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Effect of 1-Methylcyclopropene (1-MCP) Treatment on Firmness and Softening Related Enzymes of 'Sekaki' Papaya Fruit During Ripening at Ambient

(Kesan Perlakuan 1-Metilsiklopropena (1-MCP) ke Atas Tekstur dan Aktiviti Enzim Berkait Perlembutan Betik 'Sekaki' Semasa Pemasakan pada Suhu Ambien)

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ABSTRACT

*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 (90 ppb for 12 h) on softening related changes were determined through physiological changes, fruit firmness and activities of the cell wall degrading enzymes including α-galactosidase, β-galactosidase, pectin methylesterase (PME) and xylanase during ripening in papaya (*Carica papaya *L. cv Sekaki). In this study, fruits were treated with 90 ppb concentration of 1-MCP gaseous vapors for 12 h in airtight container maintained at 28°C. After the treatment fruits were placed at ambient temperature (28°C). Papaya treated with 1-MCP experienced a significant delayed in skin color development, weight loss and reduced firmness loss compared with 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 decline in fruit firmness. With 1-MCP application, fruit experienced a delay in activity of cell wall degrading enzymes but continued to increase until later stage of ripening. Thus it may be suggested that 1-MCP treatment may aid in delaying softening-related process and thereby extended the postharvest life and maintained the quality of the 'Sekaki' papaya fruit.*

Keywords: 1-methylcyclopropene (1-MCP); cell wall degrading enzymes; softening-related changes

ABSTRAK

*Kelebihan penggunaan 1-metilsiklopropena (1-MCP) didapati merencat penghasilan etilena dan sekaligus melambatkan pemasakan buah daripada berlaku. Perlakuan 1-MCP (90 ppb, 12 jam) terhadap perubahan berkaitan perlembutan telah dikaji melalui perubahan fisiologi, ketegaran tisu buah dan pengukuran aktiviti α-galaktosidase, β-galaktosidase, pektin metilesterase (PME) dan xilanase yang merupakan enzim berkait perlembutan betik (*Carica papaya *L. cv Sekaki). Betik 'Sekaki' dalam kajian ini diberikan perlakuan 1-MCP pada kepekatan 90 ppb dan ditempatkan di dalam bekas kedap udara selama 12 jam pada suhu ambien (28°C). Hasil menunjukkan perlakuan 1-MCP memperlahankan perubahan warna kulit buah, kehilangan berat dan mengurangkan kehilangan ketegaran tisu buah secara signifikan berbanding buah tanpa perlakuan 1-MCP. Kehilangan ketegaran dikatakan seiring dengan peningkatan aktiviti enzim berkait perlembutan buah. Melalui perlakuan 1-MCP, peningkatan aktiviti enzim ini dapat ditekan sepanjang penyimpanan. Walau bagaimanapun, aras aktiviti enzim perlembutan buah yang dikaji meningkat secara berterusan namun dengan lebih perlahan jika dibandingkan dengan buah tanpa perlakuan 1-MCP. Hasil kajian ini mencadangkan perlakuan 1-MCP berupaya melambatkan kemerosotan ketegaran buah selaras dengan penindasan aktiviti enzim perlembutan buah sekaligus memanjangkan hayat simpanan di samping memelihara kualiti betik 'Sekaki'.*

Kata kunci: 1-metilsiklopropena (1-MCP); enzim perlembutan buah; perubahan berkait perlembutan

INTRODUCTION

Papaya (*Carica papaya* L.) fruit, has a short shelf life (Lazan et al. 1995) and papaya ripening is dependent on the ethylene action which later accompanied by softening process. Being classified as a climacteric fruit, the rise in ethylene production parallels the respiration rate and peaks at the same time as the respiratory climacteric (Paull & Chen 1983). Fruit softening is a major aspect of the ripening process and considered to be a consequence of cell wall modifications (Jeong & Huber 2004). In this situation, both ethylene and the integrity of cell walls are closely involved in one way or another.

