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Effect of 1-Methylcyclopropene Released from 3-Chloro-2-Methylpropene and Lithium Diisopropylamide on Quality of Harvested Mango Fruit

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Abstract: A simple way to generate active 1-methylcyclopropene (1-MCP) was developed by reaction of 3-Chloro-2-methylpropene (CMP) and Lithium Diisopropylamide (LDA) in the presence of water and the effects of the resultant 1-MCP gas on fruit quality, pericarp chlorophyll fluorescence and contents of ethanol and acetaldehyde in the pulp was investigated at a 3 day interval following subsequent storage for 12 days at 25°C. Retarded yellowing of fruit pericarp but increased disease incidence was observed in the CMP and LDA treated fruit. Furthermore, decrease in fruit firmness was delayed by 3 days in the CMP and LDA treated fruit. Compared to the untreated control, Titrable Acidity (TA) content in the pulp of CMP and LDA treated fruit decreased faster throughout the whole storage period while Total Soluble Solids (TSS) was lower within the first storage of 9 days but higher in the later storage and TSS/TA ratio increased suddenly at the end of storage. Apparently higher potential and actual quantum yield of photosystem II (F_v/F_m and Yield) was tested in the CMP and LDA treated fruit. Promoted ethanol and acetaldehyde production were observed during most of the storage time while lower acetaldehyde level were tested by the end of the storage in the CMP and LDA treated fruit than the control fruit. These results indicated that application of combined CMP and LDA was a simple and feasible way and has great potential to delay ripening of Zihua mango.

Key words: Mango, 1-methylcyclopropene, quality chlorophyll fluorescence, ethanol, acetaldehyde, China

INTRODUCTION

Mango (*Mangifera indica* L.) fruit is famous for its attractive appearance, pleasant flavour and rich nutrition. As a climacteric fruit grown in the tropical and subtropical areas, mango fruit is highly perishable. Green, physiologically mature mango fruit after harvest ripe within 6-7 days and then become overripe and decay within 15 days at 25°C. In recent years, physical, chemical and biological measures have been developed to extend storage life of mango fruit while commercial application of these technologies is restricted due to complex or time-consuming operation, high cost or the safety in regard to human health and environment as well as limited validity (Tharanathan *et al*., 2006).

Gaseous 1-methylcyclopropene (1-MCP) is an efficient inhibitor of ethylene action through binding to ethylene receptors with an affinity approximately 10 times greate than ethylene (Blankenship and Dole, 2003). 1-MCP can inhibit ripening of most postharvest crops at nl L^{-1} levels. This chemically nontoxic substance is environmental friendly and harmless to the public health. In 1999, commercial products of 1-MCP (EthylBloc for ornamental crops and SmartFresh for edible crops) were certificated in America and approved for food use by the Environmental Protection Agency (EPA) in 2002. Thus, 1-MCP is widely investigated in climacteric and non-climacteric fruit, vegetables and flowers (Watkins, 2006).

1-MCP treatment maintained fruit firmness, inhibited activities of antioxidant enzymes (Singh and Dwivedi, 2008; Wang *et al*., 2009), delay the express of pectate lyases which degrade pectins (Chourasia *et al*., 2006), thus increased the number of days to ripening. 1-MCP could delay ripening of harvested mango fruit (Hofman *et al*., 2001) while the effective concentration was quite variable which could be 0.1 μl L^{-1} in thin-pericarp cultivars such as Guifei (Wang *et al*., 2006) or 200 μl L^{-1} in thick-pericarp ones such as Zihua (Jiang and Joyce, 2000). Furthermore, the desirable effect

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of 1-MCP treatment depend largely on the characteristics of the crops, temperature, treatment duration, fruit maturity, time from harvest to treatment and application times which should all been taken into consideration (Blankenship and Dole, 2003; Watkins, 2006).

Commercial application of 1-MCP to horticultural products is based on release of gaseous 1-MCP from a 1-MCP/a-cyclodextrin complex after addition of water or base solution in closed environments to ensure exposure of the crops to the chemical for several hours. Hotchkiss *et al.* (2007) reported the usage of 1-MCP in combination with Modified Atmospheres (MA) by release of 1-MCP from a packaging film matrix.

