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Integrated application of 1-methylcyclopropene and modified atmosphere packaging to improve quality retention of litchi cultivars during storage

Karen De Reuck, Dharini Sivakumar*, Lise Korsten

Postharvest Technology Group, Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria 0002, South Africa

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ABSTRACT

The effect of 1-MCP application on overall quality retention of 'Mauritius' and 'McLean's Red' litchi under modified atmosphere packaging (MAP) was investigated. Fruit was packed in biorientated polypropylene bags and exposed to different concentrations of 1-MCP (300, 500 and 1000 nL L⁻¹) within the packaging, heat sealed and stored at 2 °C for 14 and 21 d. Of the three concentrations, 300 nL L⁻¹ was most effective in preventing browning and retention of colour in both cultivars after 14 and 21 d cold storage. The effect of 1-MCP (300 nL L⁻¹) was more promising on 'McLean's Red' than 'Mauritius'. 1-MCP (300 nL L⁻¹) significantly reduced the polyphenol oxidase (PPO) and peroxidase (POD) activity, retained membrane integrity, anthocyanin content and prevented the decline of pericarp colour values, L*, a* and b* during storage. At higher concentrations, 1-MCP showed negative effects on membrane integrity, pericarp browning, PPO and POD activity in both cultivars. 1-MCP (1000 nL L⁻¹) significantly suppressed fruit respiration and retained the SSC/TA and firmness. Thus, application of 1-MCP in combination with the use of MAP can extend the storage life of 'McLean's Red' up to 21 d.

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1. Introduction

Litchi is a popular subtropical fruit of high commercial value and anthocyanin pigment is responsible for the attractive red colouration of the litchi skin. Pericarp browning and decay limit the storage life of litchis. The browning mechanism in litchi is reported to be a result of oxidation of anthocyanins by polyphenol oxidase (PPO) (Huang et al., 1990; Jiang, 2000) or peroxidase (POD) (Zhang et al., 2005). Commercially a strong antioxidative treatment, sulphur dioxide (SO₂) fumigation, has been adopted to block the oxidation reactions involved in browning. Due to harmful effects caused by the allergic reactions of sulphur residues, the European Community permits a maximum concentration of only 10 µg g⁻¹ in the edible portion of the fruit (Ducamp-Collin, 2004).

Modified atmosphere packaging (MAP) can be beneficial to maintain high humidity, essential for prevention of water loss and browning of the litchi pericarp (Kader, 1994). A modified atmosphere (17% O₂ and 6% CO₂) created in biorientated polypropylene films has enabled the retention of litchi fruit quality during storage by reducing pericarp browning (Sivakumar and Korsten, 2006). In MAP it is essential that there is no disease or pericarp browning of the fruit, since the pre-sorting of fruit before sale is not practicable

in large-scale marketing chains. Kruger et al. (2005) identified the potential of using 1-MCP treatment in combination with MAP on quality retention of 'Mauritius' litchi. Qu et al. (2006) reported that the application of 1-MCP at 1 mL L⁻¹ reduced the browning and disease index in 'Huaizhi' fruit stored at 28–33 °C and 95–100% RH for 6 d. All these observations support the use of 1-MCP application on litchi quality retention during storage. Kruger et al. (2005) used a prochloraz[®] treatment with 1-MCP and MAP (punnets) to prevent fruit decay during storage. However, the use of chemical fungicides can result in unfavorable effects on public health, the environment, and could induce resistance in pathogens. Furthermore, investigations by Kruger et al. (2005) did not include changes in litchi fruit physiology due to different concentrations of 1-MCP treatments.

The objective of this study was to investigate the potential of 1-MCP as an integrated treatment with MAP (biorientated polypropylene) to extend the storage life and quality retention for up to 21 d of fruit of two cultivars, 'Mauritius' and 'McLean's Red' grown in South Africa.

2. Materials and methods

2.1. Fruit treatment and storage

Early season litchi (*Litchi chinensis* Sonn.) fruit, 'Mauritius' and 'McLean's Red', were picked at commercial maturity from Geldenhuis plantations in Tzaneen, South Africa. Although the fruiting

* Corresponding author. Tel.: +27 12 4204097; fax: +27 12 4204588.
E-mail address: dharinisivakumar@yahoo.co.uk (D. Sivakumar).

pattern is different in both cultivars, the trials were conducted during the same growing seasons. After harvest, fruit were sorted for uniform size, colour stage and absence of mechanical damage. A completely randomized design was used in this experiment. Fruit were divided into four lots each of 500 fruit, and each lot was divided into 10 replicates each containing 50 fruit per designated storage time per cultivar.

