International Food Research Journal 18(4): 1251-1259 (2011)

Physiological changes and cell wall degradation in papaya fruits cv. 'Kaek Dum' and 'Red Maradol' treated with 1- methylcyclopropene

[1,2]Krongyut, W., [1,2*]Srilaong, V., [1,2]Uthairatanakij, A., [1,2]Wongs-Aree, C., [3]Esguerra, E. B. and [1,2]Kanlayanarat, S.

[1]Postharvest Technology Program, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkok 10140 Thailand
[2]Postharvest Technology Innovation Center, Commission of Higher Education, Bangkok 10400
[3]Postharvest Horticulture Training and Research Center, Crop Science Cluster, College of Agriculture, University of the Philippines Los Baños, College, Laguna 4031, Philippines

Abstract: The differential response of two papaya cvs. 'Kaek Dum' and 'Red Maradol' to 1-methylcyclopropene (1-MCP) applied at 100 nl l^{-1} at <10% yellow peel color was evaluated based on physiological changes and rate of fruit softening in relation to the activities of cell wall degrading enzymes and solubilization of cell wall polysaccharides. Ethylene production, respiration rate, softening, activities of cell wall degrading enzymes and the solubilization of cell wall polysaccharides were lower in 'Red Maradol' compared to 'Kaek Dum'. In both cultivars, 1-MCP suppressed ethylene production and respiration rate, delayed the onset of the climacteric and retarded the softening process. The delay in the softening of 1-MCP-treated fruit was greater in 'Red Maradol' compared with 'Kaek Dum' coincident with the low activities of polygalacturonase (PG) and β-galactosidase. Consequently, changes in the pectic, hemicellulosic and cellulosic fractions were reduced. These results account for the slower rate of softening of 'Red Maradol' than 'Kaek Dum' papaya.

Keywords: Papaya, 1-methylcyclopropene, polygalacturonase, β-galactosidase, cell wall degradation

Introduction

Papaya is an important fruit in Thailand and the two commercial cultivars in the market are 'Kaek Dum' and 'Red Maradol'. Marketability of the fruit is largely determined by the rate of softening which enhances susceptibility to mechanical damage as well as attack of insect pests and diseases thus impacting greatly on consumer acceptability (Sañudo-Barajas *et al.*, 2009). Moreover, once the fruit starts to soften, handling, transport and distribution become difficult.

Softening is one of the most ethylene-sensitive processes that occur during ripening (Lelievre *et al*., 1997; Fabi *et al*., 2007). Among the techniques aimed at retarding softening, the use of 1-methylcyclopropene (1-MCP) appeared promising. 1-MCP blocks ethylene perception by competitively binding to the hormone's receptor (Blankenship, 2001) causing a halt on the processes that lead to softening and color development that may be wholly or partially ethylene-dependent (Flores *et al*., 2001; Moya-León *et al*., 2004). In papaya, it has been established that the response to, and the successful use of 1-MCP was dependent on cultivar and stage of maturity. Ergun and Huber (2004) and Fabi *et al*. (2007) demonstrated that 'Sunrise' and 'Golden' papaya exposed to 1-MCP at the pre-ripe stage (10-20% yellow surface coloration) can be stored for 4-5 days before it reaches edibility. On the other hand, 'Gold' and 'Rainbow' papaya treated at the same stage can be stored longer, that is, for 21 days (Manenoi *et al*., 2007). However, a softening disorder characterized by a rubbery texture when ripe among 1-MCP treated papaya that would affect marketability was reported (Fabi *et al*., 2007; Manenoi and Paull, 2007; Manenoi *et al*., 2007). Bron and Jacomino (2009) and Shiga *et al*. (2009), on the other hand, did not report any rubbery texture in 'Golden' papaya fruit treated with 1-MCP. These observations demonstrated the differential response of cultivars to 1-MCP treatment.

Softening results from modification of the cell wall polysaccharides by cell wall degrading enzymes including polygalacturonase (PG), β-galactosidase (β-gal), and pectin methylesterase (PME) (Paull and Chen, 1983; Lazan *et al*., 1995; Brummell *et al*., 2004; Manenoi and Paull, 2007; Sañudo-Barajas *et al*., 2009). Moreover, investigations have been undertaken to link cell wall degradation and softening with changes in activities of cell wall degrading enzymes in papaya in response to 1-MCP treatment

*Corresponding author.
Email: *varit.sri@kmutt.ac.th*
Tel: +66 2470 7726; Fax: +66 2452 3750

(Sañudo-Barajas *et al*., 2009; Thumdee *et al*., 2010). While it has been demonstrated that response to 1-MCP treatment differ among cultivars, it is worth investigating the responses of two commercial Thai cultivars of papaya that vary in their rate of normal softening. 'Kaek Dum' is a fast softening cultivar while 'Red Maradol' softens slowly.

This study was conducted to determine the differential response of 'Kaek Dum' and 'Red Maradol' to 1-MCP in terms of physiological changes and rate of softening. To gain insights on the mechanism of softening between the two cultivars, the changes in the activities of cell wall degrading enzymes such as PG, β-gal and PME as well as changes in the solubility of cell wall polysaccharides in response to 1-MCP treatment were determined.

Materials and Methods

Plant material

Papaya fruit cvs. 'Kaek Dum' and 'Red Maradol at color break stage (<10% peel yellow) were harvested from an orchard in Nakornpathom province, Thailand. Fruit were transported to the laboratory at King Mongkut's University of Technology Thonburi, Bangkok, within 2 h from harvest. Fruit were sorted as to uniformity in size and freedom from visual symptoms of any disease or blemishes, and then washed with running tap water. For disease control, fruit were dipped in 650 ppm Imazalil solution for 5 min and air dried.

