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RESPONSE OF 'CARABAO' MANGO (*Mangifera indica L.*) TO 1-METHYLCYCLOPROPENE IN SACHET

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ABSTRACT

1-Methylcyclopropene (1-MCP) was tested on 'Carabao' mango harvested at 105 days after flower induction. Mature green mangoes of uniform quality were sanitized with 200 µL L^{-1} NaOCl, air dried, and packed inside newspaper-lined bamboo baskets, then treated with different concentrations of 1-MCP (0, 1, 10, and 140 µL L^{-1}) in sachets (EthylBloc™) for 24 h under ambient room conditions (27.1±2.0 °C; 87.0±8.6% RH). After treatment, the mangoes were stored in a cold room (19.0±1.2 °C, 90.7±9.3% RH) and evaluated for ten days for weight loss, firmness, total soluble solids (TSS), visual quality, stem-end rot, anthracnose, and peel color (subjective index; *L*, a*, b**, chroma, and h°). Results showed that 140 µL L^{-1} 1-MCP was able to maintain firmness and low TSS of the fruit for six days after treatment. Mango fruit treated with 1-MCP also had a better visual quality than the untreated samples until six days. 1-MCP was not able to slow down the peel color changes of the fruit but maintained the skin lightness (*L**) particularly by 10 µL L^{-1} 1-MCP for six days. 1-MCP did not affect the fruit weight loss and occurrence of latent infection such as stem-end rot and anthracnose.

Keywords: 1-MCP, delayed ripening, firmness, peel color, total soluble solids, visual quality

INTRODUCTION

'Carabao' mango is the Philippines' prime variety and is one of the sweetest cultivars in the world (Castillo-Israel et al., 2015). The country ranked seventh among exporters of fresh and dried mangoes with a 4% share of the global markets in 2015 (Fernandez-Stark et al., 2017). Although the Philippines has been a great player in the export industry for some time, just like any other fresh produce, the shipment of mangoes has been a challenge due to its relatively short shelf life. The long-distance farms, packhouses, and distribution hubs, as well as shortcomings in the supply chain, impose constraints on its postharvest life. In addition, controlling its ripening process is a challenge because of the stimulation of ethylene biosynthesis prior to its harvest maturity at ~100 days after flower induction (DAFI) (Castillo-Israel et al., 2014a). Mango fruit should be harvested upon reaching the maturity of 105 to 130 DAFI (Philippine National Standard [PNAS], 2009). Harvesting during the mango season is at 105 to 115 DAFI while 120 to 130 DAFI is suggested during the off-season.

1-Methylcyclopropene (1-MCP) is widely used to lengthen the shelf life of many horticultural produce (Blankenship & Dole, 2003). It is an ethylene-blocker that prevents ethylene-dependent mechanisms such as ripening and senescence in many horticultural crops. As a preharvest treatment, the dual application of 1-MCP, first at 100 then at 115 DAFI was able to control the ripening of 'Carabao' mango and extend its shelf life up to four days (Castillo-Israel et al., 2014a). 1-MCP is used more often as a postharvest chemical application in mangoes. For instance, the shelf life of 'Maha Chanok' mango fruit fumigated with 1,000 nL L^{-1} 1-MCP for 12 h was extended for up to 14 days in ambient conditions (27 °C, 80% RH) (Chutichudet et al., 2016). The same treatment resulted in the least incidence of decay during the entire storage period. The use of 1,000 or 2,000 nL L^{-1} 1-MCP in 'Baneshan' mangoes resulted in better fruit quality and longer shelf life of up to 36 days at 12.5±1 °C (Kumar et al., 2015). The application of 1-MCP before hot water treatment also showed a greater ability to reduce the rate of softening in 'Keitt' mangoes (Ngamchuachit et al., 2014). 1-MCP maintained firmness and slowed down color changes in 'Nam Dokmai' (Penchaiya et al., 2006) and 'Kensington Pride' mangoes (Razzaq et al., 2015). Even a low concentration of 100 nL L^{-1} 1-MCP for 12 h resulted in the lowest respiration rates in 'Tommy Atkins' (Cocozza et al., 2004). Jiang and Joyce (2000) also reported that 1 to 100 nL L^{-1} 1-MCP was effective in 'Zihua' mangoes.

