

Combination of 1-methylcyclopropene treatment and controlled atmosphere storage retains overall fruit quality and bioactive compounds in mango

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Abstract

BACKGROUND: Postharvest application of fungicide prochloraz and hot water dip are commercially practiced to control postharvest diseases in mangoes. Due to the increasing consumer demand for organically produced fruit, the search for natural environmental friendly alternative products and processes becomes important for the fruit industry.

This study evaluated the combined effect of 1-MCP (500 nL L⁻¹) and controlled atmosphere storage conditions (CA-1, 5% O₂ + 5% CO₂ or CA-2, 3% O₂ + 8% CO₂) on the maintenance of

fruit quality and bioactive compounds on hot water treated mangoes (cv. Kent) during postharvest storage.

RESULTS: In comparison to the 1-MCP+CA-1 treatment the 1-MCP+CA-2: reduced the incidence of anthracnose, weight and firmness loss; delayed the skin and flesh colour development; prevented the increase of soluble solids concentration/titratable acidity ratio, ethanol and acetaldehyde content; maintained the ascorbic acid, carotenoid, total phenolic content, flavonoid content, antioxidant scavenging activity in hot water treated mangoes. The untrained panel preferred 1-MCP+CA-2 treated fruit than the fruit subjected to other postharvest treatments adopted in this investigation.

CONCLUSION: Our investigation suggests that the combined effect of 1-MCP and CA-2 storage can be recommended as an alternative treatment to replace prochloraz application for hot water treated mangoes and it can be adopted commercially for organic export markets.

Keywords: 1-MCP; fruit quality; postharvest diseases; antioxidant; controlled atmosphere storage; mango

1. INTRODUCTION

The mango, *Mangifera indica* L. is an exotic fruit and popular among the consumers around the world due to its excellent flavour, attractive fragrance, beautiful colour, taste and nutritional value¹. 'Kent' is a popular cultivar among the European and Japanese consumers due to its attractive skin and flesh colour, higher sugar content and reduced fiber in the flesh². The rapid flesh softening after harvest, wounding of skin during harvesting and bad postharvest handling practices leads to postharvest decay during storage and affect the mango fruit quality³. Anthracnose (*Colletotrichum gloeosporioides* Penz. and Sacc.) and stem-end rot (*Lasiodiplodia theobromae* or *Dothiorella dominicana* or *Botryosphaeria* spp.) are the predominant postharvest

diseases that affect mango fruit quality ^{4, 5}. Control of these fungal diseases is prerequisite to extending the storage life and marketing period of mango fruit in both domestic and export markets. Control of postharvest decay due to quiescent infection is achieved commercially in South Africa by combination of hot water and fungicide prochloraz treatment ⁵. The heat treatment provides eradication activity while the residual protection is provided by prochloraz treatment. However, a residual protection against postharvest diseases is important after removal from cold storage at the retailer's market shelf.

Due to global concern over the often indiscriminate use of pesticides and its hazardous side effects on environment and human health, more stringent product registration requirements have been developed. Resistance to prochloraz in *Pseudocercospora herpotrichoides* isolates has been reported by Cavelier et al.⁶ Considerable variability in sensitivity among the *C. gloeosporioides* isolates from mango has been reported by Arauz ¹. Prochloraz solution for postharvest dip treatment are prepared in large tanks (~1500 L volume) and maintained for several days before the residue is disposed. Toxic waste disposal is costly exercise and hazardous waste poses serious environmental problems. Due to the increasing consumer demand for organically produced fruit, the search for natural environmental friendly alternative products and processes becomes important for the fruit industry.

Postharvest disease control through maintenance of host resistance can be achieved by shipping or storing fruit after harvest in controlled atmosphere storage (CA) ¹. On the other hand CA storage has been reported to extend the storage life of different mango cultivars by delaying fruit softening, postponing the onset of climacteric fruit ripening and changes related to quality parameters ⁷⁻¹¹. The application of CA with a reduced O₂ concentration of around 3 – 5 % and an elevated CO₂ concentration of 5 – 10 % are the suggested atmosphere regimes for a successful CA system for mango fruit ^{12, 13}.

The application of ethylene antagonist 1-methylcyclopropen (1-MCP) is a potentially useful tool for commercial application to reduce ripening process, maintain quality and extend shelf life of fresh produce ¹⁴. 1-MCP has been shown to delay fruit softening associated with rapid ripening in different mango cultivars ¹⁵⁻²⁴. However, mixed observations were reported on association of 1-MCP application and fruit decay. Therefore, 1-MCP must be considered as component of integrated control strategy and not be relied to give an adequate control ²⁵. The 1-MCP application was reported to retain the bioactive compounds (total phenolic compounds, ascorbic acid) and enhanced the anti-oxidant activity in tomato ²⁶.

