Efficacy of 1-methylcyclopropene post-cutting treatment on fresh-cut papaya (Carica papaya L. cv. ‘Sinta’) storage quality using two packaging forms

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Abstract
The study was conducted to determine the efficacy of 1-methylcyclopropene (1-MCP) post-cutting treatment on fresh-cut ‘Sinta’ papaya (Carica papaya L. cv. ‘Sinta’) in maintaining its storage quality using two packaging forms commonly used commercially namely PET plastic tray wrapped with LDPE stretchable plastic film, and PET clamshell plastic containers. Fresh-cut ‘Sinta’ papaya cubes at Peel Colour Index 5 (yellow with tinge of green) were packaged using plastic tray wrapped with plastic film and clamshell plastic containers. 1-MCP gas was introduced post-cutting inside the packaging to a final concentration of 2.5 N L⁻¹. The fresh-cuts were stored at 10°C and 95% RH. Samples were evaluated for headspace C₂H₄ (HS-C₂H₄), polygalacturonase (PG) activity, total reducing sugars, firmness, color (as luminosity), water-soaking, visual quality rating (VQR) and microbial load. In both packaging types, lower PG activity and total reducing sugars were observed in 1-MCP treatments compared with controls at certain storage days. In plastic tray-film packaging, lower HS-C₂H₄ levels were observed in 1-MCP treated fresh-cuts compared with the control. In clamshell packaging, significant differences in water-soaking, luminosity and VQR at days 2 and 3 were observed between 1-MCP treated and control fruits. All of the treatments, on day 2 of storage, complied with European Union countries’ limits on aerobic plate counts (7 log), yeasts and molds counts (5 log) and coliform counts (3 log). To the best of our knowledge, this is the first study on the efficacy of 1-MCP post-cutting treatment on fresh-cut papaya of the ‘Sinta’ variety and also the use in a 1-MCP study of the packaging forms mentioned.

Introduction
Papaya is one of the economically important fruit crops in the Philippines (BPI, 2011). The Philippines ranks eighth in the world in total papaya production (PCARRD-DOST, 2003). With the globalized economy, the papaya industry must keep up with the latest trends in product types to be competitive both in export and local market. An emerging area for fruits is fresh-cut processing into fresh-cuts, 1-MCP treatment’s role will be to reduce the stimulatory effect of ethylene in the senescence stage especially after cutting when ethylene production shoot up in response to stress. Many studies have reported favorable results with 1-MCP treatment of fresh-cuts fruits such as in apples, mango, banana, papaya, pineapple, kiwi fruits, melon, avocado, etc. 1-MCP treatment, generally, contributes to delay in softening, color change, reduction of weight loss and water soaking, retention of nutritious compounds like ascorbic acid and lycopene (Budu and Joyce, 2003).

The positive effects endowed to whole fruits have encouraged the exploration of 1-MCP application into fresh-cut fruits. Since usually ripe fruits are the ones processed into fresh-cuts, 1-MCP treatment’s role will be to reduce the stimulatory effect of ethylene in the senescence stage especially after cutting when ethylene production shoot up in response to stress. Many studies have reported favorable results with 1-MCP treatment of fresh-cuts fruits such as in apples, mango, banana, papaya, pineapple, kiwi fruits, melon, avocado, etc. 1-MCP treatment, generally, contributes to delay in softening, color change, reduction of weight loss and water soaking, retention of nutritious compounds like ascorbic acid and lycopene (Budu and Joyce, 2003).

The principal problem restricting the shelf-life
of fresh-cut papaya is water soaking or translucency characterized by glassy texture of the flesh. Occurrence of this disorder has been associated with ethylene induction of cell wall degrading enzymes. Thus, inhibition of ethylene action would curtail water soaking. This was demonstrated in a study by Nimitkeatkai et al. (2008) where fresh-cut ‘Khage Dum’ papaya treated with 500 nL L⁻¹ had less severe water soaking than untreated slices. The same was observed by Ergun et al. (2006) with 1-MCP treated ‘Galia’ melons which suffered diminished water soaking than the control.

