

## Storage quality of fresh-cut Philippine ‘Carabao’ mango (*Mangifera indica* L. cv. ‘Carabao’) fruits with 1-Methylcyclopropene (1-MCP) post-cutting treatment

<sup>1</sup>\*Castillo-Israel, K.A.T., <sup>1</sup>Gandia, J.B.L., <sup>1</sup>Velez, A.C.G., <sup>2</sup>Absulio, W.L. and <sup>1</sup>Bainto, L.C.

<sup>1</sup>Food Science Cluster, College of Agriculture, University of the Philippine Los Banos, College, Laguna, Philippines

<sup>2</sup>Postharvest and Seed Sciences Division, Crop Science Cluster, College of Agriculture, University of the Philippine Los Banos, College, Laguna, Philippines

### Article history

Received: 2 March 2015

Received in revised form:

22 April 2015

Accepted: 29 April 2015

### Abstract

The effects of 1-methylcyclopropene (1-MCP) post-cutting treatment on the storage quality of fresh-cut Philippine ‘Carabao’ mango (*Mangifera indica* L. cv. ‘Carabao’) were determined. Gaseous 1-MCP at a concentration of 1  $\mu\text{L L}^{-1}$  based on the package volume was injected into packed fresh-cut mangoes at stage 5 maturity. The packaging form used was polyethylene terephthalate (PET) tray overwrapped with low density polyethylene (LDPE) cling wrap film. Samples were stored at 5°C and monitored for storage quality parameters. Ethylene concentration inside the package varied during storage where considerably lower levels were observed at day 5 with the 1-MCP treated samples. CO<sub>2</sub> concentration was significantly lower only at day 1. O<sub>2</sub> concentration was lowered by 1-MCP until day 4. Visual quality rating and browning index were not affected by 1-MCP while less water-soaking was observed in the 1-MCP treated samples throughout storage. Higher pH and total soluble solids were obtained in 1-MCP treated samples while total acidity was lower. No significant variations in color were noted between the treatments. 1-MCP was found to cause a decline in total plate count at day 5 while yeasts and molds count did not vary. No coliforms were detected all throughout storage. Shelf-life of fresh-cut ‘Carabao’ mango was determined to be 7 days based on the limits set by European Union(EU) countries for aerobic bacteria and yeasts and molds count. 1-MCP was found to impart better storage quality of fresh-cut ‘Carabao’ mango, hence can be a potential tool in extending its shelf-life. To the best of our knowledge, this is the first study on the application of 1-MCP of fresh-cut mango of the ‘Carabao’ variety.

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### Keywords

‘Carabao’ mango  
Headspace ethylene  
Fresh-cuts  
1-methylcyclopropene  
Post-cutting treatment

### Introduction

Fresh-cuts belong to the minimally processed type of processed foods (Lamikanra, 2002). Production of fresh-cuts involves peeling, trimming, deseeding and cutting of fruit and/or vegetables into specific sizes. Fresh cuts must look fresh and have the sensory properties – aroma, taste, texture and visual appeal, which are associated with freshly prepared produce. The production of fresh-cut fruits is becoming more common and important since consumers are also becoming more aware of the importance of healthy eating habits, and have less time for food preparation (Olivas and Barbosa-Cánovas, 2005). The fresh-cut produce sector has responded to these rising consumer needs for food safety, convenience and quality, and is currently at different stages of development (James and Ngarmsak, 2010). Tropical fresh-cut fruits have attracted many consumers and consume these products as snack. Fresh-cut products market involves restaurants, retail outlets, supermarkets, and

even individual households (Lamikanra, 2002; James and Ngarmsak, 2010).

