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Delayed softening of papaya (*Carica papaya* L. cv. Sekaki) fruit by 1-methylcyclopropene (1-MCP) during ripening at ambient and low temperature storage conditions

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Abstract

Ripening of climacteric fruit such as papaya (*Carica papaya* L. cv.Sekaki) fruit depends on the ethylene action which later accompanied by softening process that can influence postharvest quality and storability of the fruit. Ethylene action inhibitors, 1-Methylcyclopropene (1-MCP) has been found to inhibit ethylene action and thus it can delay the fruit ripening process. The effects of 1-MCP on softening related changes were determined through skin color, weight loss, fruit firmness and activities of the cell wall degrading enzymes including α-galactosidase, β-galactosidase and pectinmethylesterase (PME) during ripening. In this study, fruit were treated with 90 ppb concentration of 1-MCP gaseous vapors for 12 hours in airtight container maintained at 20 ^{o}C. After treatments the fruit were randomly divided into two different storage temperature conditions; ambient temperature (28 ^{o}C) and low temperature (10 ^{o}C). Papayas at 10 ^{o}C conditions were packaged in polyethylene plastic stored for 4 weeks (28 days) and taken out on day 29 and left to ripen at ambient temperature (28 ^{o}C).Fruit treated with 1-MCP experienced a significant delayed in skin color development, weight loss and reduced firmness loss compared to the fruit without 1-MCP treatment. As softening progressed, activity of the cell wall degrading enzymes in fruit without 1-MCP treatment increased significantly coincident with a rapid declined in fruit firmness for both storage conditions. With 1-MCP, fruit demonstrated a delay in activity of cell wall degrading enzymes but continued to increase until 100 % yellow. Furthermore, the treated 1-MCP papaya fruit stored at 10 ^{o}C can retain shelf life for 5 days at ambient temperature without any spoilage from chilling injury (CI).Thus it may be concluded that the 1-MCP treatment may aid in delaying the softening process and further storage at low temperature extended the postharvest life and maintained the quality of the papaya fruit.

Keywords: 1-methylcyclopropene (1-MCP); *Carica papaya*; cell wall degrading enzymes; firmness; softening related changes.
Abbreviations: 1-MCP (1-Methylcyclopropene), CI (chilling injury), EDTA (ethylenediamminetetraacetate), LT (low temperature), PME (pectinmethylesterase), RT (room temperature).

Introduction

Papaya (*Carica papaya* L.) fruit, as with most climacteric fruit, have a short shelf life (Lazan et al., 1995). In climacteric fruit, such as papaya, the rise in ethylene production parallels the respiration rate and peaks at the same time as the respiratory climacteric (Paull and Chen 1983). Papaya fruit is susceptible to overripening caused by ethylene and ripening process is associated with dramatic changes in fruit texture.Texture or softness is an important physical attribute that has been associated with quality and storage life of fruit (Ali et al., 2004). Fruit softening is a major aspect of the ripening process and considered to be a consequence of cell wall modifications (Jeong and Huber 2004).In this situation, both ethylene and the integrity of cell walls are closely involved in one way or another. Ethylene antagonists, including 1-methylcyclopropene (1-MCP) binds ethylene receptors in an apparently noncompetitive way (Sisler and Serek 1997), have been identified useful for attenuating ethylene effect in plant tissues (Blankenship and Dole 2003). Papaya postharvest losses occurred due to overripening which lead to physiological disorders. Papaya treated with 1-MCP delays ethylene production, climacteric respiration, skin color development and softening of papaya without affecting total soluble solids and fruit weight loss (Manenoi et al., 2007; Shiga et al., 2009). 1-MCP is an effective inhibitor of ethylene action in a broad range of fruit, vegetables and floriculture crops. Papaya treated with 1-MCP a day after harvest demonstrated an increase days to reach the ripe stage, a delay in softening and reduction in ethylene production and respiration (Manenoi et al., 2007). This alteration of softening by 1-MCP provides an approach to determine how fruit ripening is regulated. Low temperature storage is the most commonly used technique to control ripening. Low temperature storage slows down enzymatic reactions such as those involved to respiration, senescence was found to be promising in minimizing loses of fruit quality. Arpaia and Kader (2002) observed that the respiratory activity of papayas, which was between 15 and 35 mL CO_2 kg^{-1} h^{-1}at 15 ^{o}C, decreased to 4-6 mL CO_2 kg^{-1} h^{-1} when fruit were transferred to 10 ^{o}C. According to Bron and Jacomino (2009), papaya respiratory activity, decreased during 20 days of storage at 11 ^{o}C, with respiration values of approximately 7 mL kg^{-1} h^{-1}. Therefore, low temperature storage can alter the process of papaya ripening. However, low temperature can be considered as a stress for papaya which is chilling sensitive to the extent of developing chilling injury symptoms after prolong periods of low temperature storage

(Ali et al., 2000). The purpose of this work was conducted to determine the effects of 1-MCP and low temperature storage on ripening of 'Sekaki' papaya fruit and the role of cell wall degrading enzymes involved in softening.