Among varieties of papaya with good potential for fresh-cut production is ‘Sinta’ papaya, a hybrid cultivar highly popular in the Philippines for its superior sensory attributes and the tree’s moderate resilience against papaya ring spot virus (UPLB-RDE, 2013). This is the first study on the application of 1-MCP on fresh cuts of the ‘Sinta’ papaya cultivar. In this study, 1-MCP was applied post-cutting to fresh-cut ‘Sinta’ papaya to determine the fruit’s physical, physiological, biochemical and microbial responses and consequently on storage quality, in two types of commonly used packaging for fresh-cuts namely, plastic tray over-wrapped with LDPE film, and clamshell plastic container. This is also the first study considering the effects of these two packaging types on fresh-cut papaya.

Materials and Methods

Plant material and sample preparation

The ‘Sinta’ papaya fruits were procured from a farm in Brgy. Soledad, San Pablo, Laguna at mature green stage after harvest. The fruits were soaked in a solution of Amistar, a broad spectrum fungicide, at 125 ppm active ingredient concentration for 5 minutes. The ripeness was assessed based on the Papaya Peel Color Index (PCI) for ‘Solo’ papaya made by the Postharvest Horticulture Training and Resource Center (PHTRC), UPLB. The papaya fruits were allowed to ripen until PCI 5 (yellow with tinge of green) for fresh-cut processing.

The papaya fruits were disinfected using chlorinated water (150 ppm), then peeled, deseeded and cut into cubes. The papaya cubes measuring approximately 1.6 ± 0.2 cm on all four sides, were packed into two types of packaging: PET plastic tray wrapped with LDPE stretchable plastic film (TF packaging) and PET clamshell (CS) packaging. Treatments were comprised of a 1-MCP treated and control packs for each of the two types of packaging. The 1-MCP powder carried the brand name ‘AnsipTM’ with 0.43% 1-MCP and density of 2.24 g/L. The plastic tray and clamshell container were both made of clear PET plastics while the plastic film was made of low density polyethylene (LDPE). Each pack had a net weight of 200 ± 1 g.

1-MCP treatment and cold storage

Right after packaging, the samples were brought to the cold storage room maintained at 10 ±1°C with relative humidity of 95%. 1-MCP gas at a concentration of 2.5 nL L⁻¹ was prepared by dissolving the calculated amount of 1-MCP powder in water inside an evacuated 1 L volumetric flask. Using a 1 mL disposable plastic syringe, the packages for 1-MCP treatments were injected with 1-MCP stock gas to a final concentration of 2.5 nL L⁻¹ based on the empty volume of the packaging. The edges of the packaging were sealed with tape to ensure that there is no gas leakage.

The sampling and analyses were conducted every day until the limit of saleability which corresponds to visual quality rating (VQR) of 3 was reached. This covered a period of 3 days. Samples were analyzed for physiological and physical parameters namely water-soaking, visual quality rating, color, firmness and headspace gas accumulation (C₃H₄ and CO₂). Biochemical parameters include total reducing sugars and polygalacturonase activity. Microbial
evaluation include aerobic plate count, yeasts and molds count and total coliform count. Experimental design followed was CRD.

