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Effect of low density polyethylene bag and 1-MCP sachet for suppressing fruit rot disease and maintaining storage quality of mangosteen (*Garcinia mangostana* L.)

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Abstract

The effect of packaging bags on fruit rot disease suppression and fruit quality retention of mangosteens (*Garcinia mangostana* L.) was investigated at 13°C. Harvested mangosteen fruit at stage 3 of maturity (full red pericarp) were packed in either a perforated polyethylene (PPE) bag, a low density polyethylene (LDPE) bag, or an LDPE bag combined with a 1-MCP sachet (EthylBloc™), with non-packed fruit used as the control. All samples were kept at 13°C for 30 days. Fruit incorporating an LDPE bag + 1-MCP sachet did not show fruit rot disease throughout the 30 days while those in a PPE bag showed the highest fruit rot disease. Fruit in the LDPE bag alone had lower fruit rot disease as compared with the control fruit. Furthermore, the physiological quality of mangosteen fruit packed in an LDPE bag in combination with a 1-MCP sachet was significantly retarded in terms of both respiration rate and ethylene production which resulted in maintaining calyx and pericarp color (ΔE), fruit firmness and reduced percentage weight loss. In the fruit flesh, whereas, packaging bags and 1-MCP did not affect total soluble solids concentration/titratable acidity (TSS/TA ratio). In conclusion, packing the mangosteen in an LDPE bag + 1-MCP sachet was able to suppress natural infection by postharvest fungi and to delay the ripening and senescence processes of mangosteen fruit during storage at 13°C for 30 days.

Introduction

Mangosteen (*Garcinia mangostana* L.) is one of the most common tropical fruit and is known as "the queen of fruit" because of its quality in color, shape and flavor (Morton, 1987). The fruit is a tropical evergreen trees believed to have originated in the Sunda Islands and the Moluccas of Indonesia (Macleod and Pieris, 1998). The fruit are better known in Southeast Asia (e.g. Thailand, Vietnam and the Philippines), where the crops originated and are widely grown. However, extending the shelf-life of the fruit after harvesting for both local and long-distance shipping is a problem for both retailers and importers. The main constraints limiting the shelf-life of mangosteen are physiological breakdown including drying or wilting of the calyx, the pericarp hardening, changes in color of the pericarp and infection of various plant pathogens which caused fruit rot disease such as *Fusarium* sp., *Colletotrichum gloeosporioides* (Penz. and Sac.), *Phomopsis* sp., *Lasiodiplodia theobromae* (Pat.) (Pranamorkith *et al*., 2003a; Sangchote and Pongpisutta, 1998; Khewkhom *et al*., 2011).

Postharvest technologies that have been used to extend shelf-life, restrict decay and maintain quality of fresh produce, include the use of modified atmosphere (MA) and modified atmosphere packaging (MAP) under low temperature storage (Del-Valle *et al*., 2009). The successful use of MAP is based on the permeation rates of films to allow O_2 and CO_2 to inhibit the respiration rates of fresh produce (Sandhya, 2010). Reducing the O_2 concentration to under 8% and/or increasing CO_2 concentration over 1% has been shown to prevent or delay fruit ripening (senescence) and/or retard biochemical and physiological changes (Sandhya, 2010). Polyethylene (PE), polyvinyl chloride (PVC) and polymeric (PM) film packaging has been shown for mangsteen fruit (Pranamorkith *et al*., 2003a; 2003b; 2003c), perforated polyethylene bags for loquat fruit (Ding *et al*., 2002) and PE bags for 'Hass' avocado (Meir *et al*., 1997) has been shown to maintain postharvest quality and prolong the shelf-life of fruit. In addition,

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MAP has been shown to delay the growth of aerobic spoilage microorganisms in fresh and fresh-cut produce (Farber *et al*., 2003). Moodley *et al*. (2002) found that PE is good packaging material for the storage of apple due to it suppression of *Penicillium expansum*. Similar results have been observed in loquat fruit and fresh strawberries (Ding *et al*., 2002; Kartal *et al*., 2012).