Different postharvest treatments such as application of edible coatings, heat treatments, 1-MCP application, biocontrol agents, modified atmosphere and CA storage have been researched to control postharvest decay and to maintain the mango fruit quality during long term storage. However, combining the two technologies are effective in creating a set of conditions conducive to extend the storage life, while reducing or eliminating the deleterious effects on overall fruit quality, nutritional compounds and to improve the consumer appeal for marketing fresh fruits. To the best of our knowledge, no research work has been reported on influences of 1-MCP and CA storage conditions on postharvest disease control, physico-chemical parameters and bioactive compounds in mango cv. Kent.

Therefore, the objective of this research was to investigate the combined effect of 1-MCP with two different CA storage conditions, on retention of fruit quality, bioactive compounds and sensory properties in hot water treated mangoes (cv. Kent), after cold storage (for 18 days at 10 °C, 80 % RH) and ripening at 25 °C for 5-12 days

EXPERIMENTAL

Fruit

Mango 'Kent' fruit (950) were hand harvested at commercial maturity during early morning from an orchard situated in Hoedspruit, Limpopo, South Africa. After harvest, fruit were de sapped and transported to the on-farm laboratory within an hour and sorted for uniform maturity stage (mature green stage; firmness 11-13.5 kg; SSC/TA ~12-13.5; skin colour C* ~20.3 – 22.4, h° ~152.3-149.3); size (each weighing ~340-370 g) and absence of mechanical damage or diseases.

Hot water treatment

Cultivar 'Kent' were subjected to standard hot water treatment adopted by the South African mango industry for postharvest disease control [50 °C for 2 min in 20 dm³ tank equipped with RKCF4 temperature controller (Vizier Systems (Pty) Ltd, Cape Town, SA), 2.5 kW immersion heating elements and a 0.37 kW pump to maintain the turbulence of the dip preparation] prior to different postharvest treatments adopted in this study.

1- MCP treatment

Fruit subjected to hot water treatment were exposed to 1-MCP (500 nL L⁻¹) (SmartFreshTM powder, 0.14 g Kg⁻¹ active ingredient; Rohm and Hass, South Africa) treatment for 12 h at 20 °C and 85 % RH according to Singh et al. ²⁷ and Singh and Dwivedi ¹⁸. Pre-treatment with 1-MCP was performed for 108-110 fruit within 3-4 h after hot water treatment in a 150 L plastic gas-tight container and thereafter, the container was vented. Fruit were removed from the chambers and placed in commercial corrugated cardboard cartons.

Storage conditions

After different postharvest treatments, 'Kent' mangoes (six replicate boxes each containing six fruit) were stored in four CA chambers (60 L) containing two different gas compositions CA-1, 5 % O₂ + 5 % CO₂ and CA-2, 3 % O₂ + 8 % CO₂ storage conditions. Initial O₂ and CO₂ levels in the cabinets were established by a flow-through system, mixing N₂ (100 %) and O₂ (99.5 %) or N₂ and CO₂ via pressure regulators, then automatically controlled and regulated by the gas analyser (FC-701, Milano, Italy). The temperature and RH within the chamber was monitored with a Gemini data-logger using GTLM data-logger software version 2.1 (Gemini Data Loggers Ltd., Chichester, UK).

Treatments

'Kent' mango were selected randomly from the same freshly harvested consignment and dipped in hot water (50 °C for 2 min) and thereafter, hot water treated fruit were subjected separately to the following treatments; 1-MCP+CA-1; 1-MCP+CA-2; stand alone-CA-1, stand alone-CA-2, stand-alone-Wax, 1-MCP+Wax, commercial treatment and untreated control. Mangoshine^(R) wax (Citrashine PVT Ltd, Johannesburg, South Africa) is commercially used for waxing mangoes in South Africa. Commercial treatment includes hot water dip at 50 °C for 5 min, followed by 20 min dipping in a water bath containing prochloraz (1.8 g kg⁻¹), hot air drying (38 °C, 5 s) and spraying with Mangoshine^(R) wax.

All the above mentioned treatments had ten replicate boxes and each box containing six fruit. The fruit subjected to all treatments were stored for 18 days (simulation of commercial conditions for distribution) at 10 °C and 90 % RH. At completion of 18 days storage, fruit were removed from cold storage and the weight loss was determined. Thereafter, fruit were ripened at 25 °C for 5 days, 72 % RH under normal atmosphere condition. Due to the variability in ripening rates fruit subjected to combination treatments were held for additional 7 days at 25 °C after

ripening. Ripening time was calculated as the number of days after removal from cold storage until the fruit reached the eating soft stage (hand firmness rating 1- firm, unripe (5-6 Kg); 2- moderately firm, ripe (4.9- 3.5 Kg); 3-slightly firm and ripe (3.4-2.5 Kg); 4- soft and ripe (2.4-1.1 Kg); 5- very soft – over ripe (0.5-1 Kg).

