

Novel Postharvest Treatment Reduces Ascorbic Acid Losses in Mango (*Mangifera indica* L.) Var. Kent

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Abstract: Problem statement: Mango is a tropical fruit that ripens very quickly; for this reason, there has been a continuous effort to develop postharvest technologies to extend its shelf life and quality. Among them, 1-Methylcyclopropene (1-MCP) is widely used because it inhibits the action of ethylene receptors. **Approach:** Changes in physicochemical parameters, bioactive compounds and cell wall degrading activities were evaluated during storage and ripening of fresh whole mangoes treated with 1-MCP (750 nL L⁻¹). Mature-green mangoes, cultivar Kent, untreated or treated with 1-MCP were evaluated for external quality, phytochemicals, Polygalacturonase (PG) and Pectin Methylesterase (PME) enzymatic activities during storage at 20°C for 2 weeks. **Results:** Concentration of ascorbic acid decreased during fruit ripening but 1-MCP-treated mangoes had reduced losses. Polygalacturonase and pectin methylesterase activities were reduced in the treated fruits as compared to untreated mangoes. Small changes in β-carotene were observed between treated and untreated fruits. **Conclusion:** 1-MCP affected the ripening process in “Kent” mango, reducing losses of ascorbic acid, this treatment is justified since it helps to maintain mango’s nutritional value during its shelf life.

Key words: Mango, 1-MCP, 1-methylcyclopropene, ripening, ascorbic acid

INTRODUCTION

Ripening of climacteric fruits such as mango (*Mangifera indica* L.) is followed by a peak in respiration and a concomitant production of ethylene. Ethylene is needed to trigger several ripening-associated processes, such as changes in color, texture, flavor and aroma of the fruit flesh (Giovannoni, 2004). Ethylene is a plant hormone that regulates many processes of growth and development, including ripening and is also an important mediator of plant responses to biotic and abiotic stresses (Wang *et al.*, 2002). This simple hydrocarbon molecule can diffuse in and out of plant tissues from both endogenous and exogenous (non-biological and biological) sources (Saltveit, 1999).

Ethylene affects the quality of harvested products and it can be helpful or harmful, depending on the produce and its ripening stage (Saltveit, 1999). Some commercial strategies used to withdraw deleterious effects of ethylene over produce are practiced. For instance, avoid exposure to ethylene, minimize ethylene production and action during produce ripening, harvest, storage and transport (Watkins, 2002). Also, by using compounds that inhibit ethylene action through interaction with ethylene receptors (Sisler and Serek, 1997).

1-MCP is a synthetic cyclic olefin capable of inhibiting ethylene action. It acts as a competitor of ethylene, blocking its access to the ethylene-binding receptors (Sisler and Serek, 1997). The affinity of 1-

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MCP for the receptors is approximately 10-times greater than that of ethylene and, therefore, compared with ethylene, thus it is active at much lower concentrations. 1-MCP is a gaseous nontoxic product that delays softening and improves post-storage quality of several climacteric fruits (Blankenship and Dole, 2003) and it is applied to extend their postharvest life. Moreover, 1-MCP is being used as a powerful tool to gain insights into fundamental processes that are involved in ripening and senescence, as well as to understand ethylene's action and responses (Watkins, 2006).

The effects of 1-MCP in fruits are variable depending on the fruit. For example, 1-MCP induced an increase in sugars (expressed as soluble solids) in papaya (Hofman *et al.*, 2001) and pineapple (Selvarajah *et al.*, 2001), but reduced sugars in kiwifruit (Boquete *et al.*, 2004) and nectarines (Bregoli *et al.*, 2005). Furthermore, 1-MCP had no effect on soluble solid contents of plums (Menniti *et al.*, 2004) and mamey sapote (Ergun *et al.*, 2005). Organic acids such as citric acid were reduced in 1-MCP-treated apple (Defilippi *et al.*, 2004) and were increased in guava (Bassetto *et al.*, 2005); malic acid in apple did not change due to 1-MCP treatment (Defilippi *et al.*, 2004; Kondo *et al.*, 2005). Respiration rates and ethylene production are reduced in fruits treated with 1-MCP most of the time (Jiang *et al.*, 2001; Dong *et al.*, 2002; Mwaniki *et al.*, 2005).

