THE RIPENING OF CARICA PAPAYA L. AS AFFECTED BY 1-METHYLICYCLOPROPENE (1-MCP)

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ABSTRACT: 1-MCP is a novel postharvest tool which has been proven to delay ripening and senescence in plant tissues; however its effects on tropical fruits are now being researched. In an attempt to extend shelf life, papaya fruits (cv. ‘Tainung #2’) were subjected to varying regimens of 1-MCP treatments to determine the best combination of concentration and duration of exposure to 1-MCP. Trials using hot water and thiobendozole were also performed in an attempt to decrease the occurrence of postharvest decay. Fruit were fumigated in airtight plastic containers with 1-MCP, and the effects of the gas were judged using a variety of parameters including ethylene and carbon dioxide evolution, skin colour, and firmness. The most successful treatment (0.05 μl 1-MCP for 15 mins. at 20°C) increased the time to ripening by as many as 8-10 days. However, this time could be increased if postharvest disease infection could be controlled more effectively.

INTRODUCTION

Papaya (C. papaya), which belongs to the Caricaceae family, is cultivated throughout the tropics and is fast becoming one of the most important tropical fruits because of expanding export markets. Locally, ‘Tainung #2’ is one of the more popular cultivars, mainly because of its size (1 to 1.5 kg) and its sweet taste. ‘Red Lady’ fruits are also sold, but they are less common because of their size (3 to 5 kg).

Physiologically, papaya is a climacteric fruit with typical respiratory and ethylene production patterns. Morphologically, it is a berry normally composed of five longitudinal carpels with the flesh surrounding a central cavity (Pauli, 1993). The fruit is extremely susceptible to common postharvest disorders, chiefly because of the stage of development at which it is harvested (with 6-10% yellow on its skin). Its fruit then becomes prone to mechanical damage during the latter processes of postharvest handling. The postharvest loss of fresh papaya due to mechanical and physiological damage, diseases, and pests has been estimated to vary between 40 to 100% in different countries (FAO, 1983).

It has been reported that fumigation with 1-methylcyclopropene (1-MCP) a volatile cyclic alkene inhibits ethylene action in various plant tissues (Sisler and Serek, 1997, 1999). 1-MCP is easily prepared, stable, and has neither a detectable odour nor toxic residual effects. Like ethylene, 1-MCP is a strained molecule, and therefore, their binding mechanisms are similar. However, 1-MCP remains bound to the receptor preventing an active ethylene-complex from being formed (Sisler and Serek, 1997). The 1-MCP treated tissues eventually regain ethylene sensitivity and it has been suggested that new receptor sites are generated (Sisler et al., 1996; Sisler and Serek, 1999).

The use of 1-MCP in interrupting ethylene’s hormonal function has been well explored in ornamental horticulture. However, little is known about its effects, and therefore potential for
practical use in controlling ripening and senescence in fruits and vegetables, especially those of tropical origin.

Unfortunately, extended shelf life after 1-MCP treatment is associated with increased severity of external rots and blemishes, such as: anthracnose (*Colletotrichum* sp.); fruit rot (*Phytophthora* sp.) and stem end rot (*Botryodiplodia* sp.). Several studies have shown that hot water treatments coupled with fungicide application have significantly reduced the appearance of rots and blemishes. Today thiobendazole is one of the few available fungicides cleared for postharvest use in papaya. However, increased consumer awareness and concern about possible harmful effects of these fungicides have led to the resurgence of heat treatments - a non-chemical alternative which will not expose consumers to any significant health risks (Couey 1989). The most popular heat regimen applied to papaya is total immersion in water at 49°C for 20 minutes.

Thus, the main objective of this study is to establish a suitable postharvest regimen, which can extend the shelf life of *Carica papaya* L. using 1-MCP, while controlling the increasing postharvest infection, which occurs with extended storage.

**MATERIALS AND METHODS**

For each trial mature papaya fruit (‘Tainung #2’) at the stage of colour break were hand harvested at a private farm in the Caura Valley in North Trinidad and packed in cardboard boxes lined with shredded paper. Fruit were transported to the Food Biology Laboratory, Department of Food Production, at The University of the West Indies, St Augustine within 1 hour of harvest. At the laboratory, all fruit were hand washed in tap water to remove field heat and surface debris. 1-MCP stocks were synthesized using a commercial preparation of 1-MCP (Agrofresh, Rohmhaas, USA) with a predetermined amount of water. Fruit were placed on a 2-inch tall stainless steel rack in a plastic bucket with the 1-MCP stock and immediately sealed for the required time. They were then stored at 20°C and 85-90% relative humidity on a trolley lined with foam of 1-inch thickness. Any fruit with noticeable postharvest disease were discarded throughout the experiment.