Results and discussion

Effects of 1-MCP on color changes, weight loss, chilling injury and firmness loss

1-MCP treated 'Sekaki' papaya kept at ambient temperature had a delay in skin color development when compared with the control fruit. The control fruit reached 100 % yellow skin in about 5 days compared with 7 days for 1-MCP treated fruit (Fig 1a). When kept at 28 ^{o}C, both non-treated and fruit treated with 1-MCP showed increases in yellow color attainment. Fruit treated with 1-MCP attain their yellow color more slowly. Hofman et al. (2001) also observed that 'Solo' papaya treated with 1-MCP showed a slower attainment of yellow skin color and reached 100% yellow skin in 20 days compared with 5 days for non-treated 1-MCP fruit. In addition, papaya treated with 1-MCP exhibited completely yellow skin color on the seventh day of storage. Whilst, papaya fruit kept at LT storage able to maintain 5 % yellow skin throughout 28 days of low temperature storage and skin color start to change when transferred to ambient temperature. Non-treated 1-MCP fruit kept LT reached the full ripe stage (100 % yellow skin) in 4 days at ambient temperature while 1-MCP treated papaya stored at LT, reached the full ripe stage in 5 days at ambient (Fig 1b). This provide additional evidence that color development is less ethylene dependent (Blankenship and Dole 2003) than softening once low temperature storage has affected ethylene production at 10 ^{o}C. 1-MCP reduced weight loss in 'Sekaki' papaya and the treated fruit experienced slower rate loss as compared in non-treated 1-MCP fruit during the storage. The non-treated 1-MCP fruit experienced 6.2 % water (fresh weight) loss on fifth day whilst 1-MCP treated fruit showed lower percentage of loss 4.5 % (Fig 2a) on the same day of ripening. Storage at LT reduced water loss significantly (0.2 to 1.0 %). However, papaya stored at LT showed a drastic water loss when being transferred to ambient temperature. On the fourth day at ambient, non-treated 1-MCP fruit kept at LT showed 5.26 % of water loss meanwhile 1-MCP treated fruit demonstrated water loss of 6.33 % on the fifth day at ambient (Fig 2b). Salvador et al. (2004) also observed that 'Rojo Brillante' persimmon treated with 1-MCP and kept at 1 ^{o}C showed a slower rate of weight loss and later exhibited a rapid weight loss after been transferred at 20 ^{o}C. In addition to enhanced water loss, chilling injury (CI) symptoms, expressed as surface lesions and mesocarp discoloration started to appear in fruit kept at LT storage (data not shown). Non-treated 1-MCP fruit at LT exhibited CI earlier on the second at ambient and all fruit developed decay rapidly in the lesions despite being relatively firmer on the fourth day at ambient. The CI symptoms were noticeable in 1-MCP treated fruit on the fifth day at ambient and the CI was less pronounced as compared to non-treated 1-MCP fruit (data not shown). These physiological disorders might be indicative of subtle damages to membrane systems (Ali et al. 2004). For 'Sekaki' papaya, CI symptoms were less noticed in fruit treated with 1-MCP while in non-treated 1-MCP fruit the CI symptoms were more pronounced throughout the ripening. According to Jiang et al. (2004), in banana CI caused the membrane of certain protein such as ethylene receptor losses its function thus resulting in failure to ripening normally. Application of 1-MCP as a blocker of ethylene receptors prior to low temperature storage was effective in reducing the CI symptoms on the skin of fruit. Previous studies showed that, 1-MCP application due to LT storage reduced CI symptoms including surface browning in avocado (Pesis et al. 2002) and apple (Watkins 2006).