Within 3 h after harvest, fruit (50) were placed in the biooriented polypropylene bag (MAP; 35 μm thickness; size 40 cm \times 18 cm; O_2 permeance $38 \times 10^{-14} \text{ mol s}^{-1} \text{ m}^{-2} \text{ Pa}^{-1}$ at 23 °C according to the manufacturer's information) (Sivakumar and Korsten, 2006). However, the gas permeance of the MAP depended on the degree of perforation that expresses the total area of the pores as a percentage of the film surface. For each cultivar a set of 10 replicates (MAP) was given one of four treatments in the MAP: (1) 300 nL L^{-1} 1-MCP, (2) 500 nL L^{-1} 1-MCP, (3) 1000 nL L^{-1} 1-MCP and (4) untreated control. 1-MCP was released into the MAP according to Kruger et al. (2005) from 1.5-mL capped eppendorf vials (the caps were perforated with needles, five perforations/cap) containing weighed amounts of SmartFresh™ powder (0.14% active ingredient; Rohm and Hass, South Africa) by adding warm water as droplets (30 °C). Each vial was vortexed and thereafter, the MAP was sealed with a heat sealer (Multivac C200, Multivac, Heidelberg, South Africa) to create a passive modified atmosphere around the fruit. The 1-MCP concentration was verified by means of gas chromatography, using iso-butylene as standard (Jiang et al., 2001). Fruit packed in MAP without 1-MCP treatment was included as a control (stand-alone MAP).

At the completion of each designated storage time (14 or 21 d) five replicates were removed from cold storage from each treatment and the effect of all treatments on gas composition around the fruit within the packaging, fruit quality parameters including decay incidence, browning index (BI), weight loss, fruit firmness pericarp colour, anthocyanin content, soluble solid concentration (SSC) and titratable acidity (TA), activities of oxidative enzymes PPO and POD, and pericarp relative leakage were determined. Another set of 30 replicates of different 1-MCP concentrations + MAP and stand-alone MAP was packed for each cultivar type as mentioned before. These fruit were stored at low temperature and the gas analysis was carried out from three replicates for 0–10 d. Head-space gases CO_2 and O_2 were measured using a PBI Dansensor CO_2/O_2 gas analyser (Checkmate 9900, Ringsted, Denmark) after removal from cold storage.

2.2. Fruit quality evaluation

Severity of browning was assessed visually as: 1 = no browning; 2 = 1–2 brown spots, acceptable marketability; 3 = some spots with browning, limited marketability; 4 = 50%; 5 = 75% and entire fruit surface brown. The browning index (BI) was calculated according to Zhang and Quantick (1997). Severity of postharvest disease was assessed on a 1–5 scale, describing the severity of postharvest fungal decay: 1 = no disease; 2 = 25%; 3 = 50%; 4 = 75% of the fruit surface affected, and 5 = entire fruit decayed.

The fruit subjected to all the treatments mentioned above were weighed before and after 14 and 21 d storage and data expressed as percentage weight loss. Fruit firmness was measured on opposite sides of each fruit (20 fruit per replicate per treatment) by a hand-held firmness tester (Bareiss Prüfgerätekombi GmbH, DKD-Kalibrierlaboratorium, Germany) (Sivakumar and Korsten, 2006).

Fruit pericarp colour was measured (20 fruit per replicate per treatment) using a Minolta Chromameter (model CR-300; Osaka, Japan), expressing CIELAB Commission International de l'Eclairage (CIE) colour space; L^* , a^* and b^* . Two spots on opposite sides of

the fruit were measured and the mean of the two measurements considered as one reading.

Anthocyanin content was determined from pericarp (10 g) peeled from 20 fruit. Pericarp tissue was quickly sliced and extracted with 15 mL HCl-methanol (0.15% HCl: 95% methanol = 15:85) for 4 h. The extract was filtered and its absorbance determined at 530, 620 and 650 nm, respectively. The anthocyanin content measurement was based on the formula: $\Delta A/\text{gFW} = (A_{530} - A_{620}) - 0.1(A_{650} - A_{620})$ using a spectrophotometer (Carl Zeiss (Jena), Jena, Germany) (Zheng and Tian, 2006).

A set of 20 fruit per replicate per treatment was randomly selected for SSC determination with a digital refractometer (Atago Co., Tokyo, Japan) and expressed in percentages. The % TA was determined by titration of 10 mL of fruit juice with 0.01 M NaOH and calculated as citric acid equivalent from 20 g aril obtained from 15 to 20 fruit per replicate per treatment (Sivakumar and Korsten, 2006).