1-MCP treatment

Fruit were placed in airtight glass chambers and exposed to 100 nl l^{-1} 1-MCP (Rohm and Haas, Philadelphia, PA, USA) for 12 h at 20 ± 2˚C. The other batch of fruit was also placed in sealed glass chambers at the same temperature and duration but without 1-MCP treatment (control). After the treatment, fruit were exposed to air and allowed to ripen at 25 ± 2˚C and 70-75% RH. Physiological changes such as ethylene production and respiration rate as well as rate of softening were assessed every two days. For the analysis of cell wall hydrolases and polysaccharide fractions, sliced pulp tissues from each fruit were immediately frozen in liquid nitrogen and stored at -20˚C prior to analysis.

Ethylene production and respiration rate

Three fruits from each treatment for each cultivar were weighed and placed individually in an airtight container (13 l for 'Kaek Dum' and 5 l for 'Red Maradol') fitted with gas sampling ports and incubated at 25 ± 2˚C for 1 h to monitor the respiration rate and ethylene (C_2H_4) production. One ml gas samples were withdrawn from the headspace of the chamber and were injected into a Shimadzu gas chromatograph Model GC-8A (Kyoto, Japan) for carbon dioxide (CO_2) measurement and a Shimadzu GC Model 14B (Kyoto, Japan) for ethylene measurement. Results are expressed as µl C_2H_4 $kg^{-1}h^{-1}$ and ml CO_2 $kg^{-1}h^{-1}$ for ethylene production and respiration rate, respectively.

Flesh firmness

Fruit firmness was determined from three points along the equator of three fruits using a texture analyzer (TA-XT2, Stable Micro Systems Ltd., UK), fitted with a flat plunger (5 mm diameter) which penetrated into the flesh at 1 cm depth at a rate of 10 mm min^{-1}. The flesh firmness was expressed as Newtons (N).

Extraction and assay of cell wall degrading enzymes

PG and PME extraction followed the procedure of Abu-Goukh and Bashir (2003) with some modifications. Briefly, 5 g of frozen pulp was homogenized in Polytron PT 2100 (Kinematica, Luzern, Switzerland) for 2 min in 10 ml of 100 mM sodium acetate buffer at pH 6.6 [composition (w/v): 0.2% sodium dithionite and 1% polyvinyl pyrollidone] and centrifuged at 15,000 × g for 20 min. The pellets were re-suspended in 10 ml of 1 M sodium acetate buffer (pH 6.0) containing 6% NaCl adjusted to pH 8.2 with 2 N NaOH and centrifuged at 15,000 × g for 20 min. The supernatant was filtered in Whatman #1 filter paper, dialysed against three changes of distilled water for 24 h, and used as PG and PME source.

PG activity was determined as described in previous studies (Pressey and Avants, 1973; Chang and Ming, 1998). The dialyzed extract (100 µl) was incubated for 1 h at 37˚C in a solution of 1% polygalacturonic acid in 0.1 M sodium-acetate (100 µl, pH 4.5), 0.15 M NaCl (100 µl). DNSA reagent (1 ml: 1% 3,6-dinitrophthalic acid monopyridinium salt, 2% NaOH and 20% Rochelle salt) was added, and the mixture was boiled for 5 min. Upon cooling, 5 ml of chilled distilled water was added, and the reducing group formed was measured at A_{540} with UV-visiblespectrophotometer (model UV-1601, Shimadzu Co., Kyoto, Japan). PG activity is expressed as µmole galacturonic acid mg^{-1} protein h^{-1}.

PME assay was done as described by Hagerman and Austin (1986) with modifications. Briefly, 0.1 ml of the dialyzed extract was added to a prepared mixture (pH 7.5, 25˚C) containing 2 ml citrus pectin (0.5% w/v, pH 7.5) with >85% degree of esterification

(Sigma Chemical Co., St. Louis, Mo., U.S.A.), 0.2 ml bromothymol blue in K_2HPO_4-KH_2PO_4, and distilled water. Reaction in the 3 ml final solution mixture was monitored at A_{620} with UV-visiblespectrophotometer (model UV-1601, Shimadzu Co., Kyoto, Japan). PME activity is expressed as µmole acetic acid mg^{-1} protein min^{-1}.

β-gal extraction and assay were adopted from previous reports of Nakamura *et al*. (2003) and Karakurt and Huber (2003), respectively. Briefly, 5 g of frozen pulp was homogenized in 15 ml sodium phosphate buffer (10 mM, pH 7.2) and centrifuged at 8,000 × g for 40 min. The resulting supernatant served as the enzyme source in which 0.2 ml was added to 0.2 ml of 6.6 mM *ρ*-nitrophenyl derivative of β-galactopyranoside in 0.1 M sodium acetate (pH 5.2) and incubated for 20 min at 30˚C. The reaction was terminated by adding 1 ml of 1 M ammonium hydroxide containing 2 mM EDTA, and the release of *ρ*-nitrophenol was monitored at A_{400} with UV-visiblespectrophotometer (model UV-1601, Shimadzu Co., Kyoto, Japan). Authentic *ρ*-nitrophenol was used as standard and β-gal activity was expressed as nmole *ρ*-nitrophenol mg^{-1} protein min^{-1}.

There were three replications for each enzyme assay which was represented by a single fruit per replication. Protein levels were measured from crude enzyme extracts using the Bradford assay (1976), with bovine serum albumin (BSA) as a standard.

Extraction, fractionation and analysis of cell wall fractions

Alcohol insoluble residues (AIR) of cell walls were extracted by immersing 20 g of frozen papaya pulp in 150 ml of boiling alcohol (95%) for 20 min. The boiled pulp was cooled, homogenized, and filtered through 4 layers of Miracloth. AIR recovered were first washed with 100 ml of 95% alcohol and then with acetone until it decolorized (Carrington *et al*., 1993). The AIR was air dried and weighed.