Besides color, fruit texture as characterized in tissue firmness, also changed with ripening. Firmness loss was relatively gradual as the fruit ripened at ambient temperature and control fruit exhibited a sharp decrease in firmness, almost reaching consumption firmness ($\leq$20 N) on the third day of storage. The highest losses in firmness were observed on the second days of storage at ambient, papaya lost almost 50 % of their initial firmness (Fig 3a). Fruit firmness is associated to an increase of pectin solubility and depolymerization of matrix polysaccharides which is believed to be a major contributor in reduced rigidity of cell walls that lead to fruit softening (Brummell 2006). Fruit receiving 1-MCP application maintained a high and constant firmness reading as compared to control until reached full ripe stage. Firmness retention in fruit treated with 1-MCP has been verified in many climacteric species, such as apricot, plum (Dong et al., 2002), avocado (Jeong et al., 2002), apple (Pre-Aymard et al., 2003; Watkins 2006) and also in non-climacteric species such as guavas (Bassetto et al., 2005) and strawberries (Jiang et al., 2001). Firmness was maintained even with the gradual loss in treated fruit which suggests that a minimum ethylene concentration is essential for a certain period in order to initiate necessary ripening process and continue the loss of firmness. Firmness losses reduced in fruit stored at LT and were still firm until 28 days of storage. Drastic firmness loss was observed when fruit been transferred into ambient temperature. Non-treated 1-MCP fruit at LT experienced almost 50 % of their initial firmness after one day of low temperature storage (Fig 3b). Chen and Paull (1986) also observed a reduction in firmness in papayas stored at 2 ^{o}C. In the present study, non-treated fruit continued to decrease in firmness after low temperature storage and reaching consumption condition after 3 days at 28 ^{o}C. 1-MCP treatment changed the pattern of papaya softening rate when the fruit did not lose firmness during low temperature storage and showed firmness values of 114.9 N after 28 days of storage at 10 ^{o}C (Fig 3b). However, a drastic decline in firmness was observed when fruit were transferred to ambient. Treated fruit with 1-MCP at LT showed a reduction in firmness more than 60 % of their initial firmness on the fourth day of storage. With this softening, the fruit treated with 1-MCP reach consumption firmness after the fourth day of storage. Application of 1-MCP prior to low temperature storage reduced ethylene-induced softening and decreased CI symptoms of the fruit. The results of the present study showed that 1-MCP was able of protecting the tissue against ethylene by blocking the binding site on the ethylene receptor, as suggested by Sisler & Serek (1997). Treatment with 1-MCP delayed ethylene-induced fruit ripening in 'Sekaki' papaya tested. The inhibitory effect of 1-MCP did not last long in fruit treated with 1-MCP at low temperature storage and thereafter the resumed normal ripening soon been transferred into ambient temperature. Over time, the fruit started to initiate and recover ethylene production and sensitivity.

Effects of 1-MCP on cell wall degrading enzymes activity

Ripening of papaya is accompanied by a relatively high softening rate which was paralleled by a gradual increase in activities of the cell wall enzymes; α-, β-galactosidase and pectin methylesterase (PME). α-galactosidase activity began

to increase on the second day with values of approximately 1.98 nkatal/g fw and coincident with the decreased in fruit firmness and continued to increase throughout fruit ripening (Fig 3a compared to Fig 4a). The greatest increase occurred between third and fourth day of storage at ambient with values of approximately 2.89 and 4.14 nkatal/g fw respectively (Fig 4a). In ripening papaya, α-galactosidase activity was correlated closely with firmness loss (Ali et al. 1998). Treatment with 1-MCP lowered the rate of α-galactosidase activity level on the second day with values of approximately 1.76 nkatal/g fw and the enzyme activity started to accelerated when fruit reached the fourth day of storage and highest activity level occurred at full ripe stage (Day 7) with values of approximately 4.1 nkatal/g fw (Fig 4a). However the enzyme activity for non-treated and treated fruit with 1-MCP at ambient increased gradually throughout ripening. During LT storage, the activity of α-galactosidase was suppressed completely and begins to increase as soon as fruit were transferred to ambient condition. Control fruit showed highest α-galactosidase level on the second and third day at ambient with values of 1.9 and 2.8 nkatal/g fw respectively and continued to increase until fruit reached later stage of ripening which was on the fifth day at ambient with values of 4.68 nkatal/g fw (Fig 4b). Whilst, 1-MCP treated fruit showed the lower rate of α-galactosidase activity level as compared to control with highest activity level occurred on the sixth day at ambient when fruit reached later stage of ripening with values of 3.38 nkatal/g fw (Fig 4b). The significance of α-galactosidase in modifying the cell wall is still uncertain but there are reports suggesting that α-galactosidase have transglycosylation activities that might be relevant to cell wall modification during fruit growth and development (Soh et al., 2006). β-galactosidase activity was present at early ripening and increased coincident with a significant decline in fruit firmness.Control fruit at ambient showed a significant increase in β-galactosidase activity on the second day and reaching a maximum value of 8 nkatal/g fw on the fifth day of storage (Fig 5a). However,the enzyme activity was delayed by 1-MCP treatment significantly until later stage of ripening (Day 7 of storage) with values of approximately 6.67 nkatal/g fw. In addition, the enzyme activity appeared later to increase gradually throughout ripening for both control and treated fruit (Fig 5a). The decline in β-galactosidase activity at the later stage of ripening does not occur in 'Eksotika' and 'Maradol' papaya and may explain why β-galactosidase was regarded as being significant in overall papaya softening (Lazan et al., 1995; Sanudo-Barajas et al., 2009). A similar pattern of β-galactosidase activity to that which occurs in 'Sekaki' papaya has been observed in 'Eksotika' and 'Maradol' papaya. Significant β-galactosidase activity in control fruit kept at LT storage was constant and did not showed any increase. After removal from LT storage, the activity started to increase slowly on second day at ambient (Fig 5b) and soon constantly increased reaching values of 6.74 nkatal/g fw on the fifth day at ambient. The 1-MCP treated fruit showed a lower β-galactosidase activity than control fruit but later the enzyme level slowly increased reaching values of approximately 6.3 nkatal/g fw on the sixth day (Fig 5b). 1-MCP application prior to storage at LT had inhibitory effects on β-galactosidase and the enzyme activity was delayed during low temperature storage. The existence of multiple β-galactosidase isoforms having different substrate specificity was reported from tomato (Moctezuma et al., 2003; Bennett and Labavitch 2008; Rugkong et al., 2010), 'La France' pear