Mangosteen is a climacteric fruit, as its respiration rate and ethylene production both rise rapidly during ripening (Noichinda, 1992). The application of 1-methylcyclopropene (1-MCP) has been proven to delay ripening and senescence of various fruit, vegetables and flower crops, including avocado (Hershkovitz *et al*., 2005), mangosteen (Piriyavinit *et al.*, 2011) and pear (Li *et al*., 2013). 1-MCP binds the ethylene receptor, preventing the expression of the genes associated with ethylene biosynthesis (El-Kereamy *et al*., 2003). 1-MCP gas must have a closed delivery system and the timing for treatment with 1-MCP for suppressing the ethylene response is between 6 to 24 h (Blankenship and Dole, 2003). However, a long fumigation period may cause a reduction in the length of the marketing period. Thus, a preparation of 1- MCP designed for use as a sachet formulation has been prepared and marketed under the trade name EthylBloc™. This commercial product releases 1-MCP gas for inhibition of the mechanism of ethylene action (Lee *et al*., 2006). Recently, it has been shown that application of 1-MCP in the sachet format affected flower abscission and flower life of potted impatiens (Burana *et al*., 2010) and fruit storage life of mangosteen (Pirinyaninit *et al*., 2011).

Recent studies have revealed that the combined application of MAP and 1-MCP controlled ripening and senescence of litchi (De Reuck *et al*., 2009), plum (Singh and Singh, 2012) and pear (Li *et al*., 2013). However, the combined effects of MAP and 1-MCP on mangosteen fruit during low temperature storage have not yet been reported. The aim of this study was to determine the effects of LDPE packaging (bag form) with the inclusion of 1-MCP sachets on fruit rot disease development and quality of mangosteen fruit during storage at 13°C.

Materials and Methods

Fruit preparation

Light greenish-yellow mangosteen fruit with 51-100% scattered pink spots (Stage 3 color index; Tongdee and Suwannagul, 1989) were obtained from Chanthaburi province, Thailand and transported at 13°C to the laboratory. These natural infected fruit samples were selected for uniformity of color, size and freedom from disease. The fruit were washed with tap water to remove plant debris and dust, and then disinfected with a fungicide solution (500 $mL.L^{-1}$ Prochloraz). The treated samples were packed in either perforated polyethylene (PPE) bags with six holes per bag (Ø 0.6 mm, 50 µm thick), low density polyethylene (LDPE) bags (25 µm thick, an oxygen transmission rate of 7800 $mL.m^{-2}\ d^{-1}\ atm^{-1}$ at 25°C, a CO_2 transmission rate of 42,000 $mL.m^{-2}\ d^{-1}\ atm^{-1}$ at 25°C and a water vapor transmission rate of 18 $g.m^{-2}\ d^{-1}\ atm^{-1}$ at 38°C; provided by the Fresh Bag Co. Ltd.), and LDPE bags + 1-MCP sachets (EthylBloc™) containing 0.014% active ingredient by weight (provided by GGD Trading (Thailand) Co. Ltd.). Non-packed fruit served as the control. In each treatment, 24-28 fruit were divided into four groups and performed as the four replications. In each replication (bag) contained 5-7 fruit weighing approx 500 g. All treatments then stored at 13°C and 90-95% relative humidity.

Fruit rot disease assessment

Disease severity of mangosteen fruit rot was observed in terms of a 0-5 index, with 0 = apparently healthy, 1 = slight infection on an individual fruit, 2 = 25% of a fruit infected, 3 = 50% of a fruit infected, 4 = 75% of a fruit infected and 5= 100% of a fruit infected. The percentage of disease incidence was calculated as: (no. of rotten fruit/total no. of fruit) x 100. The disease index was calculated as $DI = \sum(df)/ND$, where d = the degree of rot severity assessed on the fruit, f = respective quantity of fruit, N = the total number of fruit examined and D = the highest degree of disease severity occurring on the scale.