2.3. Measurement of oxidative enzymes activity and relative leakage

Pericarp tissues (10 g) from 20 fruit per treatment per replicate were homogenized in 20 mL of 0.05 M potassium phosphate buffer (pH 6.8) and 0.6 g of polyvinylpyrrolidone (Sigma) at 4 °C. After filtration of the homogenate through a cotton cloth, the filtrate was centrifuged for 20 min at 19,000 \times g and 4 °C. The supernatant was then collected as the crude enzyme extract. PPO activity was assayed by measuring the oxidation of 4-methylcatechol as the substrate according to the method of Jiang (2000) at 410 nm. POD activity was assayed according to Zhang et al. (2005) in a reaction mixture of 3 mL containing 25 μL of enzyme extract, 2 mL of 0.05 M phosphate buffer (pH 7.0), 0.1 mL of 1% H_2O_2 and 0.1 mL of 4% guaiacol. The increase in the absorbance at 470 nm, due to the guaiacol oxidation, was recorded for 2 min. Protein content was determined according to Bradford (1976). One unit of enzyme activity was defined as an increase in absorbance unit per minute at 25 °C. There were three replicates per treatment.

Pericarp from 20 fruit per replicate per treatment and a set of 30 peel discs were cut using a 10 mm cork borer from the equatorial region of the fruit pericarp. The pericarp peel discs were prepared and the conductivity was measured using a conductivity meter (HI76300 EC214, Hanna Instruments, Johannesburg, South Africa) according to Lichter et al. (2000).

2.4. Statistical analysis

The experiment was repeated twice and the data of each cultivar analysed separately using a bifactorial model (time of storage \times kind of treatment with respect to 1-MCP concentration) ANOVA. The mean values of the significant interactions were compared by Fisher's protected *t*-test L.S.D. (least significant difference) at the 1% level using the statistical program GenStat (2005). Pearson's correlation coefficients were calculated to determine the strength of the linear relationships between browning index, PPO, POD activity, anthocyanin content, Hunter colour values and the 1-MCP concentrations separately per cultivar and per day of storage.

3. Results and discussion

3.1. Effect of 1-MCP and MAP integrated treatments on gas composition around the fruit

Both cultivars showed similar patterns of CO_2 and O_2 levels within the packaging with respect to different treatments (Fig. 1). The equilibrium-modified atmosphere (steady state) was attained within the packaging after 5 d in 'Mauritius' and 3 d in 'McLean's

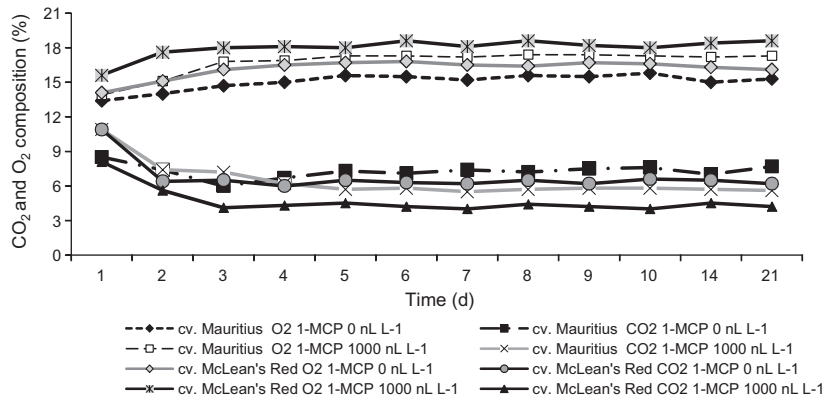


Fig. 1. Effect of 1-MCP + MAP on gas composition around (A) 'Mauritius' and (B) 'McLean's Red' litchi fruit at 2 °C. Values represent the means of gas measurements in three replicate bags. Means within 95% confidence interval.

Red'. 'Mauritius' showed slightly higher CO₂ concentrations within the packaging than 'McLean's Red', indicating that 'Mauritius' has a higher rate of respiration. Fruit treated with 1-MCP at higher concentrations (500 or 1000 nL L⁻¹) showed higher O₂ compositions within the packaging. Most climacteric fruit display decreased respiration rates upon application of 1-MCP, although higher respiration rates have been detected in 1-MCP treated ripe figs (Sozzi et al., 2005). During the steady state, litchi respiration (O₂ consumption and CO₂ production) was balanced by O₂ and CO₂ diffusion through the film: the O₂ and CO₂ concentrations reached values of ~18% and ~4% ('McLean's Red'), and ~17% and ~5% ('Mauritius'), respectively in 1000 nL L⁻¹ 1-MCP + MAP.

3.2. Effect of 1-MCP and MAP integrated treatments on incidence of browning and decay

It is evident from this study that integrated treatments of 1-MCP (300 nL L⁻¹) + MAP revealed an absence of pericarp browning in 'McLean's Red' after 14 and 21 d storage at 2 °C (Fig. 2). However, pericarp browning was not observed in 'Mauritius' in 1-MCP (300 nL L⁻¹) + MAP or stand-alone MAP after 14 d. Pericarp browning increased with increasing concentration of 1-MCP in 'Mauritius' in MAP after 14 d. Although BI was observed to increase in fruit in the integrated treatment with higher 1-MCP concentrations in both cultivars after 21 d, 'Mauritius' showed higher pericarp BI than 'McLean's Red'. In 'Mauritius' the pericarp browning was expressed as more yellowish brown. In both cultivars, the stand-alone MAP showed significantly (*P* < 0.001) reduced BI than the 1-MCP (500 or 1000 nL L⁻¹) + MAP integrated treatment after 21 d cold storage.