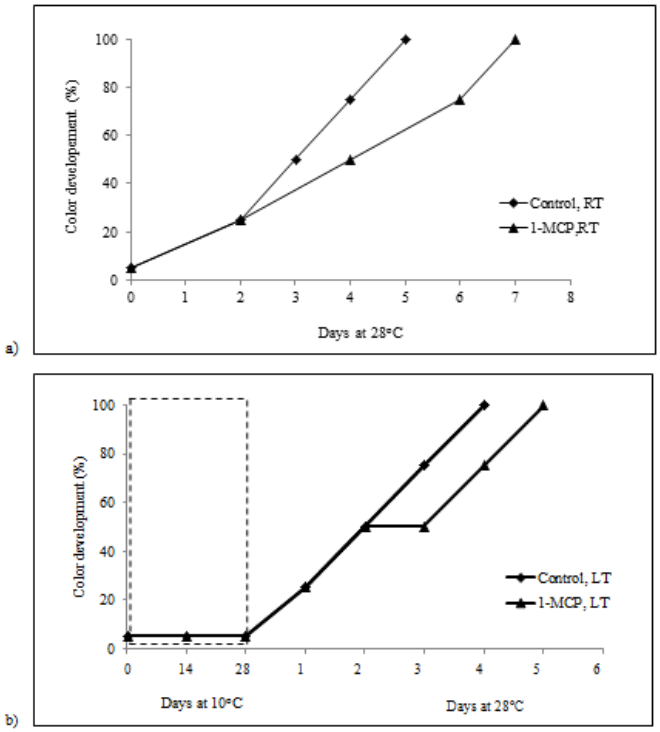

Fig 1. Skin color development of 'Sekaki' papaya stored at 28 ^{o}C (a) and stored at 10 ^{o}C for 28 days, followed by storage at 28 ^{o}C (b). Dotted box indicate the low temperature storage period.

(Mwaniki et al., 2005), papaya (Othman et al., 2011) and carambola (Balasubramaniam et al., 2005). Among three papaya β-galactosidase isozymes (β –gal I to III), β –gal II had been suggested to play essential role in papaya fruit softening and this isoform was detected being expressed continuously in papaya until ripening (Ali et al., 1998). Pectin methylesterase (PME) activity in control fruit at ambient increased rapidly in the early stage of 'Sekaki' papaya storage and continued to increased until the later stage of ripening, mainly after the third day (Fig 6a). Similar pattern of activity also observed in 1-MCP treated fruit at ambient and PME activity tend to constant up but in slower rate. The pattern detected in this study does not agreed with those of Thumdee et al. (2010) where treated 1-MCP papaya ripens at ambient tended to have higher PME activity than non-treated 1-MCP fruit. Highest PME peak in control occurred during final day of storage with values of approximately 221.45 nequal/s/g fw but 1-MCP treatment delayed it and managed to slow down accelerated PME activity with values of activity 167.3 nequal/s/g fw on the seventh day of storage (Fig 6a). According to Chen and Paull (1983), PME is readily detected before ripening and the activity increases during the softening progress. The increase in PME activity during ripening was reported in kiwi, peach (Bennett and Labavitch 2008), avocado (Wakabayashi et al., 2000) and papaya (Manrique and Lajolo 2004). PME activity in LT storage was slightly different than ambient fruit whereby rate activity of control fruit at LT accelerated in the early stage of ripening as soon as been transferred to ambient. Later the activity remained fairly constant with values of

