershkovitz *et al.*, 2005), its effects on cv. 'Pollock' is unknown. At present 1-MCP is not available or used commercially on fresh produce in Sri Lanka. We investigated the effect of 1-MCP on postharvest quality of avocado cv. 'Pollock' and the development of postharvest fungal diseases under tropical ambient conditions. We also examined whether 1-MCP affects the innate antifungal activity in the fruit peel during ripening.

MATERIALS AND METHODS

Plant material

Avocado cv. 'Pollock' fruits were handpicked at their physiological maturity from the fields at Fruit Research Centre, Gannoruwa, Peradeniya (7.2667° N, 80.6000° E) around 9:00 a.m. Fruits visibly free from disease and defects were selected and transported to the Plant Pathology Laboratory at the Department of Botany, University of Peradeniya, Sri Lanka within one hour after harvest and wiped with sterile distilled water to remove dust and debris, allowed to air-dry and used for subsequent experiments.

Effect of 1-MCP treatment on postharvest quality of avocado cv. 'Pollock'

1-Methylcyclopropene treatment

A set of six replicate fruits weighing approximately 3 kg was placed together inside a sealed fiberglass chamber for the exposure to 1-MCP gas. EthylBloc® powder (0.14% active ingredient) was mixed with water in order to release 1-MCP gas. A vessel containing 1M KOH was kept in the chamber to absorb CO₂ from respiration. The appropriate volume of 1-MCP gas to achieve the final concentration 300 nll⁻¹ was injected through a rubber septum into the chamber (after Jiang *et al.*, 2001). The fruits were incubated for 20 hours at room temperature, 27± 2 °C. Another set of six fruits was incubated under the same conditions without 1-MCP treatment to serve as the control. After 20 hours, 1-MCP treated and non-treated fruits were taken out of the chambers and stored at room temperature (27± 2 °C) and relative humidity 65±2 %. For antifungal activity studies, a total of 24 fruits were either gassed in the same manner or kept as controls for destructive analysis of fruit peel tissues at different stages of ripening.

Assessments

Fruits were assessed for peel colour, fresh weight, firmness, glossiness and disease development until they reached the end of shelf life based on 5-10% (fruit area) rot development. Fungal rot development was estimated visually as area covered by the disease, expressed as a percentage value where 0 % indicates no disease and 100% indicates

the total coverage of fruit with the disease. Fungal spp. present in the diseased areas were isolated onto Potato Dextrose Agar (PDA) medium (Wanigasekara, 2009).

The peel colour was assessed visually using a self-prepared colour chart considering amount of green/yellowness across the fruit on a scale of 0–4, where 0 = dark green; 1 = breaker (definite colour break from dark green to yellow); 2 = more green than yellow (75% green and 25% yellow); 3 = yellow green (50% green and 50% yellow); 4 = more yellow than green (80% yellow and 20% green). Fruit firmness was rated as hand firmness on a scale from 1 - 4, where, 4 = hard, 3 = moderately hard, 2 = moderately soft, 1 = soft. Weight of each replicate fruit was measured daily (\pm 0.01 g). The reduction of weight was expressed as percentage weight loss. Glossiness of fruit peel was visually estimated on a self-prepared index; 3 = high, 2 = moderate, 1 = low

Effect of 1-MCP treatment on antifungal activity in avocado

Extraction of antifungal compounds from the fruit peel

Three fruits per treatment (1-MCP treated and control fruits) were randomly selected at different stages of ripening (for analysis of peel tissues). The outer skin (about 1-2 mm thick) was cut out from 1-MCP treated and non-treated avocado fruits at different stages from the day of harvest to the day of full ripening (yellow) stage. The tissue segments were stored at -20 °C for 1-2 days. For extraction of antifungal compounds, 30 g of peel tissue was homogenized twice with two 50 ml portions of diethylether [(C₂H₅)₂O; Scientific Ltd, U.K.] for 5 minutes separately using a homogenizer (ULTRA TURREX; T 25 basic; IKA LABORTECHNIK) operated at its highest speed (5). The two extract portions were combined and filtered through Whatman No.1 filter paper. The filtrate was collected and evaporated *in vacuo* at 210 rpm in a rotary evaporator (Stuart RE 300) at 40 °C (Sivanathan and Adikaram, 1989).

