International Food Research Journal 25(3): 1001-1006 (June 2018)
Journal homepage: *http://www.ifrj.upm.edu.my*

Effect of 1-MCP on ethylene regulation and quality of tomato cv. Red Ore

Poyesh, D.S., Terada, N., Sanada, A., Gemma, H. and *Koshio, K.

Tokyo University of Agriculture, Sakuragaoka, Setagaya-ku, Tokyo 156-8502, Japan

Article history

Received: 19 January 2017
Received in revised form:
27 February 2017
Accepted: 28 February 2017

Keywords

1-Aminocyclopropane-1-Carboxylic Acid Oxidase (ACO)
1-Aminocyclopropane-1-Carboxylic Acid Synthase (ACS)
Color
Respiration
Softening

Abstract

1-MCP is a new tool for regulating ethylene and preserving the quality of fresh produce. Tomato fruit *Solanum lycopersicum* cv. Red Ore harvested at green and pink stages was used to examine the effect of 1-MCP on their ethylene production and quality. After treating with 1-MCP for 24 hours, ethylene, ACS, ACC, CO_2, color, firmness and weight loss were measured. Differences in ethylene production at mature green and pink stages, might be due to the effect of 1-MCP on ethylene production through changed activity of ACS, result in blocking of autocatalysis (mature green) and autoinhibition (pink stage). The shelf lives were improved in 1-MCP-treated fruit at both stages of harvest as indicated by the decrease in respiration, softening, and weight loss, along with the delay in color change (a-value). Our results newly suggest that 1-MCP treatment can have different affects on tomato ethylene production depending on maturation stages (green or pink), but contribute to prolong the shelf life in both stages of tomato fruit.

Introduction

Ethylene is a natural plant growth regulator that affects growth and developmental processes of plants including ripening and senescence (Abeles *et al*., 1992). It is a hydrocarbon that can move into and out of plant tissues easily from endogenous and exogenous sources (Saltveit, 1999) and has been the subject for many researchers to study its biosynthesis and action in plants (Lelivre *et al*., 1998; Saltveit, 1999; Giovanni, 2001; Watkins, 2002; Adams-Philips *et al*., 2004). First, Dimity Neljubov (1879-1926) discovered that ethylene is a biologically active gas that affects plant growth (Abeles *et al*., 1992). The effects of ethylene production can be either beneficial or deteriorative depending on the products, ripening stages, and its usage (Saltveit, 1999). Therefore, controlling ethylene biosynthesis and/or ethylene sensitivity can be a key factor to keep the freshness of harvested climacteric fruits, possibly in the simple and less costly method, so that it can also be applicable in developing countries as they have less access to low temperature storage facilities.

1-Methylcyclopropene (1-MCP) is a chemical compound with a molecular weight of 54 and a formula of C4H6, which is reported to interact with ethylene receptors, and as a result inhibits the ethylene-depending responses (Sisler and Blankenship, 1996; Sisler and Serek, 1997, 2003 and Guill' en *et al*., 2007). The affinity of 1-MCP is approximately 10 times greater that of ethylene Recently, application of 1-MCP in commercial scale on edible crops is under taken by AgroFresh, Inc., a subsidiary of Rohm and Hass under the trade name SmartFresh® (Blakenship and Dole, 2003).

Although almost 20 years have passed since 1-MCP was discovered and patented by Ed Sisler and Sylvia Blankenship (Sisler and Blankenship, 1996), it is still a research target. Since it will take decades to improve infrastructures and technologies in order to reduce the post-harvest losses in developing countries, one of the possible and promising solutions could be the use of chemicals such as 1-MCP to reduce the loss after harvest, as it is less costly acts in trace level, easy to apply, and widely accessible. Therefore, the aim of this research is to investigate the effect of 1-MCP on tomato storability at two stages of harvest (green and pink), focusing on ethylene production.

Materials and Methods

Plant materials

Tomato, *Solunum lycopersicum* (cultivar. Red Ore) fruit harvested at green and pink stages were brought to the Laboratory of Tropical Horticultural

*Corresponding author.
Email: *koshio@nodai.ac.jp*

Science, Tokyo University of Agriculture (Setagaya campus). Twenty uniform fruit (10 fruit in each treatment arm) were selected based on ethylene production with almost the same level of emission. The experiments were been conducted during the years 2014-2016.