Quality assessment

Color changes of the fruit pericarp and calyx were measured using a Minolta Colorimeter (Model RC 300). The values of a^*, b^* and L^* were measured from each replication and the hue angle calculated (with red-purple at an angle of 0°, yellow at 90° and bluish-green at 180°). The color differences in terms of calyx and peel color changes (ΔE) were calculated using the formula: $(\Delta E) = ((L^*_t-L^*_0)^2 + (a^*_t-a^*_0)^2 + (b^*_t-b^*_0)^2)^{1/2}$. The L^* scale represented lightness or darkness of the fruit pericarp, a^* scale represents the green to red as it trends to negative and positive values, and b^* scale represents the blue and yellow color contrast; the t suffix indicates these value at time t and the 0 suffix the initial values. The color readings were taken twice at the equatorial region of each fruit and averaged to give a value for each fruit.

Pericarp firmness was measured with a Texture Analyzer (TA-XT2, Stable Micro-system, England) equipped with a 5 mm diameter probe. The

measurements were taken in the equatorial fruit zone to a depth of 10 mm at a rate of 10 mm.min^{-1} and force was expressed in Newtons (N).

Total soluble solids (TSS) and total acidity (TA) were determined in the flesh juice of mangosteen. The white flesh of the arils with the enclosed seeds was wrapped in cheese cloth and squeezed by hand to separate the juice from seeds. TSS was measured with a hand-held refractometer (PAL – 1, Tokyo, Japan) calibrated with distilled water. TA was measured by titrating a 9 mL aliquot of the flesh juice with 0.1 M NaOH, phenolphthalein was used as the indicator. TA was expressed as a percentage of citric acid per 100 mL. The TSS/TA ratio was calculated.

Weight loss of mangosteen fruit was measured by weighing each package containing 6 fruit with the balance and the percentage weight loss calculated.

Respiration rate and ethylene production

Four fruit randomly selected from the bags in a treatment were weighed and kept in airtight 1,200 mL plastic boxes at 13°C (90-95% RH). The air was flushed for 2 h before closing the container. 1 mL of the headspace gas was then taken using a hypodermic syringe and CO_2 and ethylene production were detected with a gas chromatograph. The respiration rate was detected with a GC-8A (Shimadzu, Japan) installed with a Porapack N column and Thermal Conductivity Detector (TCD). Helium was used as the carrier gas at a flow rate of 30 mL.min^{-1} and column temperature was maintained at 50^{o}C. Ethylene production was measured by a GC-14B (Shimadzu, Japan) installed with a Flame Ionization Detector (FID) and a 2 m x 4 mm stainless steel column packed with 80-100 mesh Porapack Q. Nitrogen was used as a carrier gas with a flow rate of 35 mL.min^{-1} and the temperature of the injector and column was maintained at 120°C and 80°C, respectively.

Internal gas (CO_2 and O_2) composition inside packaging bags

Internal gas composition inside the packaging bags was measured using a CO_2 and O_2 analyzer (CharpaTech Center Co., Ltd.). A syringe was injected into an LDPE bag or into an LDPE bag containing a 1-MCP sachet. Gas composition inside the package was expressed as a percentage of CO_2 and O_2.

Statistical analysis

The experiment was arranged as a completely randomized design (CRD) with 4 replicates (bags). All parameters were recorded every 5 days until 30 days. Analysis of all data was conducted using SAS software. Analysis of variance was used to compare more than two means, the least significant difference (LSD) and Duncan's multiple range test (DMRT) were used for mean separation. Differences of $p \leq 0.05$ were considered significant.

Results

Fruit rot disease

Disease incidence was first observed on day 25 in mangosteen packed in PPE and in LDPE bags (28.6% and 14.3%, respectively), and on day 30 in the control fruit (16.7%) (Table 1). Fruit packed in LDPE bags combined with 1-MCP sachets did not appear to have any postharvest diseases throughout the 30 day storage period. Disease severity gradually increased during the storage period. The highest disease severity was observed on day 30 in PPE bags packed fruit (score 0.5) followed by LDPE bags (score 0.3) and un-treated fruit (score 0.2).

Quality assessment

Color changes of the fruit pericarp and calyx during the storage period is shown in Figures 1A and 1B, respectively. The fruit pericarp developed from light greenish-yellow with scattered pink spots (Stage 3) to the purple black stage (Stage 6) during storage. The ΔE values of fruit pericarp and calyx increased gradually from day 0 to day 30 in all treatments. However, it was found that the LDPE bag + 1-MCP treatment delayed color development of both the fruit pericarp and calyx when compared with other treatments.