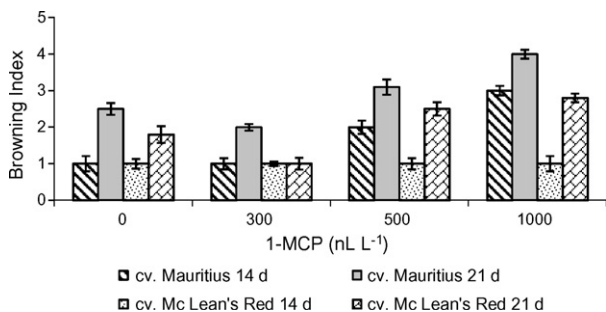


Fig. 2. Effect of 1-MCP + MAP on browning index of 'Mauritius' and 'McLean's Red' litchi pericarp at 2 °C. Values represent the means of five replicate bags each containing 50 fruit, and vertical bars indicate standard deviation of the means.

Both cultivars had disease free fruit after 14 and 21 d cold storage in 1-MCP (300 and 500 and 1000 nL L⁻¹) + MAP and stand-alone MAP. It should be noted 'Mauritius' packed in stand-alone MAP at market shelf conditions (14 °C) during experiments in 2004 did not show decay (Sivakumar and Korsten, 2006). However, 'McLean's Red' in stand-alone MAP during experiments in 2005 revealed ~11.5% decay incidence in market shelf conditions (Sivakumar et al., 2007). Although stand-alone MAP can reduce decay incidence during simulated marketing conditions, a 'protectant' is needed to protect the fruit from decay during temperature changes from 2 to 14 °C. Different integrated treatments have been investigated with anti-browning and biocontrol agents to protect fruit from decay in MAP (Sivakumar et al., 2008), and these treatments were effective for domestic marketing chains up to 18 d.

Furthermore, mixed observations were reported on the association of 1-MCP and fruit decay. Absence of decay has been shown in 1-MCP treated plums (Valero et al., 2003), but 1-MCP application increased decay incidence in strawberry (Jiang et al., 2001). The induction of decay in strawberry was reported to be dose dependent since higher concentrations of 1-MCP (500 and 1000 nL L⁻¹) induced decay incidence by inhibiting the beneficial metabolic pathway by lowering phenolic compounds that contribute to natural defense mechanisms (Ku and Wills, 1999). Meanwhile, increasing the concentration of 1-MCP to 500 and 1000 nL L⁻¹ + MAP resulted in progressive decay incidence with a severity score '2' when the storage life was extended to 30 d in 'Mauritius' (data not shown). This might be attributed to the inhibition of defense mechanisms mediated by phenylalanine ammonia-lyase enzyme (PAL) activity. Furthermore, Qu et al. (2006) reported inhibition of PAL enzyme activity in 'Huaizhi' with 1 mL L⁻¹ 1-MCP application. It is evident from the reports of Qu et al. (2006) that the disease index was low when the PAL enzyme activity was high. However, further investigation is needed with respect to different concentrations of 1-MCP treatments and PAL enzyme activity.

3.3. Effect of 1-MCP and MAP integrated treatments on weight loss, fruit firmness and SSC/TA

The high RH (85–90%) within the MAP enabled the reduction of weight loss in all treatments (data not shown). Integrated treatments with 1-MCP (500 or 1000 nL L⁻¹) + MAP showed significantly (*P* < 0.001) higher fruit firmness than 1-MCP (300 nL L⁻¹) + MAP and stand-alone MAP after 21 d storage in both cultivars (Fig. 3A). Although litchi is a non-climacteric fruit and ethylene does not play a major role in postharvest fruit ripening and fruit softening, 1-MCP (500 or 1000 nL L⁻¹) reduced the loss of firmness after 21 d storage in both cultivars. Fruit from the 1-MCP (300 nL L⁻¹ + MAP) and