1-methylcyclopropene (1-MCP) is an ethylene antagonist that binds ethylene receptors in an apparently non-competitive way (Sisler & Serek 1997), and have been identified as useful for attenuating ethylene effect in plant tissues (Blankenship & Dole 2003). It delays ethylene production, climacteric respiration, skin color development and softening of papaya without affecting the total soluble solids and fruit weight loss (Manenoi et al. 2007; Shiga et al. 2009). This alteration of softening by 1-MCP provides an approach to determine how fruit ripening is regulated. The purpose of this work was to determine the relationship between 'Sekaki' papaya softening and activity of cell

wall degrading enzymes during ripening. In particular, we emphasized the effects of 1-MCP in delaying softening during ripening by suppressing the cell wall degrading enzymes activities and thereby lengthen the postharvest life and preserve the quality of the 'Sekaki' papaya fruit.

MATERIALS AND METHODS

FRUIT SOURCE AND TREATMENTS

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 fruits were uniform in size and free from external defect and were then transported to Universiti Kebangsaan Malaysia, Bangi (Malaysia).

The fruits were treated according to Lazan et al. (1995), the fruits were rinsed with water, air dried, soaked in 0.02% prochloraz for 5 min and left to dry. Half of the fruits were placed into airtight chambers (15 L) and exposed to 90 ppb of 1-MCP (SmartFresh, Agrofresh Rohm and Haas, Philadelphia, USA) for 12 h at 28°C. The other half of the fruits were placed in the similar airtight chamber with the same temperature conditions but without the 1-MCP treatment. After the treatment, the untreated fruit (control) and the fruit treated with 1-MCP were kept at ambient temperature (28°C).

COLOR CHANGES, WEIGHT LOSS, FIRMNESS DETERMINATION

The 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 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 fruits. For tissue sampling, the entire mesocarp were cut into small cubes (~1 cm^3), frozen in liquid nitrogen and kept at -70°C until needed for analysis.

CRUDE ENZYMES EXTRACTION AND ASSAYS

For enzymes analysis, crude enzymes were extracted at 4°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 NaCl, 13 mM EDTA, 10 mM β-mercaptoethanol and 2% (w/v) polyvinylpyrrolidone (PVP-40) and the protein crude extract was then left for 1 h with occasional stirring. Supernatant was recovered by centrifugation (Sorval RC-5B Superspeed) at 29 000×g for 30 min (Ali et al. 1998).

Assays for α-galactosidase and β-galactosidase performed 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 pH3, 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°C. As for β-galactosidase, 0.52 mL 0.1 M sodium citrate pH4.1 and 0.4 mL 13 mM p-nitrophenil-β-D-galactopiranosidase were used in the assay. The reaction was stopped by adding 2 mL of 0.2 M sodium carbonate and the amount of p-nitrophenol formed was determined from the absorption at 415 nm.

The assay for pectin methylesterase was performed 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 pH7.3 for 10 min. The assay mixture consisted of 0.5 mL of undesalted crude extract and 25 mL 1% pectin. The xylanase assay mixture which comprised of 0.17 mL of 0.1 M sodium citrate pH5.0, 0.12 mL of 0.1% bovine serum albumin, 0.09 mL of 2% oat xylan (Sigma) and 0.04 mL desalted crude extract was incubated for 10 min at 40°C and the reducing sugar released was estimated by the method of Gross (1982).

Boiled enzyme was used as a control in all the assays. All the enzymes activities were expressed as nkatal g^{-1} fresh weight, except for PME which was expressed as inequivalent carboxyl group formed s^{-1} g^{-1} fresh weight. Six replications were used, each of which consisted of a single fruit according to ripening stage. The same fruit was used during the entire enzyme analysis period.

STATISTICAL ANALYSIS

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).

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 as compared with the control fruit. Control fruit reached 100% yellow skin in about 5 days compared with 7 days for 1-MCP treated fruit (Figure 1(a)). When kept at 28°C, both control and fruit treated with 1-MCP showed increased in yellow color attainment. The 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 (Figure 1(a)).

1-MCP reduced weight loss in 'Sekaki' papaya and the 1-MCP treated fruit experienced slower rate loss as compared with control fruit during the storage. The control fruit experienced 6.2% water (fresh weight) loss on fifth day whilst 1-MCP treated fruit showed lower percentage of loss (4.5%) (Figure 1(b)) on the same day of ripening. The 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 (≤20 N) on the third day of storage. The highest losses in firmness were observed on the second day of storage at ambient, papaya lost almost 50% of their initial firmness (Figure 1(c)). 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 with 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) and apple (Watkins 2006).