Detection of antifungal activity in fruit peel-Cladosporium Bioassay

The peel extracts obtained were tested for the presence of antifungal compounds using *Cladosporium* bioassay coupled with thin layer chromatography (TLC) (Sivanathan and Adikaram, 1989). About 5 mg of the peel extract was dissolved in 1 ml of 95% ethanol and 100 μ l aliquots of dissolved extracts were spotted on TLC plates (20x20 cm; Whatman 250 μ m; aluminium-backed; UV fluorescing). The plates were then developed in chloroform: methanol (90:10 v/v) and air-dried overnight. Sporulating cultures on PDA plates (about 14 days old) of *Cladosporium* sp. were flooded with Czapek- Dox nutrient solution and the mycelia were scraped off using a sterile spatula. The

mycelial suspension was filtered through sterile glass wool to remove the hyphal fragments. The filtrate of conidia was evenly sprayed using an atomizer (Knf; Neuberger; D-79112) onto the developed TLC plates. The plates were then incubated in a moist chamber for 2-3 days under room temperature (26 \pm 2 °C). The presence of antifungal compounds was detected by the lack of mycelia in those zones. The diameter of the inhibition zones was recorded in cm² using graph paper.

Data analysis

The experiments were laid out in a completely randomized design (CRD). Each treatment comprised six replicate fruits. All the experiments were repeated twice. Data were analyzed using statistical package Minitab version 16 (Kruskal-Wallis for nonparametric data and two sample t-test for parametric data).

RESULTS

Effect of 1-MCP treatment on postharvest quality of avocado cv. 'Pollock'

A number of postharvest quality parameters of 1-MCP treated avocado cv. 'Pollock' fruits were compared with non-treated fruits. Fungal rot development (5-10% of total area of fruit) was the shelf-life determining parameter for the fruit (Table 1). The most prominent disease observed throughout the study was stem end rot and the causative agent was identified as *Botryodiplodia theobromae*. Pathogens causing anthracnose was not isolated even after several attempts. 1-MCP treatment significantly reduced the severity of natural stem end rot development as compared with the control fruits (Fig. 1).

Upon 1-MCP treatment the number of days needed for the appearance of stem end rot on fruit also delayed by 5 days (Fig. 2C). 1-MCP treatment effectively doubled the fruit shelf life compared to non-treated fruit (Table 1). Based on the observations, time taken for stem end rot to cover 5-10% of the total area of fruit was considered as the end of shelf life (Table 1) and occurred before other ripening related changes including peel colour (Fig. 2A), fruit firmness (Fig. 2B).

1-MCP treated fruits exhibited significant ($p < 0.05$) reduction in weight loss, higher retention of firmness and surface glossiness throughout the storage period (Fig. 1). Peel colour development was also significantly delayed (Fig. 2A). Fruit colour changed from dark green to pale yellow by fully ripe stage in 'Pollock' avocados. By the end of the shelf life (4 days) of non-treated fruits, the quality of 1-MCP treated fruit was significantly higher ($p < 0.05$) for all parameters tested (Table 1).