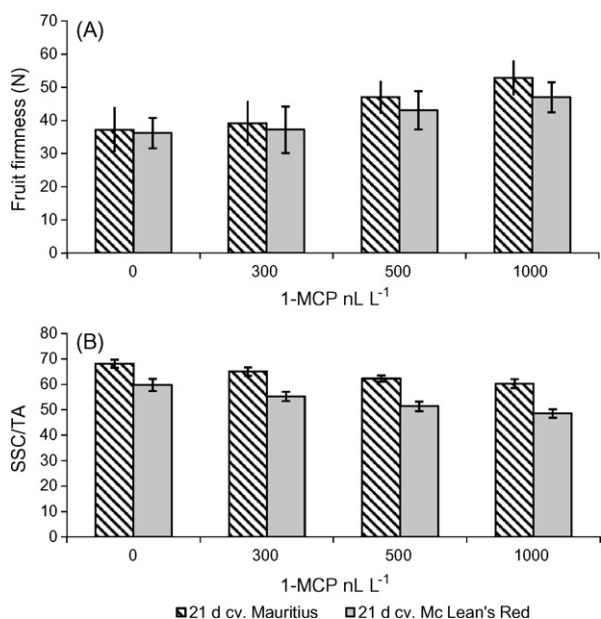


Fig. 3. Effect of 1-MCP+MAP on (A) fruit firmness (B) SSC/TA in 'Mauritius' and 'McLean's Red' litchi fruit at 2 °C. Values represent the means of five replicate bags [from 20 fruit (pericarp) per replicate per treatment] and vertical bars indicate standard deviation of the means.

stand-alone MAP showed acceptable firmness. The fruit firmness observed in fruit subjected to 1-MCP (300 nL L⁻¹ + MAP) treatment was higher than that in SO₂ fumigated fruit (Sivakumar and Korsten, 2006). Reduction in loss of firmness with 1-MCP treatment has been reported in guava (Bassetto et al., 2005) tomato (Guillen et al., 2006), and other climacteric fruit.

Non-significant variation in SSC/TA was observed with respect to all treatments in both cultivars stored up to 14 d. However, after 21 d storage the SSC/TA significantly ($P < 0.001$) increased. This effect was higher in 'Mauritius' than in 'McLean's Red' (Fig. 3B). Slightly higher SSC/TA in 'Mauritius' than in 'McLean's Red' at harvest explains the difference observed after 21 d with respect to all treatments. In both cultivars, 1-MCP (500 or 1000 nL L⁻¹) treatments prevented the increase in SSC/TA after 21 d. The 1-MCP (500 and 1000 nL L⁻¹) reduced respiration, resulting in a decline of SSC/TA by keeping the SSC unchanged. However, 1-MCP (300 nL L⁻¹) retained the SSC/TA better than stand-alone MAP as shown in Fig. 3B. It was reported in our previous studies that the SSC increased up to 19–20% and TA increased to 0.8–1% in SO₂ fumigated fruit, resulting in a lower SSC/TA ratio (Sivakumar and Korsten, 2006). The SSC/TA ratio indicates litchi taste and flavour: fruit subjected to 1-MCP (300 nL L⁻¹ + MAP) treatment retained their good taste and flavour (data not presented).

3.4. Effect of 1-MCP and MAP integrated treatments on pericarp colour and anthocyanin content

Changes in colour parameters during storage were cultivar dependent. The freshly harvested 'McLean's Red' showed higher L*, a* and b* values than 'Mauritius' fruit (Table 1). The L* value showed significant differences for both cultivars ($P < 0.001$) after 14 d of cold storage with respect to different treatments; the L* value also decreased with time of storage (data not shown). After 21 d cold storage, 'Mauritius' and 'McLean's Red' showed a significant ($P < 0.001$) decline in L* (darker fruit) in 1-MCP (500 or 1000 nL L⁻¹) + MAP or stand-alone MAP. However, the 1-MCP (300 nL L⁻¹) + MAP showed significantly ($P < 0.001$) higher L* (brighter) in both cultivars. A significant ($P < 0.001$) decrease in

Table 1
Fruit properties at harvest for the two South African litchi cultivars in this study.

Parameters	Mauritius	McLean's Red
Weight (g)	23.8	20.2
Colour L*	38.2–40.12	41.23–42.3
Colour a*	28.42	33.89
Colour b*	26.3–27.6	27.5–28.24
Fruit firmness (N)	50–58	37–38
SSC/TA	60–55.6	50.68–47.5
Anthocyanin content (Δ A/g fresh weight ⁻¹)	1.85–2.25	3.12–4.5