1-methylcyclopropene (1-MCP) is an ethylene antagonist which acts by binding to ethylene receptors in tissues. The affinity of 1-MCP with the receptor is 10x greater than that of ethylene, thus competing with ethylene. Once bound to receptors, it inhibits ethylene ripening responses (Blankenship and Dole, 2003). 1-MCP presents the advantages of high efficacy at relatively low concentrations and short exposure periods for prolonged periods after a single exposure and low phytotoxicity (Sisler, 2006). 1-MCP has been proven to be an effective tool in extending the shelf-life of intact produce, and in the recent years, have been applied also to fresh-cuts. Various applications of 1-MCP on fresh-cut/minimal processing systems have been reported. Results are quite variable in terms of whether 1-MCP provides a benefit, no effect or negative effect on the shelf-life and quality retention of fresh-cut products (Toivonen, 2008). Factors which affect responses of fresh-cuts

\*Corresponding author.

Email: [kattcastillo@yahoo.com](mailto:kattcastillo@yahoo.com)

to 1-MCP include storage temperature, condition of raw product, cultivar, harvest maturity, duration of storage before cutting and the 1-MCP treatment approach (Toivonen, 2008).

Mango is a good candidate for fresh-cut processing because the fruit might have greater appeal if they were peeled, sliced and flesh removed from seeds for immediate consumption. Very little is known about the potential shelf-life, quality changes, or microbial population of fresh-cut mangoes (Rattanapanone *et al.*, 2001). Application of postharvest technologies to maintain quality of fresh-cut mangoes include controlled atmosphere (CA) on 'Tommy Atkins', 'Haden' and 'Palmer' varieties (Limbanyen *et al.*, 1998) and 'Tommy Atkins' and 'Kent' varieties (Rattanapanone *et al.*, 2001); high CO<sub>2</sub> atmospheres on 'Carabao' and 'Nam Dokmai' (Poubol and Izumi, 2005); and polysaccharide coatings on 'Tommy Atkins' (Goodner *et al.*, 2004). Studies on the application of 1-MCP on fresh-cut mango has also been carried out using 'Keitt' and 'Kent' varieties (Vilas-Boas and Kader, 2007). No study has been yet reported on the application of 1-MCP on fresh-cut 'Carabao' mango.

The Philippine 'Carabao' mango was listed in the 1995 Guinness Book of World Records as the sweetest fruit in the world defeating other countries that also produce tropical mangoes. This citation opened a great opportunity for the country to establish domestic market and bright potential to compete in the world market both in fresh or processed forms (Dela Cruz, 2006). Hence, this mango variety was given attention in this study. This study aimed to determine the effects of 1-MCP post-cutting application on the storage quality of fresh-cut 'Carabao' mango and evaluate its potential as a tool and extending its shelf-life.

## Materials and Methods

### Fresh-cut processing

'Carabao' mango samples were obtained from a farm in Barangay Siranglupa, Calamba, Laguna. The samples were allowed to ripen until Peel Color Index 5 (yellow with traces of green) of the color scale developed by Postharvest Horticulture Training and Research Center, UP Los Banos, Philippines, prior to fresh-cut processing.

The fruits were washed then sanitized by soaking in 200 ppm chlorine solution for 2 minutes. The solution for washing was prepared by mixing 15 mL 5.25% hypochlorite in 1 gallon water. The fruits were air dried, sliced to remove the cheeks, and sliced further to produce 1 inch strips of mango slices which

were removed by scooping them from the peel. The fresh-cuts were directly packed into previously sanitized and dried PET trays at 150 g per pack. The packs were then overwrapped with LDPE clingwrap film.

1-MCP was prepared by weighing the calculated amount of 1-MCP powder (AnSIPTM, 0.43% 1-MCP) to prepare 1 uL L<sup>-1</sup> 1-MCP gas. The powder was weighed into an evacuated 1L volumetric flask and added with a few drops of water to dissolve the powder. The 1-MCP gas prepared was injected using disposable plastic syringe into the packs at a concentration of 1 uL L<sup>-1</sup> based on the volume of the empty tray. The holes made by injection were resealed using tape. Samples were then stored at 5°C and monitored for quality parameters during storage.