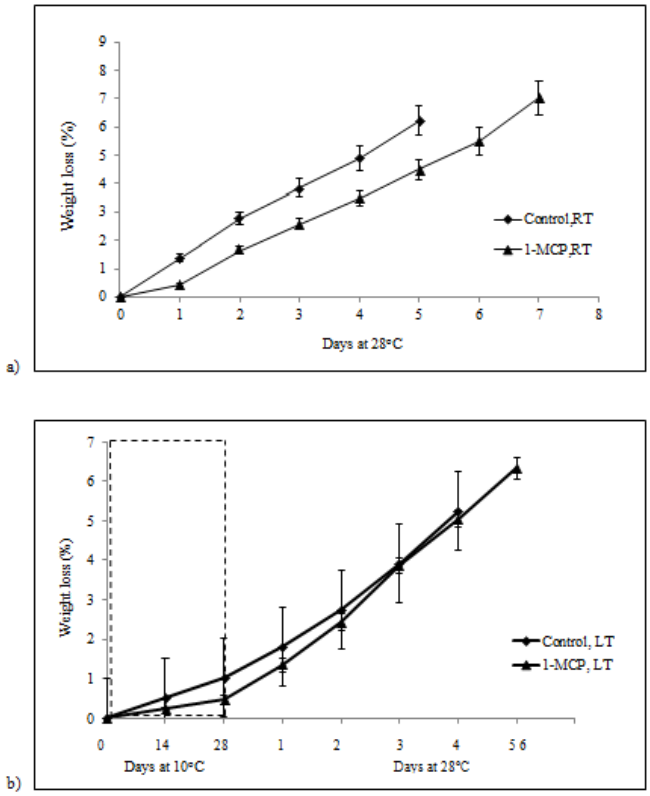

Fig 2. Weight loss of 'Sekaki' papaya papaya stored at 28 ^{o}C (a) and stored at 10 ^{o}C for 28 days, followed by storage at 28 ^{o}C (b). Dotted box indicate the low temperature storage period. Vertical bars represent S.E of the mean (n=6).

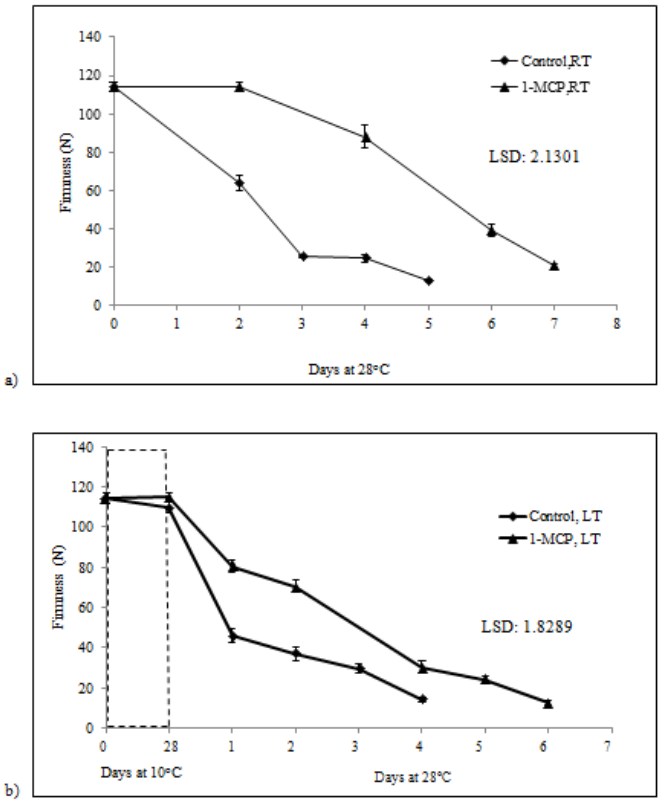

Fig 3. Firmness loss of 'Sekaki' papaya papaya stored at 28 ^{o}C (a) and stored at 10 ^{o}C for 28 days, followed by storage at 28 ^{o}C (b). Dotted box indicate the low temperature storage period. Vertical bars represent S.E of the mean (n=6).

approximately from 74.4 to 117.9 nequal/s/g fw on the first day until third day at ambient before accelerated significantly during full ripe stage and tend to show higher activity than control fruit at ambient with values of activity 233.6 nequal/s/g fw (Fig 6b). Both 1-MCP treatment and storage at LT suppressed PME activity with values of activity from 56.3 to 59.3 nequal/s/g fw on the first day until third day at ambient and the level constantly increased until the fifth day of storage with values of approximately 161 nequal/s/g fw (Fig 6b). PME activity levels peaked during full ripe stage and gradually increased but lower as compared to non-treated 1-MCP fruit. Fruit softening is a major aspect of the ripening process in papaya and is considered to be a consequence of compositional and structural changes in cell wall that involves pectin solubilization and depolymerization and accompanied by increasing hydrolyses enzyme activity such as β-galactosidase, PME and α-galactosidase (Ali et al., 1998; Chen and Paull 2003; Thumdee et al., 2010).