Direct production of gaseous 1-MCP can meet well a commercial scale to control fruit ripening. Considering the unavailability and/or relative high cost of commercial 1-MCP, a simple way to generate the active 1-MCP referring to the method of Magid *et al.* (1971) was developed in the present study. Two chemicals 3-chloro-2-methylpropene (CMP) and Lithium Diisopropylamide (LDA) were combined and then reacted with H_2O resulting from fruit respiration, prior to the active 1-MCP was released. The effect of the combined treatment of CMP and LDA on fruit ripening and quality of mango was investigated.

MATERIALS AND METHODS

Mature green Zihua mango fruit were harvested from an orchard located in Guangzhou Fruit Research Institute in August, 2008. Fruit were selected for uniformity of size, color and freedom from defects. After dipped for 10 sec in 1% sodium hypochlorite solution, the fruit were air dried and randomly divided into two groups (150 fruit group^{-1}). One group was put into glass jars (19 cm in diameter, 26 cm in height and 7.2 L in capacity; 15 fruit jar^{-1}) each with a small beaker at the bottom. A volume of 0.1 mL 3-chloro-2-methylpropene (CMP) (Aldrich, Inc. USA) + 0.3 mL lithium diisopropylamide mono (tetrahydrofuran) complex solution (LDA) (Aldrich, Inc. USA) were injected into the beakers before the jars were sealed.

The concentration of the active 1-MCP produced by CMP and LDA was tested as the method below. The control fruit were also sealed into jars for the duration of the corresponding CMP and LDA treatments. The sealed jars were kept for 12 h at 25°C then 5 fruit were packaged in a low density polyethylene bag (0.015 mm thick). All the fruit was stored at ambient temperature (25°C) for 12 days. About 15 fruit was taken at each sample time for the following evaluations and analyses. The experiment was repeated twice and similar results were obtained.

Determination of the active 1-MCP concentration generated by CMP and LAD: The active concentration of 1-MCP generated by CMP and LAD was measured 7 h after CMP and LAD was injected and the jar was sealed. A Gas Chromatography (GC) machine (GC-2010, Shimazu, Japan) equipped with a capillary column (Rtx-Wax, 30 mm ×0.32 mm ×20 μm) and a Flame Ionization Detector (FID) was used. A headspace gas of 1 mL drawn by a gas-tight syringe was injected into GC. Temperature of the injection port and detector were 220 and 230°C, respectively. Temperature program of the column oven was as follows: 120°C, 3°C min^{-1} -150°C and then 10°C min^{-1} -200°C. Iso-butylene was used as the standard gas to calculate 1-MCP concentrations as reported by Jiang and Joyce (2000). The tested concentration of 1-MCP generated by 0.1 mL CMP + 0.3 mL LDA mL LDA were 138 ul L^{-1}.

Evaluation of peel color and disease incidence: Peel color of individual fruit was estimated by measuring the extent of the total yellow area on each fruit pericarp on the following scale: 0, green; 1, ≤1/3 yellow; 2, 1/3-2/3 yellow and 3, ≥2/3 yellow. The color index was calculated using the formula: Σ(yellowing scale/the highest scale x percentage of corresponding fruit within each class).

Disease development was recorded as the proportion (%) of fruit surface with anthracnose or rot spot as follows: 0, no lesion; 1, ≤1/8 lesion; 2, 1/8-1/4 lesion; 3, 1/4-1/2 lesion and 4, ≥1/2 lesion. The disease index (%) was calculated by the formula: Σ(disease scale/the highest scale x percentage of corresponding fruit within each class).

Detection of chlorophyll fluorescence: Chlorophyll fluorescence measurements were carried out at 25°C with a portable fluorometer (PAM2100, Heinz Walz GmbH, Germany). A fiber-optic adapter (2010-A, Walz) was used to fix the distance between the fiber optic terminus and the fruit exocarp. Measurements were taken in two opposite positions of each fruit at the same location in the fruit surface and then averaged. The maximal efficiency of PSII photochemistry, Fv/Fm = (Fm-Fo)/Fm was determined using the fast actinic test (Run 2-procedure on the fluorometer) with the measuring beam set to a frequency of 600 Hz after the fruit was dark-adapted for 20 min whereas the measurement of actual quantum yields of photosystem II, Yield = ϕPSII = (Fm'-Ft)/Fm' were performed with the measuring beam automatically switching to 20 kHz during the saturating flash (20 sec).