colour a* and b* value was observed in both cultivars after 14 d storage in 1-MCP (500 or 1000 nL L⁻¹) + MAP. Both cultivars in 1-MCP 300 nL L⁻¹ + MAP showed higher a* and b* values after 14 d. Although changes in a* and b* were observed in 'McLean's Red' after 14 d storage, these changes were not observed visually. During long-term storage (21 d) both cultivars showed a decline in a* and b* values. Both cultivars showed a significant ($P < 0.001$) decline in a* and b* and higher BI after 21 d cold storage in 1-MCP (500 or 1000 nL L⁻¹) + MAP. Changes in a* values with respect to integrated treatments and storage time are given for both cultivars in Fig. 4A because the a* value was considered to represent the red colour of the pericarp (Ducamp-Collin et al., 2008). The magnitude of 1-MCP on colour loss during storage depended on the a* value at harvest, therefore, the efficacy of 1-MCP at higher concentrations was lower in 'McLean's Red' since the colour values were higher at harvest (Table 1). The interaction of storage time and 1-MCP treatment was significant ($P < 0.001$) for a*, b* and L* values in both cultivars. Colour changes with respect to a* and b* were reduced after 21 d in both cultivars with 1-MCP (300 nL L⁻¹) + MAP. This observation supports the findings of Guillen et al. (2006) on tomatoes, where the lower 1-MCP concentration (0.5 μ L L⁻¹) reduced the colour changes with respect to a* during storage. However, different observations were reported with respect to colour changes and 1-MCP treatments. In sweet cherries, 1-MCP did not influence postharvest colour changes or stem browning (Gong et al., 2002).

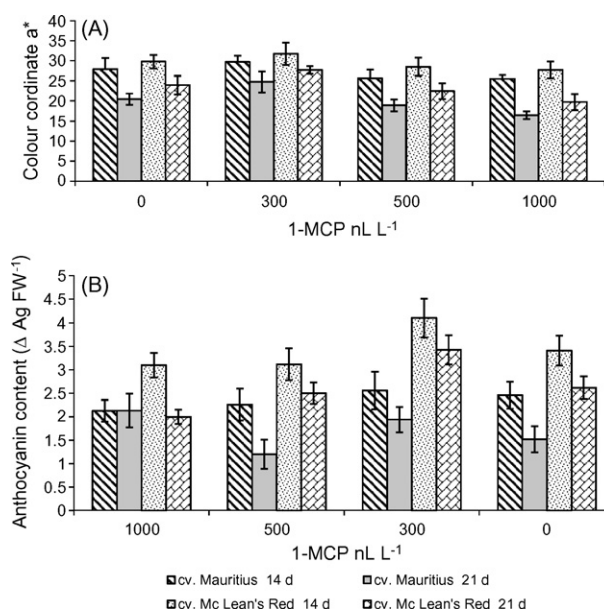


Fig. 4. Effect of 1-MCP+MAP on (A) colour value a* coordinate (B) anthocyanin content of 'Mauritius' and 'McLean's Red' litchi pericarp at 2 °C. Values represent the means of five replicate bags, a* was measured from 20 fruit in each replicate bag per treatment Anthocyanin content was measured from five replicate carrier bags [from 20 fruit (pericarp) per replicate per treatment] and vertical bars indicate standard deviation of the means.

In climacteric fruit, such as apricots, 1-MCP treated fruit may be greener and exhibit less colour change than untreated controls (Fan et al., 2000). A similar observation was reported in peaches (Kluge and Jacomino, 2002). Furthermore, the colour changes in apricot and plums are not affected by 1-MCP (Dong et al., 2002).

The anthocyanin content was reduced during storage for both cultivars (Fig. 4B). However, 'McLean's Red' showed higher anthocyanin content after 14 and 21 d storage than 'Mauritius'. The difference between the cultivars was observed at the freshly harvested stage (Table 1). Although both cultivars were grown under similar conditions in the same orchard, the difference in anthocyanin concentration could be due to the genetic control of anthocyanin levels (Matthew et al., 2005). The anthocyanin content declined with increasing BI in both cultivars. This decline was higher in 'Mauritius' after 14 and 21 d, and in 'McLean's Red' after 21 d. The 1-MCP treatment at 300 nL⁻¹ significantly ($P < 0.001$) reduced the loss of anthocyanin content in the pericarp of both cultivars during storage. However, a significant ($P < 0.001$) decrease in anthocyanin content was observed in both cultivars in the integrated treatments with higher concentrations of 1-MCP (500 or 1000 nL⁻¹). The anthocyanin content in the integrated treatments with 1-MCP at higher concentrations was lower than in the stand-alone MAP. The interaction of storage time and 1-MCP treatment was significant ($P < 0.001$) for anthocyanin content in both cultivars. In strawberry fruit, the anthocyanin content usually increases during storage (3 d at 20 °C). However, the application of 1-MCP 1000 nL⁻¹ can reduce the increase in anthocyanin content (Jiang et al., 2001). This finding supports the observed negative effect of 1-MCP at higher concentrations on anthocyanin content in both litchi cultivars during storage. The inhibitory effect of 1-MCP at 1000 nL⁻¹ could be due to a lower PAL enzyme activity (Jiang et al., 2001), a key enzyme in the biosynthesis of phenolics (Cheng and Breen, 1991). Furthermore Qu et al. (2006) observed an increase in BI while the PAL enzyme activity declined in 'Huaizhi' litchi stored at 28–33 °C. Therefore, the reduction in anthocyanin content in 'Mauritius' and 'McLean's Red' fruit could be due to a reduction in PAL enzyme activity. However, further investigations on PAL enzyme activity in both cultivars with respect to different concentrations of 1-MCP would be beneficial to explain the retention of anthocyanin content at 300 nL⁻¹ 1-MCP application.