The effect of different postharvest treatments on incidence of postharvest diseases was observed up to 12 days after ripening after removal from the cold storage. Overall fruit quality was evaluated by determining the skin and flesh colour, fruit firmness, soluble solid concentration (SSC) and titratable acidity (TA) on the 5th day after ripening for all treatments adopted in this investigation. Due to the variability in ripening rates observed in 1-MCP treated fruit the sensory evaluation by the untrained panel was carried out on the 10th day after ripening. The ascorbic acid, off-flavour producing volatiles (ethanol and acetaldehyde) and bioactive compounds (carotenoids, total phenolic, flavonoid content) and antioxidant scavenging activity were determined on the 10th day after ripening at optimum eating quality stage.

Fruit quality evaluation

Fruit (replicate boxes) subjected to all the treatments mentioned above were weighed before and after 18 days cold storage and after ripening at 25° C for 5 days. Data was expressed as percentage weight loss. Incidence of anthracnose was recorded as the ratio of fruit showing disease symptoms against the total number of fruit treated. Severity of anthracnose was demined according to a visual scale 0-no symptom, 1-10 % of fruit surface, 2-25 %, of fruit surface, 3-50 % of fruit surface, 4-75 % of fruit surface, 5- 100 % of fruit surface. Skin and flesh colour were measured (20 fruit per treatment) using a Minolta Chromameter (model CR-400; Osaka, Japan) colour analyser calibrated to a white porcelain reference plate. The

colour space coordinates L^* , a^* , b^* , hue angle (h°) arctangent (b^*/a^*), and chroma $(a^{*2} + b^{*2})^{1/2}$. Two spots on opposite sides of the fruit were measured and the mean of the two measurements were considered as one reading. Fruit firmness was assessed for 20 fruit per treatment on opposing sides of each fruit with a Chatillon penetrometer (Chatillon and Sons, New York, USA) equipped with a 6 mm diameter plunger capable of penetrating through the peel into the flesh.

A set of 20 fruit per replicate per treatment was randomly selected for SSC and TA, determination. The SSC was determined with a digital refractometer (Atago Co., Tokyo, Japan) and expressed in percentages. Percentage Titratable acidity (TA) was determined by titration of mango juice (10 mL) with 0.01 mol L⁻¹ NaOH solution to pH 8.1. The acidity was expressed as citric acid equivalent and the SSC/TA ratio was determined. Ascorbic acid and carotenoid content were determined from ten replicate fruit per treatment. Ascorbic acid content in the fruit juice was determined from 20 g flesh using 2,6-dichlorophenolindophenol titrimetric method. The results were expressed as mg 100 g⁻¹ FW. Carotenoid content was determined according to Lichtenthaler and Buschmann²⁸. Frozen mango flesh (0.3 g) was added to 10 mL of aqueous ethanol/ butylated hydroxytoluene solvent [5 ml:90 mL butylated hydroxytoluene (100 mg L⁻¹)] and diethyldithiocarbamate (200 mg L⁻¹) antioxidants to prevent carotenoids degradation extracted with 1 mL of acetone. Then the mixture was homogenized for 1 min and incubated at 4 °C in darkness for 1.5 h to extract the pigments. The homogenate was centrifuged at 16,000 × g for 15 min and 200 µL of supernatant were placed in 96-well plates. Ethanol/antioxidant solvent was used as a standard to calibrate the spectrophotometer. The absorbance was read at 470 nm (UV/VIS 1601 spectrophotometer (Shimadzu, Kyoto, Japan)). The concentration of total carotenoids was expressed as mg 100 g FW⁻¹.

Extraction of polyphenols

Mango fruit (20 g) from ten fruit per treatment of was homogenized separately with 15 mL of methanol/water (8 mL:100 mL v/v), using an Ultra-Turrax homogenizer (Ultra-Turrax, IKA Werk, Germany). The homogenate was sonicated for 15min and centrifuged at 10,000 rpm at 5 °C for 15 min. Thereafter, the sample was vacuum filtered through Whatman No. 1. The method was repeated twice for maximum extraction of polyphenols. The volume of the collected extracts was made up to 50 mL with methanol and further dilutions were made up with methanol /water (8 mL:100 mL v/v). The final concentration of the extract was 0.4 g mL⁻¹ of the original mango pulp and was used for total phenols, flavonoids and 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) assays ²⁹.

Total phenolic and flavonoid content

Total phenolic content (TPC) of the fruit extracts was determined using the Folin–Ciocalteu assay described by Singleton and Rossi ³⁰ with some modifications. A 40 µL aliquot of diluted fruit extract was mixed with 1.8 mL of Folin–Ciocalteu reagent. After 5 min of equilibrium time at 25 °C, 1.2 mL of (7.5 g 100 ml⁻¹) Na₂ CO₃ solution was added to the extract. The solutions were mixed and allowed to stand for 1 h at 25 °C and thereafter, the absorbance was measured at 765 nm using a UV/VIS 1601 spectrophotometer (Shimadzu, Kyoto, Japan). Total phenolic compounds were calculated using a standard curve of gallic acid and expressed as mg of gallic acid equivalents (GAE) 100 g FW⁻¹.