Pericarp firmness decreased sharply from day 0 to day 10, and then decreased slightly from day 15 to day 30 during the storage period (Figure 2A). LDPE and LDPE + 1-MCP treatments to maintained firmness better than either the PPE treatment or the unpacked control. On day 30, firmness of fruit packed in LDPE and LDPE + 1-MCP were 16.4 and 18.7 N, respectively, while that in the control fruit was 16.8 N.

TSS concentration in all samples tended to increase (a range of 14.8 – 18.2 °Brix) whereas of TA (4.5-7.4%) slightly decreased; however, there were no significant differences among of treatments throughout the storage period (data not shown). The TSS/TA ratio did not changed significantly from the initially day (3.07) to the last day of storage (3.12) in all treatments. This indicates that all packaging types did not clearly affect on the TSS or the TA values (Figure 2B).

Weight loss tended to increase in all treatments (Figure 2C). From day 0 to day 30, the weight loss of PPE packed fruit, LDPE packed fruit and LDPE plus

Table 1. Effects of perforated polyethylene (PPE) bags, low density polyethylene (LDPE) bags and LDPE bags + 1-MCP sachet on disease incidence, disease severity and disease index of mangosteen fruit during storage at 13°C, 90-95% RH. Non-packed fruit were used as the control

Treatments	Days of storage					
	25	30	25	30	25	30
	Disease incidence (%)		Disease severity (Score)		Disease index (DI)	
Control	0.0^{c}	16.7^{c}	0.0	0.2^{ab}	0.000	0.012
PPE	28.6^{a}	50.0^{a}	0.3	0.5^{a}	0.009	0.019
LDPE	14.3^{b}	28.6^{b}	0.2	0.3^{ab}	0.009	0.015
LDPE+1-MCP	0.0^{c}	0.0^{d}	0.0	0.0^{b}	0.000	0.00
F-test	**	**	NS	*	NS	NS
C.V. (%)	14.9	5.2	163.3	81.8	144.7	7.4

a, b, c Different superscripts in the same column indicated that means were significantly different.
NS means non-significantly different.
* means significant at $P \leq 0.05$.
** means significant at $P \leq 0.01$.

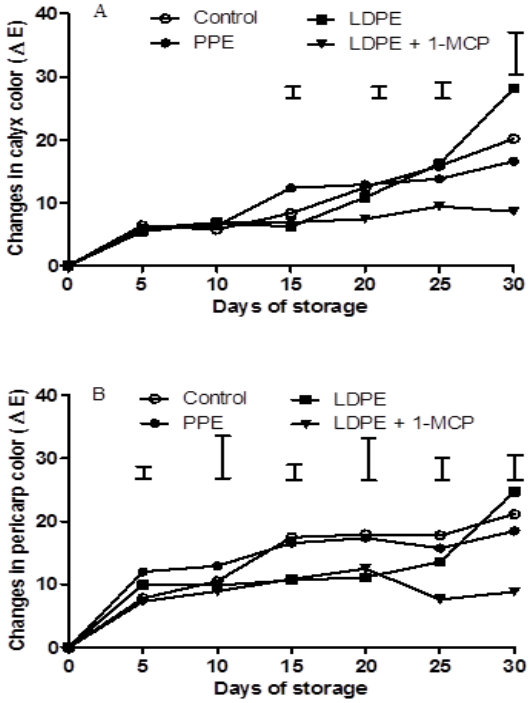

Figure 1. Effect of perforated polyethylene (PPE) bags, low density polyethylene (LDPE) bags, LDPE bags + 1-MCP sachet insert; non packed used as the control fruit, on (A) changes in calyx color (ΔE) and (B) changes in pericarp color (ΔE) in mangosteen fruit during 30 days of storage at 13°C, 90-95% RH. The bars represent SD at p=0.05

1-MCP packed fruit were 1.92%, 0.30% and 0.20%, respectively, which were all significantly lower than that in the control fruit (5.13%). This result indicates that the LDPE bag (with or without the MCP insert) was the best package to prevent weight loss in mangosteen fruit.