1-MCP applications

Tomato fruit was treated with 1 µl L^{-1} concentration of 1-MCP which prepared from Smart Fresh® made by Agro Fresh, Inc. for 24 hours, while non-treated fruit as the experimental control were kept in airtight chambers as suggested by Blankenship and Dole, 2003; Watkins, 2006 and Guillen, 2009. The application concentration of 1-MCP was decided as 1 µl L^{-1} based on our preliminary experiment indicating that1 µl L^{-1} was the most effective among three tried different concentrations of 1-MCP (0.5, 1 and 1.5 µl L^{-1}). All the fruit was kept at ambient temperature throughout the duration of the experiment.

Ethylene measurement

Each fruit was placed inside the 550ml glass jar and incubated under dark condition for an hour at room temperature (25°C). Ethylene production was analyzed after 1, 6, 12, 24 hours and thereafter measured every 48 hours. Head space gas of 1ml was taken out by a plastic syringe and the amount of ethylene was analyzed by Shimadzu GC-FID, Model GC-14B equipped with a Sunpack A column (Shinwa Kako, 2.1 m×3.2 mmφ, glass column filled with porous polybeads) and FID: injector 180°C, column 80°C, detector 200°C. Ten replication were prepared for each treatment.

In addition, ethylene production was also monitored by CI-900 Portable Ethylene Analyzer, which was developed by ICT international. Ten fruit was incubated in the chamber immediately after the treatment and the data were recorded automatically.

1-Aminocyclopropane-1-Carboxylic Acid Synthase (ACS) and Oxidase (ACO) activity

In vitro ACS activity was measured using Inge Bulens *et al*. (2011) method with some modifications. The tomato samples were frozen 24 hours after treatment with liquid nitrogen and stored at -80° C in freezer manufactured by Nihon Freezer. Co. Ltd, Japan. ACC was extracted from frozen crushed tomato samples in Tricine buffer solution, centrifuged with 10000 rpm (round per minute) at 4°C and supernatant was collected in a 15 ml falcon tube. The supernatant was reacted with SAM chloride for 2 hours by shaking gently, then the reaction was stopped using ethanol. The internal standard solution (deuterium-labelled ACC 1 mM) of 50 µl was added to the samples, and analyzed with GC-MS (QP2010PLUS: Shimadzu) after trimethylsilylation according to the method of Yin *et al*. (2010). The ACS activity was calculated by the amount of ACC production, which was derived from the added SAM.

ACO activity was perceived indirectly by the level of ethylene production; after measurement of ethylene, we prepared the discs of 1 cm in diameter from both control and 1-MCP treated tomato, washed with deionized water, then put one group as control in 1 mM AVG (aminovinylglycine) while the other group were put in the same concentration of AVG for 30 minutes, and shifted into 10 mM of ACC (1-aminocyclopropane-1-carboxylic acid) solution for 1 hour. Then, we put every disk into small test tube and measured ethylene using 1ml of headspace gas by GC-FID as mentioned above. Later on we calculated the activity of ACO by subtracting the ethylene production value of the disks of control (treated only with AVG) from the one treated with both AVG and ACC.

Respiration rate measure

Respiration Rate measure as CO_2 release was analyzed after 1, 6, 12, 24 hours and thereafter measured every 48 hours by GC-TCD (Gas Chromatography-Thermal Conductivity Detector, Shimadzu Japan) using the headspace method. Each fruit was placed inside the 550 ml glass jar and incubated under constant dark condition for an hour at room temperature. The headspace gas of 1 ml was taken out by a plastic syringe and the amount of CO2 was analyzed by Shimadzu GC-14B equipped with a column (Shinwa Kako, 2.1 m×3.2 mmφ, glass column filled with porous polybeads) and TCD: injector 150°C, column 40°C, detector 150°C.