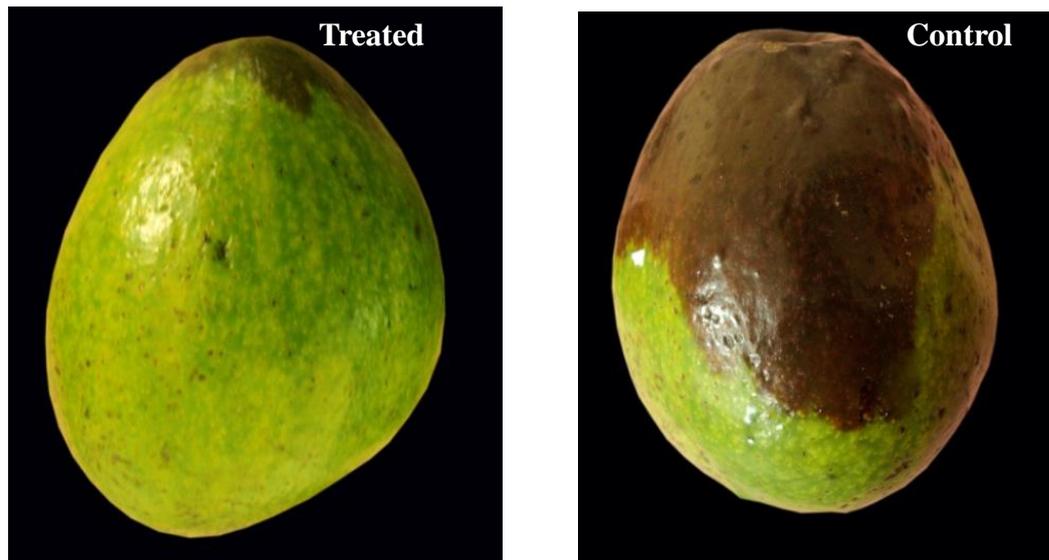


Figure 1. Effect of 1-MCP treatment on natural stem end rot development in avocado cv. 'Pollock' after 8 days of storage under tropical ambient conditions.

Table 1. Effect of postharvest 1-MCP treatment on quality parameters of avocado cv. 'Pollock' fruits at 4 days after harvest (end of the shelf life of non-treated fruits).

Treatment	% Weight loss	Colour	Firmness	Glossiness	%Area under disease	Shelf life (Days)
1-MCP treated	2.61a	0a	4b	3b	0a	9a
Control	4.13b	1b	3a	2a	8b	4b

Values indicated in each column are means of six replicates.

Values in each column followed by a different letter are significantly different at $p < 0.05$ ($n=6$).

5-10% rot development was considered as the level for end of shelf life.

Table 2. Antifungal activity of diethylether extracts of 1-MCP treated and non-treated (Control) avocado cv. 'Pollock' fruit peels (100 μ l spot) run in chloroform: methanol (90:10 v/v) in one dimensional thin layer chromatography bioassayed with *Cladosporium* sp. at different time periods (in days) after harvest

Rf value	Antifungal activity (area of fungal inhibition cm^2)								
	Day 0	Day 3		Day 5		Day 8		Day 11#	Day 14#
	Treated + Control	Treated	Control	Treated	Control	Treated	Control	Treated	Treated
0.75	9.46	6.6	3.1	4.42	2.78	2.33	1.16	1.6	0.62
0.18	~0.23	~0.21	~0.08	~0.09	~0.04	~0.08	~0.04	~0.08	~0.07

Day 3, third day after harvest; Day 5, control at colour break; Day 8, control at fully ripe; Day11, treated at colour break; Day14, treated at fully ripe

by this time the untreated fruits (Control experiment) have been deteriorated and discarded

Effect of 1-MCP on the antifungal activity of fruit peel

Thin layer chromatograms prepared with diethyl ether extracts of peel tissues obtained from 1-MCP treated and non-treated fruits and sprayed over with *Cladosporium* sp. produced two inhibition areas at Rf 0.75 and 0.18 (Fig. 3).

Antifungal activity detected at Rf 0.75 was more prominent than that at Rf 0.18 for both 1-MCP treated and non-treated fruit (Fig. 2). The largest area of antifungal activity was detected at the day of harvest (day 0). Regardless of the 1-MCP treatment a gradual decline in peel antifungal activity at Rf 0.75 occurred during storage (Fig. 2; Table 2).