Most of the above studies used gaseous 1-MCP in air-tight sealed chambers with doses ranging from 1 to 10,000 nL L^{-1} for 12 to 24 h. This manner of application makes the adoption of 1-MCP at the commercial level difficult and very limited (Osuna-Garcia et al., 2015). To be used conveniently with produce during postharvest storage, a 1-MCP trapping system using α-cyclodextrin component has been sold under the trade name EthylBloc® (Lee et al., 2006). It uses an inert matrix material to contain 1-MCP while inside a sachet. The release mechanism of 1-MCP involves three stages: 1) the 1-MCP molecules are released into the free space within the matrix of the adsorbing agent in the sachet pouch, 2) the 1-MCP molecules pass through the film microstructure into the headspace containing the fresh produce, and 3) the 1-MCP molecules penetrate the surface of the fresh produce (Lee et al., 2006).

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Several studies on the development of 1-MCP release system through different sachet materials (Lee et al., 2006), heat pressed polymer films (Hotchkiss and Watkins, 2007), and cellulose paper packaging materials (Hu et al., 2017) were reported. Some were evaluated on its effect on fresh produce. 1-MCP in sachet extended the shelf life of broccoli florets stored at 12 °C by retarding yellowing and vitamin C losses (Yamashita et al., 2006). Also, 1-MCP sachets positioned in packages of poinsettia cuttings provided a viable means for reducing the leaf abscission following a 72 h-shipment at sub-optimal temperatures of 10 to 26 °C (Faust & Lewis, 2005).

Although several studies have been conducted with 1-MCP in relation to various mango varieties, the postharvest effect of 1-MCP, especially in sachet, on 'Carabao' mango has not yet been reported. Thus, in this study, the effect of 1-MCP in sachet was tested on 'Carabao' mango, and the optimum concentration of 1-MCP for delaying fruit ripening was also determined.

MATERIALS AND METHODS

Plant material preparation and treatment

'Carabao' mangoes (60 kg) at 105 days after flower induction (DAFI) were harvested from Digos City, Davao del Sur and transported to the Southern Philippines Fresh Fruit Corporation (SPFFC), a mango export company, where it was purchased. The maturity of the fruit was determined by counting the days after spraying potassium nitrate to induce flowering in mango trees. Commercially mature green mangoes with uniform size and quality were brought to the Postharvest Biology Laboratory in the University of the Philippines Mindanao, Davao City. The fruit were sanitized with 200 µL L^{-1} NaOCl for 3 min, air-dried, and treated with 1-methylcyclopropene (1-MCP) within 24 h from harvest.

Fruit samples weighing 5 kg per replication were randomly distributed among four treatments: 1) control, 2) 1 µL L^{-1} 1-MCP, 3) 10 µL L^{-1} 1-MCP, and 4) 140 µL L^{-1} 1-MCP. The amount of 1-MCP powder for 1 and 10 µL L^{-1} concentrations in the package was calculated based on the Ideal Gas Law (Semat & Katz, 1958) and packed in pilon cloth. The original sachet packaging had a concentration of 140 µL L^{-1} 1-MCP (EthylBloc™ 0.014% 1-MCP, AgroFresh, Inc., USA). Mimicking the wholesalers' practice of traditional ripening, one 1-MCP sachet was inserted in the middle of mangoes piled inside a bamboo basket (V= 7.46 L) lined with sheets of newspaper (Figure 1). The mangoes were covered with newspaper and the rim of the bamboo basket was securely tied with polypropylene twine. The baskets were held in ambient room conditions (27.1±2.0 °C; 87.0±8.6% RH) during treatment for 24 h. After treatment for 24 h, the baskets were opened and the quality (weight loss, firmness, total soluble solids (TSS), visual quality, degree of stem-end rot and anthracnose, and peel color) of the fruit was evaluated at the opening of the baskets. All fruit samples were stored in a CoolBot-equipped cold room (19.3±2.9 °C, 87.6±14.9% RH) for the duration of storage.

Quality evaluation

The weight loss, firmness, TSS, and visual quality were measured at initial and 3, 6, 8, and 10 days after treatment. Meanwhile, the degree of stem-end rot, anthracnose, and peel color were determined at the same time with the other parameters and daily after six days to capture the onset of the disease and changes in peel color. Weight loss was calculated as the proportional difference of final weight from the initial weight. Fruit firmness was measured using a pressure tester (Fruit Tester FT 327 Wagner Instruments, USA) on both sides of the mango fruit. The TSS content was determined by extracting the juice from mango flesh and placing one to two drops of it on the prism surface of a digital refractometer (Atago PAL-1 Atago, Japan). The values were expressed in % Brix.