AIR (100 mg) was sequentially extracted (Carrington *et al.*, 1993; Maclachlan and Brady, 1994) using 20 ml each of the following solvents: distilled water; 50 mM EDTA in 30 mM sodium acetate buffer (pH 6.5); 50 mM sodium carbonate; 1 M KOH; and 4 M KOH in 20 mM $NaBH_4$. Extraction in each of these solvents containing NaN_3 (0.02% final concentration) was accomplished with agitation at 150 rpm for 10 h at room temperature followed by centrifugation for 20 min at 12,000 rpm. The supernatants designated as water-, EDTA-, and Na_2CO_3-soluble fractions were pectin-containing, while the 1 M and 4 M KOH-soluble fractions were hemicellulose-containing. The final insoluble material (cellulose residue) was collected by centrifugation as above, washed twice with 20 ml of distilled water and dialyzed. All fractions were subsequently dialyzed for 12 h against two changes of distilled water at 4˚C. In the case of Na_2CO_3-, and 1 M and 4 M KOH-soluble fractions, these were neutralized first with glacial acetic acid prior to dialysis. The pectin contents in water-, EDTA-, and Na_2CO_3-soluble fractions were determined by measuring the uronic acid content following the colorimetric method of Blumenkrantz and Asboe-Hansen (1973) at A_{520} with D-galacturonic acid as a standard. The total neutral sugar in hemicelluloses (KOH soluble fractions) and the acid-solubilised sugar content of the insoluble residue (cellulosic residue) were determined by using the anthrone assay of Dische (1962) at A_{620}. Glucose was used as a standard.

Statistical analysis

Experiments were laid out in a completely randomized design. Comparisons of means were made using the Duncan Multiple Range Test at $P < 0.05$. Statistical analysis was carried out using the SAS system for Windows version 6.12 (SAS Institute, Inc. Cary, NC).

Results and Discussion

Ethylene production, respiration rate and flesh firmness

Consistent with the previous reports on suppression of ethylene production with 1-MCP treatment (Fabi *et al*., 2007; Manenoi *et al*., 2007; Bron and Jacomino, 2009; Sañudo-Barajas *et al*., 2009), 'Kaek Dum' and 'Red Maradol' papaya fruits similarly exhibited a significant reduction in ethylene production when treated with 1-MCP at 10% yellow peel color (Figures 1A and B). Between the two cultivars, suppression of ethylene production was greater in 'Kaek Dum' than 'Red Maradol' and no peak was observed. In the case of 'Red Maradol', ethylene production was only suppressed by 1-MCP but not the attainment of the climacteric peak. In this cultivar, peak ethylene production of 1-MCP-treated fruit occurred at the same time as the control fruit (Figure 1B). The high ethylene production on the 4th day in both the control and 1-MCP-treated 'Red Maradol' fruit was followed by an abrupt decline. In 'Kaek Dum', decline in ethylene production was observed only in the control fruit (Figure 1A). In the case of 1-MCP-treated fruit, ethylene production exhibited a continuous increase, the highest production of which occurred on the 8th day. The sharp increases in ethylene production in both cultivars indicate that the autocatalytic ethylene

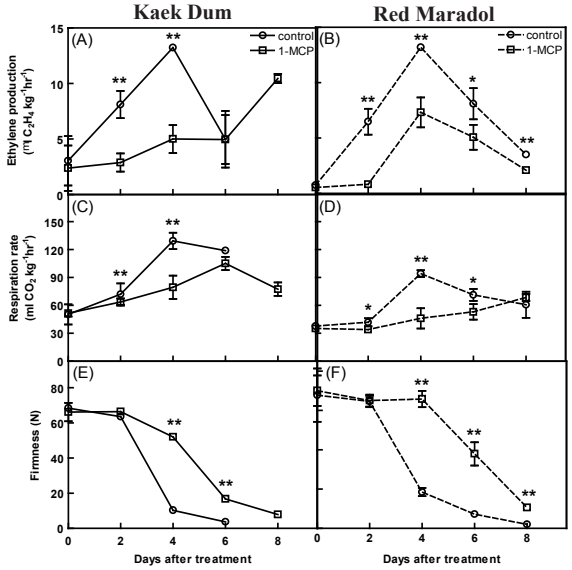

Figure 1. Changes in ethylene production (A and B), respiration rate (C and D), and flesh firmness (E and F) in control and 1-MCP treated fruits of 'Kaek Dum' and 'Red Maradol' during ripening at 25 ± 2°C. Data are means ± SD (n = 3). The asterisks indicate significant differences between treatment at the same day ($^{*}P<0.05$, $^{**}P<0.01$).

production was not completely inhibited in contrast to the report of Guillén *et al*. (2006) in tomato fruits where there was a complete absence of this sharp increase in ethylene production with 1-MCP treatment.

From Figures 1C and D, it will be noted that 'Kaek Dum' had higher rates of respiration than 'Red Maradol'. In both cultivars, 1-MCP likewise significantly retarded respiration rate and delayed the onset of the climacteric peak. This delay and suppression in respiration by 1-MCP has been reported in papaya (Fabi *et al*., 2007; Manenoi *et al*., 2007; Bron and Jacomino, 2009; Sañudo-Barajas *et al*., 2009). As in ethylene production, climacteric peak occurred on the 4th day in the control fruits in both cultivars followed by a decline. 'Kaek Dum' exhibited slight increases which peaked on the 4th day followed by a decline. On the other hand, 'Red Maradol' exhibited a continuous increase in respiration rate though at a slow rate. The delay in climacteric resulted in a 2-day extension of the shelf-life of treated fruits compared to the control. In 'Kaek Dum' shelf life was 8 days in 1-MCP-treated fruit and 10 days for 'Red Maradol' (data not shown).