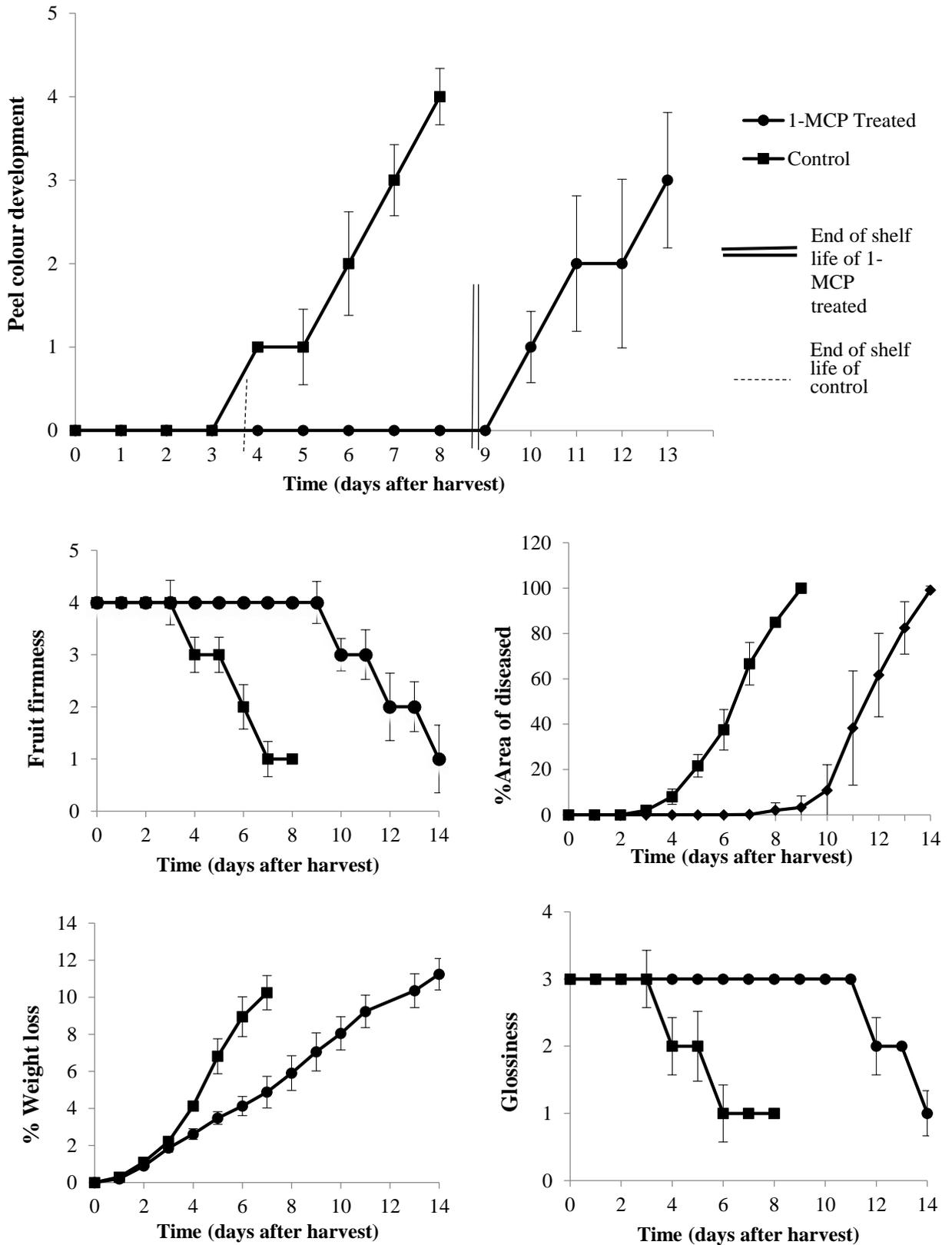


Figure 2. Effect of 1-MCP treatment on peel colour, fruit firmness, % area of disease, fresh weight and glossiness. Peel colour [0 = dark green, 4 = more yellow than green (A)]. Fruit firmness [1 = soft, 4 = hard (B)]. % Area of disease [1 = no disease appearance and 100 = full disease development (C)]. Percentage weight loss (D). Glossiness [1 = low; 3 = high (E)] Where greater than symbol size, vertical bars represent the standard error of mean.