Visual quality was assessed using a 1-5 rating scale designed by Ekman et al. (2019) described as 1= excellent, no symptoms of deterioration; 2= good, minor symptoms of deterioration that are not objectionable; 3= fair, evident deterioration but not serious, the limit of saleability; 4= poor, serious deterioration, the limit of usability; 5= extremely poor, unusable. The onset and degree of stem-end rot and anthracnose were evaluated using the

Figure 1. 'Carabao' mangoes piled inside a bamboo basket with 1-MCP sachet placed in the middle (A), covered with newspaper sheets, and tied with polypropylene twine (B).

scale: 1= no discoloration at the stem-end/visible spots; 2= slight infection, 1-5% on the surface; 3= moderate infection, 6-10%; 4= moderately severe infection, 11-25%; 5= severe infection, >25% (Ekman et al., 2019). Peel color was assessed using the index: 1= green; 2= breaker, trace of yellow at the stem-end; 3= turning, more green than yellow; 4= more yellow than green; 5= yellow with a trace of green; 6= fully yellow (Ekman et al., 2019). Further, the peel color was quantitatively measured using a color meter (Chroma Meter CR-400, Konica Minolta Optics, Inc., Japan) by taking the average of three measurements on the surface. The values were expressed as the color space L* (lightness), a* [green (–) or red (+)], b* [blue (–) or yellow (+)], chroma, and hue angle (°) defined by the International Commission on Illumination (CIE).

The days to reach saleability, saleable days, days to reach the table ripe stage, and shelf life were also determined. The days to reach saleability was reckoned by counting the days when the mangoes reach the peel color index of ≥ 5, a visual quality rating of ≤ 3, and no diseases (Lacap et al., 2019). Saleable days refer to when the fruit was considered marketable (i.e., the time when the fruit was deemed ripe until the end of shelf life). The days to reach the table ripe stage was determined by counting the days when the fruit reached the full yellow stage (peel color index of 6), soft texture, and characteristic aroma. The shelf life was determined by counting the time from the day of harvest until it goes past the limit of saleability (i.e., a visual quality rating of > 3 and the presence of disease).

Statistical analysis

The experiment was laid out in a Completely Randomized Design with three replications for each treatment. Each replication had nine fruit samples. Data were analyzed using Analysis of Variance (ANOVA) at $P<0.05$. Differences in means were detected using Fisher's Least Significant Difference at $P<0.05$ (Statistical Tool for Agricultural Research [STAR] 2.0.1, International Rice Research Institute, Philippines).

RESULTS AND DISCUSSION

1-MCP treatment did not affect the fruit weight loss

Weight loss in 'Carabao' mango was not affected by 1-methylcyclopropene (1-MCP) treatment (Figure 2A). Both treated and untreated mangoes lost only 0.5% to 3% of their weight over the course of 10 days. The low weight loss could be attributed to the favorable storage conditions (i.e., temperature of 19.0±1.2 °C and relative humidity, RH, at 90.7±9.3%). The high RH lowered the vapor pressure deficit between the fruit and the environment which led to a low fruit transpiration rate thus the low weight loss. Vapor pressure deficit is responsible for moisture loss from the fruit as it tries to compensate for the difference between the amount of moisture in the air and the amount of moisture the air could potentially hold when it is saturated (Wollaeger & Runkle, undated).

1-MCP maintained fruit firmness during early storage

Fruit firmness was affected by 140 µL L-1 1-MCP especially at three days after treatment where it maintained its initial firmness (9 kgf) while the other fruit already softened by two-fold (Figure 2B). After three days, mangoes treated with 140 µL L^{-1} 1-MCP were the firmest among the treatments with 8 kgf, followed by those treated with 10 and 1 µL L^{-1} 1-MCP with 5.2 and 4.2 kgf, respectively, while the untreated mangoes were less firm at 3.2 kgf. This suggests that the use of a high concentration, particularly at 140 µL L^{-1}, may help delay fruit softening as a consequence of the delayed ripening process. This could be a result of 1-MCP blocking ethylene from the receptors and preventing the expression of genes that code for the enzymes related to ripening such as polygalacturonase (PG), pectin-methylesterase, and β-glucanase that breaks down cell wall polymers such as cellulose, hemicellulose, and pectin which causes softening in fruit (Payasi et al., 2009; Bouzayen et al., 2010). Castillo-Israel (2012) reported that PG activity in mango fruit was inhibited by 1-MCP at the breaker and more green than yellow stages of maturity. In addition, several studies have shown that 1-MCP delayed the peak of climacteric phase, decreased ethylene production, maintained flesh firmness longer, and delayed color development in various crops (Osuna-Garcia et al., 2015).