The effects of 1-MCP’s on the fruits were judged using a variety of parameters including ethylene and carbon dioxide evolution, skin colour, firmness. Ethylene (*C*₂*H*₄) and carbon dioxide (*CO*₂) evolution were determined by gas chromatography (Finnigan, Model #9001; Austin Texas). Up until Day 5 (in Trial #2), the fruit were incubated in an airtight container and after 3 hours a 0.3 ml sample of gas was extracted from the headspace with a syringe via a rubber septum. On day 6 (in Trial #2 and throughout Trial #3) 0.3 ml samples were also withdrawn from the fruits’ cavity. The actual amounts of *C*₂*H*₄ and *CO*₂ produced were calculated against standard gas mixtures and expressed in ml/kg.

Skin colour was measured using a Minolta colorimeter (Model CR-200, Minolta Corp, Ramsey, N.J.) calibrated with a white calibration plate (CR-A43). Colour was expressed as L*a*b*, where increasing ‘L’ values represent increasing lightness, ‘a’ values were positive for red and negative for green, and ‘b’ values were positive for yellow and negative for blue (Singha et al., 1991). Flesh firmness was determined on the cut surface 1-inch thick slice of papaya using a Koehler digital penetrometer (Model #K 19550, Koehler Instrument Company, N.Y.). Values were expressed in mm 2sec⁻¹, where larger values represented softer fruit.

Each experiment design used was of completely randomized design with a factorial arrangement of variables. Data were subjected to Analysis of Variance using MINITAB (minitab
12) and the level of significance determined by the F-test. Comparison of the means was done using the least significant difference (LSD) method at the 5% level.

Trial #1

**Hot water treatment**

Fruit were subsequently treated with either hot water (49°C for 20 mins) and/or thiobendazole, air dried, fumigated with 5 μl⁻¹ 1-MCP for 14 hours and stored at 20°C on a trolley lined with foam of 1 inch thickness. Hot Water Treatment: Fruit were completely immersed in a hot water bath constructed from a 150 L drum equipped with two VWR Scientific thermo-regulators (VWR Scientific, model # 1122, Niles, Illinois) on opposite ends. To keep fruit submerged, a padded stainless steel grill was placed at water level.

**Thiobendazole (TBZ) Treatment**

Fruit were totally immersed in a solution of 1000 ppm thiobendazole for 2 minutes and left to air dry.

**Hot Water and TBZ Treatment**

Fruit were first subjected to the hot water treatment, which was immediately followed by complete immersion in 1000 ppm TBZ.

Fruit that were not treated with hot water or TBZ were observed as the control. Five papayas, which were fumigated with air, were also stored under identical conditions, to be compared with the controls (1-MCP only).

**RESULTS AND DISCUSSION**

At the end of this trial it was observed that hot water (49°C / 20 mins) treatment was the most successful in delaying the development of post harvest diseases in Tainung #2 papaya. Control fruit (5 μl⁻¹ 1-MCP only) remained unaffected for a maximum of 8 days before rapidly succumbing to stem end rots and external lesions infected with *Colletotrichum* sp, *Phytophthora* sp. and some other secondary organisms that were not identified. By day 16 all fruit had succumbed to at least one postharvest infection and was discarded (Figure 1).

When fruit fumigated with 1-MCP were compared to fruit that were fumigated with air and stored for the same period, it was apparent that the 1-MCP did in fact arrest the ripening process in the treated fruit (Figure 1). 1-MCP treated fruit did not lose their initial green skin colour and the flesh became spongy as the observation period lengthened. Carbon dioxide was detected in the headspace of the incubation container throughout the trial; however, no ethylene was recorded. None of the 1-MCP treated fruit attained any of the characteristics associated with ripened papaya fruit.

1-MCP has been found to delay the respiratory climacteric in fruits (Golding et al., 1998; Feng et al., 2000) and decrease the rate of carbon dioxide production in treated tissue (Fan and Mattheis, 2000), who reported that climacteric respiration requires continuous ethylene action. 1-MCP can also delay the onset of the ethylene climacteric because it strongly inhibits the usual increase in activity of the ethylene biosynthetic enzymes ACC synthase and ASS oxidase (Natasuka et al., 1997). Natasuka et al., (1998) and Mullins et al., (2000) further hypothesized
that 1-MCP could inhibit the positive feedback regulation of both ACC synthase and ASS oxidase genes during fruit ripening. Sisler et al., 1999; Golding et al., 1998; Harris et al., 2000, also reported on the ability of 1-MCP to prevent total degreening since completion of the process involves enzymes whose biosynthesis may be irreversibly disrupted by 1-MCP.

Rupasinghe et al., (2000), and Feng et al., (2000) reported success in delaying softening in apples and avocados respectively with 1-MCP. Enzyme activity was low throughout the storage period (up to 18 days). Despite the lower activity however, they noted that fruit treated with 1-MCP ripened and softened normally. In Trial #1 none of the 1-MCP treated fruit softened normally leading to the conclusion that the 1-MCP treatment was too severe.