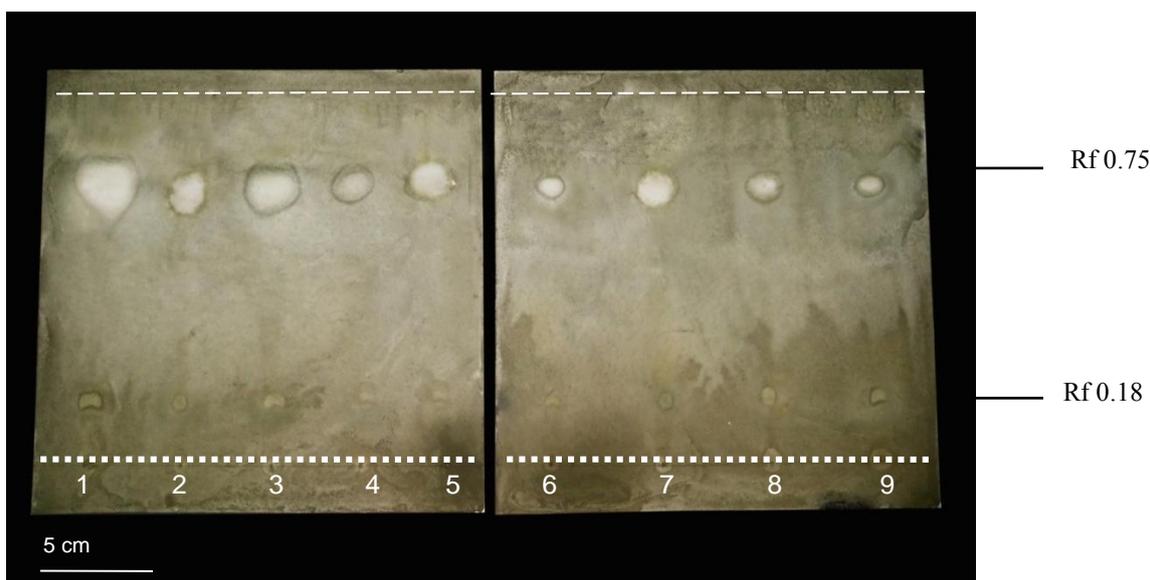


Figure 3. One dimensional thin layer chromatography (TLC) of crude diethyl ether extracts of 1-MCP treated and control fruit peels of 'Pollock' avocado (100 μ l spot) run in chloroform: methanol (90: 10 v/v) and bioassayed with *C. cladosporioides*. Lane 1, at the day of harvest (before 1-MCP treatment); lane 2, third day after harvest control; lane 3, third day after harvest treated; lane 4, control at colour break (day 5); lane 5, treated (day 5); lane 6, fully ripe control (day 8); lane 7, treated (day 8); lane 8, treated at colour break (day 11); lane 9, fully ripe treated (day 14). Dotted line= origin. Dashed line= solvent front.

This decline in antifungal activity was much faster in control fruits than in 1-MCP treated fruit and more extensive at the fully ripe stage as indicated by areas of fungal inhibition 1.11 and 2.33 cm^2 , respectively. Loss of antifungal activity (Fig. 3) paralleled the other ripening-related changes (Fig. 2). However antifungal activity in terms of area of fungal inhibition for treated fruit was smaller than for control fruit at their respective colour break stages, 2.33 cm^2 , 3.1 cm^2 and at fully ripe stage, 0.62 cm^2 , 2.78 cm^2 (Table 2). However, the antifungal activity maintained in treated fruit over time was sufficient to delay disease onset.

DISCUSSION

The ability of 1-MCP to inhibit ethylene action is due to irreversible binding to ethylene receptors in tissues (Sisler and Serek, 1999). Application of 1-MCP extends shelf life of a number of avocado cultivars including 'Ettinger', 'Fuerte', 'Reed' (Feng *et al.*, 2000), 'Hass' (Hofman *et al.*, 2001), 'Simmonds' (Jeong *et al.*, 2002) and 'Pinkerton' (Hershkovitz *et al.*, 2005). According to those studies, the effectiveness of 1-MCP treatment depended upon the 1-MCP concentration used, duration of exposure to 1-MCP, cultivar studied and temperature under which avocados were stored. The concentration of 1-MCP and duration used in our experiments at 300 nl^{-1} and 20 h exposure was similar to the optimum levels found by Jeong *et al.* (2002) for West Indian avocado variety 'Simmonds'. Delay in ripening of 1-MCP

treated fruits was characterized mainly by a significant reduction in the rate of fruit softening.

The current study is the first report of 1-MCP effects on the ripening behavior of avocado cultivar 'Pollock'. The number of days taken to develop stem end rot disease to 5-10% of the total area of fruit was considered to determine the shelf life, as the disease spread occurred more rapidly than fruit softening during the process of ripening. Most of the previous reports of 1-MCP effects on avocado were for storage at 20 $^{\circ}\text{C}$ and this current study with cv. 'Pollock' shows that the fruit shelf life can be doubled when stored under tropical ambient conditions (27 ± 2 $^{\circ}\text{C}$). 1-MCP treatment also delayed the softening of avocado cv. 'Pollock' fruits significantly. This may be attributed to reduced activity of cell wall degrading enzymes, polygalacturonase (PG) and cellulase in avocado fruits upon 1-MCP treatment (Jeong *et al.*, 2002).