Storage at LT is used widely to extend postharvest fruit life and maintain quality (Sun et al., 2010). The use of low temperature storage means to delay ripening by reducing ethylene production, respiration rate, softening, the increase in total soluble solid (TSS) and the reduction in total acidity (TA) (Diaz-Mula et al., 2011). Most plant cell metabolism was suppressed in low temperature condition. In this present study, tissue firmness in papaya was maintained during 28 days at 10 ^{o}C storage and declined firmness occurred when fruit been transferred to ambient (Fig 3b). One that contributes to delayed firmness loss was the activity of cell wall degrading enzymes been suppressed when fruit stored at LT. In carambola, storage at 5 ^{o}C and 10 ^{o}C suppressed the firmness loss, delayed the pectin modification in cell wall, while it also retarded the increase in the activities of PME and β-galactosidase (Ali et al., 2004). Kovacs and Szerdahelyi (2002) also reported that β-galactosidase activity in apricot markedly retarded when stored at LT storage. Similar effects of low temperature storage in suppressing PME activity have also been observed for 'Fuji' apple (Wei et al., 2010). Papaya stored at 5 ^{o}C exhibited an increase in PME activity for two days and later the enzyme activity was maintained throughout the low temperature storage period (Karakurt and Huber 2003). On the other hand Rugkong et al. (2010) observed that PME activity was not affected by low temperature storage even though PME gene expression was reduced in chilled fruit during low temperature storage. The softening of climacteric fruit such as papaya correlated well with the ethylene emission. 1-MCP application inhibited ethylene production thus reduced the ethylene responses by suppressing the synthesis of degradation enzymes (Blankenship & Dole 2003). In 'Fuji' and 'Golden Delicious' apple, 1-MCP application retarded the polygalacturonase (PG), PME and β-galactosidase activity levels and thus delayed fruit softening (Wei at al., 2010). 1-MCP inhibition might occur at enzymic level, since it has been reported that 1-MCP suppressed ethylene production in tomato during ripening by strongly inhibiting the increase in ACS and ACO enzyme activity (Nakatsuka et al., 1997). In this present study, 1-MCP suppressed β-galactosidase, PME

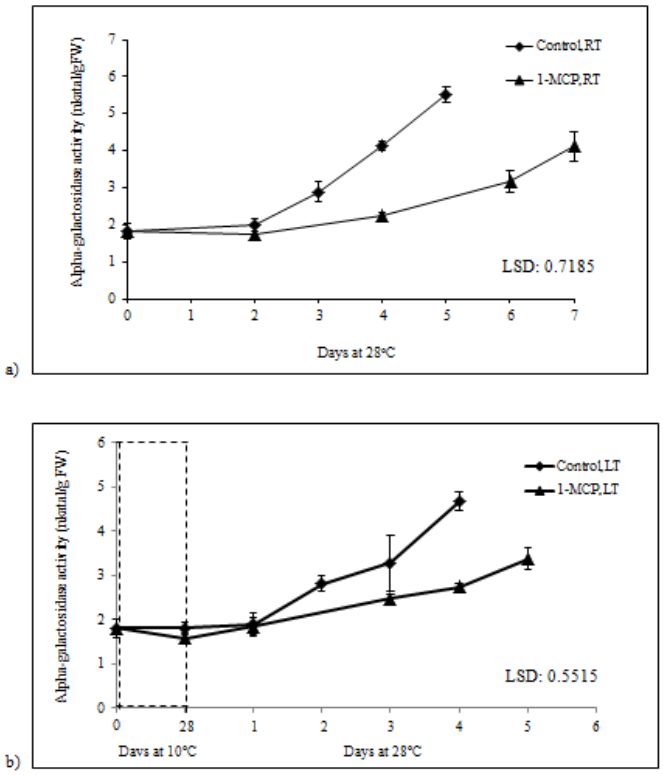

Fig 4. α-galactosidase activity of 'Sekaki papaya' stored at 28 °C (a) and stored at 10 °C for 28 days, followed by storage at 28 °C (b).Dotted box indicate the low temperature storage period. Vertical bars represent S.E of the mean (n=6).