As for bioactive compounds, ascorbic acid content was higher in 1-MCP treated mango "Tommy Atkins" as compared to the untreated mango (Del Monte *et al.*, 2004). However, there were no significant changes in ascorbic acid content of mango "Guifei" (Wang *et al.*, 2006) and chayote (Cadena-Iniguez *et al.*, 2006) after 1-MCP treatment. β -carotene was little affected in 1-MCP treated "Spring Belle" peach fruits (Caprioli *et al.*, 2009).

Softening is a result of several metabolic changes during maturity and ripening in fruits and vegetables. Softening in climacteric fruit ripening is generally attributed to the degradation of the cell wall, particularly to the solubilization of pectins (Lohani *et al.*, 2004). These ultra structural and chemical changes may be the result of de novo synthesis of cell wall hydrolases, such as Polygalacturonase (PG), Pectin Methylesterase (PME), Pectate Lyase (PL) and cellulose, during fruit ripening (Brummell and Harpster, 2001).

1-MCP delays softening of most fruits, although some crop species are insensitive to this compound (Blankenship and Dole, 2003). For example, 1-MCP effectively delayed softening of banana (Watkins, 2006) and "Tonewase" and "Saijo" persimmon fruits (Harima *et al.*, 2003). Firmness is the measured parameter related to softening; in 1-MCP treated

"d'Anjou" pear, firmness was higher than in non-treated fruits (Argenta *et al.*, 2003). Better firmness was also observed in several 1-MCP treated fruits, including avocado (Feng *et al.*, 2000), strawberry (Jiang *et al.*, 2001), guavas (Bassetto *et al.*, 2005), plum cultivars (Martinez-Romero *et al.*, 2003), pear (Hiwasa *et al.*, 2003; Ekman *et al.*, 2004), watermelon (Mao *et al.*, 2004), banana (Lohani *et al.*, 2004), kiwifruit (Boquete *et al.*, 2004), apple (Defilippi *et al.*, 2004) and nectarine (Bregoli *et al.*, 2005).

In this context, while PME activity decreases in fruits during ripening, PG activity increases. However, the levels of enzymatic activity of PG and PME were lower in 1-MCP treated banana compared to the untreated fruits (Lohani *et al.*, 2004). In addition, PME provides substrate for PG and together they act mainly on the cell wall middle lamella (Koch and Nevins, 1989). In plums, exo-PG and endo-glucanase activities were lower in 1-MCP treated fruits when compared with untreated fruits; whereas Pectin Esterase (PE) and endo-PG were similar in treated and untreated plums (Dong *et al.*, 2001). Hiwasa *et al.* (2003) reported that ethylene is required for both initiation and progression of softening in pear fruit and they found that 1-MCP reduced the accumulation of PG1 and PG2 mRNAs of and endo- β -glucanase in the fruits (Hiwasa *et al.*, 2003). The reduction in accumulation of these transcripts paralleled the pattern of fruit softening. In contrast, in avocado exposed to 1-MCP, PG activity was reduced during ripening and cellulase activity was also low throughout the storage period. Despite the lower enzymatic activities of fruit treated with 1-MCP, they ripen and soften normally (Feng *et al.*, 2000). The aim of this study was to evaluate the effects of 1-MCP application on "Kent" mango fruits over external quality parameters, bioactive compounds and two cell-wall degrading enzymatic activities.

MATERIALS AND METHODS

Plant material and tissue sampling: Mature-green mango (*Mangifera indica* L. cv. Kent) (180 days after blooming) fruit of uniform size (approximately 500 g) were selected and hand harvested from the production region of Bacobampo, Sonora, Mexico (27°04'37.96" N and 109°27'05.56" W) and transported within 12 h to the laboratory in Hermosillo. Fruits had an average firmness of 137 N, 11.9 °Brix and color parameters L = 71.6, a = 2.7 and b = 72.5. Upon arrival, fruits were washed with chlorinated water (200 ppm sodium hypochlorite), air dried and the 1-MCP treatment was applied at 20°C.