Total flavonoid content (TFC) was measured according to Dae-Ok and Chang ³¹. Crude fruit extract (1 mL) was mixed with 4 mL of deionised water, 300 µL NaNO₂ (5%) and allowed to stand for 5 min at 25 °C. Thereafter, 300 µL of AlCl₃ (10 g 100 ml⁻¹) and extracts were reposed by 1 min and 2 mL of NaOH (1 mol L⁻¹) were added to the extract. The volume was made up to 10 mL with deionised water. The mixture was shaken vigorously and the absorbance of the mixture was read at 510 nm using a UV/VIS 1601 spectrophotometer (Shimadzu, Kyoto,

Japan). The TFC were expressed on a fresh weight basis as mg of quercetin equivalents/100 gFW⁻¹.

DPPH free radical-scavenging assay

Antioxidant scavenging activity of the mango extract was measured using DPPH (Sigma–Aldrich, Johannesburg) scavenging assay according to Ribeiro et al.³² The aliquots (100 µL) of test sample (pulp extract at 0.05 µg mL⁻¹, at 1000, 2000, 3000, 4000 and 5000 µg mL⁻¹), were mixed with 5.0 mL of 0.1 mM DPPH in methanol. The control samples contained all the reagents except the extract. After vortexing for 1 min, the reaction mixture was allowed to stand in the dark for 30 min at 25 °C. Thereafter, the absorbance was measured at 515 nm using a model UV/VIS 1601 spectrophotometer (Shimadzu, Kyoto, Japan). Triplicate measurements were recorded and their percentage inhibition value was calculated according to the following equation:

$$\text{Scavenging activity (\%)} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} \times 100$$

Ethanol and acetaldehyde content

Mango flesh 1.5 g, (five replicates per treatment) was placed in 20 mL capacity amber-coloured tubes and incubated at 65 °C in a water bath for 15 min. Headspace samples of 1 mL were injected into a gas chromatograph (Agilent 6890N GC equipped with a flame ionization detector FID, Oven temperature 100 °C; injector temperature 110 °C; FID temperature 180 °C and N₂ was used as carrier gas; 2 m 1/8 in. Column- chromosorb stainless steel packed with 80/100 µm mesh Porapak 101). Retention times and standard curves of ethanol and acetaldehyde in water solutions were used for peak identification and

quantification. Fruit subjected to CA-2, 1-MCP+CA-2, Wax, 1-MCP+Wax, commercial treatment and untreated control were analysed for ethanol and acetaldehyde content. Results were expressed as chromatographic area (c.a.).³³

Sensory analysis by an untrained

Fruit were placed on white plates (16 fruit x 5 plate per treatment) and 16 untrained panelists assessed the fruit quality for flesh colour, off-flavour development, ripe aroma, taste and overall acceptance based on a structured hedonic scale from 1 to 10. Off-flavour was assessed, where 1=no off flavour and 10= very strong off flavour. Flesh colour, 1=pale yellow and 10=orange-yellow (based on the intensity of colour development). Fruit taste, 1= bad and 10= excellent; texture, 1= very soft and 10= firm; overall acceptance, 1=bad and 10=excellent. The sample presentation was randomised and the panelists were 60 % females and 40 % males, with ages ranging from 20-50.

Statistical Analysis

The experiments were designed as a completely randomised design and were repeated twice in order to confirm the observations. Data were subjected to analysis of variance (ANOVA) using the GenStat for Windows (2004) statistical package. Fisher's protected least significant difference (LSD) at ($P<0.05$) level of significance was performed. Pearson's correlation analysis was conducted to analyze the data obtained for the different treatments to study the relationships between antioxidant capacity and ascorbic acid carotenoid or TPC or TFC.