3.5. Effect of 1-MCP and MAP integrated treatments on oxidation enzymes activity and integrity of the pericarp membrane system

PPO activity increased up to 14 d, and thereafter a decline in PPO activity was observed in both cultivars (Fig. 5A). This observation supports the finding of Tian et al. (2002) in an unspecified Chinese cultivar, where PPO activity of the litchi pericarp was shown to be higher before pericarp browning occurred and then decreased rapidly during storage. The PPO activity was ~50% higher in 'Mauritius' than 'McLean's Red' after 14 d storage in 1-MCP (300 nL⁻¹)+MAP. The integrated 1-MCP treatments with 500 and 1000 nL⁻¹ 1-MCP showed significantly ($P < 0.001$) higher PPO activity in fruit stored up to 14 and 21 d in both cultivars. It is also evident from the findings of Qu et al. (2006), that 'Huaizhi' fruit treated with 1 mL⁻¹ 1-MCP showed higher PPO activity than the untreated control fruit during storage at 28–33 °C for 6 d. The PPO activity in both cultivars was lower than the POD activity. This observation supports the findings of Ducamp-Collin et al. (2008). The POD activity was low up to 14 d and its activity increased after 21 d (Fig. 5B), and showed similar trends to PPO in 1-MCP+MAP integrated treatments. Fruit in 1-MCP (300 nL⁻¹)+MAP had lower POD activity than other treatments. The interaction of storage and 1-MCP treatment was significant ($P < 0.001$) for PPO and POD activity in both cultivars. There are two different biochemical processes

that contribute to browning: one involves the action of enzymes and the other involves changes in the red pigment molecules.

The integrity of membrane systems can be expressed as relative leakage rate. The increased relative leakage rate observed with storage time shown in Fig. 5C is due to the senescence of the pericarp. However, fruit subjected to 1-MCP (300 nL⁻¹)+MAP showed a lower relative leakage rate than other treatments. The effect of 1-MCP (300 nL⁻¹)+MAP on membrane system integrity was higher in 'McLean's Red' than 'Mauritius' (Fig. 5C). The relative leakage increased in the pericarp in integrated treatments with increasing 1-MCP concentrations. The integrated 1-MCP treatments with 500 and 1000 nL⁻¹ 1-MCP showed a higher relative leakage than stand-alone MAP treatment in 'Mauritius' after 14 d storage. After 21 d storage, 1000 nL⁻¹ 1-MCP showed a higher relative leakage rate in both types of cultivars and 'Mauritius' showed a higher relative leakage rate than 'McLean's Red'. The interaction of storage time and 1-MCP treatment was significant ($P < 0.001$) for relative leakage in both cultivars. Under long-term storage conditions, loss of membrane integrity has been reported as a result of pericarp senescence (Duan et al., 2004). The loss of cell mem-