Changes in chlorophyll and carotenoid pigments also contribute to the colour changes that take place in avocado during fruit development and also after harvest (Cox *et al.*, 2004). 1-MCP treatment resulted in retention of green colour in cv. 'Pollock' fruits for a significantly longer period. This can be attributed to reduction in chlorophyllase enzyme activity, which regulates the loss of green colour, in many avocado cultivars including 'Hass', 'Pinkerton' and 'Ettinger' (Hershkovitz *et al.*, 2005).

1-MCP treated 'Pollock' fruits retained their glossiness significantly longer than non-treated fruits remaining unchanged until the end of shelf life (10 days after harvest), another quality affecting consumer preference. Delay in postharvest decline of epidermal wax content also occurs with other climacteric fruits such as apples upon 1-MCP treatment (Dong *et al.*, 2012). Rate of loss of fresh weight in cv. 'Pollock' fruits was also significantly reduced by 1-MCP treatment. Reduction of ethylene production is commonly associated with 1-MCP treatment in many climacteric fruits including avocado (Feng *et al.*, 2000). This reduction in the rate of weight loss can largely be attributed to delay in the onset of respiratory climacteric in 1-MCP treated fruit as with many climacteric fruits including banana (Sisler and Serek, 1997), mango (Hofman *et al.*, 2001), apples (Fan *et al.*, 1999) and avocado (Lemmer *et al.*, 2002).

The antifungal activity in fruit peel was mainly due to a compound separated at Rf_{0.75} on TLC, bioassayed with *Cladosporium* sp. Independent of the 1-MCP treatment, the antifungal activity declined with time during storage under the tropical ambient conditions. However, the reduction in antifungal activity was slower in 1-MCP treated fruits and coincided with the ripening related changes in the fruit.

Gradual reduction of antifungal activity during ripening has been reported for many other cultivars of avocado. The antifungal activity of cultivars 'Hass' and 'Fuerte' against the anthracnose fungus *Colletotrichum gloeosporioides* was attributed to the presence of a diene (1-acetoxy-2-hydroxy-4-oxo-heneicosa-12, 15-diene) in the fruit peel (Prusky *et al.*, 1982). Sivanathan and Adikaram (1989) found four compounds with antifungal activity in cultivar 'Green' fruit peel. The four compounds were denoted as AV I (Rf = 0.75), AV II (Rf = 0.70), AV III (Rf = 0.32) and AV IV (Rf = 0.30). According to IR and HNMR spectral studies AV II was similar to the antifungal diene previously isolated by Prusky *et al.* (1982). It is likely that the major antifungal compound separated at Rf = 0.75 for 'Pollock' fruits is the same as AVI, reported by Sivanathan and Adikaram (1989) as we followed the same extraction procedure for compound extraction and used the same solvent system to develop the TLCs used by them. Absence of antifungal activity in 'Pollock' fruits at Rf values 0.3, 0.32 and 0.70 could be due to cultivar differences. Karni *et al.* (1989) found that the decrease in *Colletotrichum* antifungal activity related to the diene was regulated by lipoxygenase, which is in turn regulated by epicatechin, an endogenous antioxidant in avocado fruit.

The decline of antifungal activity coincided with the progression of stem end rot in both 1-MCP treated and non-treated 'Pollock' fruit. Although the stem end rot symptoms appeared before the other signs of ripening-related changes became apparent, a marked

reduction in antifungal activity was detected in fruits coincident with the initiation of the rot. It may be that the antifungal compounds have some role in suppressing the stem end rot, but not to the level they play in the anthracnose pathosystem due to passive entry of *B. theobromae* through stem end scar as opposed to the direct penetration of the intact fruit by *C. gloeosporioides*, the anthracnose fungus which appears to be latent on the fruit until the ripening process results in the decline observed in the skin antifungal diene (Prusky, 1997).

It can be concluded that 1-MCP is effective in extending the storage life of avocado cv. 'Pollock' fruit significantly under tropical ambient conditions mainly through delaying the stem end rot development and this treatment could be beneficial to the avocado supply chain in Sri Lanka.

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