At 10% yellow peel color, 'Red Maradol' was firmer than 'Kaek Dum' (Figures 1E and F). During ripening, the decline in fruit firmness was faster in 'Kaek Dum', about six fold (from 68 to 10 N), compared to only 4-fold (from 68 to 18 N) in 'Red Maradol' consistent with the typical characteristic of 'Kaek Dum' as a fast ripening cultivar. 1-MCP treatment retarded the decline in firmness of both cultivars and was more apparent in 'Red Maradol' where softening was greatly retarded during the first 4 days after 1-MCP treatment (Figure 1F). After this period, a rapid decrease in firmness was noted though 'Red Maradol' was still firmer than 'Kaek Dum' until the 8th day. In the case of the control fruits, rapid softening occurred within 4 days in both cultivars with 'Kaek Dum' becoming very soft on the 6th day and unacceptably soft (less than 3 N) on the 8th day, while firmness of 'Red Maradol' was still acceptable on this day.

One of the typical responses of papaya fruit to 1-MCP treatment is inhibition of softening (Fabi *et al*., 2007; Manenoi *et al*., 2007; Thumdee *et al*., 2010) similar to persimmon (Luo, 2007) and cantaloupe (Huber *et al*., 2007). The inhibition of softening was more apparent during the early stages of fruit ripening, in the case of our experiment, during the first four days of early ripening. As ripening progressed however, and with the observed increases in ethylene production even with 1-MCP treatment (Figures 1A and B), fruit began to soften fast. Blankenship and Dole (2003) noted that fruits exposed to 1-MCP treatment eventually initiate and recover ethylene production and sensitivity which was attributed either to the synthesis of new receptor sites or the dissociation of 1-MCP from the receptor. As shown in Figure 1A, ethylene production of 'Kaek Dum' was highest on day 8 which resulted in very soft fruit while that of 'Red Maradol' exhibited a decline on the 6th and 8th day after 1-MCP treatment. This further supports the observation that softening is one of the most ethylene-sensitive processes (Lelievre *et al*., 1997).

Fruit softening of papaya cvs. 'Gold', 'Rainbow' and 'Sunset' treated with 100 nl l^{-1} 1-MCP at color break stage was delayed and rubbery texture at the ripe stage was obtained (Fabi *et al*., 2007; Manenoi *et al*., 2007; Thumdee *et al*., 2010). In the cultivars that we tested however, 1-MCP applied at the same concentration and stage of ripeness delayed only the softening of the fruit and did not cause the occurrence of rubbery texture at the ripe stage. In our preliminary experiments, inhibition of softening and rubbery texture was obtained only when these cultivars were treated with higher concentrations (150 and 200 nl l^{-1}) of 1-MCP at 25 ± 2°C. Thus, for the Thai cultivars 'Kaek Dum' and 'Red Maradol', the suitable concentration of 1-MCP that should be applied at 10% yellow peel color is 100 nl l^{-1}.

1-MCP application did not delay the peel and pulp color development in both cultivars ($P > 0.05$) (data not shown) in contrast to the reported retardation of color change of Bron *et al*. (2006) and Manenoi *et al*.

(2007). However, even though peel and pulp color changes were not inhibited with 1-MCP treatment, firmness values of both cultivars remained fairly high unlike in the control fruits which softened fast as the peel changes color.

The increase in total soluble solids (TSS) content of papaya fruit during ripening was not significant (data not shown) since papayas have low starch content (about 0.5%) (Selvaraj *et al*., 1982). Moreover, TSS in treated fruits of both cultivars was unaffected by 1-MCP treatment ($P > 0.05$) (data not shown) similar to the results obtained by Fabi *et al*. (2007).

Effect of 1-MCP treatment on activities of cell wall degrading enzymes

PG, β-gal, and PME enzymes have a major role in textural changes in papaya fruits (Lazan *et al*., 1995; Ali *et al*., 2004), especially PG and β-gal are reported to be largely ethylene-dependent (Brummell and Harpster, 2001). When fruit were harvested at 10% yellow peel color, the activities of PG, β-gal, and PME were already detectable (Figure 2). Between the two cultivars, the activities of PG, β-gal and PME were higher in 'Kaek Dum' than 'Red Maradol'. The activities of these enzymes continuously increased with ripening of the control fruits in both cultivars accompanied by a decrease in firmness (Figures 2A-D and Figures1E and F) except for PME activity which remained almost unchanged (Figures 2E and F). This suggests that among the cell wall degrading eznymes, these two enzymes play a role or are related to softening of 'Kaek Dum' and 'Red Maradol' papaya fruits as observed also in banana (Xue *et al*., 1995) and pear (Ning *et al*., 1997). The increase in the activities of PG and β-gal were consistent with the increase in ethylene production (Figures 1A and B) and cell wall degradation (Figure 3) in control and treated fruits of both cultivars. This indicates that ethylene has a major role on the activity of enzymes involved in cell wall degradation (Brummell and Harpster, 2001). PME may not necessarily provide a major contribution in the softening of both cultivars as also observed in 'Eksotika' (Lazan *et al*., 1995) and 'Sunset' (Thumdee *et al*., 2010) papaya fruits. However, we thought that the softening of 'Kaek Dum' and 'Red Maradol' may be result from endoxylanase, which is strongly correlated with firmness loss during ripening (Paull and Chen, 1983; Manenoi and Paull, 2007; Thumdee *et al*., 2010). We did not monitor the activity of this enzyme in the present study, and will be an interesting subject of future investigations.