Results and Discussion

Incidence of anthracnose

It is evident from this investigation that the inhibitory potency of different treatments on the anthracnose incidence and severity of hot water treated mango fruit after ripening (on the 12th day, hand firmness scale rating 4-5) was as follows: commercial treatment (5.2 %, severity scale 1) > 1-MCP+CA-2 (14.3 %, scale 1) > 1-MCP+CA-1 (20.67 %, scale 2) untreated control fruit (40.3 %, scale 3) (Fig. 1 A and B). Although the commercially adopted prochloraz fungicide treatment showed significant reduction of anthracnose control, the 1-MCP+CA-2 treatment applied to reduce the anthracnose incidence to a great extent than the untreated control and other postharvest treatments. However, 1-MCP+CA-2 treated fruit held at 25° C for 14-16 days showed less anthracnose incidence (30 %, scale 2) than the untreated control fruit (65 %, scale 4). According to Rangel et al. ²², 1-MCP reduced the activity of polygalacturonase (PG) and cellulase and delayed the fruit softening in 'Ataulfo' mango. The inhibitory effect of 1-MCP on PG and cellulase would have extended the action of natural defense mechanism in mango fruit. It is also possible that the 1-MCP molecules bound in the skin can inhibit the PG activity of *C. gloesporioides* and preventing the pathogenesis as suggested by Spotts et al. ²⁵ It is evident from this investigation that the 1-MCP treatment showed a synergistic effect on reducing the incidence of anthracnose in hot water treated mangoes under higher CO₂ composition, 8 % (CA-2 storage conditions). Furthermore, different observations were reported on the association of 1-MCP and fruit decay under normal atmosphere conditions. Absence of decay has been shown in 1-MCP treated plums ³⁴. 1-MCP delayed the decay development in apricots ³⁵, stem-end gray mold rot (*Botrytis cinerea*) in d'Anjou pear ²⁵. According to Jiang et al. ³⁶, 100 and 250 nLL⁻¹ 1-MCP treatments delayed the decay development in strawberries. On the other hand CA storage (higher CO₂) (3 % O₂ and 10 % CO₂) was reported to reduce the incidence of anthracnose in hot water treated cv. Tommy Atkins, and the CA storage showed a residual effect on the development of

anthracnose at ambient temperature for 7 days in normal atmosphere ³⁷. In our study the 1-MCP+CA-2 treatment revealed a residual effect on the development of anthracnose at 25 °C for 12 days in normal atmosphere in hot water treated fruit and this combination treatment can be recommended for the organic export markets.

Weight loss and fruit firmness

In consumer's point of view weight, firmness, colour, and aroma are important parameters during purchasing mangoes, and the rapid changes in these quality parameters limit the storage life. On that basis the weight loss was significantly higher in untreated control fruit (~ 9 %) after ripening on the 5th day (Fig. 2). It is evident from this investigation that the 1-MCP treatment in combination with CA storage or wax treatment showed a synergistic effect in reducing weight loss in cv. Kent. Additionally, the combined effect of 1-MCP treatment with CA-2 storage condition significantly reduced the weight loss in hot water treated 'Kent' mangoes. The 1-MCP under CA storage with higher CO₂ reduced the weight loss in non climacteric litchi fruit 'Mc Lean's Red' ³⁸. This observed weight loss reduction in hot water treated 'Kent' mango subjected to 1-MCP+CA-2 can be attributed to the reduced rate of respiration, which reduces its loss of organic matter and the metabolic water. The effect of 1-MCP+Wax was significantly higher in reducing weight loss in 'Kent' mangoes than the commercial treatment.

The fruit softening was related to loss of firmness. The retention of fruit firmness showed the following trend after ripening on the 5th day in hot water treated mangoes; 1-MCP+CA-2 > 1-MCP+Wax > commercial treatment and 1-MCP+CA-1 > untreated control (Fig. 2). 'Fruit softening' is considered as a result of degradation of protopectin. Decrease in protopectin levels and the increase in soluble pectin levels were observed in 1-MCP treated mango fruit with delayed fruit softening ^{20, 24}. Furthermore, the CA storage conditions with increasing CO₂

composition (<10 %) has been shown to decrease ethylene production and ripening by affecting the ethylene biosynthetic pathway³⁹. Therefore, the combination of 1-MCP pre-treatment and CA-2 storage (10 % CO₂) had served synergistically to reduce fruit softening via maintaining the firmness in hot water treated 'Kent' mangoes.

Fruit skin and flesh colour

The effect of different treatments after ripening on skin and flesh colour changes were expressed by L*, C* and h°. The skin colour change during ripening in 'Kent' mango is from green to yellow. The skin L* and C* values increased while h° value decreased with ripening (on the 5th day) in untreated control fruit (Fig. 3 A). The L* value is not shown in the text. 1-MCP treatment was reported to delay the colour development in mango 'Nam Dokma'²⁰. Generally flesh colour changes in mango were reported as a reliable parameter to note the extent of fruit ripening⁴⁰. The C* value, a measure of colour intensity and increase of chroma during ripening could be linked to carotenoid biosynthesis⁴¹. Higher h° value means retention of greenish yellow skin colour. The decrease in h° value during storage was also reported to correspond with the increase in carotenoids synthesis during fruit ripening. The increase of carotenoids is associated with climacteric increase in respiration and ethylene production⁴².