### Headspace gas (ethylene, CO<sub>2</sub>, O<sub>2</sub>) analyses

Levels of headspace carbon dioxide, oxygen and ethylene were obtained by gas chromatography using Shimadzu Gas Chromatograph (Model GC-8A) equipped with thermal conductivity detector (TCD) and silica gel column for CO<sub>2</sub> and O<sub>2</sub>, while for C<sub>2</sub>H<sub>4</sub> analysis, flame ionization detector (FID) and alumina column was used. Settings for the GC-FID were as follows: 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. The GC-TCD has the following settings: injection port temperature - 90°C, column temperature - 50°C, gas pressure - 1.25 kg/cm<sup>2</sup>. Amounts of headspace gases were calculated as:

$$C_2H_4, O_2 \text{ or } CO_2 (uL L^{-1}) = \frac{\text{peak height of sample}}{\text{peak height of standard}} \times \text{std } C_2H_4, O_2 \text{ or } CO_2 \text{ concn } (1 uL L^{-1})$$

### Visual quality rating (VQR)

The VQR of the fresh-cut mango samples were observed and evaluated using the following scale: 9,8 – excellent, field fresh; 7,6 – good, minor defects; 5,4 – fair, moderate defects; 3 – poor, serious defects, limit of saleability; 2 – limit of edibility; 1 – non-edible under usual conditions.

### Water-soaking and browning

The degree of water-soaking and browning were observed and evaluated separately using the following scale: 1 – none; 2 – 1-10% water-soaking/browning; 3 – 11-25% water-soaking/browning; 4 – 26-50% water-soaking/browning; 5- >50% water-soaking/browning.

### pH

The pH of the fresh-cut mango samples was

determined using a calibrated pH meter. The sample was prepared by homogenizing 20 g sample in 80 mL distilled water.

#### Total soluble solid (TSS)

Total Soluble Solids was measured using a digital refractometer (Milwaukee MA871 Refractometer 0-85% °Bx). A drop of the filtered homogenized sample was placed on the mirror of a calibrated refractometer and the brix reading was taken.

#### Titratable acidity (TA)

Sixty milliliters of distilled water and 1-2 drops of phenolphthalein indicator was added to 10 mL of the filtrate. Ten mL of the filtrate was added with 60 mL of distilled water and 1-2 drops of phenolphthalein indicator. This was titrated against a standard 0.1 N NaOH solution. TA was calculated as:

$$\%TA = VN \times \frac{\text{meq wt of predominant acid}}{\text{wt of equivalent aliquot in g}}$$

Where

wt of equivalent aliquot in g = (wt of sample in g / vol of juice + dH<sub>2</sub>O) x vol of aliquot

V = volume in mL of NaOH used

N = concentration in Normality of NaOH used

meq wt of predominant acid = 0.064 mg/meq citric acid anhydrous

#### Color evaluation

Color of pineapple fruit slices was measured using a chromameter (Konica-Minolta CR-10) for luminosity, L\* and chroma, c. Measurements were done on each side of the fruit slice.

#### Microbiological analyses (total plate count, yeasts and molds count, coliform count)

Three fresh-cut packs per type of fruit were chosen at random for each analysis period. From each fresh-cut product, 25 grams was taken aseptically and put into a sterile plastic bag with a 225 mL diluent (0.1% peptone water) using a sterile spoon. Then serial dilution was done aseptically until the desired dilutions were obtained. Three dilutions were done: 10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup>. Pour plating was done in duplicates from each of the three dilutions. One mL sample was plated with 10-20 mL of the previously sterilized medium. For the determination of the Total Plate Count (TPC), Plate Count Agar (PCA) was used as the medium and the incubation time is 48 h. In the determination of yeasts and molds count, Potato Dextrose Agar (PDA) was used and the incubation time is 96 h. Lastly, in the determination of coliform count, the Violet Red Bile Agar (VRBA)

was used and the incubation time is 18-24 h. After the incubation period, the microbial count was obtained by counting all the colonies on each plate. Lastly, the estimated colony forming units per mL (CFU/mL) was calculated using the guidelines for counting and reporting standard plate counts (SPC):

$$N = [ \sum(C_1 + C_2) ] (DF)$$

Where: N = total number of colony forming units per mL (cfu/mL)

C<sub>1</sub> = average number of colonies counted on the lower dilution

C<sub>2</sub> = average number of colonies counted on the higher dilution

$$DF = 1/d$$

d = dilution factor corresponding to the first dilution

#### Statistical analysis

Statistical analysis of the data gathered were done through the use of SAS program using Least Significant Difference (LSD).