That 'Kaek Dum' is a fast softening cultivar is evident from the pattern of increase in PG and β-gal activities during ripening where it was higher and faster than that in 'Red Maradol'. Treatment with 1-MCP suppressed the increase in activity of the cell wall degrading enzymes (Figure 2). The treated 'Kaek Dum' and 'Red Maradol' fruits had significantly lower levels of PG and β-gal ($P < 0.05$) compared with the control (Figures 2A-D). Thus, the maintenance of tissue firmness during the first four days after 1-MCP treatment can be attributed to the suppression of activities of cell wall degrading enzymes, particularly PG and β-gal. The slight increase in the activities of PG detected on the 6th day of storage and the moderate increase in β-gal activity coincided with fast decline in firmness (Figures 1E and F and Figures 2C and D).

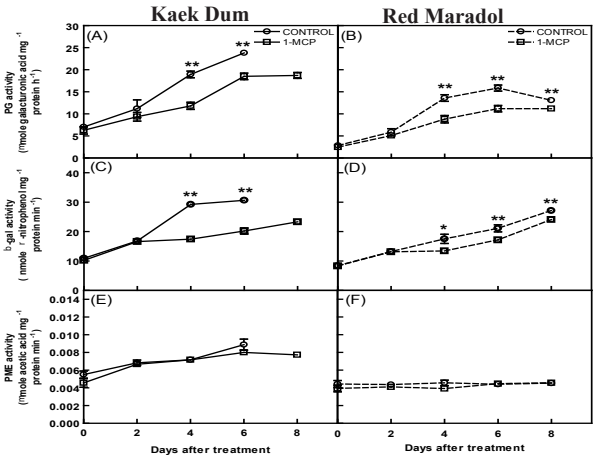

Figure 2. Activities of PG (A and B), β-gal (C and D), and PME (E and F) in control and 1-MCP treated fruits of 'Kaek Dum' and 'Red Maradol' during ripening at 25 ± 2°C. Data are means ± SD ($n = 3$). The asterisks indicate significant differences between treatment at the same day ($^{*}P<0.05$, $^{**}P<0.01$).

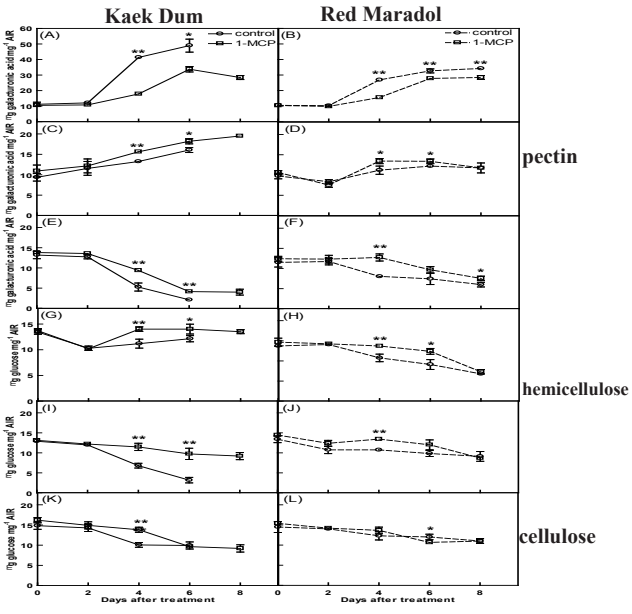

Figure 3. Contents of galacturonic acid in pectin extracts and total neutral sugar in matrix glycan extracts and cellulosic residue in control and 1-MCP treated fruits of 'Kaek Dum' and 'Red Maradol' during ripening at 25 ± 2°C. The AIR were extracted sequentially with water (A and B), EDTA (C and D), Na_2CO_3 (E and F), 1 M KOH (G and H), 4 M KOH (I and J) and cellulosic residue (K and L). Data are means ± SD ($n = 3$). The asterisks indicate significant differences between treatment at the same day ($^{*}P<0.05$, $^{**}P<0.01$)

With regard PME activity, it was higher in 'Kaek Dum' than 'Red Maradol (Figures 2E and F) consistent with the observed faster softening of the former cultivar. 1-MCP treatment did not affect the pattern of change in PME activity in both cultivars. Only a slight increase in PME activity was observed on day 2 in 'Kaek Dum' after which no apparent increase was observed. In 'Red Maradol', PME activity did not change as the fruit ripened and eventually softened.

As shown in Table 1, the activities of PG and β-gal correlated with the decrease in firmness in control fruit of both cultivars similar to the results in 'Sunset' cultivars (Thumdee *et al*., 2010). The PG activity in 'Kaek Dum' and 'Red Maradol' of non-MCP-treated fruit showed significant ($P < 0.05$) negative correlation ($r = -0.967^{*}$ and $r = -0.954^{*}$) with fruit firmness. A significant ($P < 0.05$) negative correlation with firmness was also obtained with β-gal activity ($r = -0.981^{*}$ and $r = -0.923^{*}$ in 'Kaek Dum' and 'Red Maradol', respectively). On the other hand, there were no significant correlations between flesh firmness and PME activity in both cultivars. When fruit softening was delayed with 1-MCP treatment, all of the cell wall degrading enzymes showed negative correlation with fruit firmness although significant correlations were obtained only with PG activity in 'Kaek Dum' ($r = -0.971^{**}$) and β-gal and PME activities in 'Red Maradol' ($r = -0.960^{**}$ and $r = -0.944^{*}$, respectively).

Modification of cell wall polysaccharides with 1-MCP treatment

The yield of AIR in the control fruits of both cultivars continually decreased as the fruits ripened and softened and that the AIR of 'Kaek Dum' was lower than that of 'Red Maradol' (data not shown). The decrease of AIR content in both cultivars indicated that large alcohol-insoluble polymers are being degraded to small alcohol-soluble polymers during ripening or might be a result of reduction in cell wall synthesis (Manrique and Lajolo, 2004; Owino *et al*., 2004). A higher recovery of AIR was obtained in 1-MCP-treated fruits compared to the control fruit, suggesting that 1-MCP treatment inhibited the degradation of large alcohol-insoluble polymers to small alcohol-insoluble polymers during ripening. The levels of AIR however, in 1-MCP-treated fruit decreased also with ripening.