Fruit treated with 140 µL L^{-1} 1-MCP had lower total soluble solids during early storage

The treatment with 140 µL L^{-1} 1-MCP in sachet slowed down the ripening of 'Carabao' mango until six days after treatment as indicated by lower total soluble solids (TSS) compared to those treated with lower 1-MCP concentrations and the control (Figure 2C). At six days of storage, mangoes treated with lower concentrations of 1-MCP (1 or 10 µL L^{-1}) as well as the control already exhibited higher TSS (9.8% to 11.4% Brix) while mangoes treated with 140 µL L^{-1} 1-MCP were still at 7.2% Brix. After eight days, the TSS levels of the mangoes started to become uniform. Maintaining the lower TSS levels after six days in mango fruit treated with 140 µL L^{-1} 1-MCP could also be attributed to the blocking effect of 1-MCP in delaying physiological and biochemical events pertaining to ripening including the degradation of starch into sugars. This is associated with the rise of ethylene in mango fruit which is marked by a substantial increase in amylase activity, reducing and non-reducing sugars, and a decrease in starch content (Lima et al., 2001). As ethylene plays a major role in the expression of ripening-related genes that code for the enzymes responsible for fruit ripening, blocking its receptors by 1-MCP will prevent triggering the cascade of ripening events (Sisler & Serek, 1997).

Fruit treated with 1-MCP had better visual quality during early storage

Mango fruit applied with 1-MCP in sachet had better visual quality than the untreated ones until six days of storage (Figure 2D). Regardless of the concentration, fruit treated with 1-MCP had a better visual quality with very good to excellent scores compared to the untreated samples. The lower visual quality scores garnered by the control mangoes were due to the progression of defects that goes along with the advancement in ripening. The fruit's visual quality started to decline after seven days with

a shelf life lasting from 10 to 12.4 days. The excellent visual quality exhibited by fruit treated with 1-MCP was due to slow deterioration of quality as ripening was delayed.

Degree of stem-end rot and anthracnose were not affected by 1-MCP

Treatment of 'Carabao' mangoes with 1-MCP in sachet did not affect the degree of stem-end rot (Figure 2E). Whether treated or not, the degree of stem-end rot was very low in the fruit with symptoms starting to appear only towards the end of the storage period. The low storage temperature probably hindered the early onset of latent infection and proliferation of microorganisms thus exhibiting low stem-end rot infection in mangoes. Similar to stem-end rot, the degree of anthracnose in mangoes was not affected by 1-MCP (Figure 2F). The mangoes developed anthracnose slowly which started to progress from slight to moderate infection only after 10 days. On the other hand, studies have shown that the ripening process in other varieties of mango was delayed by up to several days by delaying fruit softening and color changes through 1-MCP (Jiang & Joyce, 2000; Cocozza et al., 2004; Wongmetha & Ke, 2012; Ngamchuachit et al., 2014; Kumar et al., 2015; Razzaq et al., 2015; Chutichudet et al., 2016; Penchaiya et al., 2016; Sakhale et al., 2018).

Degreening of mango skin color was not affected by 1-MCP

1-MCP in sachets did not affect the peel color development of 'Carabao' mango (Figure 3). Based on the subjective index (Figure 3A) and objective color measurements (Figures 3B-F), the peel color did not vary. Degreening of the mango skin progressed with time reaching the table ripe stage after 8 to 9 days. Mangoes applied with 10 µL L^{-1} 1-MCP showed lighter peel color on the sixth day of storage however, this did not show a consistent trend over the course of storage (Figure 3B). Greenness (a*), yellowness (b*), and chroma (color intensity) were similar. Over time, fruit showed color development from green to yellow (Figure 3C-E). Figure 3F shows the hue angle of the mango peel color which lies on the second quadrant of the Hunter L*a*b* System, which is