MCP treatment and storage: A powder containing 0.14% of 1-MCP as active ingredient was used for fruit treatment (SmartFresh, AgroFresh Rohm and Haas, Philadelphia, PA). Two groups of fruit were treated, as follows: 50 mL of distilled water at 50°C were added to the flask containing a predetermined amount of powder (for untreated fruits, the flask did not contain powder) and was stirred until its complete dissolution, giving a 1-MCP concentration of 750 nL L⁻¹. 1-MCP application was performed by placing the fruit into hermetic chambers (202-L polyethylene boxes) and exposing them to the gas for 12 h at 20°C. After the treatment period, the chambers were opened and fruits were kept at 20°C for 2 weeks. At 3-day intervals, fruits were sampled to analyze physicochemical parameters, β -carotene, ascorbic acid and PG and PME activity.

Physicochemical parameters: Six fruit/treatment/sampling were used to measure pulp color by means of two readings on opposite sides along the equatorial region of the fruit using a colorimeter (Minolta CR-300, Osaka, Japan). Results were expressed as hue color angle and chroma. After that, weight loss was determined by the difference between the initial and final weights of each replicate using a digital Balance Voyager V0120 (Ohaus, USA). Pulp firmness was determined with a 10 mm point digital penetrometer (Chatillon DMF50, USA) carrying out two readings per fruit on opposite sides along the equatorial regions and the results were expressed in Newton (N). Sample juice was taken from these fruits to measure Titratable Acidity (TA); 5 mL of juice diluted with 50 mL of water was titrated with 0.1 N NaOH to pH 8.2 and expressed as percentage of citric acid, using an automatic titrator (Mettler DLG7, Switzerland). Total Soluble Solids (TSS) concentration was determined by direct reading of mango juice drop in a digital refractometer (Atago PR-101, Atago Co. Ltd., Tokyo, Japan) and results were expressed as °Brix.

Respiratory rate and ethylene production: Respiratory rate and ethylene production were evaluated daily, as previously reported (Watada and Massie, 1981), by incubating one fruit of known mass and volume in a 3.98 L hermetic flask during 1 h. One milliliter of headspace was withdrawn with a syringe and injected to a gas chromatograph Varian Star 3400 (Varian, USA). CO₂ and C₂H₄ concentration was calculated comparing the peak areas with known standards. Respiratory rate was expressed as mL CO₂ kg⁻¹ h⁻¹ and ethylene production as μ L C₂H₄ kg⁻¹ h⁻¹.

Bioactive compounds: β -carotene was determined as described by Mejia *et al.* (1988) and expressed as

milligrams per 100 g of pulp. Fresh tissue (1 g) was homogenized (Ultra Turrax T-25 Basic S1, Ika, Werke, USA) at 13500 rpm for 3 min with 15 mL of tetrahydrofurane, containing 0.4% butylated hydroxytoluene. The mixture was centrifuged for 15 min at 14 000 rpm, filtered through a 0.22 μ m filter and analyzed by HPLC using a Microsorb RP-C18, 3 μ m (4.6 mm \times 10 cm) column with a 3 cm guard column (Supelco, Mexico) and acetonitrile/methanol/tetrahydrofurane (58:35:7) as the mobile phase at a flow rate of 1.0 mL min⁻¹. β -carotene was detected by ultraviolet absorption at 460 nm.

Ascorbic acid was determined as described by Doner and Hicks (1981). Three samples per treatment were analyzed with a Varian 9012 (Varian, Mexico) liquid chromatography equipped with an L-4000 UV detector and an L-6000 pump. Ascorbic acid was detected using a water-NH₂ type °Bondapak analytical column (3.9 \times 300 mm, 10 μ m), 10 μ L loop injector. The mobile phase was acetonitrile: KH₂PO₄ 1 M (75:25 v/v), at a flow rate of 1.5 mL min⁻¹ and the detector wavelength was set at 268 nm. Ascorbic acid results were expressed in milligrams of ascorbic acid per 100 g of pulp.

PG activity: PG activity was measured from mango crude extract prepared by homogenizing 10 g of frozen mango pulp with 20 mL of 1% NaHSO₃ (pH 6.0) in an Ultra Turrax T25 at 24 000 rpm during 1 min. The homogenate was filtered twice and washed with 20 mL of 1% NaHSO₃, resuspended in 15 mL of 1 M NaCl and agitated during 3 h at 4°C. The activity was measured as the reducing groups released from polygalacturonic acid (Gross, 1982). Protein concentration was determined in the extracts according to the dye-binding Bradford (1976) assay, using bovine serum albumin as the standard.