**Measurement of headspace ethylene (HS-C₄H₂) and carbon dioxide (HS-CO₂)**

Headspace gas was withdrawn using 1mL syringe. The HS-C₄H₂ was determined using gas chromatograph equipped with flame ionization detector (GC-FID). The GC-FID has the following settings: injection port temperature- 120°C, column temperature - 100°C, column length - 2.0 m, inner diameter: 3.0 mm, and gas flow rate: 35 mL/min. For HS-CO₂ and HS-O₂, a gas chromatograph with thermal conductivity detector (GC-TCD). The GC-TCD has the following settings: injection port temperature - 90°C, column temperature - 50°C, gas pressure - 1.25 kg/cm². Amounts of headspace gases were calculated as:

\[
\text{C}_4\text{H}_4\text{CO}_2 = \frac{\text{peak height of sample x std. C}_4\text{H}_4\text{CO}_2 \text{ concentration (ppm)}}{\text{peak height of std. (1ppm)}}
\]

**Water soaking or translucency**

This was based on the estimated collective surface area that exhibit translucency or glassy appearance. The degree of water soaking was rated based on the scale set by the researcher as follows: (1) none (0%), (2) slight (5-10%), (3) moderate (11-15%), (4) above moderate (16-30%) and (5) severe (>30% of fruit surface).

**Color measurement**

Color as luminosity \(L^+\) was measured using Capsure Palette Chromometer. Each side of the papaya cube was measured for color. Three cubes per replicate were sampled.

**Visual quality rating (VQR)**

This parameter reflects the overall acceptability of a sample’s appearance. Sample quality was evaluated applying the scores described: (9,8) excellent, field fresh, (7,6) good, defects minor, (5,4) fair, defects moderate, (3) poor, defects serious, limit of saleability, (2) limit of edibility and (1) non-edible under usual conditions. This is the VQR scale established and being used by PHTRC.

**Total reducing sugar**

To determine the total reducing sugar, the Nelson-Somogyi method with minor modification by Krishnaveni et al. (1984) was employed. Twenty (20) g fresh sample was soaked in 40 mL hot 80% ethanol for 10 minutes, homogenized and filtered using ordinary filter paper. Extraction was repeated and the filtrates pooled together to obtain the total volume.

Before the assay, the alcohol was evaporated by keeping the extract on a water bath at 80°C. Ten (10) mL of water was added to dissolve the sugars. A 0.1 ml aliquot of alcohol-free extract was obtained and added with 2 ml of distilled water. One (1) mL of Copper Reagent A (2.5 g Na₃CO₃, 2 g NaHCO₃, 2.5 g KNaC₇H₂O₆•4H₂O) and 20 g Na₂SO₄ dissolved to 100 mL with dH₂O and Copper Reagent B (15 g Cu₂SO₄ and 1 drop of H₂SO₄ dissolved to 100 mL with dH₂O) mixture (24 parts: 1 part) was added to each tube. The tubes were heated in a boiling water bath for 10 minutes. After cooling the tubes, 1 mL of arsenomolybdate reagent was added. Distilled water was added to increase the volume of the test solutions to 10 mL. The individual absorbance was measured at 620 nm. Reducing sugars (%) was obtained using glucose as standard.

**Polygalacturonase (PG) activity**

Fifty (50) grams of sample was homogenized in 4 parts cold (4°C) 6% NaCl solution for 2 minutes. The homogenate was filtered using cotton. The filtrate was centrifuged (5500 rpm, 4°C, 20 min) to get a clear supernatant. To determine the PG activity of the supernatants, the method developed by Acranante et al. (1991) and modified by Jiang et al. (2003) was used. The enzyme solution (0.5 mL) was mixed with 1.5 mL of substrate solution composed of 0.225 g polygalacturonic acid dissolved in 50 mL 40 mM acetate buffer with pH of 5.0. A 2 mL blank enzyme solution was prepared from heat-denatured enzyme solution. The tests mixtures and the corresponding blanks were incubated in a rotating water bath at 37°C temperature for 2 h. Next, 15 mL of distilled water was added to 1mL of each of the reaction mixtures. An aliquot of 0.1 mL from each diluted mixture was tested for reducing sugar through the Nelson-Somogyi method. PG activity was measured by the amount of sugars released using the following equation:

\[
\text{PG Activity} = \frac{\text{sugar value(mg)} \times 10^{-3} \text{g}(\text{160})}{\text{(U/g FW)}} = \frac{\text{(4.875ml/g FW)}}{(\text{2hr})(0.5ml)}
\]