The h° value (flesh) in 'Kent' was ~ 95.5-98.0 at harvest. A remarkable decline in h° value (~ 60.2- 65.5) was noted at fully ripened stage on the 5th day. The h° value (flesh) in 1-MCP and CA-2 treated fruit was higher than the untreated control fruit on the 5th day (Fig.3B) and this indicates that the 1-MCP+CA-2 application had residual effect on ripening and carotenoid synthesis. This is clearly shown by the significant difference in carotenoid content (flesh) between the fruit subjected to 1-MCP+CA-2 treatment and the untreated control or commercial treatment (Fig. 3B). On the other hand, higher CO₂ composition in CA-2 conditions

was shown to affect the C^* and h° (skin and flesh) after cold storage at 25 °C. This observation supports the observation of Kim et al. ³⁷, on hot water treated ‘Tommy Aitkin’ mangoes stored under CA storage (3 % O₂ and 10 % CO₂). Therefore, the 1-MCP treatment in combination with CA storage served synergistically to reduce the colour development of the skin and flesh in hot water treated ‘Kent.’ However, the effectiveness of the adopted postharvest treatments on delaying skin and flesh colour development was higher with 1-MCP+CA-2 than 1-MCP+Wax or commercial treatment. There is no significant differences in h° , C values and carotenoid content between the fruit subjected to 1-MCP+CA-2 treatment and commercial treatment on the 10th day (at 25 °C, ripening) (no data shown).

SSC/TA ratio

The SSC/TA was significantly higher in untreated fruit (Fig. 4). Generally the SSC tends to increase and the TA decreases as mango fruit ripens ⁴³. A decline in SSC/TA ratio was noted in combination treatments with 1-MCP. Although treatments; 1-MCP+CA-2 or 1-MCP+Wax significantly reduced the SSC/TA ratio in hot water treated ‘Kent’, it is clearly evident that the effect of 1-MCP+CA-2 was higher in delaying the increase of SSC/TA by inhibiting the biochemical reactions related to ripening. The observed decline in SSC/TA ratio was mainly due to the increase of TA. The higher TA in 1-MCP treated fruit could be due to the inhibitory effect of 1-MCP on the loss of existing acids during ripening ⁴⁴. The 1-MCP treatment delayed the decrease in TA in cultivars ‘Guifei’ ⁴⁵ and ‘Tommy Atkins’ ⁴⁵. The CA-2 stand-alone storage condition adopted in this study was shown to increase the TA in ‘Kent’. Similar observation was reported in ‘Delta R2E2’⁹ and ‘Tommy Atkins’ ³⁷ under 3 % O₂ + 8 % CO₂ and 3 % O₂ + 10 % CO₂ CA storage conditions respectively.

Therefore, this study clearly demonstrated that the 1-MCP treatment followed by CA-2 storage enabled to maintain the SSC/TA ratio in hot water treated 'Kent' mango fruit and synergistically extended its residual effect on retention of fruit quality parameters at 25 °C in normal atmosphere conditions (market shelf) up to 5 days. However there is no significant difference in SSC/TA ratio was noted between the fruit subjected to 1-MCP+CA-2 treatment and commercial treatment on the 10th day (at 25 °C, ripening) (no data shown).

Bioactive compounds

Ascorbic acid content decreased during ripening after cold storage (no data shown). The 1-MCP application in combination treatments showed an inhibitory effect on the decline of ascorbic content during ripening in this investigation. However, the decrease in ascorbic acid content was significantly retarded by 1-MCP+CA-2 in hot water treated mangoes (Fig. 5). Ascorbic acid plays an important role as an antioxidant in the detoxification process that results from the formation of different ROS, the hydrogen peroxide (H₂O₂), superoxide radicals (O₂⁻), and hydroxyl radicals (OH•) inside the plant cell⁴⁶. Higher TA is responsible for the stability of ascorbic acid in fruit⁴⁷, this explains the retention of ascorbic acid content in our study in fruit subjected to 1-MCP+CA-2 treatment that showed increased TA.

It has to be noted that the protective benefits of the functional compounds are due to their ability to quench free radicals, and thereby preventing the abnormal oxidative changes in the human body⁴⁷. Research findings showed that the phenolic compounds (equivalents of gallic acid) decrease as fruit ripen^{10,48}. However, TPC appear to be responsible for the maintenance of antioxidant capacity in both, whole and fresh cut mangoes²⁹. Figure 5 shows changes in TPC and TFC in cv. Kent subjected to different postharvest treatments in this study. Among all the postharvest treatments adopted in this investigation, the application of 1-MCP and CA-2 storage

significantly retarded the decline of TPC and TFC during ripening. The 1-MCP and CA-2 treatment could be activating the phenylalanine ammonia lyase (PAL). PAL is one of the key enzymes used in the synthesis of phenolic compounds in plant tissues. According to González-Aguilar,⁴⁹ the PAL activity in 'Haden' mango was strongly correlated to the phenolic content of the fruits.