Hardness, color and weight

The fruit firmness was measured with non-destructive firmness meter Multilateral Tester Model 2519-104 manufactured by INSTRON Company, indicating the force (N) required for pressing the fruit skin 1 mm/second using Φ 1 cm metal plug. The L, a, and b values of fruit color have been measured by the Handy Colorimeter NR- 3000 Osaka Japan. Weight losses of fruit were recorded from each replication in all treatments at periodical intervals, and cumulative losses in weight were calculated and expressed in percentages

Results

Ethylene production

The data of ethylene production in tomato obtained from GC-FID suggest that ethylene production was decreased by 1-MCP treatment in mature green tomato fruit as early as 6 hours after treatments and it was significantly different at 1 % (Tukey's test) at 48, 72, 96 hours after treatment as compared with control (Figure 1.A). On the contrary, ethylene production was increased in tomato at pink stage, as early as 12 hours after 1-MCP treatment; it was significantly higher in Tukey's test at 48 and 72 hours (at 1% level), and 96 hours (at 5% level) after treatment as compared with control (Figure 1. B).

The abovementioned results from GC-14B (equipped with FID column) were in conformity with the results that came out from automatic ethylene detector CI-900 Ethylene Portable Analyzer. Both instruments showed the same tendency: less ethylene production in case of 1-MCP treated mature green fruit and higher ethylene production in case of 1-MCP treated pink fruit (Figure 2. A and B).

ACO and ACS activities

It was shown that 1-MCP treatment didn't affect significantly at ACO activities at both stages of harvest, namely mature green and pink stage of tomato (Figure 3, C and D). However, 1-MCP treatment significantly decreased the activity of ACS at mature green tomato at 1% (Tukey's test) as compared to the control (Figure 3 A and B). Adversely, 1-MCP increased the activity of ACS in tomato at pink stage at 1% (Figure 3, upper panel).

Respiration rate measure

It was revealed that 1-MCP treatment decreased the respiration shown in CO_2 production. The tomato fruit harvested at mature green and treated with 1-MCP, significantly decreased CO_2 production at 0, 24, and 48 hours after treatment at 1 % (Tukey's test) and 6 and 12 hours at 5% as compared with control which produced higher amount of CO_2, respectively (Figure 1. C). Similarly, the tomato fruit harvested at pink stage and treated with 1-MCP, produced significantly less amount of CO_2 at 6, 12, 24, and 48 hours after treatment at the 5% level as compared with control (Figure 1. D).

Hardness

The data suggested that 1-MCP delayed softness of tomato fruit on both stages of harvest (green and pink stages)(Figure 5. A and B). The fruit harvested at mature green and treated with 1-MCP were significantly firmer at 1% level at 144, 192, 240 and 288 hours after treatment (Tukey's test). Similarly, the fruit harvested at pink stage and treated with 1-MCP, was significantly firmer at the 1 % level at 144,192, and 240 hours; it was significant at 5% level after 288 hours as compared with control (Tukey's test).

Color

It was revealed that 1-MCP could maintain the

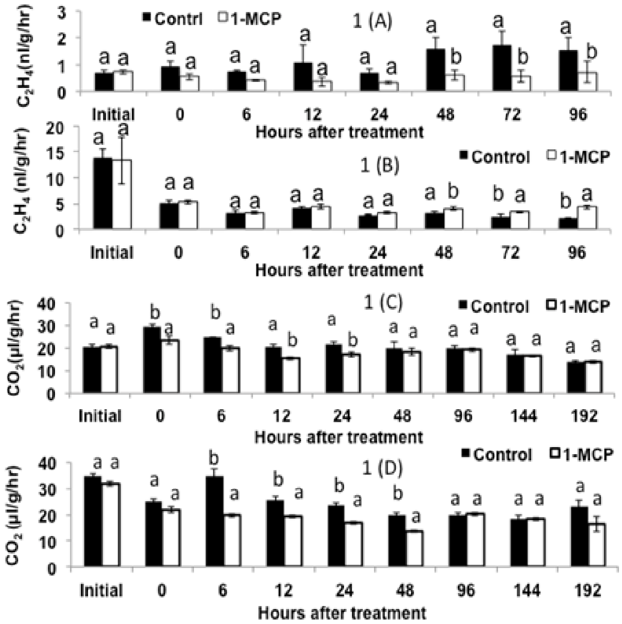

Figure 1. Effect of 1-MCP treatment on ethylene production in tomato at green (A) and pink (B), and CO2 production at green (C) and pink (D) stages respectively. Ethylene production was measured by GC-FID after one-hour incubation. Respiration was measured by GC-TCD after one-hour incubation. Results represent means ± SE (n=10). Values with different superscripts indicate significant difference (p< 0.05)