**Firmness measurement**

A hand-held penetrometer (MF push-pull scale; max. limit of 100 lbs max; graduation of 0.5 lbs) was used. The penetrometer had the flat type plunger. To get the firmness, the penetrometer plunger was pressed perpendicular against the sample enough only to sink the whole plunger disk.
Microbiological analyses

Twenty-five (25) grams of analytical unit per replicate of a sample treatment was aseptically taken out from the package. Then, 225 ml of sterile 0.1% peptone water was added and the sample was homogenized. Decimal dilutions of $10^{-2}$, $10^{-3}$, and $10^{-4}$ from the original dilution were made.

Plating for aerobic plate count (APC), yeasts and molds (YM), and coliform count

The method by AOAC (1990) was used. One (1) mL of aliquot from each dilution was transferred to each of the duplicate plates for APC, YM and coliform count. Consequently, 12-15 mL of plate count agar (PCA) for APC, potato dextrose agar (PDA) for YM and violet red bile agar (VRBA) for coliform detection and enumeration were cooled to 45°C then poured into the plates. The plates were agitated to uniformly mix the inoculum and the agar medium. When the agar had solidified, the plates were incubated in an inverted position at 35°C for 24-48 h for APC and coliforms and at ambient temperature for 3 to 5 days for yeasts and molds. Pinpoint size colonies in the PCA and PDA were also counted. On the other hand, only colonies with purple-red color, 0.5 mm or larger and surrounded by precipitated bile acids were counted in the VRBA medium.

Interpretation of results

Statistical analysis of the data was conducted with the aid of SPSS version 20 software. The continuous and ratio data such as headspace ethylene and CO$_2$ were interpreted through Welch-Anova post hoc Games-Howell while ordinal data such as the visual quality rating (VQR) were analyzed through Kruskall-Wallis post hoc Mann-Whitney at 95% confidence level (CL).

Results and Discussion

Headspace ethylene (HS-C$_2$H$_4$) and CO$_2$ (HS-CO$_2$)

Headspace ethylene in all treatments declined during storage. Lower HS-C$_2$H$_4$ were observed in 1-MCP treated fresh-cuts packaged in plastic tray overwrapped with film (TF) compared with the control until day 3 storage (Figure 2A), though were not statistically significant. In the clamshell (CS) packaging, almost the same values were obtained for both 1-MCP treatment and control. The decline in ethylene production during storage is due to the post-climacteric status of the ‘Sinta’ papaya when it was processed. At PCI 5, the climacteric peak of ethylene production had already commenced and is expected to take a decline right after. 1-MCP application at this stage is expected to suppress ethylene action as well as its autocatalytic biosynthesis. Also, in the case of fresh-cuts, 1-MCP gassing late in the ripening stage would be beneficial to somehow offset the effect of residual ethylene and cushion the reinvigorated ethylene production due to the stress from cutting (Mao et al., 2007). This was observed in the TF treatment which exhibited lower HS-C$_2$H$_4$. The same was observed in other climacteric fresh-cut fruits like kiwi fruit (Mao et al., 2007), partially ripe ‘Kent’ mango (Vilas-Boas and Kader, 2006), ‘Solo’ papaya (Manenoi et al., 2007) and ‘Raf’ tomato (Guillen et al., 2006).

In CS treatment however, this was not observed. The type of packaging is a factor that led to this discrepancy. The TF packaging reflected a more efficient gas entrapment which could have retained the 1-MCP at a longer period thus a longer exposure time for the fruits. In CS packaging however, loose points in the package could have led to gas escape. This situation stressed the normal un-hermetic characteristic of clamshell packaging for food. This was also evidenced by the much lower values of headspace gases in CS packaging than in TF.