#### Results and Discussion

The action of 1-MCP as an ethylene inhibitor was clearly demonstrated in its post-cutting application to fresh-cut 'Carabao' mango as evidenced by the lowering of headspace ethylene at day 5 storage (Figure 1A). Accumulation of headspace ethylene was exhibited by the 1-MCP treatment starting at day 2 until day 5. However, the concentration of ethylene was much lower (0.2%) compared with the control where the peak of ethylene accumulation reached to about 0.39%. This illustrates that fresh-cut mango flesh responds positively to 1-MCP as ethylene production was hampered. 1-MCP has been shown to inhibit ethylene biosynthesis in intact 'Carabao' mango fruits pre-harvest (Castillo-Israel *et al.*, 2014). This is because the upsurge in ethylene production in 'Carabao' mango takes place at about 10 days before harvest maturity (Cua, 1989). 1-MCP postharvest application to intact 'Carabao' mango fruits were however ineffective because the upsurge in ethylene production had already occurred prior to its application (Castillo-Israel, 2012). Alves *et al.* (2004) also demonstrated the lesser susceptibility of 'Tommy Atkins' mangoes to 1-MCP in more advanced maturity stage. On the other hand, this study has illustrated the sensitivity of fresh-cut mango flesh 1-MCP even at the ripe stage. This is probably because the barrier which is the thick waxy

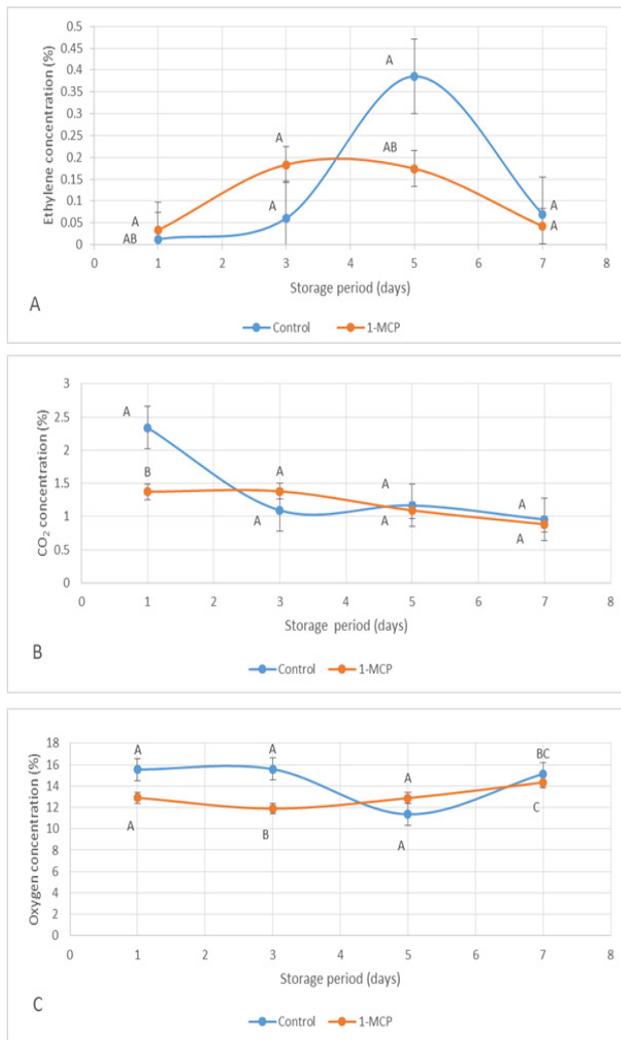


Figure 1. Headspace ethylene (A), CO<sub>2</sub>(B) and O<sub>2</sub>(C) concentrations of fresh-cut ‘Carabao’ mango treated post-cutting with 1 nL L<sup>-1</sup> 1-MCP and stored at 5°C. Values with the same letter symbols are not significantly different based on LSD at P=0.05. Each data point is a mean of 3 replicates