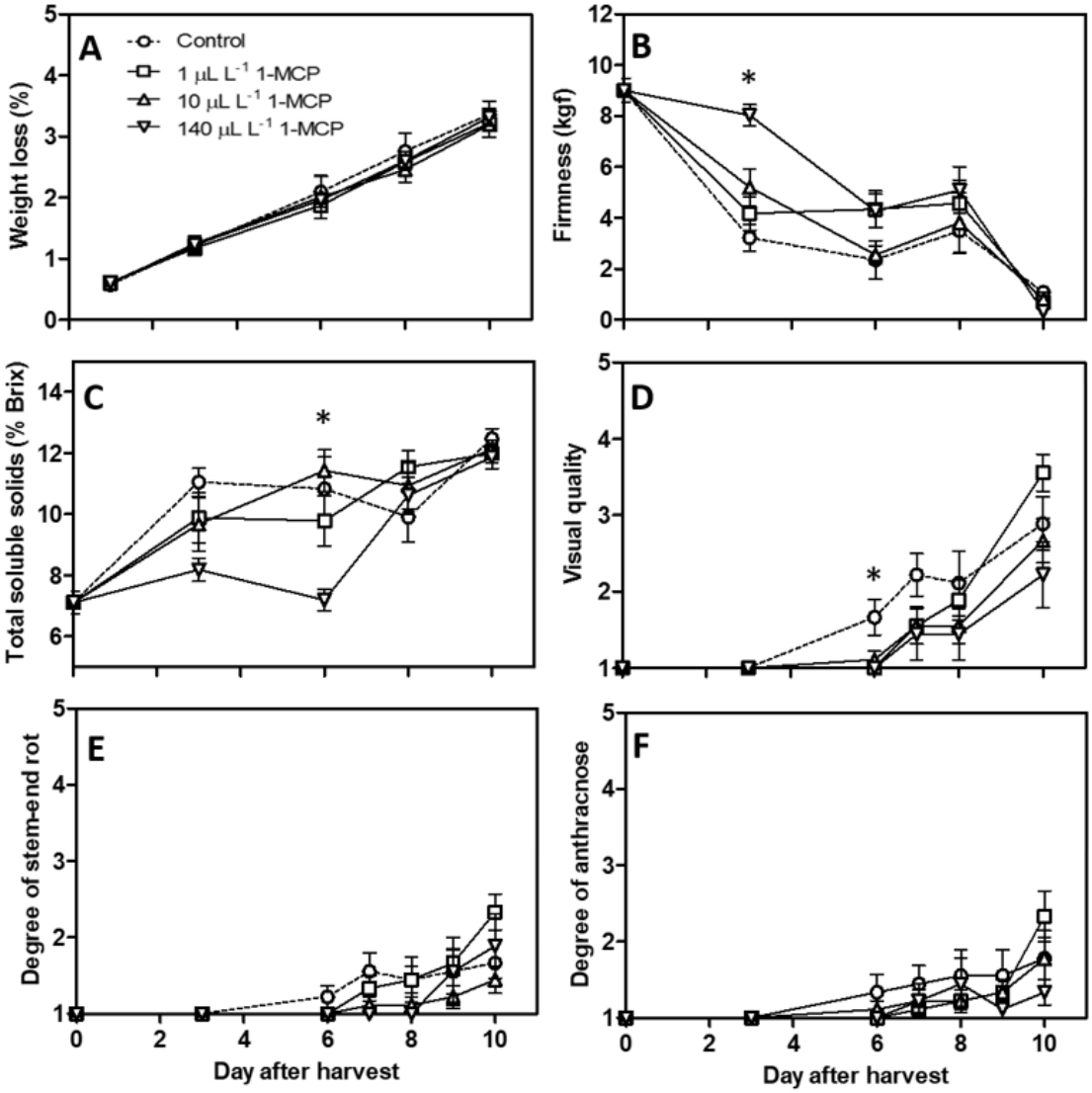

Figure 2. Weight loss (A), firmness (B), total soluble solids (C), visual quality (D), degree of stem-end rot (E), and degree of anthracnose (F) of 'Carabao' mango treated with 1-methylcyclopropene (1-MCP) in sachet for 24 h followed by storage in 19.0±1.2 °C and 90.7±9.3% RH. An asterisk indicates where there is a significant difference among the treatments using LSD at $P<0.05$. Error bar represents the Standard Error of the Mean (n= 3).