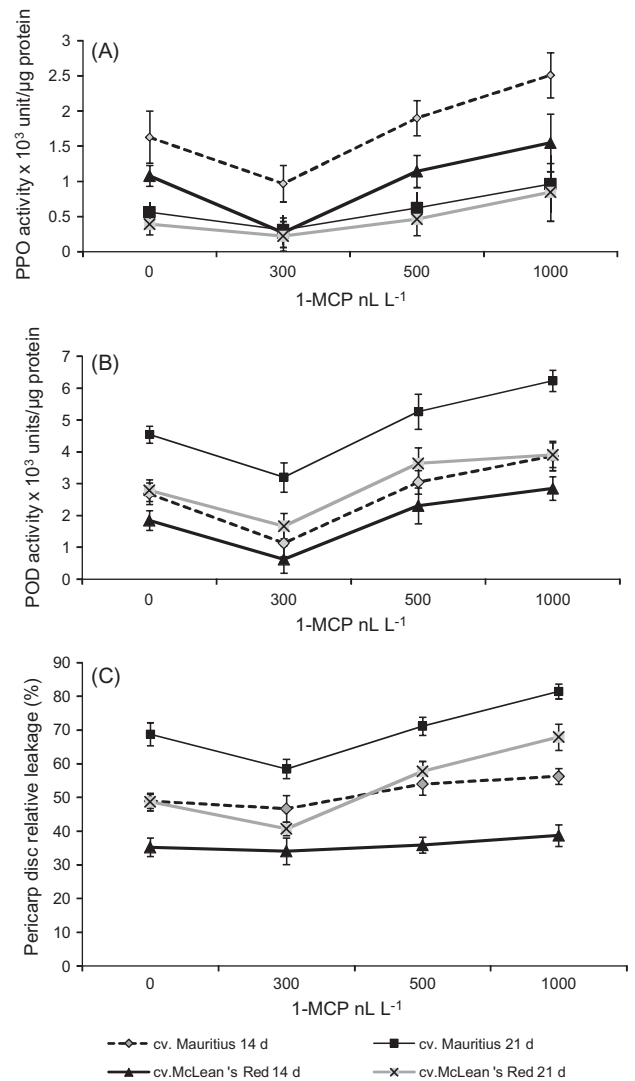


Fig. 5. Effect of 1-MCP + MAP on (A) PPO, (B) POD activity and (C) pericarp relative leakage in 'Mauritius' and 'McLean's Red' litchi pericarp at 2 °C. Values represent the means of five replicate carrier bags [from 20 fruit (pericarp) per replicate per treatment] and vertical bars indicate standard deviation of the means.

Table 2

Pearson's correlation coefficients between 1-MCP concentrations and Hunter colour values and browning related parameters in 'Mauritius and McLean's Red' litchi fruit at 2 °C for 14 and 21 d.

Parameter	Mauritius		McLean's Red	
	14 d	21 d	14 d	21 d
Hunter colour values				
L	-0.501*	-0.654*	-0.597	-0.697*
a	-0.668*	-0.674*	-0.677	-0.702*
b	-0.680*	-0.780*	-0.640	-0.680*
Browning related parameters				
Browning Index	0.757*	0.803*	ns	0.698*
PPO	0.701*	0.772*	0.567	0.739*
POD	0.624*	0.725*	0.634	0.649*
Anthocyanin content	-0.695*	-0.740*	-0.490	-0.627*
Membrane leakage	0.729*	0.727*	0.621	0.657*

*Significant at $P < 0.01$, ns- non significant.

brane integrity is known to be a result of malfunction of membrane lipid biosynthesis and membrane repair due to shortage of ATP, resulting in ion leakage and cellular decompartmentalisation (Qu et al., 2006). Consequently, browning reactions will take place when anthocyanins come into contact with the oxidizing enzymes PPO and POD. According to Qu et al. (2006), a stable energy charge is essential to maintain normal metabolism in harvested litchi fruit: the application of 1-MCP at 1 mL⁻¹ helped to minimize the change in energy charge during storage compared to untreated control fruit. However, this study reveals that 1-MCP is effective at a lower concentration (300 nLL⁻¹) in maintaining membrane integrity. Further studies are needed to determine the ATP:ADP ratio and energy charge during 300 nLL⁻¹ 1-MCP application in 'Mauritius' and 'McLean's Red' to conclude its effect on the retention of membrane integrity.

3.6. Correlation analyses

The correlation analysis data obtained for 21 d storage revealed linear relationships between the 1-MCP concentration and BI, relative leakage, PPO and POD activity for both cultivars (Table 2). However, negative correlations were observed between 1-MCP concentrations and the colour values L*, a* b* and anthocyanin content.

Although there is uncertainty about the role of C₂H₄ in pericarp browning of harvested litchi (Pang et al., 2001), the BI increased in C₂H₄ treated 'Huaizhi' fruit during storage at 28–30 °C for 6 d. Respiration in the pericarp also increased after a dip in ethephon (50 g L⁻¹) in 'Guiwei' fruit, but this increase was not observed in the aril and did not induce cyanide-insensitive respiration, one of the features of non-climacteric fruit. According to Qu et al. (2006), C₂H₄ treated fruit showed lower energy charge while the untreated control revealed higher energy charge. However, the 1-MCP treatment reduced the change in energy charge, maintaining normal metabolism. The higher concentrations of 1-MCP treatments were associated with an increase in lignin concentration and it was more pronounced in 'Mauritius' (data not shown) and similar observations were reported by Qu et al. (2006). Taking all of the above into consideration, 1-MCP at the lower concentration (300 nL L⁻¹) is effective with modified atmosphere packaging to prevent pericarp browning and senescence related mechanisms and the loss of fruit quality due to senescence of the aril. The release of 1-MCP through polypropylene film is negligible (Hotchkiss and Watkins, 2007). Although, we assume there is no significant change in 1-MCP concentration within the biorientated polypropylene carrier bag based on Hotchkiss and Watkins (2007), this needs to be investigated in the future. The colour retention and absence of pericarp browning

in 'McLean's Red' reveals that 'McLean's Red' fruit are better suited for integrated treatments. However, we consider the use of non-uniform coloured, late seasonal fruit, and the time delay between harvesting and packing operations, to be the limiting factors for this treatment.

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