peel, had been removed, exposing the ethylene receptors on the mango flesh directly to 1-MCP. Other studies on fresh-cut 1-MCP applications have also shown sensitivity to 1-MCP as reflected by lowered ethylene production such as apples (Calderon-Lopez *et al.*, 2005) and ‘Hayward’ kiwifruit (Vilas-Boas and Kader, 2007).

Effects on respiration rate was observed through the accumulation of headspace CO<sub>2</sub> and O<sub>2</sub>(Figure 1B and 1C). Low CO<sub>2</sub> and O<sub>2</sub> concentrations were obtained in 1-MCP treatments only at the initial stage of storage (day 1 for CO<sub>2</sub> and days 1-3 for O<sub>2</sub>). 1-MCP seemed to exert its effect on respiration only at the initial stages of storage and is not sustained longer possibly due to the eventual dissociation of 1-MCP with ethylene receptors. Respiration rates were also found to be suppressed by 1-MCP in fresh-cut watermelon (Mao *et al.*, 2005), though the

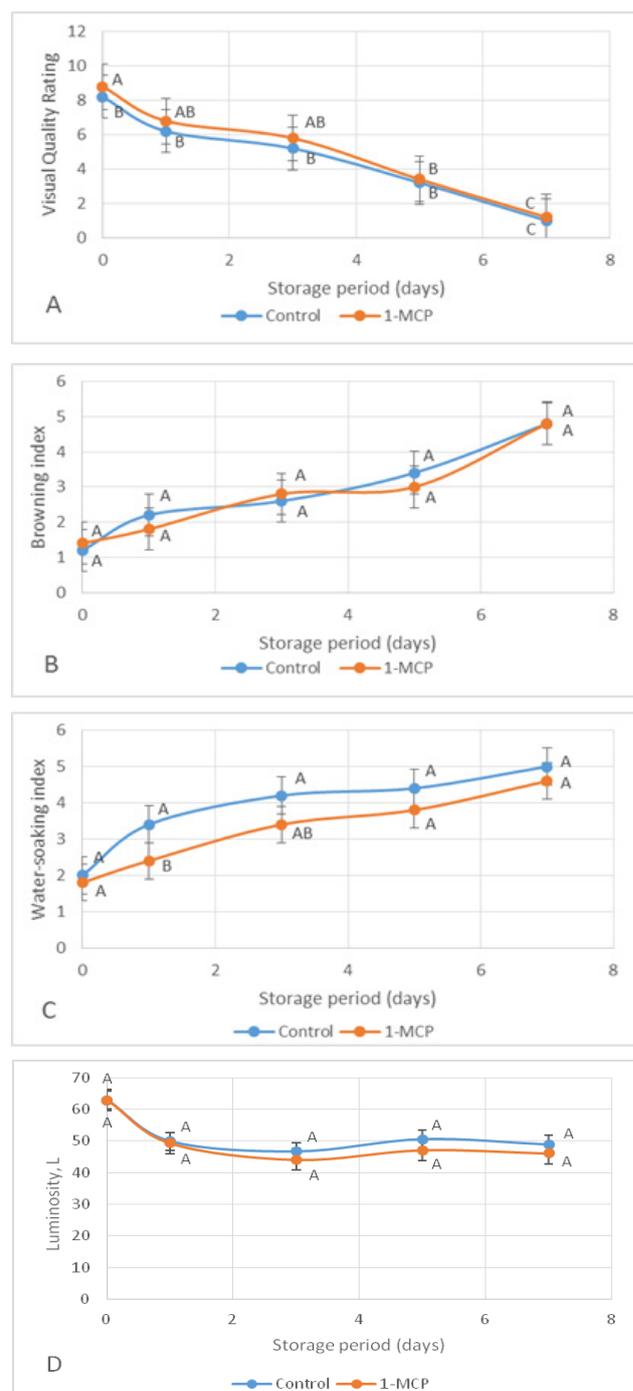


Figure 2. Visual Quality Rating (A), Browning Index (B), Water-soaking Index (C) and Luminosity (D) of fresh-cut ‘Carabao’ mango treated post-cutting with 1 nL L<sup>-1</sup> 1-MCP and stored at 5°C. Values with the same letter symbols are not significantly different based on LSD at P=0.05. Each data point is a mean of 3 replicates