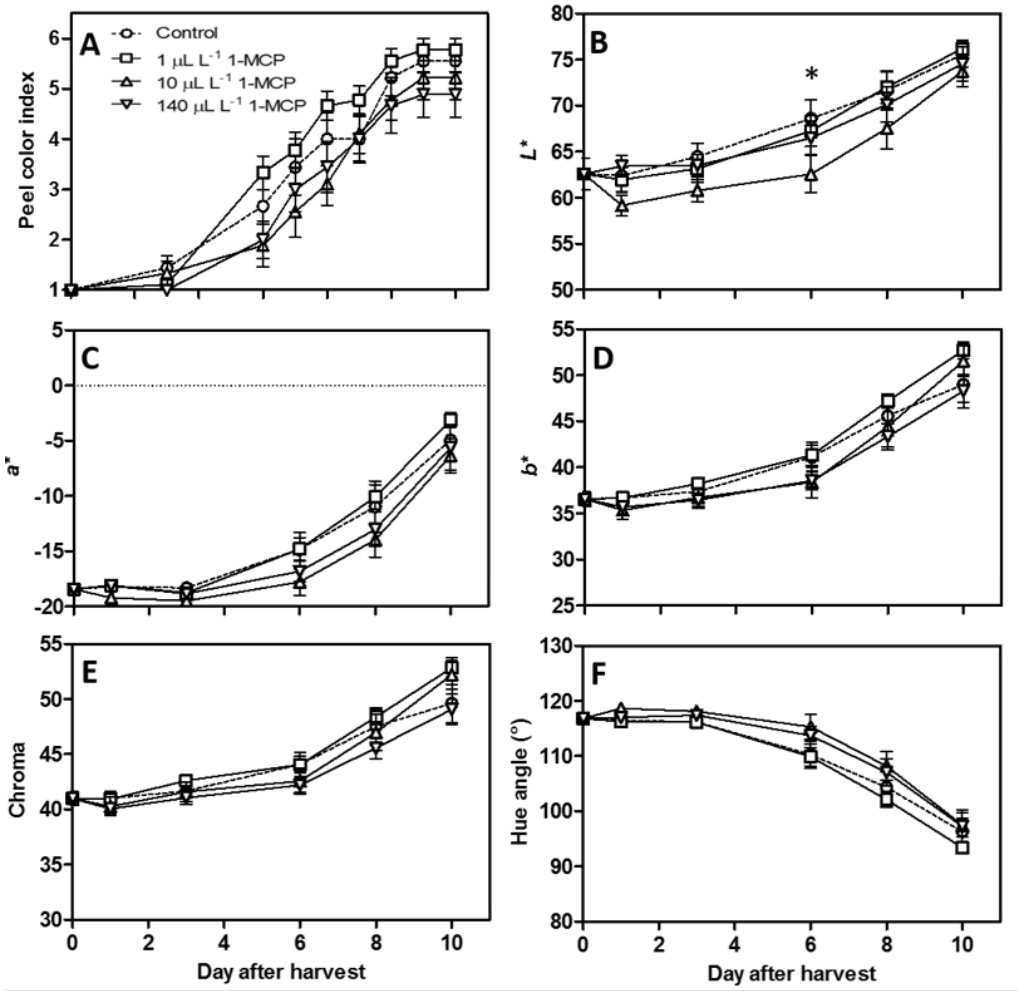

Figure 3. Peel color index (A), L* (B), a* (C), b* (D), chroma (E), and hue angle (F) of 'Carabao' mango treated with 1-methylcyclopropene (1-MCP) for 24 h followed by storage in19.0±1.2 °C and 90.7±9.3% RH. An asterisk indicates where there is a significant difference among the treatments using LSD at P<0.05. Error bar represents the Standard Error of the Mean (n= 3).

between yellow (90 °C) and green (180 °C) color. The hue angle shows a declining trend over time depicting the color development from green to yellow; however, this also did not differ among treatments. Although the concentration of 1-MCP in sachet was much higher than what is usually used in fumigation (e.g., 1 to 1000 nL L-1), its effect on peel color was not pronounced in this study. This could be because 1-MCP gas was not completely trapped around the mangoes for sufficient time to penetrate the produce as the newspaper lining could be permeable to 1-MCP gas.