Respiration rate and ethylene production

Packaging in bags resulted in a suppression of respiration compared with the un-packed fruit (Figure 3A). In the control fruit, the respiration rate sharply increased and reached a peak on day 5 (455.7 mg CO_2. kg^{-1} h^{-1}), then progressively decreased throughout the storage period. In contrast, the rate of respiration in PPE-packed fruit rapidly decreased to day 5, then reached a peak by day 15 (261.9 mg CO_2.kg^{-1} h^{-1}) the subsequently declined. However, the respiration rate in fruit packed with LDPE alone and in combination with 1-MCP initially decreased rapidly until day 10, increased until day 20 (174.0 and 155.7 mg CO_2.kg^{-1} h^{-1}, respectively) and then subsequently declined.

The ethylene production of mangosteen fruit increased in all samples for the first 5 days, then decreased by day 10 and slightly increased again on day 15-25 of storage. The peak of ethylene production in the control fruit was observed on day 5 (22.0 µL.C_2H_4. kg^{-1} h^{-1}); however, this was not significantly different to the other treatments. However, from day 20 to 25, ethylene production in the control fruit was significantly higher than the rates in various packages by 9.9 and 10.2 µL.C_2H_4.kg^{-1} h^{-1}, respectively. In comparison, ethylene production by perforated PE-packed fruit and fruit packed in LDPE reached a peak at day 5 (21.3 and 18.2 µL.C_2H_4.kg^{-1} h^{-1}, respectively). However, the lowest ethylene production was found in LDPE incorporating a 1-MCP sachet on day 5 (12.0 µL C_2H_4.kg^{-1} h^{-1}) (Figure 3B).

Internal gas (CO_2 and O_2) inside the storage package

In fruit storage at 13°C, the internal gas carbon dioxide (CO_2) concentration inside the package increased throughout the storage period. From day 0 to day 30, carbon dioxide concentration in the LDPE bag and in the LDPE bag + 1-MCP sachet increased from 0.03 to 8.95 and from 0.03 to 6.41%, respectively. The concentrations of oxygen inside the package markedly decreased in LDPE and LDPE + 1-MCP treatments to 2.68 and 1.12%, respectively over the 30 days storage period while those in the control and PPE treatments remained unchanged (Figure 3C and 3D).

Discussion

Mangosteen is a climacteric fruit that develops purple skin color when it ripens, similar to the dark purple changes found in grapes (El-Kereamy *et al.*,

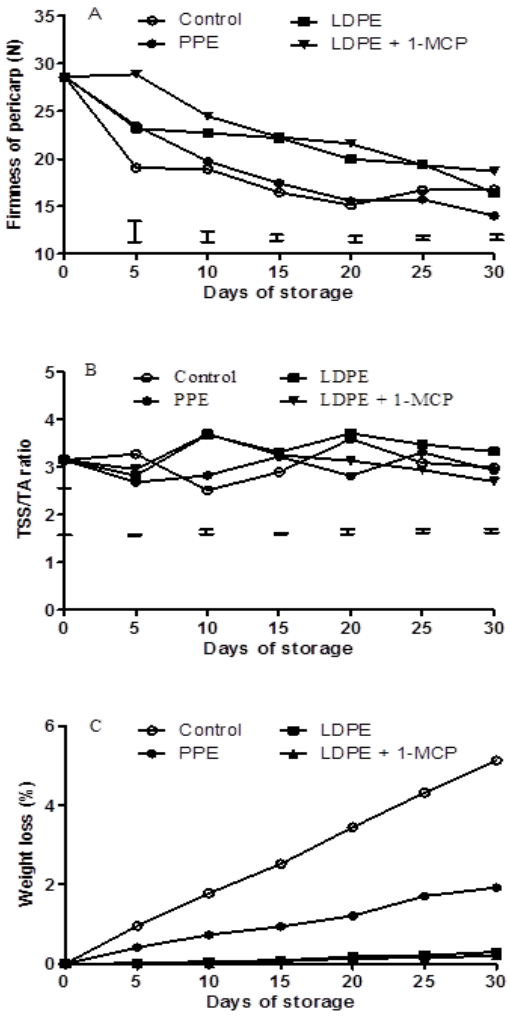

Figure 2. Effect of perforated polyethylene (PPE) bags, low density polyethylene (LDPE) bags and LDPE bags + 1-MCP sachet insert, with non packed fruit used as the control, on (A) pericarp firmness, (B) total soluble solids concentration/titratable acidity (TSS/TA ratio), and (C) weight loss in mangosteen fruit during storage time at 13°C, 90-95% RH. The bars represent LSD at p=0.05