The increase in water soluble pectins (WSP) in 'Golden' papaya fruit (Manrique and Lajolo, 2004; Shiga *et al*., 2009), banana (Duan *et al*., 2008), fig (Owino *et al*., 2004) and nectarines (Ortiz *et al*., 2010) was generally ascribed to the action of PG acting in concert with other hydrolytic enzymes (Sethu *et al*., 1996). This was observed also in the Thai cultivars 'Kaek Dum' and 'Red Maradol'. The faster softening of 'Kaek Dum' compared with 'Red Maradol' can be attributed to the higher solubilization of cell wall polysaccharides (Figure 3) especially the water-soluble pectins (Figure 3A). In 'Kaek Dum', WSP significantly increased from about 11 µg galacturonic acid mg^{-1}AIR at harvest to about 50 µg galacturonic acid mg^{-1}AIR on day 6 compared with only about 33 µg galacturonic acid mg^{-1}AIR in cv. 'Red Maradol' on the same day (Figures 3A and B). There was a significantly higher amount of water-soluble pectin in the control fruits than in the 1-MCP treated fruits in both cultivars (Figures 3A and B) consistent with the observed decrease in fruit firmness.

In the study of Chin *et al*. (1999), the levels of chelator (EDTA)-soluble and Na_2CO_3 fractions decline with ripening. Our results however, showed that EDTA-soluble fraction increased during ripening of cv. 'Kaek Dum' although it was lower in 1-MCP-treated than the control fruit. In 'Red Maradol', EDTA-soluble fraction initially decreased but as ripening progressed, this increased before decreasing again (Figure 3D). The contents of cell wall polysaccharide fractions that are soluble in EDTA, Na_2CO_3, and KOH of both cultivars were higher in 1-MCP treated fruits compared to the control (Figures 3C-J) and the acid-solubilised sugar content of the cellulose residue was higher in 1-MCP treated fruits when compared with control fruit of 'Kaek Dum' while the result in 'Red Maradol' was unclear between control and treated fruit (Figures 3K-L) and the reverse was true in the case of water-soluble fraction indicating limited solubilization of the cell wall as effected by 1-MCP treatment as has been reported in 'Golden' (Shiga *et al*., 2009) and 'Sunset' (Thumdee *et al*.,

Table 1. Pearson linear correlations between fruit firmness and hydrolase activities in control and 1-MCP treated fruits of cvs. 'Kaek Dum' and 'Red Maradol' papaya fruits during ripening at 25 ± 2˚C

Enzyme	Correlation between firmness and hydrolase activities							
	Kaek Dum				Red Maradol			
	Control fruit		1-MCP treated fruit		Control fruit		1-MCP treated fruit	
	Coefficient (r)	Sig (2-tailed)	Coefficient (r)	Sig (2-tailed)	Coefficient (r)	Sig (2-tailed)	Coefficient (r)	Sig (2-tailed)
PG	-0.967	0.033*	-0.971	0.006**	-0.954	0.012*	-0.781	0.119
β-gal	-0.981	0.019*	-0.860	0.062	-0.923	0.025*	-0.960	0.010**
PME	-0.829	0.171	-0.764	0.133	-0.773	0.125	-0.944	0.016*

* Correlation is significant at the 0.05 level.
** Correlation is significant at the 0.01 level.

2010) papaya fruit treated with 1-MCP. The levels of Na_2CO_3-soluble fraction exhibited a continuous decrease with ripening in both cultivars whether subjected to 1-MCP or not (Figures 3E and F). This is an indication of a shift in the extractable levels of different pectin fractions during ripening which is a reflection of sequential degradation of the cell wall polysaccharides (Chin *et al*., 1999). These increases in the contents of water- and EDTA- soluble pectins are prevalent during the ripening-associated softening of carambola (Chin *et al*., 1999), papaya (Manrique and Lajolo, 2004), durian (Khurnpoon *et al*., 2007) and blueberry (Vicente *et al*., 2007).

The hemicellulose fractions soluble in 1 M KOH and 4 M KOH were higher in 1-MCP treated fruits compared to control fruit in both cultivars (Figures 3G-J). In cv. 'Kaek Dum', the level of 4 M KOH soluble hemicellulose decreased which corresponds to an increase in the extractable level of 1 M KOH soluble hemicellulose during ripening (Figures 3G and I). In the case of 'Red Maradol', slight but continuous decrease in 1 M and 4 M KOH soluble fractions was obtained (Figures 3H and J).

The total neutral sugar content in cellulose residue of 'Kaek Dum' and 'Red maradol' decreased in the control and 1-MCP treated fruits (Figures 3K and L). The decrease in the acid-solubilised sugar content of the insoluble residue has been described in 'Sunrise Solo' papaya fruit (Manrique and Lajolo, 2004), 'Golden' papaya fruit (Shiga *et al*., 2009), cvs. 'Akatsuki' and 'Odoroki' peach fruits (Yoshioka *et al*., 2010) declined during ripening. This in contrast to the report of Paull *et al*. (1999) and Sañudo-Barajas *et al*. (2009) where cellulose content increased as fruit ripened.