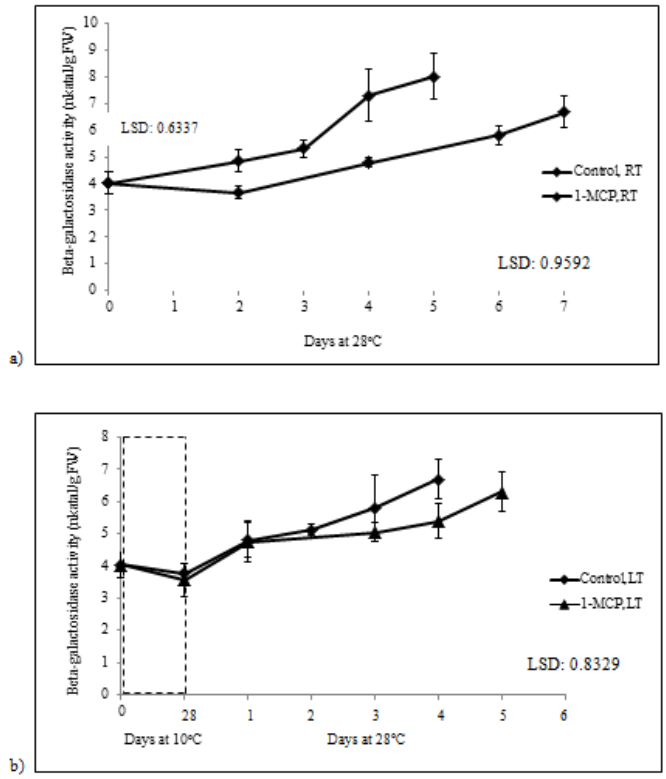

Fig 5. β-galactosidase activity of 'Sekaki papaya' stored at 28 °C (a) and stored at 10 °C for 28 days, followed by storage at 28 °C (b).Dotted box indicate the low temperature storage period. Vertical bars represent S.E of the mean (n=6).

and α-galactosidase in papaya, then increased until later stage of ripening (Figs 4, 5 and 6) so we assume that these cell wall degrading enzymes activity was delayed by 1-MCP but not prevented and thus papaya resumed normal ripening. This is thought that 1-MCP binds permanently to receptors present at the time of treatment and resumption of ethylene sensitivity is due to appearance of new binding sites on the ethylene receptors or disassociation of 1-MCP from the receptor though there is little supporting data for either possibility (Blankenship & Dole 2003; Chervin et al., 2004). According to Tatsuki et al. (2007), suggested that the amounts of ethylene production and of ethylene receptors present when 1-MCP is applied and the amounts that are induced after 1-MCP treatment are the keys to 1-MCP efficacy. In addition, 'Sekaki' papaya treated with 1-MCP (90 ppb) was found to soften completely without developing 'rubbery' texture when ripe whereas Manenoi et al. (2007) reported that papaya fruit treated with 50-1000 nL L^{-1} 1-MCP at color break had a rubbery texture when reached 100 % skin yellowing.

Materials and methods

Papaya fruit (*Carica papaya* L.) cv. Sekaki were harvested from private farm in Pagoh, Johor (Malaysia) at stage 2 (in which yellow color covers 5% of the skin's surface).The selected fruit were uniform in size and free from external defect and were then transported to Universiti Kebangsaan Malaysia, Bangi (Malaysia). Fruit were treated according to Lazan et al. 1995.The fruit were rinsed with water, air dried, soaked in 0.02 % prochloraz for 5 minutes and left to dry.

Half of the fruit were placed into airtight chambers (15 L) and exposed to 90 ppb of 1-MCP (SmartFresh, Agrofresh Rohm and Haas, Philadelphia, USA) for 12 hours at 28 °C. The other half of the fruit was placed in the similar airtight chamber with the same temperature conditions but without the 1-MCP treatment. After the treatment, half of the untreated fruit and half of the fruit treated with 1-MCP were stored at 10 °C for 28 days, while the remaining fruit were kept at 28 °C for ripening. After the low temperature storage period, fruit were removed from storage and kept at 28 °C until the ripening process was complete. In summary, the experiment was composed of the following treatments:
a. Fruit not treated with 1-MCP and kept at 28 °C; b. Fruit treated with 1-MCPand kept at 28 °C; c. Fruit not treated with 1-MCP and stored at 10 °C for 28 days and then kept at 28 °C thereafter; and d. Fruit treated with 1-MCP, stored at 10 °C for 28 days and then kept at 28 °C thereafter. During storage at ambient, skin color, weight loss, fruit firmness and cell wall degrading enzymes activities were analyzed according to ripening stage. At day 0 (right before low temperature storage), day 28 (after low temperature storage) and after fruit transferred to ambient, skin color, weight loss, fruit firmness and cell wall degrading enzymes activities were also analyzed according to ripening stage. Fruit skin color was recorded as color indices/ripening stages according to the papaya maturity. Stage 1: mature green; stage 2: light green (5 % yellow skin); stage 3; yellowish green (25 % yellow skin); stage 4: yellow (50 % yellow skin); stage 5: yellowish orange (75 % yellow skin); stage 6: orange (100 % yellow skin) (Federal Agricultural Marketing Authority