PME activity: PME preparation of crude extract enzyme was done according to Wicker *et al.* (1987). Briefly, frozen mango pulp was homogenized in a 1:5 ratio (w/v) of pulp to 0.25 M Tris-Cl, 0.3 M NaCl, pH 8.0, at high speed for 15 s using a blender. The mixture was stirred for 1 h at room temperature and centrifuged at 16.000 g for 25 min at 4°C. The supernatant, constituting the crude enzyme extract, was stored at -20°C in small aliquots until needed. Analyses were conducted in triplicate on the enzyme extract at 25°C. Briefly, PME activity was measured potentiometrically by measuring free carboxyl groups formed as a result of enzyme action on pectin (Banjongsinsiri *et al.*, 2004). The reaction mixture in the standard method assay consisted of 5 mL of PME sample and 50 mL of a 1%

citrus pectin solution containing 0.1 M NaCl. During hydrolysis at 30°C, the pH was maintained at 7.5 by the addition of 0.045 N NaOH using an automatic pH-titrator (Mettler DL25, USA). One unit of PME activity was defined as the amount of enzyme that released 1 μ mol of carboxylic acid group per minute.

Statistical analysis: A variance analysis (two-way ANOVA) was performed using STATISTICA 8.0 software (Tulsa, OK, USA) and comparison of the treatments was made by means of Tukey's multiple range test at a significant level of $p \leq 0.05$.

RESULTS

Fruit quality, CO₂ and ethylene were not affected by the 1-MCP treatment: Skin color and firmness losses are the most appreciable changes that occur in mango fruit at temperatures above 10°C. These changes depend on the cultivar, storage conditions and postharvest treatment applied to produce. In the present study, the 1-MCP treatment applied to "Kent" mango did not affect TA, TSS, firmness, respiratory rate and ethylene production during storage at 20°C for 2 weeks (data not shown).

Bioactive compound contents of mango were affected by the 1-MCP treatment: Figure 1 shows that β -carotene levels decreased and then increased at the end of the storage period (2 weeks) in both treated and untreated mangoes. In 1-MCP treated mangoes the β -carotene levels decreased from 5.5-2 mg 100 g⁻¹ Fresh Weight (FW) then increased to 6.9 mg 100 g⁻¹ FW by day 15; while untreated mangos started at 4.2 mg 100 g⁻¹ FW then decreased to 2.1 and increased to 6.3 mg 100 g⁻¹ FW at the end of the storage period.

Figure 2 shows the Ascorbic Acid (AA) content of mangoes during storage at 20°C. The AA content in 1-MCP treated mangoes did not change significantly during twelve days of storage. Initial AA content of 1-MCP treated "Kent" mangoes was 31 mg 100 g⁻¹ FW, 28 mg 100 g⁻¹ FW on day 12 and the final AA content was 20 mg 100 g⁻¹ FW at 15 days after treatment. AA content in untreated mangoes started at 30 mg 100 g⁻¹ FW, decreased to 24 mg 100 g⁻¹ FW on day 9 and the final AA content was 17 mg 100 g⁻¹ FW after 15 days of harvest. Even though AA levels in mangoes decreased during storage, 1-MCP treatment prevented ascorbic acid losses from days 1-12, where the differences between treated and untreated mangoes were statistically different ($p \leq 0.05$).

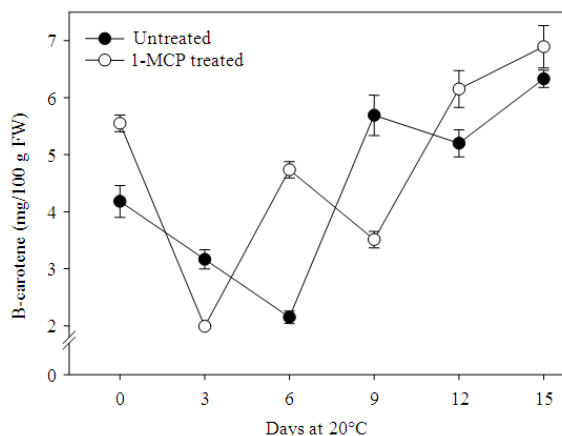


Fig. 1: β -carotene content of mango pulp untreated and 1-MCP treated and stored at 20°C for 15 days. Data are the mean \pm SE of three replicates