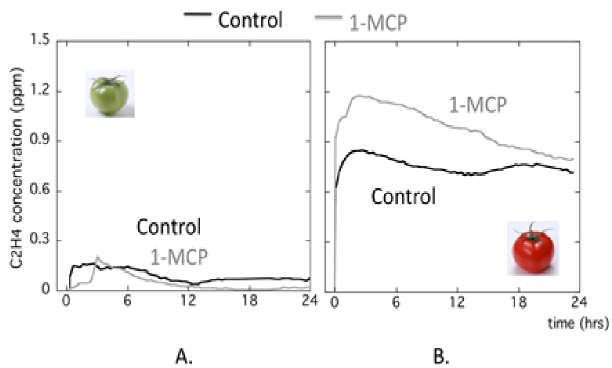

Figure 2. Effect of 1-MCP treatment on ethylene production in tomato at green (A) and pink (B) stages respectively. Ethylene production was measured by CI-900 immediately after treatment. Ten fruits were incubated in the chamber and the data were recorded automatically.

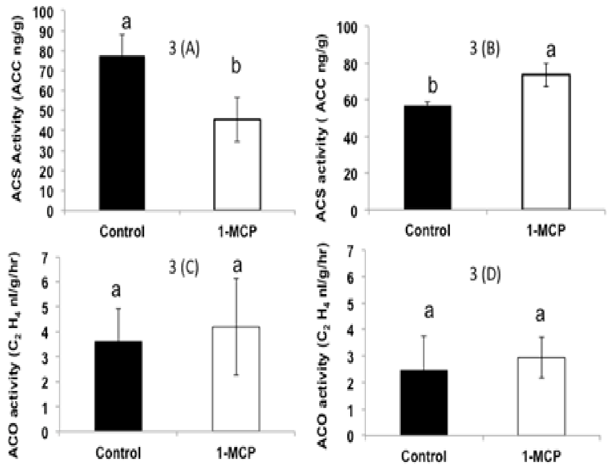

Figure 3. Effect of 1-MCP treatment on ACS at green 3(A) and pink 3(B) stages and ACO at green 3(C) and pink 3 (D) respectively. ACS and ACO activity was measured by GC-MS as the amount of ACC being produced after the addition of SAM and the amount of ethylene being produced after the addition of ACC, respectively. Ten discs 1 cm in diameter of tomato were used for the measurements. Results represent means ± SE (n=10). Values with different superscripts indicate significant difference (p< 0.05)

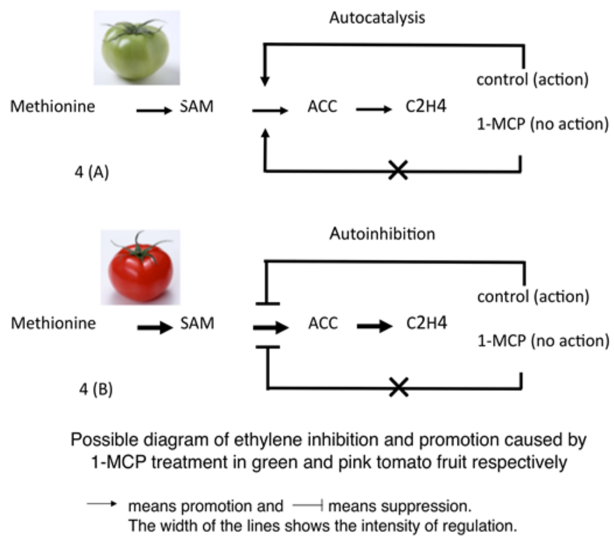

Figure 4. Possible diagram of ethylene inhibition and promotion caused by 1-MCP treatments in mature green (A) and pink (B) stages respectively. The effects of 1-MCP on tomato fruit were observed in different two ways: blocking autocatalysis ethylene production in green mature fruit due to reduced ACS activity and blocking autoinhibition in pink tomato fruit due to increased ACS activity (cv. Red Ore). On the other hand, the activity of ACO remained insignificant at both stages respectively.