Assessments of Titrable Acidity (TA), Total Soluble Solids (TSS) and firmness: Pulp tissues were homogenized in a grinder and then filtered with 8 layer gauze. The filtrate was collected for analyses of TSS and

TA contents. TSS was assayed using a hand-held refractometer (J1-3A, Guangdong Scientic Instruments) while TA was determined by titration with 0.1 M NaOH up to pH 8.65. Results were expressed in percentage. The TSS/TA ratio was also calculated. Fruit firmness was measured by a fruit sclerometer (GY-1, Hangzhou Top Instruments). Fruit were peeled and measurements were then taken near the stem and head in two opposite sides of each fruit.

Determinations of ethanol and acetaldehyde production: The above-mentioned GC was used to determine ethanol and acetaldehyde production of fruit pulp. Fruit juice was extracted and stored in plastic tubes containing 0.4 g NaCl mL^{-1} juice at -20°C. Frozen juice was thawed under ambient conditions and 15 mL aliquot was then transferred to a 50 mL conical flask and sealed with a soft rubber stopper. Samples were incubated at 80°C for 10 min. A headspace gas of 1 mL drawn by a gas-tight syringe was injected into GC. The injection port and detector temperature were 210 and 220°C, respectively. The column oven temperature program was 40°C (5 min), 10°C min^{-1} -65°C (8min), 30°C min^{-1}-210°C (10 min). Quantitative analyses of ethanol and acetaldehyde were carried out by using standard aqueous solutions and by making the corresponding standard curves under the same condition.

Data analyses: The experiment design was completely randomized. Data represented the means of at least 3 replicates and analyzed by one way Analysis Of Variance (ANOVA) using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). Significant differences between means were tested using Least Significance Difference (LSD) or Duncan's new Multiple Range Test (DMRT) procedure at the 5% level.

RESULTS AND DISCUSSION

Changes of peel color, disease index and firmness: After 6 days of storage, fruit turned yellow while disease, mainly anthracnose and stem end rot development increased rapidly (Table 1). Obviously lower color index but higher disease index was observed in the CMP and LDA treated fruit in this storage period. Considerably higher rots rate by 1-MCP was also founded by Hofman *et al.* (2001). The treatment delayed fruit softening by 3 days. However, a more rapid decrease in firmness occurred after 6 days of storage in the CMP and LDA treated fruit than the control fruit.

Changes of chlorophyll fluorescence: Chlorophyll fluorescence is an indirect indicator of the physiological status of green tissues (Maxwell and Johnson, 2000). Chlorophyll fluorescence measurement has the advantage of detecting cellular injury resulting from senescence in advance of the development of visible symptoms (De Ell, 1999). Fv/Fm and Yield referring to potential and actual quantum yield of photosystem, respectively were reported in assessing storability of fruit and vegetables (Bron *et al.*, 2004; Schofield *et al.*, 2005). Jacobi *et al.* (2001) suggested a remarkable positive correlation between chlorophyll fluorescence values and quality of harvested mango fruit. Markedly higher Fv/Fm and Yield value in the CMP and LDA treated fruit than that in the control fruit was observed (Fig. 1a, b) which suggested that the postharvest ripening of mango fruit could be postponed by the treatment.

Changes of TSS, TA and TSS/TA ratio: Total Soluble Solids (TSS) increased at the beginning of storage and the highest TSS content was observed on the 6th day while the increase from of TSS content was postponed significantly by the CMP and LDA treatment (Fig. 2a), which was coincide with the result of Wang *et al.* (2006) by 1-MCP treatment. Titrable Acidity (TA) dropped quickly after harvest. The lower TA was obtained in the CMP and LDA treated fruit (Fig. 2b). TSS/TA ratio was stable in the first 3 days of storage and then increased slowly in the control fruit but rose rapidly in the CMP and LDA treated fruit (Fig. 2c). Increased loss of TA content

Table 1: Effect of combined CMP and LDA on changes in color, disease incidence, firmness of Zihua mango fruit stored at 25°C