authors suggested that 1-MCP alone would unlikely benefit the storage duration of the said commodity. Respiration rates were likewise not influenced by 1-MCP in fresh-cut ‘Kent’ and ‘Keitt’ mango slices (Vilas-Boas and Kader, 2007). In ‘Carabao’ mango, the effects on respiration was also similarly not prominent throughout storage.

Preparation of slices involves a complex

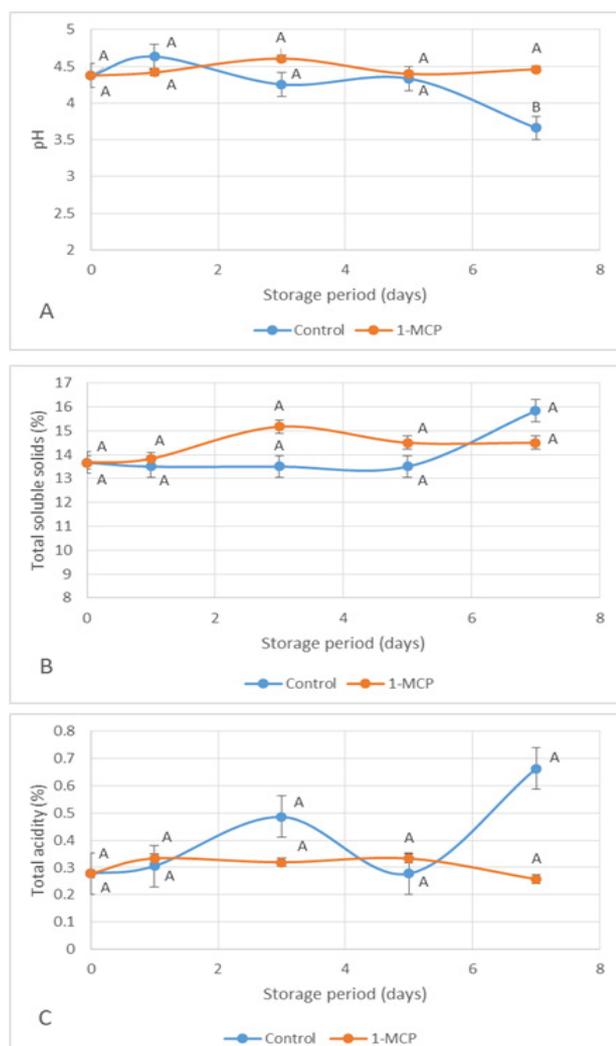


Figure 3. pH (A), Total Soluble Solids (B) and Total Acidity (C) of fresh-cut 'Carabao' mango treated post-cutting with 1 nL L<sup>-1</sup> 1-MCP and stored at 5°C. Values with the same letter symbols are not significantly different based on LSD at P=0.05. Each data point is a mean of 3 replicates

interaction of processes including wound-induced respiration and greater browning potential due to damage of tissues that allows contact of enzymes and substrates at the tissue surface (Calderon-Lopez *et al.*, 2005). This leads to accelerated deterioration of visual quality and development of water-soaking and browning on the fruit surfaces. Physical changes such as visual quality and browning were not significantly affected by 1-MCP treatment (Figure 2A and 2B). The observed effects of 1-MCP on physiological parameters such as ethylene production and respiration rate were not visually translated as improved visual quality of the fresh-cuts. The lowering of ethylene and respiration rate incurred by 1-MCP may not be enough to cause a detectable visual improvement of the fresh-cut 'Carabao' mango. In fresh-cut 'Kent' and 'Keitt' mangoes, however, softening and browning were delayed by 1-MCP post-cutting application (Vilas-Boas and Kader, 2007).