As conveyed by the HS-CO$_2$ graph in Figure 2B, the treatments except TF-MCP produced diminishing amount of CO$_2$ until day 3 and significant rise on day 4. This decline in respiration also occurred in fresh-cut kiwi fruit and mango, ‘Grand Nain’ banana (Vilas-Boas and Kader, 2006) and watermelon (Saftner et al., 2007). According to Saftner et al. (2007), the
decrease in respiration was probably due to recovery from the physical damage inflicted by cutting and to excision-associated drop in resistance of the tissue to CO$_2$ gas. The increase in CO$_2$ production at the latter days of storage was attributed to the high microbial population on the fresh-cuts.

Color (L$_*$), water soaking and visual quality rating (VQR)

Measurements of colour as luminosity, L$_*$ (Table 1) reflects the degree of water soaking. The decreasing trend in luminosity of the ‘Sinta’ papaya treatments concurred with what prevailed in fresh-cut ‘Maradol’ papaya stored at 10°C wherein the luminosity decreased from day 0 to day 4 (Rivera-Lopez et al., 2005). In fresh-cut ‘Kent’ mango, luminosity likewise decreased during the 4 days storage. A lowering in luminosity signals intensification of darkening (Vilas-Boas and Kader, 2006). This darkening in papaya is a result of water-soaking (textural change) rather than because of enzymatic browning. Luminosity values at days 2 and 3 for TF-MCP were higher compared with TF-control which indicates more intense darkening in the control. This means that 1-MCP was able to suppress water soaking in TF packaging. The same was observed in half-ripe fresh-cut ‘Khage Dum’ papaya stored at 10°C, 1-MCP treatment (500 nL L$^{-1}$ for 6h at 10°C) after cutting and both before and after cutting tapered the degree of water soaking (Nimitkeatkai et al., 2008).

There was comparable progression of translucency or water soaking between the TF treatments (Figure 4A). In CS packaging, there was an observed significant increase in water soaking of the control at day 2. This retardation of water soaking caused by 1-MCP at day 2 coincides with the significantly higher level of total reducing sugars of the same treatment at the same day compared with the control. These reducing sugars are released from pectin degradation, leading to cell wall breakdown physically manifested as translucency.

The TF treatments showed relatively equal rates of visual quality deterioration until day 3 (Figure 3B). Though TF packaging provided a longer contact time of 1-MCP with the fruits, the confinement of other gases such as CO$_2$ seemed to overrode the effect of 1-MCP which resulted to faster deterioration and microbial growth. On CS treatments, no significant difference in VQR was observed until day 2. On day 3, CS-MCP had significantly higher VQR than the control. This indicates a slight delay in deterioration of papaya treated with 1-MCP and packaged in clamshell containers.

Polygalacturonase (PG) activity, total reducing sugars (TRS) and firmness

PG activity of the TF-MCP and TF-control varied significantly at days 2 and 3, the value for TF-MCP being higher (Figure 4A). 1-MCP seemed to enhance PG activity in this case. However, in other studies such as of Sañudo-Barajas et al. (2009), 1-MCP treatment of ‘Maradol’ papaya with 20-25% yellowing suppressed the increase in PG activity. In the experiment conducted by Jeong and Huber (2004) on avocado fruit, 1-MCP treatment denied the actions
of both the endo-β-1,4-glucanase and PG which led to higher firmness retention (Paliyath et al., 2008). For the CS packaged treatments, PG activity significantly differed only at day 2, where a higher value was also obtained in CS-MCP. On the other hand, the decreased PG activity from day 1 to day 3 compared to the initial value for all treatments could have been innate to papaya as Paull and Chen (1997) found the PG activity of ‘Solo’ papaya weakened at the post-climacteric or senescence stage. The TRS levels of TF treatments vary significantly at day 1 but for the CS treatments, at days 1 to 3 (Figure 4B). TRS were generally higher in the presence of 1-MCP. Reducing sugars are products of the action of PG on pectin. The high PG activity at day 2 was reflected as high TRS level at day 3, when the full action of PG was reflected the following day as hydrolyzed free sugars. These support the observed action of 1-MCP on PG.