Days after harvest	Treatments	Color index	Disease index	Firmness ($\times 10^5$ Pa)
0	Control	0	0	10.2^{a}
	CMP and LDA treatment	0	0	10.2^{a}
3	Control	0	0	10.1^{a}
	CMP and LDA treatment	0	0	10.2^{a}
6	Control	$13.6\%^{ab}$	$15.4\%^{b}$	9.7^{b}
	CMP and LDA treatment	$7.8\%^{b}$	$29.2\%^{a}$	10.1^{a}
9	Control	$51.4\%^{a}$	$33.3\%^{b}$	5.7^{b}
	CMP and LDA treatment	$27.8\%^{b}$	$46.2\%^{a}$	5.7^{b}
12	Control	$68.5\%^{a}$	$40.3\%^{b}$	4.9^{a}
	CMP and LDA treatment	$63.0\%^{a}$	$61.1\%^{a}$	4.1^{b}

Data within a column with different letters are significantly different at the 5% level

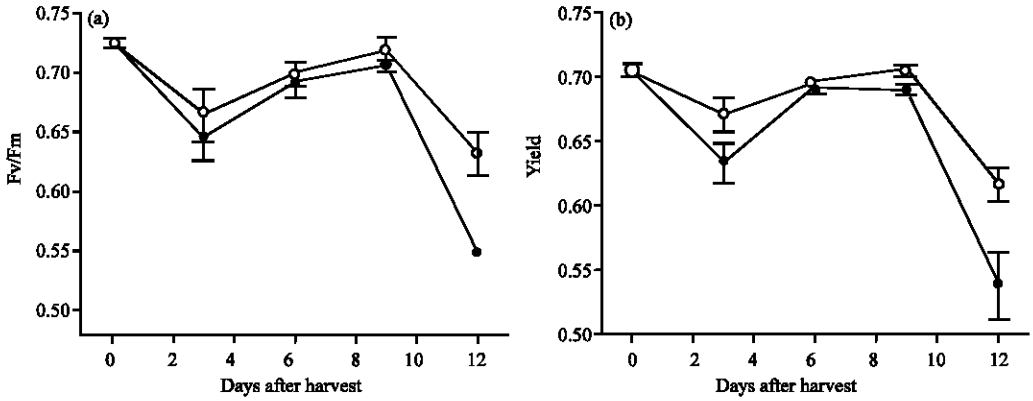

Fig. 1: Effect of the combined CMP and LDA on Fv/Fm and Yield in mango fruit stored at 25°C. Each point represents the means±SE (Standard Error) of three replicates. (●) Control; (○) Combined CMP and LDA