The application of 1-MCP has reduced ethylene-induced softening and the results of the present study showed that 1-MCP was able to protect the tissue against ethylene by blocking the binding site on the ethylene receptor as suggested by Sisler and Serek (1997). The treatment with 1-MCP delayed ethylene-induced fruit ripening in 'Sekaki' papaya was tested. However, the inhibitory effect of 1-MCP did not last long in fruit treated with 1-MCP because the fruit started to initiate and recover the ethylene production and sensitivity.

EFFECTS OF 1-MCP ON CELL WALL DEGRADING ENZYMES ACTIVITY

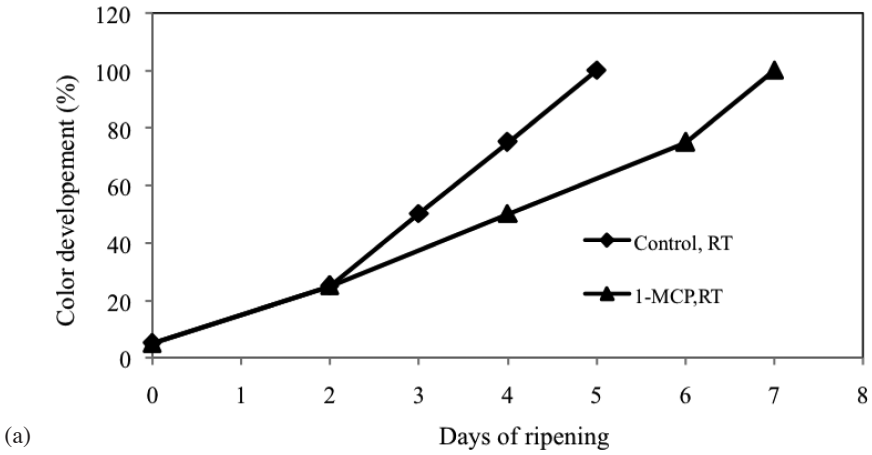
Ripening of papaya is accompanied by a relatively high softening rate which was paralleled by a gradual increase in activities of the cell wall enzymes; α-, β-galactosidase, pectin methylesterase (PME) and xylanase. α-galactosidase activity began to increase on the second day with values of approximately 1.98 nkatal/g fw (Figure 2(a)) and coincident with the decreased in fruit firmness and continued to increase throughout fruit ripening. 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 (Figure 2(a)).

In a 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 accelerate 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 (Figure 2(a)). However the enzyme activity for control and treated fruit with 1-MCP at ambient increased gradually throughout ripening. 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).

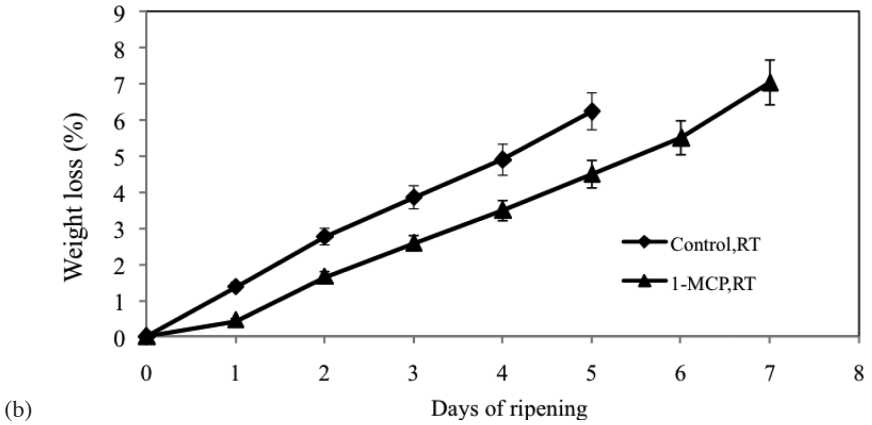
β-galactosidase activity was present at early ripening and increased coincident with a significant decline in fruit firmness. Control fruit at ambient showed a significant increase in β-galactosidase activity on the second day and reaching a maximum value of 8 nkatal/g fw on the fifth day of storage (Figure 2(b)). 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 (Figure 2(b)).

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. The existence of multiple β-galactosidase isoforms having different substrate specificity was reported from papaya (Othman et al. 2011) and among the 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 increase until the later stage of ripening, mainly after the third day (Figure 2(c)). Similar pattern of activity was also observed in 1-MCP treated fruit at ambient and PME activity tend to increase but at 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 tend to have higher PME activity than control 1-MCP fruit. Highest PME peak in non-treated 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 (Figure 2(c)). According to Paull and Chen (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 & Labavitch 2008) and avocado (Wakabayashi et al. 2000).