2003), Chinese strawberry (Zhang *et al.*, 2005) and in 'Hass' avocado (Cox *et al*., 2004). Calyx color and a fresh appearance is a postharvest quality measure of exported mangosteen. At harvest, all calyces are fresh green, then tend to wilt and turn brown. The application of MAP successfully extends the postharvest shelf-life of whole and fresh-cut produce by suppressing their respiration rate and weight loss while delaying color changes (Kader, 1986; Waghmare *et al*., 2013). Recently, 1-MCP treatment has been reported to retard softening, color development, suppress respiration rate and ethylene production in pear fruit (Watkins, 2006; Villalobos-Acuna *et al*., 2011; Liu *et al.*, 2013). The results reported in this study are similar to previous findings on a number of other crops. For example, Ding *et*

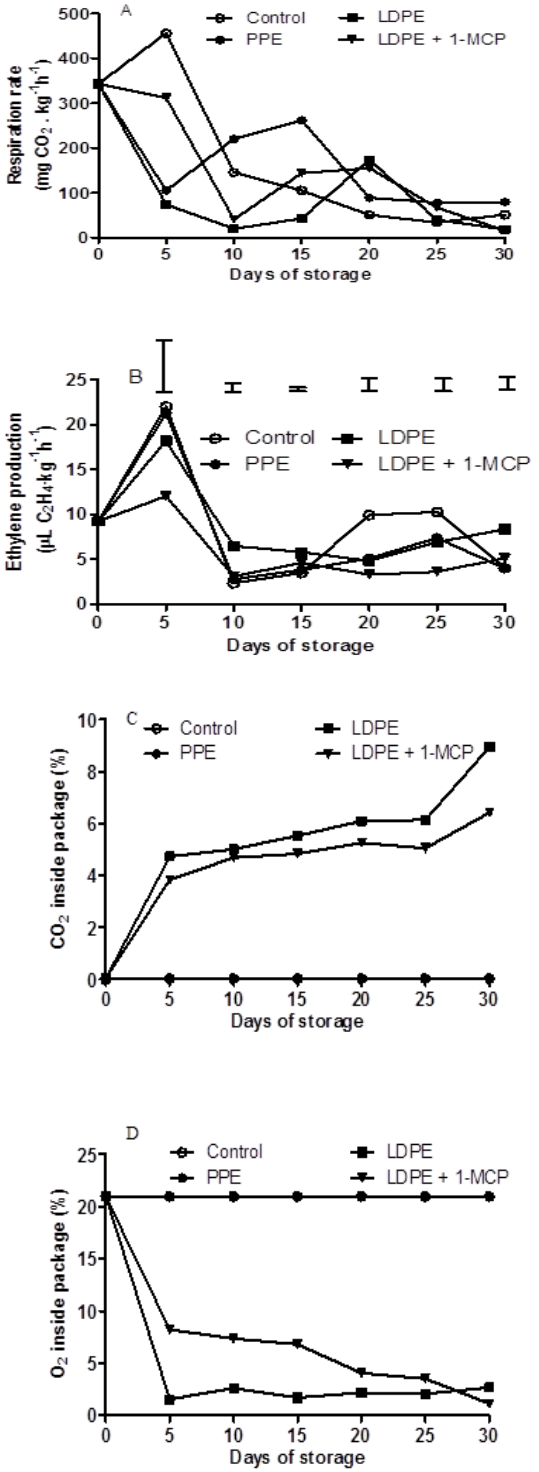

Figure 3. Effect of perforated polyethylene (PPE) bags, low density polyethylene (LDPE) bags, LDPE bags + 1-MCP sachet insert, non packed used as the control fruit, on (A) ethylene production, (B) respiration rate, (C) CO_2 inside package (%) and (D) O_2 inside package (%) in mangosteen fruit during storage time at 13°C, 90-95% RH. The bars represent LSD at p=0.05