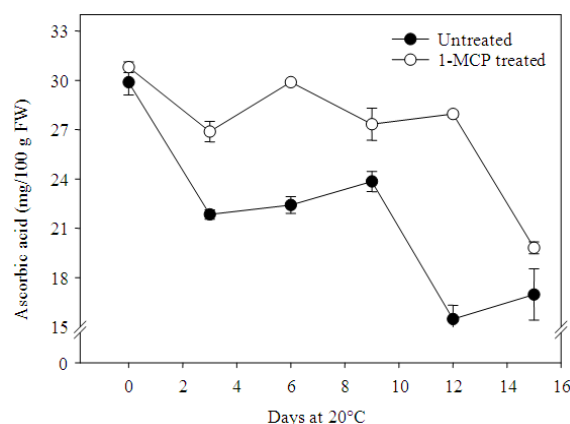


Fig. 2: Ascorbic acid content of mango pulp untreated and 1-MCP treated and stored at 20°C for 15 days. Data are the mean \pm SE of three replicates

PG and PME enzymatic activity: PG and PME are two key enzymes related to softening during fruit ripening. Figure 3 shows the PG activity profile in mango. In this study, PG activity gradually increased in 1-MCP-treated (2.7-6.1 units mg⁻¹ protein) and untreated (3.0-8.2 units mg⁻¹ protein) mangoes during ripening throughout the 15 day period. In 1-MCP treated mangoes PG activity was statistically similar ($p \leq 0.05$) to PG activity from untreated mangoes during the first 9 days of storage. However, PG activity of treated and untreated mangoes from day 12-15 was statistically different ($p \leq 0.05$). After 2 weeks at 20°C, PG activity for 1-MCP-treated mangoes was 6.1 units mg⁻¹ protein and 8.2 units mg⁻¹ protein for untreated ones.

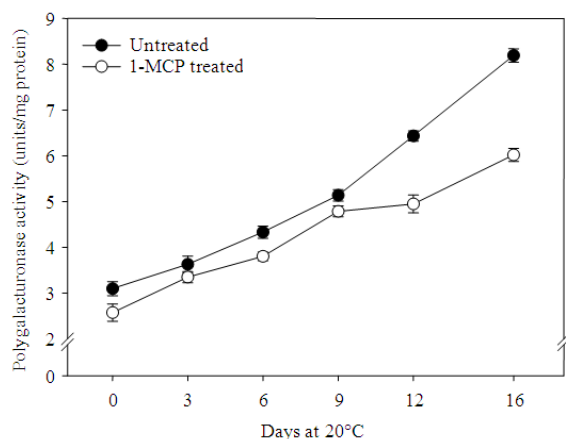


Fig. 3: PG activity from the pulp of mango “Kent” harvested at the mature green stage, treated with 1-MCP and stored at 20°C during 15 days. Data are the mean \pm SE of three replicates

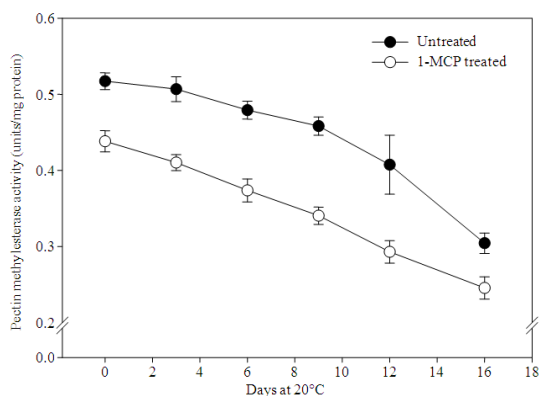


Fig. 4: PME activity of mango “Kent” harvested at the mature green stage, treated with 1-MCP and stored at 20°C during 15 days. Data are the mean \pm SE of three replicates

The PME activity profile is shown in Fig. 4; it gradually decreased in both treated and untreated mangoes during ripening (storage period). The PME activity was statistically different ($p \leq 0.05$) between 1-MCP-treated and untreated mangoes along this study. PME activity started at 0.52 and ended at 0.29 units mg^{-1} protein in untreated mango fruit, whereas 1-MCP-treated mangoes started at 0.44 units mg^{-1} protein and ended with 0.26 units mg^{-1} protein.