(a)



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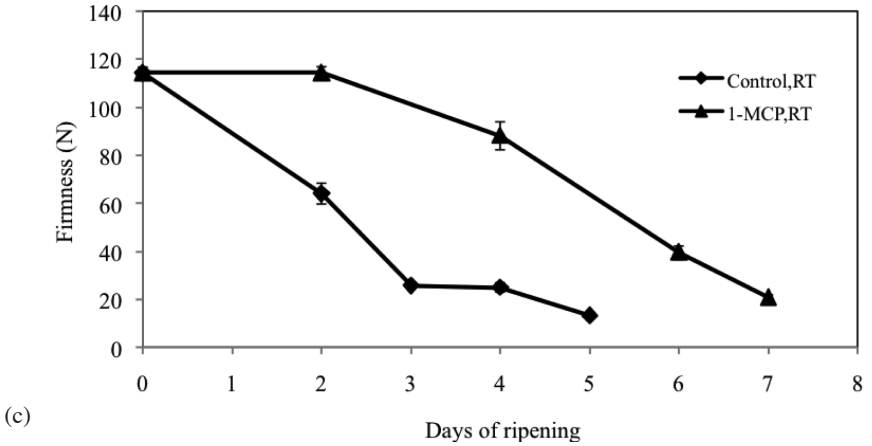
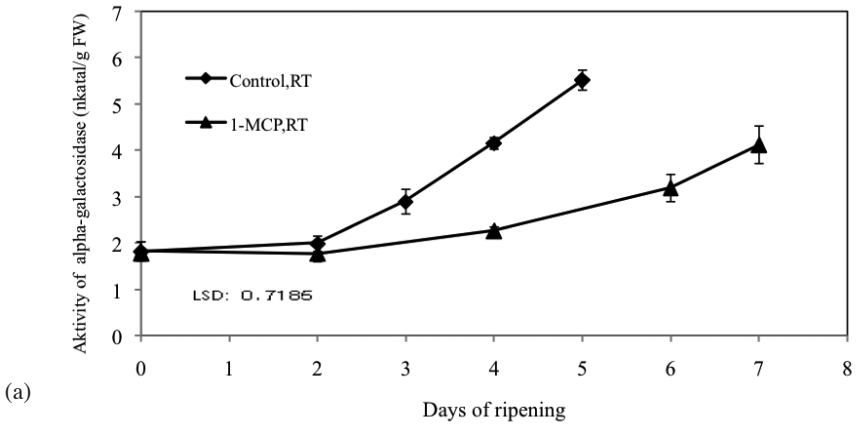


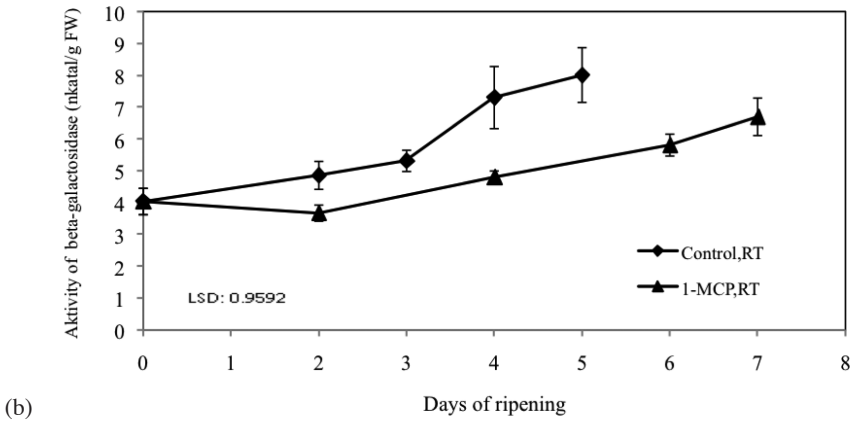
(c)

FIGURE 1. Skin color development (a) weight loss (b) and firmness loss (c) of control and 1-MCP treated 'Sekaki' papaya stored at 28°C. Vertical bars represent S.E. of the mean (n=6)