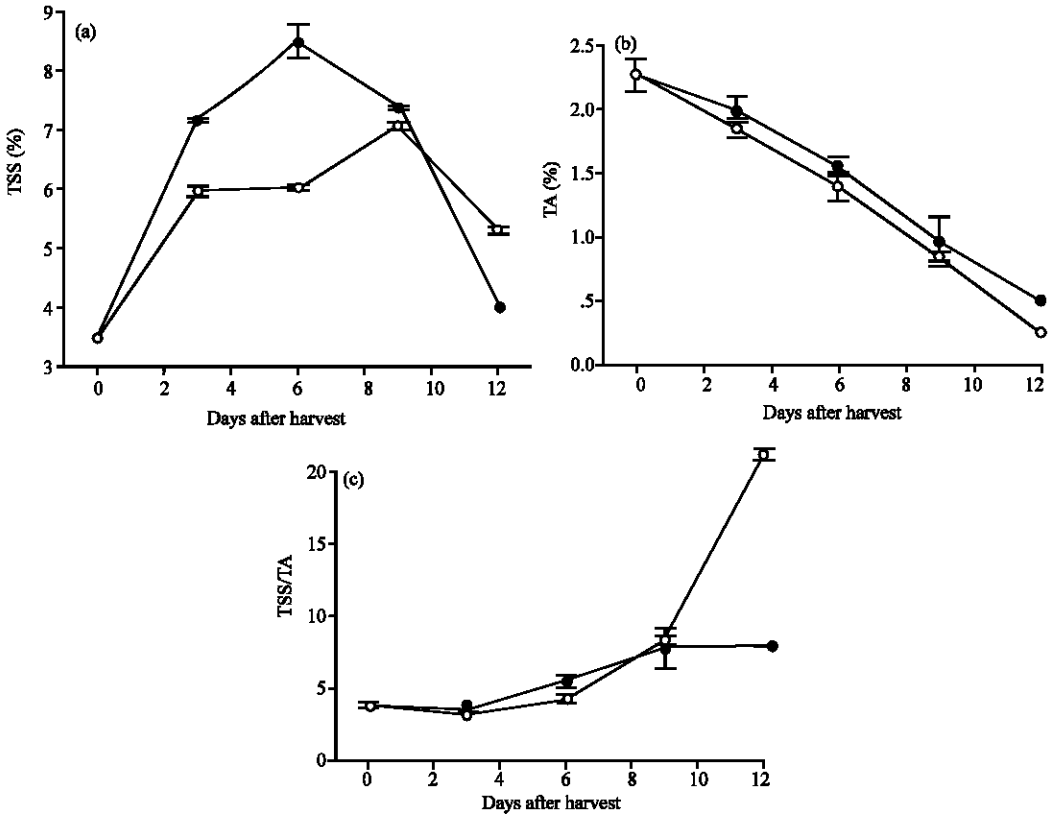

Fig. 2: Effect of the combined CMP and LDA on contents of Titrable Acidity (TA), Total Soluble Solids (TSS) and TSS/TA ratio in mango fruit stored at 25°C. Each point represents the means±SE of three replicates. (●) Control; (○) Combined CMP and LDA

in the CMP and LDA treated fruit may be due to anaerobic respiration which consumed more acid substrates compared with the control fruit. This assumption can be supported by enhanced ethanol and acetaldehyde production (Fig. 3a, b).

Changes of ethanol and acetaldehyde (AA) production: The presence of ethanol and acetaldehyde is an indicator of anaerobic respiration (Echeverria, 1988). As shown in Fig. 3, the two fermentative metabolites had the same change tendency. In the control fruit, ethanol and

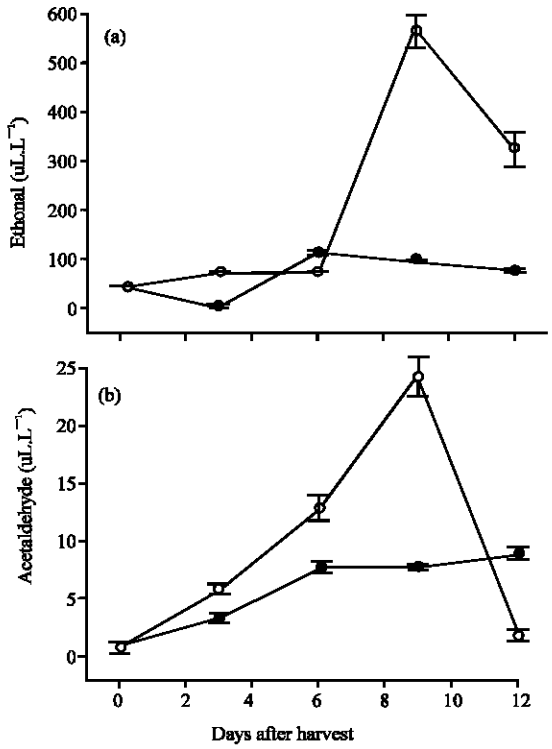

Fig. 3: Effect of combined CMP and LDA on ethanol and acetaldehyde production in mango fruit stored at 25°C. Each point represents the means±SE of three replicates. (●) Control; (○) Combined CMP and LDA

acetaldehyde production went up slightly during the most of storage time. In contrast, the two compounds increased sharply immediately after treatment and reached a peak on the 9th day in the CMP and LDA treated fruit and then dropped quickly.

At the end of storage time, acetaldehyde product was lower but ethanol production was still higher in the CMP and LDA treated fruit than in the control.

Subtropical fruit including mango have been proposed to be among the most sensitive to anaerobiosis damage (Pesis, 2005), thus the higher rots rate in the treated fruit than in the control fruit may be due to the accumulation of ethanol and acetaldehyde.

Furthermore, ethanol and acetaldehyde contribute significantly to inhibit ripening (Burdon *et al*., 1996). The delayed ripening of harvested mango fruit was consistent with the enhanced production of the two compounds in this study. These results suggested that they played important role in regulation the ripening and senescence of harvested mango fruit.

CONCLUSION

Preparation of active 1-MCP by a simple reaction of CMP and LAD was proven to be successful. Yellowing and softening of mango fruit were retarded while TSS, TA content and TSS/TA ratio were lower before the 6 days of storage in the CMP and LAD treated fruit compared with the control fruit, suggesting a potential role of 1-MCP in delaying ripening of Zihuamango fruit in the early storage period at ambient temperature which was supported by higher potential and actual quantum yield of photosystem II.In addition, higher rots rate was correlated with enhanced ethanol and acetaldehyde production in the treated fruits than in the control fruit. Therefore, to obtain a extend shelf life of mango fruit by the CMP and LAD treatment, measures should be taken carefully to consider the control of both yellowing and disease development.