Firmness values were generally higher for the 1-MCP treatments but did not vary significantly except for the CS treatment at day 3 (Figure 4C). Though PG activity was enhanced by 1-MCP, this did not immediately translate physically as enhanced softening. In other fruits, 1-MCP treatment of kiwi and partially ripe ‘Kent’ and ‘Keitt’ mangoes slices reduced the softening (Vilas-Boas and Kader, 2006). The action of PG was rather manifested as water soaking, which is more evident in papaya.

Microbial quality
The aerobic plate count of the TF-MCP was significantly lower than the control at day 2 while those of the CS treatments did not vary significantly (Table 2). Yeasts and molds were however significantly higher in TF-MCP compared with the control at day 2. This illustrates possible indirect control of deterioration by 1-MCP by affecting microbial growth. Pertaining to the microbial quality, 1-MCP application according to Toivonen (2008) can regulate senescence associated decay and microbial proliferation.

Pathological disorders may be abated or worsened by 1-MCP treatment. In citrus fruits, incidence of mold rots and stem rots rose (Marcos et al., 2005). Janisiewics et al. (2003) reported that 1-MCP treated apples had lower resistance to bitter rot and blue mold compared to untreated fruit. For plums, 1-MCP treated fruits showed less brown rot infection caused by Monilinia laxa (Menniti et al., 2004). In strawberry, low concentrations of 1-MCP imparted the fruit with higher immunity against decay but 1-MCP concentrations of 500-1000 nL L⁻¹ accelerated the decay (Jiang et al., 2001).

In the shelf-life study by O’Connor-Shaw et al. (1994), the fresh-cut papaya kept at 10°C and evaluated on day 2 had an APC and coliform count

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<th>Treatments</th>
<th>Day 0</th>
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<td>Plastic tray-film (Control)</td>
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Microbiological counts are in log. Values within the same column accompanied by a common letter are not significantly different based on Welch ANOVA post hoc Games-Howell at 95% CL.
of 8 log and 6 log, respectively. These are higher compared to the counts obtained by this current study. Meanwhile, the APC, YM and coliform counts of all the treatments settled within the 7 log, 5 log and 3 log limits, respectively, of the Spanish legal authorities and other EU countries (Sperber and Doyle, eds., 2009). On day 3 however, the counts are expected to exceed the limits, thus will already be unfit for consumption. Thus based on microbial quality, the shelf-life of the fresh-cut ‘Sinta’ papaya is 2 days when stored at 10°C. The study of O’Connor-Shaw et al. (1994) also determined the shelf-life of fresh-cut papaya stored at 4°C and 13°C at 2 days.

Conclusion

Fresh-cut ‘Sinta’ papaya has shown positive responses to 1-MCP post-cutting treatment. In plastic tray overwrapped with film packaging, lower headspace \( \text{CH}_4 \) levels were observed in 1-MCP treated fresh-cuts. Significantly lower total reducing sugars were also obtained in 1-MCP treatment at days 1 and 2 while significantly higher PG activity was obtained in the 1-MCP treatment. Bacterial growth was significantly retarded by 1-MCP at day 1. No significant differences in headspace \( \text{CO}_2 \), water-soaking, VQR and luminosity were noted.

In clamshell tray packaging, significant differences in water-soaking and VQR at days 2 and 3, respectively, were exhibited by 1-MCP treatment. 1-MCP treatments were also observed to have significantly lower headspace \( \text{CO}_2 \) at days 1 and 3. No significant differences in the other parameters were noted. Results of this study have demonstrated that 1-MCP elicits positive effects on some storage quality parameters of fresh-cut ‘Sinta’ papaya though responses in both packaging types varied. Hence, 1-MCP could be a potential tool in maintaining the storage quality of fresh-cut ‘Sinta’ papaya using both packaging types.

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