Xylanase activity was low at early ripening then slowly increase on the second day of storage with values of activity approximately 4 nkatal/g fw. This enzyme activity consistently continued to increase throughout ripening (Figure 2(d)). The highest activity occurred during the final day of storage (Day 5) with values of activity 5.29 nkatal/g fw. Fruit treated with 1-MCP demonstrated a delayed in xylanase activity but gradually to increase on second day of storage with values of activity 3.05 nkatal/g fw until reached later stage of ripening (Day 7) with values of activity approximately 4.76 nkatal/g fw (Figure 2(d)). According to Thumdee et al. (2010), xylanase activity in papaya was found to increase throughout the ripening, following 1-MCP treatment the enzyme activity was suppressed. On the other hand, Chen and Paull (2003) reported that xylanase activity in papaya and tomato peaks with polygalacturonase (PG) activity at a time when changes in hemicellulose component are more pronounced;



(a)



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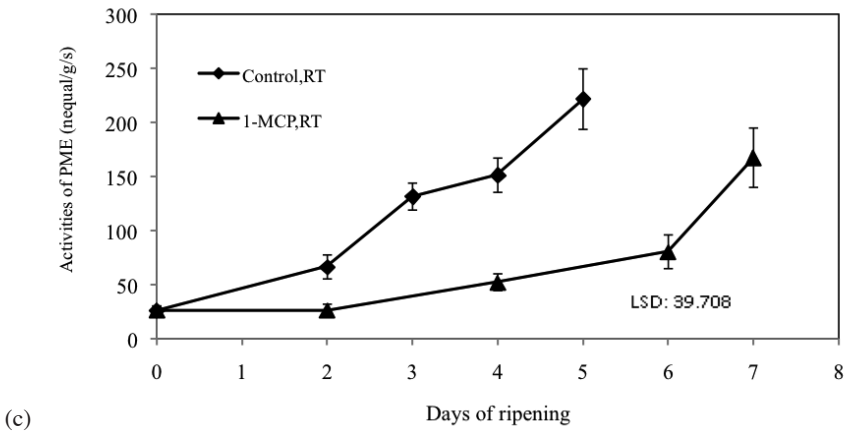

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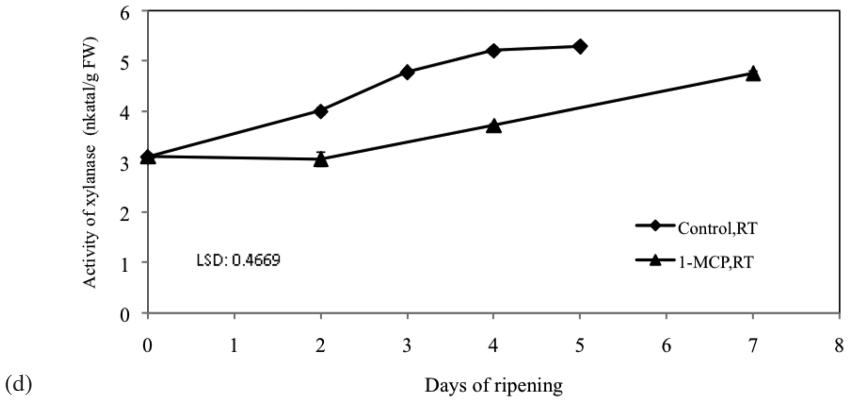

(d)

FIGURE 2. Activities of α-galactosidase (a) β-galactosidase (b) pectin methylesterase (c) and xylanase (d) of control and 1-MCP treated 'Sekaki' papaya stored at 28°C. Vertical bars represent S.E. of the mean (n=6)

40-60% yellow skin stage and showed a decline during late ripening stage.

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). 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, α-galactosidase and xylanase in papaya, then increased until the later stage of ripening so we assume that the cell wall degrading enzymes activity was delayed by 1-MCP but not prevented and thus papaya resumed normal ripening.

1-MCP binds permanently to the receptors present at the time of treatment and resumption of ethylene sensitivity. This is due to the 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).

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.

Conclusion

The choice of 'Sekaki' papaya as the subject of research was 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) was able to delay the attainment of full yellow coloration, retard loss of tissue firmness in 'Sekaki' papaya. It also suppressed the increase in cell wall degrading enzymes activities. In addition, 1-MCP treated fruits were able to soften completely at later ripening stage.

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