1-MCP was not able to slow down the peel color changes in the fruit but maintained the skin lightness (L*) especially by 10 µL L^{-1} 1-MCP until six days of storage. The color changes from green to yellow during ripening are due to chlorophyll degradation (Bouzayen et al., 2010). In the case of 'Carabao' mango, 1-MCP has been shown to inhibit ethylene biosynthesis in fruit at pre-harvest however, the postharvest application seemed less effective because the upsurge in ethylene production had already occurred prior to its application (Castillo-Israel et al., 2014a). Attempts to delay the ripening of 'Carabao' mango fruit at the pre-climacteric stage have been a challenge because ripening initiates in the mesocarp prior to full maturation and ethylene production showing a peak at about 10 days before attaining its harvest maturity (Cua, 1989). Castillo-Israel et al. (2014a) also reported that upsurges in internal ethylene and ethylene production in 'Carabao' mango were observed at 100 days after flower induction (DAFI), suggesting that 1-MCP should be applied prior to this stage. After harvest, ethylene evolution rates from the intact 'Carabao' mango start to increase at two days after harvest with a peak at three days (Nuevo et al., 1984).

Fruit treated with 140 µL L^{-1} 1-MCP had longer saleable days and shelf life

As ripening was delayed in mango fruit treated with 140 µL L^{-1} 1-MCP, it took a longer time to reach a saleable stage (11.1 days) and in return had longer shelf life (12.4 days) than the mangoes applied with lower concentration of 1-MCP with days to saleability of 8.4 to 9.1 days and shelf life of 10 to 11.1 days (Figure 4). Meanwhile, the untreated fruit had 8.4 days to reach saleability and a shelf life of 11.9 days. Those treated with 140 µL L^{-1} 1-MCP also took a longer time to reach the table ripe stage (12.9 days), though it was not significantly different with the rest of the treatments (10.7 to 12.2 days) and the control (11.1 days). The number of saleable days (1.9 to 3.6 days) did not vary among treatments as the onset of stem-end rot (6.3 to 10.4 days) and anthracnose (8.1 to 11.4 days) occurred at about the same time which contributed to the limit of

saleability of the mangoes. The fruit's shelf life (10 to 12.4 days) did not differ among treatments as it developed stem-end rot and anthracnose all at the same time (Figure 2E-F).

In this study, 1-MCP was applied in mangoes harvested at 105 DAFI which is the beginning of the recommended harvest maturity. It is surmised that most ethylene receptors are already occupied at 105 DAFI due to the observed upsurges in internal ethylene and ethylene production even before reaching the recommended harvest maturity (Castillo-Israel et al., 2014b). Further, when 1-MCP (10 µL L-1) was applied pre-harvest at 100 DAFI and reapplied at 110 DAFI, it regulated the ethylene biosynthesis in 'Carabao' mango fruit as 1-MCP retarded the ethylene autocatalysis. Yang & Hofman (1984) reported that 1-MCP caused a reduction of ethylene levels in tissues, delayed the onset of the respiratory peak, and suppressed the production of 1-aminocyclopropane-1-carboxylate (ACC), the rate-limiting step in an ethylene feedback mechanism.

Hence, the time of application of 1-MCP in 'Carabao' mango is important in achieving ethylene-blocking effects (Vasquez-Celestino et al., 2016). The time at which ethylene detaches from receptors, degradation of the receptors, the synthesis of new receptors, and the possibility that 1-MCP dissociates from receptors are factors that determine the effectivity of 1-MCP treatment (Castillo-Israel et al., 2014a). It is also possible for the 1-MCP molecules to attach when new receptors are synthesized by the fruit as it matures. Kevany et al., (2007) showed that receptors are broken down by ethylene and new receptors are synthesized as the fruit maturity advances. At this time, 1-MCP could attach to newly-synthesized receptors before ethylene binds to it. This was confirmed by Castillo-Israel et al. (2014b) when pre-harvest reapplication of 1-MCP at 110 DAFI was more effective than those treated only once with 1-MCP. It was assumed that new ethylene receptors are synthesized at 110 DAFI, and 1-MCP reapplication at this time effectively blocks ethylene from binding to the new receptors. 1-MCP was also able to demonstrate its ethylene-inhibitory effects when applied to fresh-cut 'Carabao' mango slices as the ethylene receptors were more exposed to it (Castillo-Israel et al., 2015). However, the ethylene levels in the different portions of the 'Carabao' mango do not differ significantly (Nuevo et al., 1984).