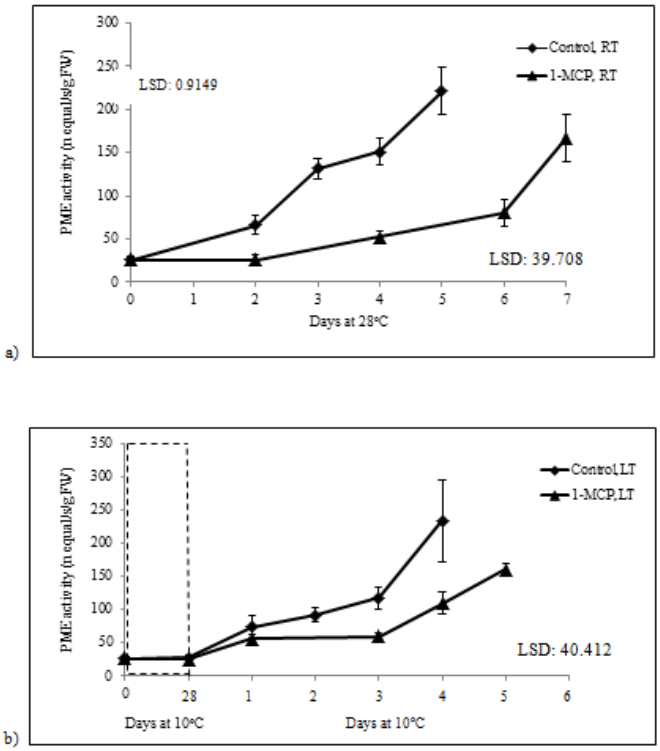

Fig 6. PME activity of 'Sekaki papaya' stored at 28 ^{o}C (a) and stored at 10 ^{o}C for 28 days, followed by storage at 28 ^{o}C (b).Dotted box indicate the low temperature storage period. Vertical bars represent S.E of the mean (n=6).

Malaysia, 2008). Weight (water) loss was expressed as percentage of fresh weight against initial weight at harvest. Fruit weight was taken every day using an electronic scale (Mettler PJ3000, Switzerland). Firmness determination was carried out prior to tissue sampling for enzyme analysis according to Chin et al. (1999). Briefly, tissues firmness determinations were made on the cuts surface located on the middle section of the fruit using a McCormick Pressure Tester (Model FT327-12, Milan, Italy). Firmness readings were made on three sites and were expressed as Newton (N). This process was repeated for six fruit. Chilling injury was expressed as percentage of fruit with skin browning (based on the number of affected fruit against total number of fruit in particular treatment) (Ali et al., 2004). For tissue sampling, the entire mesocarp were cut into small cubes (~1 cm^3), frozen in liquid nitrogen and kept at -70 ^{o}C until needed for enzyme analysis. For enzymes analysis, raw enzymes were extracted at 4 ^{o}C. About 10 g of tissue was homogenized (Edmund Buhler 7400, Tubingen, Germany) in 10 ml 0.1 M sodium citrate, pH 4.6, containing 1 M NaCi, 13 mM EDTA, 10 mM β-mercaptoethanol and 2 % (w/v) polyvinylpyrrolidone (PVP-40) and the protein crude extract was then left for 1 hour with occasional stirring. Supernatant was recovered by centrifugation (Sorval RC-5B Superspeed) at 29 000 x g for 30 min (Ali et al., 1998). Assays for α-galactosidase and β-galactosidase were according to Soh et al. (2006) and Chin et al. (1999) respectively. α-galactosidase was assayed in a reaction mixture containing 0.52 ml 0.1 M sodium citrate pH 3, 0.4 ml 0.1 % (w/v) bovine serum albumin and 0.4 ml 4 mM substrate p-nitrophenil-α-D-galactopiranosidasa (Sigma) and 0.08 ml of undesalted crude extract incubated for 15 min at 37 ^{o}C. As for β-galactosidase, 0.52 ml 0.1 M sodium citrate pH 4.1 and 0.4 ml 13 mM p-nitrophenil- β-D-galactopiranosidase was used in the assay. The reaction was stopped by adding 2 ml 0.2 M sodium carbonate and the amount of p-nitrophenol formed was determined from the absorption at 415 nm. Six replication were used, each of which consisted of a single fruit according to ripening stage.

Assay for pectin methylesterase was according to Lazan et al. (1995). PME was determined by titrating the release of carboxyl groups by the action of PME on the substrate with 0.1 M NaOH to pH 7.3 for 10 min. The assay mixture consisted of 0.5 ml of undesalted crude extract and 25 ml 1 % pectin. Boiled enzyme was used as a control in all the assays. All the enzymes activities expressed as nkatal g^{-1} fresh weight, except for PME which was expressed as nequivalent carboxyl group formed s^{-1} g^{-1} fresh weight. Six replication were used, each of which consisted of a single fruit according to ripening stage. The same fruit were used during the entire enzyme analysis period. The data were analyzed for significance difference by applying variance analysis (ANOVA) using the SAS statistical package (SAS, Institute Inc. Cary, NC). Data represented in the figures were subjected to mean separation by the LSD (Least Significance Difference) test (p = 0.05).

Conclusion

The choice of 'Sekaki' papaya as the subject of research due to different cultivar of papaya might provide new insights and advance understanding into plant ethylene responses including softening related changes. We concluded that 1-MCP (90 ppb) able to delay the attainment of full yellow coloration, retard loss of tissue firmness in 'Sekaki' papaya. It also delayed the increased in cell wall degrading enzymes activities. In addition, 1-MCP treated fruit were able to soften completely at later ripening stage.

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