Antioxidant capacity or scavenging activity is a desirable attribute for marketing the potential health benefits of fresh fruit and vegetables. The antioxidant scavenging activity was reported to decline with ripening³⁷. However, the application of 1-MCP treatment in combination of CA-2 storage improved the retention of antioxidant scavenging activity in hot water treated mangoes as shown in Fig. 5. Wang et al.⁵⁰ and Gardner et al.⁵¹ showed that the antioxidant activity of fruits might be attributed mainly to the content of phenols. A correlation was noted between the DPPH and TPC ($r^2 = 0.82$). Correlations were significant at 0.05 % level. A moderate correlation was observed between the DPPH and TFC ($r^2 = 0.56$) at 0.05 % level. However, correlation was not observed between ascorbic acid content and antioxidant activity in cv. Kent in our study. On the contrary, investigations were shown moderate correlations between the ascorbic acid content and antioxidant activity⁵². The amount of total phenol, flavonoid, ascorbic acid content and the antioxidant capacity was reported to vary among different cultivars, climatic conditions, cultural practices, stage of maturity and postharvest handling and storage.⁵³

Off-flavor volatiles and sensory data

Fruit were taken for sensory and off flavour evaluation on the 10th day after ripening. The acceptable ripening stage for sensory and of flavour analysis was determined by flesh colour (60-70 chroma and 50-65 h⁰) and fruit firmness 3.4-0.5 Kg (3-5 scale hand firmness rating). Ethanol and acetaldehyde are by products of fruit fermentation. The presence of ethanol and acetaldehyde is associated with off-flavours and odours, which reduce the eating quality

of the fruit. Production of ethanol in 'Khaew Sawoey' mangoes during CA storage at 5 % CO₂ and 3 % O₂ was previously reported ⁵⁴. In our investigation the ethanol and acetaldehyde production was observed in fruit held at CA-2 conditions or in stand-alone wax treatment. This was probably due to increased alcohol dehydrogenase and pyruvate decarboxylase activity under anoxic atmosphere ⁵⁵. The application of 1-MCP treatment reduced the formation of ethanol and acetaldehyde in fruit held at CA-2 (1-MCP+CA-2) and the ethanol and acetaldehyde concentration (c.a) were lower than the levels observed in commercially treated fruit (hot water and prochloraz) as shown in Fig.6. According to Kuyen et al. ⁵⁶ hot prochloraz dip treatment in 'Kensington Pride' mango exhibited higher ethanol content after cold storage and ripening. Ethanol and acetaldehyde production in fruit can reduce fruit quality by contributing to off-flavour development when present at amounts greater than their flavour threshold values. After ripening (10th day), the preference of untrained panelists for fruit subjected to different treatments are shown in Fig.7A-D. Although panelists indicated higher values for sweetness and flesh colour in fruit subjected to commercial treatment, the 1-MCP+CA-2 treatment retained the fruit firmness and reduced the off-flavour development (Fig. 7D). On the other hand the fruit from commercial treatment showed higher scores for ripe aroma (Fig. 7A). Furthermore, it is evident from this investigation (shown in Fig.6) that the higher ethanol and acetaldehyde content were responsible for the observed off-flavour in fruit stored under CA-1, CA-2 or untreated control or waxed fruit. The observed differences in off-flavour volatile in commercial treatment and wax treatment is due to the remarkable reduction of anthracnose incidence in fruit underwent commercial treatment that included the prochloraz dip. Panelists' commented moderately ripe aroma in fruit from 1-MCP+CA-2 treatment.

Although this study does not include the information on the effect of 1-MCP+CA-2 treatment on major aroma compounds, it is evident from the reports that the 1-MCP

application affects the aroma production associated with ripening⁸. The inhibitory effect on aroma production was shown in Gala apples in combination treatment with 1-MCP+CA-2⁵⁷. The overall acceptance was higher for 1-MCP+CA-2 treated mangoes and it indicated that the panelists preferred slightly firm fruit with less ripe aroma (not overripe aroma) and acceptable sweetness.

Conclusion

Our study demonstrated the combination treatment of 1-MCP+CA-2 (3% O₂ + 8% CO₂) for hot water treated mangoes delayed the ripening, as evident by the physico-chemical changes reported in this investigation. The combination treatment 1-MCP+CA-2 can be recommended as an alternative treatment for prochloraz application for hot water treated mangoes cv Kent and it can be applied commercially for health conscious export markets. However, semi-commercial export trials and cost benefit analysis has to be fulfilled prior to commercialisation of this technology.

Furthermore, the effectiveness of 1-MCP treatment is cultivar depended and related to the quantity of ethylene receptors present in the skin, the ability to bind 1-MCP⁵⁸ and to infiltrate into the inner tissue of the fruit⁴⁵. These factors can affect the positive performance of different cultivars. The recommendation for CA treatments depends on cultivars. The impact of 1-MCP treatment can vary within a cultivar according to growing geographical conditions or regions and⁵⁹.