CONCLUSION

This study demonstrated the effect of 1-MCP in sachet on 'Carabao' mangoes that were harvested at 105 days after flower induction (DAFI), considered as an early harvest. 1-MCP was added in the middle of commercially mature fruit packed in bamboo baskets following the traditional method of ripening 'Carabao' mango. Based on the results, 140 µL L^{-1} 1-MCP in sachet was able to maintain firmness and total soluble solids of the fruit until six days of storage. Mango fruit applied with 1-MCP in sachet also had a better visual quality than the untreated ones for up to six days. 1-MCP was not able to slow down the peel color changes in the fruit but maintained the skin lightness (L*) particularly by 10 µL L^{-1} 1-MCP until six days of storage. Therefore, under the conditions of the study, the use of 140 µL L^{-1} 1-MCP sachet for 24 h in mangoes harvested at 105 DAFI and packed in lined bamboo baskets best delayed the ripening characteristics such as softening and conversion of starch to sugar until six days of storage in cool conditions (19.0±1.2 °C, 90.7±9.3% RH).

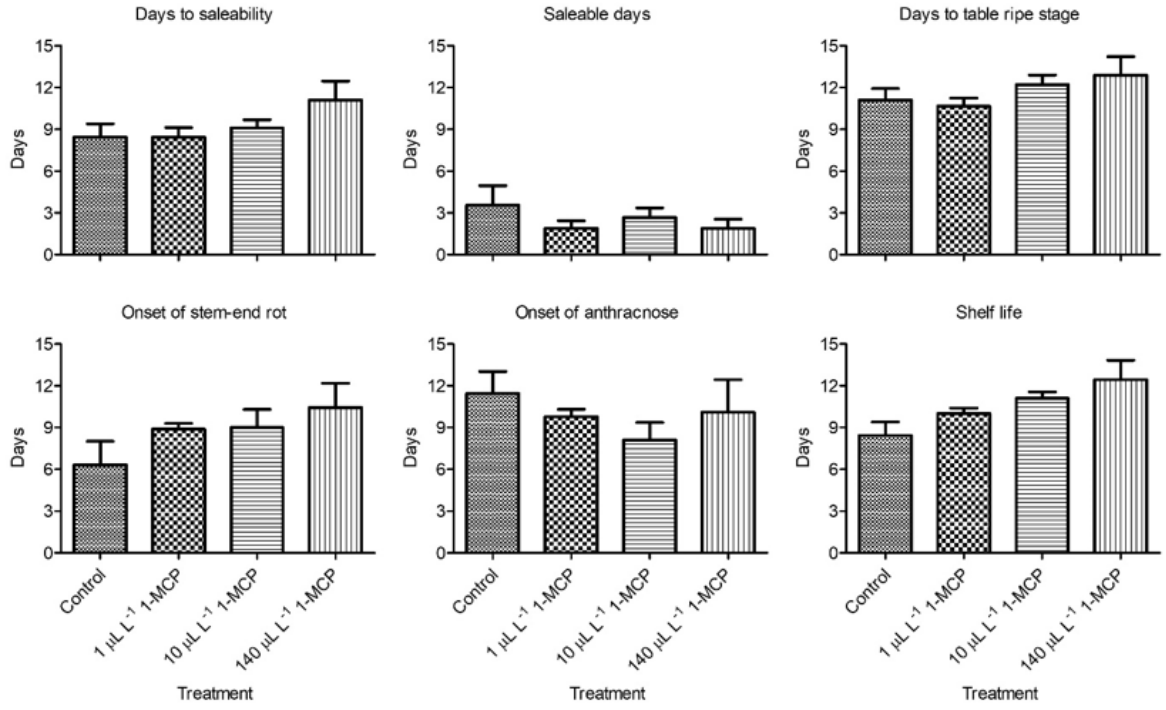

Figure 4. The days to reach saleability, saleable days, days to reach table ripe stage, onset of anthracnose, onset of stem-end rot, and shelf life of 'Carabao' mango treated with 1-methylcyclopropene (1-MCP) for 24 h followed by storage in 19.0±1.2°C and 90.7±9.3% RH. Error bar represents the Standard Error of the Mean (n= 3).

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