The extensive amount of decay development observed in the 1-MCP treated papayas was similar to the findings of Ku et al., 1999 (strawberries). This may be explained by the fact that ethylene activates pathogen defense mechanisms in plants, including phytoalexin and lignin biosynthesis, and the activation of antifungal hydrolases such as chitinases and glucanases, so small amounts of endogenous ethylene aid to maintain a basic level of resistance towards environmental and pathological stresses (Ecker and Davis, 1987; Boller, 1988).

Trial #2

The fruit were treated with 0.0, 0.5, 1.0 or 1.5 μl⁻¹ 1-MCP (as described earlier) for either 1 or 3 hours in a factorial arrangement. Those fumigated with air were recognized as the control. The fruit were subsequently stored at 20-25°C and 85-90% RH.

RESULTS AND DISCUSSION

By day 6, the control fruit had attained optimal ripeness which lasted as long as 15 days and quickly succumbed to postharvest disease or became over ripe as evidenced by their total loss of chlorophyll (L* ≥ 60, a* ≥ 4.41, b* ≥ 41.1) and reduction in firmness (87.11mm/2sec). Figure 2 (a), and (b) clearly show that by as early as day 3 the control fruit were at a much more advanced state of chlorophyll degradation and respired at a greater rate than any of the treated fruit, even those subjected to the lowest concentration of 1-MCP (0.5 μl⁻¹) for the shortest period of time (1 h).

Both the concentration of 1-MCP applied and the duration of the fumigation significantly affected the amount of ethylene (p≤0.001) and carbon dioxide (p≤0.05) detected in the fruits’ cavities. Concentration and duration of exposure also had a highly significant effect on loss of firmness (p=0). All the remaining fruit lost their green colour by Day 15, but did not develop the usual orange-red skin colour. The highest L* and a* values recorded were 56.73 and 8.13 respectively, which were recorded on the control fruit since Day 6.

These observations suggested that the perception and/or production of ethylene was inhibited by 1-MCP thus delaying the onset of ripening. However, the poor development of quality after 15 days of storage, and the lack of significant differences between treatments suggest that even the lowest concentrations used were to severe. As such, both variables (concentration and duration of exposure) were manipulated in a subsequent trial in an attempt to discern their effects on the efficacy of 1-MCP action and to find a suitable fumigation regimen for papaya fruit.
Trial #3

The fruit were treated with 0.0, 0.05 and 0.25 μl⁻¹ 1-MCP for 2, 15 or 90 minutes in a factorial arrangement. Those fumigated with air were recognized as the control. The fruit were subsequently stored at 20-25°C. Observation continued until the supply of fruit was exhausted and analyses were performed at 4-day intervals.

RESULTS AND DISCUSSION

Controls achieved optimal ripeness by Day 8 as evidenced by skin colour (L*=63.1 and a*=11.2), loss of firmness (96.7 mm/2 sec) and ethylene (7.37 ml kg⁻¹) and carbon dioxide (24.62 ml kg⁻¹) evolution (Figure 3). However at this time, no significant difference was detected between the control and any of the treated fruit.

When the results obtained treated fruit were analysed in isolation, significant differences could be detected. Time, duration of exposure and concentration of 1-MCP all influenced skin colour L*, however no interactions between any of these factors were observed. On the other hand skin colour a* was influenced by the interaction between time and duration of exposure.

Another physical attribute of the papaya affected by the interaction between time and duration of exposure was loss of firmness. Even up to day 12, the fruit treated for 90 minutes had in no way begun to achieve the desired texture and those treated for 2 minutes had surpassed it. The most desirable texture was that of the fruit treated for 15 minutes.

CONCLUSION

The most suitable postharvest regimen for prolonging the shelf life of papaya is immersion in hot water (49°C for 20 minutes) followed by 0.05 μl⁻¹ 1-MCP for 15 minutes. While the fruits subjected to the other treatments did last as long, they were unsuitable either because they had passed the stage of optimal ripeness (2-minute treatments) or they had not yet attained the desired physical and biochemical standards achieved by the untreated papaya fruits but had developed extensive disease infection.

Another conclusion that can be drawn from the above observations is that manipulating the length of time that the fruit is exposed to 1-MCP may be a more effective and economical approach since it allows extremely minute concentrations to be applied successfully.
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Figure 1. Percentage decay of Tainung #2 papaya fruit during storage at 20°C
Figure 2a. Skin Colour ‘a’ of 1-MCP treated papayas

Figure 2b. Carbon dioxide evolution of 1-MCP treated papayas
Figure 3. Loss of firmness in 1-MCP treated papaya