Conclusions

'Kaek Dum' is a fast softening cultivar since the activities of cell wall degrading enzymes were higher compared with 'Red Maradol' which is a slow softening cultivar. These cultivars exhibited differences in response to 1-MCP with 'Red Maradol' responding more favorably to 1-MCP treatment than 'Kaek Dum'. 1-MCP delayed the onset of climacteric ethylene production and respiration rate and retarded the softening process of both cultivars. Delayed softening of 1-MCP treated fruit coincided with the low activities of the cell wall-degrading enzymes especially PG and β-gal enzymes. 1-MCP also reduced the solubilization of the uronic acid in pectic polymer, total neutral sugar in hemicellulosic polymers and acid-solubilised sugar content in cellulosic polymers in both cultivars. Further investigations are needed on expression of genes encoding these enzymes, and analysis of glycosidic linkage to elucidate the mechanism of differential softening of these two cultivars.

Acknowledgements

This work was funded by the Commission on Higher Education through a grant to Miss Witchaya Krongyut under the Strategic Scholarship/ Fellowships Frontier Research Network. Support from the Postharvest Technology Innovation Center at King Mongkut's University of Technology Thonburi (KMUTT) is also acknowledged.

References

Abu-Goukh, A.A. and Bashir, H.A. 2003. Changes in pectic enzymes and cellulase activity during guava fruit ripening. Food Chemistry 83 (2): 213-218.

Ali, Z.M., Chin, L. and Lazan, H. 2004. A comparative study on wall degrading enzymes, pectin modifications and softening during ripening of selected tropical fruits. Plant Science 167 (2): 317-327.

Blankenship, S. 2001. Ethylene effects and the benefits of 1-MCP. Perishables Handling Quarterly 108: 2-4.

Blankenship, S.M. and Dole, J.M. 2003. 1-Methylcyclopropene: a review. Postharvest Biology and Technology 28 (1): 1-25.

Blumenkrantz, N. and Asboe-Hansen, G. 1973. New method for quantitative determination of uronic acids. Analytical Biochemistry 54 (2): 484-489.

Bradford, M.M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. Analytical Biochemistry 72 (1-2): 248-254.

Bron, I.U. and Jacomino, A.P. 2009. Ethylene action blockade and cold storage affect ripening of 'Golden' papaya fruit. Acta Physiologiae Plantarum 31 (6): 1165-1173.

Bron, I.U., Jacomino, A.P. and Pinheiro, A. 2006. Influence of ripening stage on physical and chemical attributes of 'Golden' papaya fruit treated with 1-methylcyclopropene. Bragantia Campinas 65 (4): 553-558.

Brummell, D.A., Cin, V.D., Crisosto, C.H. and Labavitch, J.M. 2004. Cell wall metabolism during maturation, ripening and senescence of peach fruit. Journal of Experimental Botany 55 (405): 2029-2039.

Brummell, D.A. and Harpster, M.H. 2001. Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. Plant Molecular Biology 47 (1-2): 311-340.

Carrington, C.M.S., Greve, L.C. and Labavitch, J.M. 1993. Cell wall metabolism in ripening fruit. VI. Effect of the antisense polygalacturonase gene on cell wall changes accompanying ripening in transgenic tomatoes. Plant Physiology 103 (2): 429-434.

Chang, W.M. and Ming, T.L. 1998. Activity of softening enzymes during storage of sugar apple (*Annona squamosa* L.) at different temperatures. Food Preservation Science 24 (5): 319-323.

Chin, H., Ali, Z. M. and Lazan, H. 1999. Cell wall modifications, degrading enzymes and softening of carambola fruit during ripening. Journal of Experimental Botany 50 (335): 767-775.

Dische, Z. 1962. Colour reactions of carbohydrates. In Whistler, R.L. and Wolfrom, M.L. (Eds). Method in Carbohydrate Chemistry, p. 475-514. New York: Academic Press.

Duan, X., Cheng, G., Yang, E., Yi, C., Ruenroengklin, N., Lu, W., Luo, Y. and Jiang, Y. 2008. Modification of pectin polysaccharides during ripening of postharvest banana fruit. Food Chemistry 111 (1): 144-149.

Ergun, M. and Huber, D.J. 2004. Suppression of ethylene perception extends shelf-life and quality of 'Sunrise Solo' papaya fruit at both pre-ripe and ripe stages of development. European Journal of Horticultural Science 69 (5): 184-192.

Fabi, J.P., Cordenunsi, B.R., Barreto, G.P.M., Mercadante, A.Z., Lajolo, F.M. and Nascimento, J.R.O. 2007. Papaya fruit ripening: response to ethylene and 1-Methylcycropene (1-MCP). Journal of Agricultural and Food Chemistry 55 (15): 6118-6123.

Flores, F., Amor, M.B., Jones, B., Pech, J.C., Bouzayen, M., Latché, A. and Romojaro, F. 2001. The use of ethylene-suppressed lines to assess differential sensitivity to ethylene of the various ripening pathways in cantaloupe melons. Physiologia Plantarum 113 (6): 128-133.

Guillén, F., Castillo, S. Zapata, P.J., Martínedz-Romero, D. Valero, D. and Serrano, M. 2006. Efficacy of 1-MCP treatment in tomato fruit 2. effect of cultivar and ripening stage at harvest. Postharvest Biology and Technology 42 (3): 235-242.

Hagerman, A.E. and Austin, P.J. 1986. Continuous spectrophotometric assay for plant pectin methylesterase. Journal of Agricultural and Food Chemistry 34 (3): 440-444.

Huber, D.J., Jeong, J. and Lee, J. 2007. Softening and Ripening of 'Athena' cantaloupe (*Cucumis melo* L. var. reticulatus) fruit at three harvest maturities in response to the ethylene antagonist 1-methylcyclopropene. Hortscience 42: 1231-1236.

Karakurt, Y. and Huber, D.J. 2003. Activities of several membrane and cell-wall hydrolases, ethylene biosynthetic enzymes, and cell wall polyuronide degradation during low-temperature storage of intact and fresh-cut papaya (*Carica papaya*) fruit. Postharvest Biology and Technology 28 (2): 219-229.