color of tomato and delay changes both in green and pink stages (Figure 5. C and D). The mature green tomato fruit, which were treated with 1-MCP, were significantly delayed in color changes after 1, 2, and 3 weeks in storage at ambient temperature as compared to the control. Similarly, the tomato harvested at pink stage and treated with 1-MCP significantly delayed changes in color at 0 hour at the 1% level and they were significant at the 5% level from week 1 to week 3 as compared with control without any treatments.

Loss in weight

The data on weight loss revealed the effectiveness of 1-MCP treatment on tomato fruit at both stages tomato fruit (data not shown). The mature green tomato treated with 1-MCP, significantly delayed loss in weight at the 1% level at 24, 48, 96, 144, 240, and 288 hours after treatment as compared with control. Similarly, the tomato fruit harvested at pink stage and treated with 1-MPC significantly prevented the loss in weight at the 1 % level at 24, 48, 96 and 144 hours and 5% level at 192, 240, and 388 hours as compared with control (Tukey's test).

Discussion

The results of this experiment show that 1-MCP possesses a practical benefit to delay ripening and decrease respiration, with delayed changing in color, retarded softness and reduced loss in weight in both stages of harvest in both mature green and pink stages. It is of great interest that the 1-MCP regulated the ethylene production in different ways between green and pink stages of tomato fruit, suggesting that the role of ethylene production in its self-regulation could be different according to the ripening stages of tomato fruit. That effect was only examined in tomato cultivar "Red Ore" in this article, but similar results were obtained in case of other tomato cultivars in our preliminary study. The data of ACO and ACS activities lead us to the possible mechanism of ethylene regulation in tomato cultivar "Red Ore"; the decrease in ethylene production at green stages, and increase at pink stages might be due to the effect of 1-MCP on ethylene production through ACS activity, which results in blocking autocatalysis (in case of mature green) and autoinhibition mechanisms (in case of pink stage)(Figure 4). The results could be reasonable if we consider that the climacteric rise could be triggered by autocatalysis phase I ethylene in case of mature green tomato fruit, while fruit over-ripening might be avoided by autoinhibition system in case of pink stage. The different reaction of tomato fruit to 1-MCP at mature green and pink stage is a new finding, and the facts were dually made sure by different ethylene measurement procedures. There have been some researchers who showed the influence of 1-MCP on ethylene biosynthesis through feedback inhibition in mature green tomato fruit

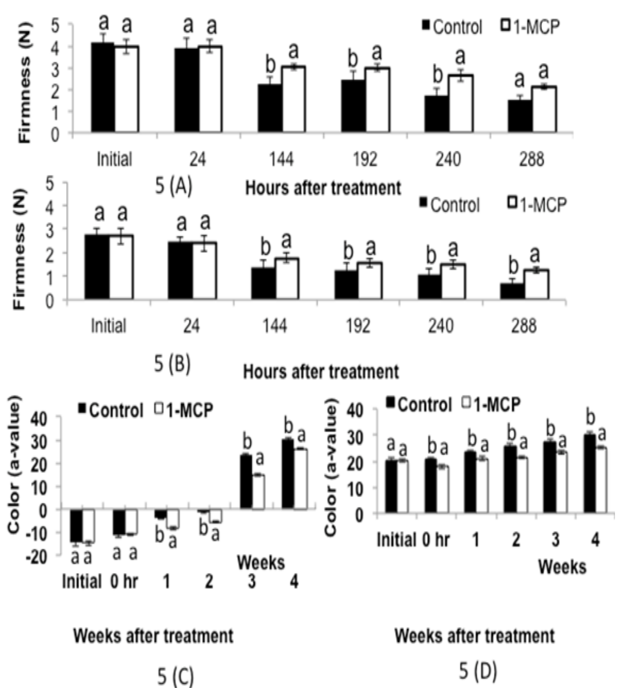

Figure 5. Effect of 1-MCP treatment on fruit firmness in tomato at mature green 5(A) and pink 5 (B) stages and color at mature green ((C) and pink (D) stages respectively. The firmness was measured by non-destructive Multilateral Tester and the color was measured by Handy Colorimeter. Results represent means ± SE (n=10). Values with different superscripts indicate significant difference ($p < 0.05$)