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REFERENCES

Blankenship, S.M. and J.M. Dole, 2003. 1-Methylcyclopropene: A review. Postharvest Biol. Technol., 28: 1-25.

Bron, I.U., R.V. Ribeiro, M. Azzolini, A.P. Jacomino and E.C. Machado, 2004. Chlorophyll fluorescence as a tool to evaluate the ripening of Golden papaya fruit. Postharvest Biol. Technol., 33: 163-173.

Burdon, J., S. Dori, R. Marinansky and E. Pesis, 1996. Acetaldehyde inhibition of ethylene biosynthesis in mango fruit. Postharvest Biol. Technol., 8: 153-161.

Chourasia, A., V.A. Sane and P. Nath, 2006. Differential expression of pectate lyase during ethylene-induced postharvest softening of mango (*Mangifera indica* var. Dashehari). Physiol. Plant., 128: 546-555.

De Ell, J.R., 1999. Applications of chlorophyll fluorescence techniques in postharvest physiology. Horticult. Rev., 23: 69-107.

Echeverria, E.D., 1988. Carbohydrate and enzyme distribution in protoplasts from Valencia orange juice sacs. Phytochemistry, 27: 73-76.

Hofman, P.J., M. Jobin-Decor, G.F. Meiburg, A.J. Macnish and D.C. Joyce, 2001. Ripening and quality responses of avocado, custard apple, mango and papaya fruit to 1-methylcyclopropene. Aust. J. Exp. Agric., 41: 567-572.

Hotchkiss, J.H., C.B. Watkins and D.G. Sanchez, 2007. Release of 1-methylcyclopropene from heat-pressed polymer films. J. Food Sci., 72: E330-E334.

Jacobi, K.K., E.A. MacRae and S.E. Hetherington, 2001. Loss of heat tolerance in Kensington mango fruit following heat treatments. Postharvest Biol. Technol., 21: 321-330.

Jiang, Y. and D.C. Joyce, 2000. Effects of 1-methylcyclopropene alone and in combination with polyethylene bags on the postharvest life of mango fruit. Ann. Applied Biol., 137: 321-327.

Magid, R.M., T.C. Clarke and C.D. Duncan, 1971. Efficient and convenient synthesis of 1-methylcyclopropene. J. Organic Chem., 36: 1320-1321.

Maxwell, K. and G.N. Johnson, 2000. Chlorophyll fluorescence a practical guide. J. Exp. Bot., 51: 659-668.

Pesis, E., 2005. The role of the anaerobic metabolites, acetaldehyde and ethanol, in fruit ripening, enhancement of fruit quality and fruit deterioration. Postharvest Biol. Technol., 37: 1-19.

Schofield, R.A., J.R. De Ell, D.P. Murr and S. Jenni, 2005. Determining the storage potential of iceberg lettuce with chlorophyll fluorescence. Postharvest Biol. Technol., 38: 43-56.

Singh, R. and U.N. Dwivedi, 2008. Effect of Ethrel and 1-methylcyclopropene (1-MCP) on antioxidants in mango (*Mangifera indica* var. Dashehari) during fruit ripening. Food Chem., 111: 951-956.

Tharanathan, R.N., H.M. Yashoda and T.N. Prabha, 2006. Mango (*Mangifera indica* L.) The king of fruits-an overview. Food Rev. Int., 22: 95-123.

Wang, B.G., J.H. Wang, X.Y. Feng, L. Lin, Y.M. Zhao and W.B. Jiang, 2009. Effects of 1-MCP and exogenous ethylene on fruit ripening and antioxidants in stored mango. Plant Growth Regul., 57: 185-192.

Wang, B.G., W.B. Jiang, H.X. Liu, L. Lin and J.H. Wang, 2006. Enhancing the post-harvest qualities of mango fruit by vacuum infiltration treatment with 1-methylcyclopropene. J. Hortic. Sci. Biotechnol., 81: 163-167.

Watkins, C.B., 2006. The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. Biotechnol. Adv., 24: 389-409.