al. (2002) observed that packaged loquat fruit with MAP showed minimal water loss as compared to PPE packed fruits. Waghmare and Annapure (2013) revealed that MAP was effective in retaining sensory

and quality characteristics by reducing weight loss, delaying color changes and inhibiting microbial infection of fresh-cut papaya. The results in our study suggest that LDPE bags which incorporated 1-MCP sachets had the greatest effect on decreasing weight loss, delaying pericarp color changes and retaining the fresh green color of the calyx. In contrast, LDPE packaging alone showed only a moderate effect on color development and weight loss compared to control fruit. These changes were much less pronounced when the fruit were packed in PPE bags. A direct association between color changes and firmness was previously determined in both mangosteen (Piviyavinit *et al*., 2011) and pear fruit (Li *et al*., 2013).

The data showed that packaging bags had a greater potential to maintain pericarp firmness than simply low storage temperature alone as in the control fruits, although TSS/TA ratio was not affected. In loquat, organic acid levels and total sugars were not significantly changed in MAP treatments (Ding *et al*., 2002).

In fruit storage at 13^{o}C, the progressive decrease in the firmness of pericarp until the end of storage at day 30 was significantly reduced under the LDPE and LDPE+1-MCP treatments. This retardation in firmness changes may have been through retarded degradation of insoluble protopectins to the more soluble pectin acid and pectin. During fruit ripening, depolymerization or shortening of the chain length of pectin substances occurs with the increase in pectinesterase and polygalactronase activities. Low oxygen and high carbon dioxide concentrations have been shown to reduce the activities of these enzymes and to have a direct association with the retention of firmness in both fruit and vegetables (Salunkhe *et al*., 1991). The current data shows that, in the LDPE bags with or without 1-MCP sachets, oxygen was lower and carbon dioxide concentrations higher compared to PPE bags and control fruits significantly affecting the physical quality of the fruit. The internal gas concentrations inside MAP can slow down respiration (Ben -Yehoshua, 1987) as reported in previous studies on 'Laiyang' pear (Garmrasni *et al*., 2010; Li *et al*., 2013), and apple (Yang *et al*., 2013). Moreover, tissue exposure to low levels of O_2 and/or high levels of CO_2 have been shown previously to inhibit ethylene biosynthesis and its action. Reducing the O_2 levels decreases ethylene biosynthesis by fresh fruits and vegetable and reduces their sensitivity to ethylene (Kader, 1986). Under limited O_2 conditions, conversion of 1-aminocyclopropane-1-carbonxylic acid (ACC) to ethylene can be completely suppressed because O_2 is a co-substrate in the oxidation of ACC to ethylene by ACC oxidase (ACO) (Yang, 1985). In this study, the data were inconclusive with regard to ethylene production. Hershkovitz *et al.* (2005) showed that the peak in ethylene production was delayed in three 1-MCP treated avocado cultivars.

Our data clearly shows lower disease infection in mangosteen fruit packed in LDPE bags compared to those in PPE bags. No disease was found in the LDPE bags that incorporated 1-MCP sachets. Similarly, treatment with 0.5 $\mu L.L^{-1}$ 1-MCP plus micro-perforated film packaging maintained the fruit quality of pear (Li *et al.*, 2013). The technique of using 1-MCP for delaying postharvest ripening and senescence processes was also used in banana (Pongprasert and Srilaong, 2014) and apple fruit (Yang *et al*., 2013). However, the present study revealed that the delay in disease infection in un-packed fruit by day 30 may cause low humidity when compared with the fruit packing in packages, thus, pathogenic infection may not develop well on the fruit surface.

Conclusions

Our data suggests that application of a 1-MCP sachet in combination with the use of LDPE bags had the greatest effects on delaying senescence processes including decreasing weight loss, delaying pericarp color changes, retaining a fresh green color of the calyx and suppressing pathogenic infection.

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