Khurnpoon, L.S., Siriphanich, J. and Labavitch, J.M. 2007. Cell wall metabolism during durian fruit dehiscence. Postharvest Biology and Technology 48 (3): 319-401.

Lazan, H, Selamat, M.K. and Ali, Z.M. 1995. β-galactosidase, polygalacturonase and pectinesterase in differential softening and cell-wall modification during papaya fruit ripening. Physiologia Plantarum 95 (1):106-112.

Lelievre J.M., Latche, A., Jones, B. Bouyazen, M. and Pech, J.C. 1997. Ethylene and fruit ripening. Physiologia Plantarum 101 (4): 727-739.

Luo, Z. 2007. Effect of 1-methylcyclopropene on ripening of postharvest persimmon (*Diospyros kaki* L.) fruit. LWT-Food Science and Technology 40 (2): 285-291.

Maclachlan, G. and Brady, C. 1994. Endo-1,4-β-glucanase, xyloglucanase, and xyloglucan endo-transglucosylase activities versus potential substrates in ripening tomatoes. Plant Physiology 105 (3): 965-974.

Manenoi, A. and Paull, R. 2007. Papaya fruit softening, endoxylanase gene expression, protein and activity. Physiologia Plantarum 131 (3): 470-480.

Manenoi, A., Bayogan E.R.V., Thumdee, S. and Paull, R.E., 2007. Utility of 1-methylcyclopropene as a papaya postharvest treatment. Postharvest Biology and Technology 44 (1): 55-62.

Manrique, G.D. and Lajolo, F.M. 2004. Cell-wall polysaccharide modifications during postharvest ripening of papaya fruit (*Carica papaya*). Postharvest Biology and Technology 33 (1): 11-26.

Moya-LeÓn, M.A., Moya, M. and Herrera, R. 2004. Ripening of mountain papaya (*Vasconcellea pubescens*) and ethylene dependence of some ripening events. Postharvest Biology and Technology 34 (2): 211-218.

Nakamura, A., Maeda, H., Mizuno, M., Koshi, Y. and Nagamatsu, Y. 2003. β-galactosidase and its significance in ripening 'Saijyo' Japanese persimmon fruit. Bioscience, Biotechnology, and Biochemistry 67 (1): 68-76.

Ning, B., Kubo, Y., Inaba, A. and Nakamura, R. 1997. Softening characteristics of Chinese pear 'Yali' fruit with special relations to changes in cell-wall polysaccharides and their degrading enzymes. Scientific Reports of the Faculty of Agriculture 86: 71-78.

Ortiz, A., Seymour, G.B., Tucker, G.A. and Lara, I. 2010. Cell wall disassembly during the melting phase of softening in 'Snow Queen' nectarines. Postharvest Biology and Technology 58 (2): 88-92.

Owino, W.O., Nakano, R., Kubo, Y. and Inaba, A. 2004. Coordinated expression patterns of genes encoding cell wall modifying enzymes during ripening in distinct anatomical tissue regions of the fig (*Ficus carica* L.) fruit. Postharvest Biology and Technology 32 (3): 253-261.

Paull, R.E. and Chen, N.J. 1983. Postharvest variation in cell wall-degrading enzymes of papaya (*Carica papaya* L.) during fruit ripening. Plant Physiology 72 (2): 382-385.

Paull, R.E., Gross, K. and Qiu, Y. 1999. Changes in papaya cell walls during fruit ripening. Postharvest Biology and Technology 16 (1): 79-89.

Pressey, R. and Avants, J.K. 1973. Two forms of polygalacturonase in tomato. Biochimica et Biophysica Acta 309 (2): 363-369.

Sañudo-Barajas, J.A., Labavitch, J., Greve, C., Osuna-Enciso, T., Muy-Rangel, D. and Siller-Cepeda, J. 2009. Cell wall disassembly during papaya softening: role

of ethylene in changes in composition, pectin-derived oligomers (PDOs) production and wall hydrolases. Postharvest Biology and Technology 51 (2): 158-167.

Selvaraj Y, Subramanyan, M.D. and Iyer C.P.A. 1982. Changes in the chemical composition of four cultivars of papaya (*Carica papaya* L.) during growth and development. Journal of Horticultural Science 57 (1): 135-143.

Sethu, K.M.P., Prabha, T.N. and Tharanathant, R.N. 1996. Post-harvest biochemical changes associated with the softening phenomenon in *Capsicum annuum* fruits. Phytochemistry 42 (4): 961-966.

Shiga, T.M., Fabi, J.P., do Nascimento, J.R.O, Petkowicz, C.L. de O., Lajolo, F.M. and Cordenunsi, B.R. 2009. Changes in cell wall composition associated to the softening of ripening papaya: evidence of extensive solubilization of large molecular mass galactouronides. Journal of Agricultural and Food Chemistry 57 (1): 7064-7071.

Thumdee, S., Manenoi, A., Chen, N.J. and Paull, R.E. 2010. Papaya fruit softening: role of hydrolases. Tropical Plant Biology 3 (5): 98-109.

Vicente, A.R., Ortugno, C., Rosli, H., Powell, A.L.T., Greve, L.C. and Labavitch, J.M. 2007. Temporal sequence of cell wall disassembly events in developing fruits. 2. analysis of blueberry (*Vaccinium* species). Journal of Agricultural and Food Chemistry 55 (10): 4125-4130.

Xue, Y., Kubo Y., Inaba, A. and Nakamura, R. 1995. Effects of humidity on ripening and texture in banana fruit. Journal of the Japanese Society for Horticultural Sciene 64 (4): 657-664.

Yoshioka, H., Hayama, H., Tatsuki, M. and Nakamura, Y. 2010. Cell wall modification during development of mealy texture in the stony-hard peach "Odoroki" treated with propylene. Postharvest Biology and Technology 55 (1): 1-7.