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Figure captions

Fig. 1 Incidence of anthracnose in mango 'Kent' fruit subjected to different postharvest treatments and stored at 10 °C for 18 days, ripened at 25 °C and held at normal atmosphere for 12 days.

HWT- Hot Water Treatment; CA-1, 5% O₂ + 5% CO₂ and CA-2, 3% O₂ + 8% CO₂, Wax- Mangoshine[®], 1-MCP (500 nL L⁻¹), UT-Untreated control, CT- Commercial treatment

UT, CA-1, CA-2, Wax, CT were ripened for 5 days; 1-MCP+CA-1, 1-MCP+Wax -ripened for 7 days and 1-MCP+CA-2 ripened for 10 days and observed up to 12 days. Means in each bar followed by the same letter are not significantly different at P < 0.05.

The data presented are the means of two experiments with six replications.

Fig. 2 Effect of different postharvest treatments on weight loss and firmness in mango 'Kent' after cold storage at 10 °C and ripening at 25 °C for 5 days

HWT- Hot Water Treatment; CA-1, 5% O₂ + 5% CO₂ and CA-2, 3% O₂ + 8% CO₂, Wax- Mangoshine[®], 1-MCP (500 nL L⁻¹), UT-Untreated control, CT- Commercial treatment. Means in each bar followed by the same letter are not significantly different at P < 0.05. The data presented are the means of two experiments with six replications.

Fig. 3 Effect of different postharvest treatments on (A). Skin colour values C*, h° of (B) Flesh colour values C*, h° and carotenoid content mango 'Kent' after cold storage at 10 °C ripening at 25 °C for 5 days

HWT- Hot Water Treatment; CA-1, 5% O₂ + 5% CO₂ and CA-2, 3% O₂ + 8% CO₂, Wax- Mangoshine[®], 1-MCP (500 nL L⁻¹), UT-Untreated control, CT- Commercial treatment. Means in each bar followed by the same letter are not significantly different at P < 0.05. The data presented are the means of two experiments with six replications

Fig. 4 Effect of different postharvest treatments on Soluble Sugar Concentration (SSC) and Titratable Acidity ratio (SSC/TA) in mango 'Kent' after cold storage at 10 °C ripening at 25 °C for 5 days.

HWT- Hot Water Treatment; CA-1, 5% O₂ + 5% CO₂ and CA-2, 3% O₂ + 8% CO₂, Wax- Mangoshine[®], 1-MCP (500 nL L⁻¹), UT-Untreated control, CT- Commercial treatment. Means in each bar followed by the same letter are not significantly different at P < 0.05. The data presented are the means of two experiments with six replications.

Fig. 5 Effect of different postharvest treatments on ascorbic acid, total phenolic and flavonoid contents and antioxidant scavenging activity in mango 'Kent' after cold storage at 10 °C ripening at 25 °C for 10 days (P<0.05).

HWT- Hot Water Treatment; CA-1, 5% O₂ + 5% CO₂ and CA-2, 3% O₂ + 8% CO₂, Wax- Mangoshine[®], 1-MCP (500 nL L⁻¹), UT-Untreated control, CT- Commercial treatment. Means in each bar followed by the same letter are not significantly different at P < 0.05. The data presented are the means of two experiments with six replications.

Fig. 6. Effect of different postharvest treatments on ethanol and acetaldehyde (off-flavor volatiles)

HWT- Hot Water Treatment; CA-2, 3% O₂ + 8% CO₂, Wax- Mangoshine[®], 1-MCP (500 nL L⁻¹), UT-Untreated control, CT- Commercial treatment. Means in each bar followed by the same letter are not significantly different at P < 0.05. The data presented are the means of two experiments with six replications.

Fig. 7. Effect of different postharvest treatments on hedonic rating for flesh colour, taste, off-flavour, firmness and overall appearance of 'Kent' mango fruit analysed by a untrained panel after 18 days storage at 10 °C and on the 10th day after ripening at 25 °C. The data presented are the means of two experiments with six replications.

(A). CA-1, (5% O₂ + 5% CO₂); 1-MCP (500 nL/L), (B). CA-2, (3% O₂ + 8% CO₂); 1-MCP (500 nL L⁻¹), (C) UT-Untreated control; (D). CT- Commercial treatment. Prior to different postharvest treatments the fruit were subjected to commercial hot water treatment.

HWT- Hot Water Treatment; CA-2, 3% O₂ + 8% CO₂, Wax- Mangoshine[®], 1-MCP (500 nL L⁻¹), UT-Untreated control, CT- Commercial treatment

Each panelists assess the samples for the traits shown using a structured hedonic scale (with anchor points 'very weak' (1) on the left hand side and 'very strong' (100) on the right hand side). The data presented are the means of two experiments with six replications.