Water-soaking which is a result of membrane breakdown, is also an ethylene-mediated process. It is a product of the activities of cell wall degrading enzymes prominently that of polygalacturonase (PG) and pectin methyl esterase (PME) and lipoxygenase (Karakurt and Huber, 2003). Water-soaking was observed to be lower in 1-MCP treated slices (Figure 2C). Water-soaking was noted to decline rapidly at day 1. At this time, the 1-MCP treated slices had a significantly lower water-soaking score of 2.5 compared with the control having a score of 3.5. Thus, the lower water-soaking observed in 1-MCP treated slices can be accounted to the lower ethylene production obtained as shown in Figure 1A. The ethylene-mediated lowering of the activities of these cell-wall degrading enzymes led to the visible delay in development of water-soaking. Delayed water-soaking were likewise noted in other fresh-cut fruits such as 'Galia' melon (Ergun *et al.*, 2007) and watermelon (Mao *et al.*, 2004).

The color parameter luminosity, L\* is also used as a measure of browning and water-soaking. A lowering in luminosity signals intensification of darkening (Vilas-Boas and Kader, 2007). Though evaluation of water-soaking showed visible differences between control and 1-MCP treatment, luminosity was not relevantly affected by 1-MCP (Figure 2D). In fresh-cut kiwi, 1-MCP did not affect L\* values but slower decrease in L\* values were observed in 1-MCP treated 'Kent' and 'Keitt' mango slices (Vilas-Boas and Kader, 2007). Delay in color changes as measured by L\* and b\* values were also noted in intact 'Nam Dokmai' mangoes.

The pH of the 1-MCP treated samples slightly increased at day 3 and was maintained thereafter. The increase in pH is a consequence of the loss of organic acids which take place during ripening. Generally, pH changes during storage of fresh-cut 'Carabao' mango treated with 1-MCP did not vary significantly with the control except at the last day of storage, day 7, where pH of the control was much lower (3.6) than the 1-MCP treatment (4.5). pH lowering can be an indicator of acid fermentation by microorganisms like lactic acid bacteria and acetic acid bacteria. During the end of storage, 1-MCP was able to maintain the pH which could possibly be a consequence of counteraction of ethylene-induced microbial growth specifically those involved in acid fermentation.

Total soluble solids (TSS) was higher in the 1-MCP treatment (15.1%) compared with the control (13.5%) at day 3. This higher TSS of the 1-MCP treatment was carried over until day 5. The higher TSS is indicative of a better eating quality of the

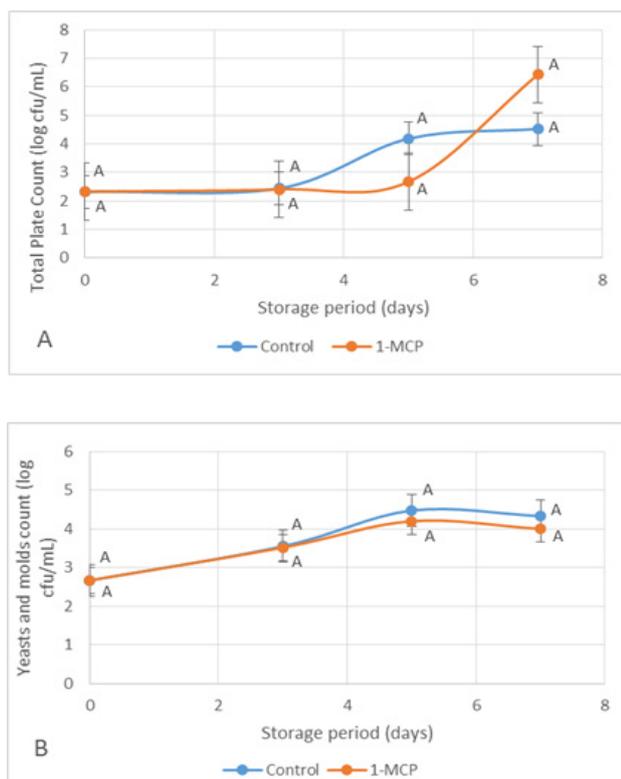


Figure 4. Total Plate Count (A) and Yeasts and Molds Count (B) of fresh-cut 'Carabao' mango treated post-cutting with 1 nL L<sup>-1</sup> 1-MCP and stored at 5°C. Values with the same letter symbols are not significantly different based on LSD at P=0.05. Each data point is a mean of 3 replicates