DISCUSSION

In a previous report (Osuna-Garcia *et al.*, 2005), 1-MCP applied at 300 nL L^{-1} dose (during 20 h at 13°C)

barely affected “Kent” mango’s respiration rate and ethylene production and did not affect either TSS or pulp color. However, treatment with 1-MCP retarded firmness losses of mango. A report for “Dashehari” mango showed that 1-MCP treatment reduced sugars and reduced firmness losses of mangoes as compared to untreated ones (Singh *et al.*, 2007). The fact that 1-MCP has different effects on appearance and other quality attributes could be due to the variety and maturity stage of the mango used, as well as on the concentration and time of exposure to 1-MCP.

Reported concentrations of 1-MCP applied to mango fruits are variable and it is necessary to make trial and error experiments to define appropriate amounts for the different cultivars. There is a report of a higher concentration (25 000 nL L^{-1}) of 1-MCP applied to “Kensington pride” mango during 14 h; although this amount prolonged mango shelf life for up to 5 days, it also enhanced stem rots in this fruit (Hofman *et al.*, 2001). In contrast, when another mango cultivar was treated during 12 h with 1-MCP at concentrations of 1000-100,000 or 200,000 nL L^{-1} , their ripening was suppressed to a higher extent (Jiang and Joyce, 2000). According to these results, we can conclude that sensitivity of mango fruit to 1-MCP is an important factor that has to be considered in order to optimize its application. These results suggest that application and evaluation of different concentrations, temperature and time of exposure to this compound to obtain the best conditions for each cultivar are needed.

Ornelas-Paz *et al.* (2007) reported that β -carotene content of different mango cultivars ranges from 0.4-2.8 $\text{mg } 100 \text{ g}^{-1}$. These authors reported that “Haden” mango has the highest carotene content with 3 $\text{mg } 100 \text{ g}^{-1}$ while “Kent” cultivar only has 1 $\text{mg } 100 \text{ g}^{-1}$. Differences in β -carotene content observed in mango could be attributed mainly to differences in maturation. The “Kent” mangoes used in the study by Ornelas-Paz *et al.* (2007) had a TSS of 16, whereas mangoes used in the present study, initially had a TSS of 12, reaching a final TSS concentration of 20, after 2 weeks at 20°C. The β -carotene content of 1-MCP treated mangoes stored for 6 and 12 days at 20°C was higher and statistically different ($p \leq 0.05$) from the content of untreated mangoes. This result suggests that 1-MCP treatment helps to keep the nutritional value of mango fruit.

The reduced losses of AA content in mango “Kent” are in agreement with a report for “Keitt” mango by Gomez and Lajolo (2008) and for “Tainong” mango (Wang *et al.*, 2009). The study of compounds that affect AA content in edible parts of plants is important since it

could lead to a better nutritional value and antioxidant capacity of food crops. Treatment of “Tommy Atkins” mangoes with 500 nL L⁻¹ of 1-MCP kept fruits with higher vitamin C contents (Alves *et al.*, 2004). These results demonstrate that this postharvest technology is useful in keeping the nutritional quality of this tropical fruit. At the moment, the mechanisms that control AA contents in tropical fruits are still unknown; therefore future studies on AA biosynthesis will lead to elucidate these mechanisms.

Yashoda *et al.* (2007) reported a gradual increase in the activity of PG in ripe “Alphonso” mango. The reduction of PG activity on 1-MCP treated “Kent” mangoes suggests that the biosynthesis or stability of this enzyme is affected by the gas; however, further studies are needed to know the mechanism involved in this event. Yashoda *et al.* (2007) reported similar PME activity in “Alphonso” mango during ripening. The PME activity shown by the 1-MCP-treated mangoes was lower than the untreated fruits, suggesting that such mechanism does occur.

CONCLUSION

As a conclusion, in this study, 1-MCP did not delay ripening and softening during postharvest in the study hereby and the main effect of 1-MCP was in reducing the losses of ascorbic acid during the postharvest stage; this by itself represents an important effect to take its use into consideration. Possible reasons for lack of effect in firmness are the dose and time of exposure. However, if a small dose is able to prevent nutritional value, it is worth investigating these effects. It would be of great interest to investigate whether this 1-MCP treatment of mangoes leads to a longer shelf life.

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