fresh-cuts since higher TSS means higher sweetness. Sugar metabolism as suggested by these observations, is probably not ethylene-dependent since 1-MCP was observed to lower ethylene production in this commodity. Differences in Total acidity (TA) was likewise insignificant throughout storage, though it can be noted that the 1-MCP treatment had an almost constant total acidity while fluctuations in TA were observed in the control. Highest TA was obtained in the control at day 7 which coincides with the low pH value obtained on the same day. Though results of various studies are inconsistent, Calderon-Lopez *et al.* (2005) generalized that 1-MCP treatment on apple slices had little or no effect on TSS and TA, and it is likely that the physiological processes involved in changes of these factors are not ethylene-dependent.

1-MCP has demonstrated to have an ability to counteract ethylene-induced microbial growth. In loquat fruits, significant inhibition of total aerobic mesophiles, psychrotrophic aerobic bacteria, yeast and molds were observed in 1-MCP-MAP combination (Oz and Ulukanli, 2011). Microbial decay rates were also found to be inhibited by 1-MCP in fresh-cut cilantro (Kim *et al.*, 2007). 1-MCP was likewise observed to counteract ethylene-induced microbial growth on fresh-cut watermelon (Zhou *et*

*al.*, 2006). Monitoring of the total plate count (TPC) during storage of the samples revealed that 1-MCP was able to delay the growth of aerobic bacteria from day 3 until day 5 with an overshoot of bacterial growth at day 7. This inhibition of bacterial growth at this storage period coincides with the inhibition of ethylene production observed. This is a probable evidence of ethylene-induced microbial growth of which 1-MCP was able to counteract being an ethylene inhibitor.

The maximum limit of mesophilic aerobic total plate count as proposed by Raybaudi-Massilia *et al.* (2007) is log 7 CFU/mL. This set limit is also followed by Spanish legal authorities and other EU countries (Sperber and Doyle, 2009). From the TPC data, the samples are still considered safe for consumption at day 7, where the count is log 5 CFU/mL, which is still below the acceptable limit of log 7 CFU/mL. Thus, it is still safe beyond 7 days, though the exact day for the limit was not determined.

Yeasts and molds growth were the same with the control and 1-MCP treatment throughout storage, which implies that ethylene does not play a role in induction of yeasts and molds in this particular commodity. The EU countries' limit on yeasts and molds count is log 5 CFU/mL (Sperber and Doyle, 2009). The samples were not able to reach the microbial safety limit for yeasts and molds at day 7 since the highest counts were only about log 4.25 CFU/mL. Thus, they can still be consumed until 7 days, though the exact day where the limit is reached was not determined. No coliforms were detected throughout storage (data not shown).

## Conclusion

This study was able to demonstrate the ethylene-inhibitory effects of 1-MCP when applied to fresh-cut 'Carabao' mango slices. 1-MCP was able to lower ethylene production and consequently delay ethylene-mediated responses such as development of water-soaking in fruit flesh slices. Visual quality and browning were not significantly affected as well as respiration rate, pH, TSS and TA. Aerobic bacteria growth was shown to be ethylene-induced as the delay in increase of TPC coincided with ethylene inhibition. 1-MCP could be an effective tool in improving the storage quality of fresh-cut 'Carabao' mango.

## Acknowledgement

The authors are grateful to the UP System Emerging Interdisciplinary Research (EIDR) Program

(OVPAE-EIDR P-19-612-102) for providing funds for this research.

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