like Wills and Ku in 2002, but they did not mention the different reaction of tomato fruit to 1-MCP. The same can be said in other reports on the influences of 1-MCP on ethylene biosynthesis in some fruits and vegetables through feedback inhibition (Blankenship and Dole, 2003). As for the efficacy of 1-MCP on tomato fruit, some reports mentioned fruit quality, and others mentioned some combinations of 1-MCP concentration (from 0.035 to 100 µl L^{-1}), duration of treatment and temperature, and application durations (from 12 to 24 h) (Hoeberichts *et al.*, 2002; Mostofi *et al.*, 2003; Opiyo and Ying, 2005).

1-MCP effects on other ripening-related factors such as respiration. 1-MCP treatment decreased the amount of CO_2 production at both mature green and pink stages as compared with control (Figure 1 C and D). This may be due to the blocking of ethylene effect of climacteric rise induction. This is in conformity with the results of the previous reports of Will and Ku (2002) and Guillen *et al.* (2006) in tomato, Abdi *et al.* (1998) in plum and Watkin (2006) in apple.

Some quality related parameters such as color, firmness, and loss in weight have been reported to be deeply related to ethylene production (Valero *et al.*, 2010). Color change in tomato fruit can be attained by chlorophyll degradation and carotenoid pigmentation and both processes are known to be promoted by ethylene production (Valero and Serrano, 2010). The result that 1-MCP retarded the color changes on both stages of harvest in our experiment is in accordance with the previous works reported by Wills and Ku (2006), as well as the case for apple, pear, green plum, kiwifruit, and avocado (Valero and Serrano, 2010). Fruit softening is prevented or delayed by 1-MCP treatment on both green and pink stages of harvest and this also could be attributed to the reduced ethylene-receptor bindings resulting in less activities of cell wall-degrading enzymes like cellulase, polygalacturonase, and pectinesterase. Similar results are reported in tomato (Wills and Ku, 2006), in apricot (Valero and Serrano, 2010), banana (Lohani *et al.*, 2004), pear (Mwaniki *et al.*, 2005) and plum (Watkin, 2006). The fact that 1-MCP prevented losses in weight in both mature green and pink stages tomato may be due to the hard skin which was attained by the increased fruit firmness. The effect of 1-MCP on reduction in weight loss is also reported in tomato (Guillen *et al.*, 2006), plums (Valero *et al.*, 2003), and avocado (Watkin, 2006). We could conclude that 1-MCP can be a promising tool to prolong shelf life of tomato fruit both in mature green and pink mature stages.

Conclusion

1-MCP treatment prolonged the shelf life of tomato fruit by blocking ethylene action regardless of ripening stage. Hence, it preserved color, firmness, reduced respiration and loss in weight. However, the mode of 1-MCP action could be different depending on the ripening stage of tomato fruit: blocking of autocatalysis of ethylene production in green mature stage and blocking of autoinhibition of ethylene production in pink stage. In addition, 1-MCP is generally recognized as safe (GRAS) based on the approval by Environmental Protection Agency (EPA) in 2002, and European Union in 2005 as non toxic chemical with negligible residue. It might be greatly useful especially in developing countries, because treating fruits and vegetables with 1-MCP doesn't need special skills and it is a less-costly method.

Acknowledgements

The authors are greatly thankful to Green Farm Lapin Co.,Ltd., especially Mr. Kiyofumi Watanabe and Mr. Yoshinobu Ariga, who kindly provided tomato fruit for our experiments.

References

Abdi, N., Holford, P., McGlasson, W.B. and Mizrahi, Y. 1997. Ripening behavior and responses to propylene in four cultivars of Japanese plum. Postharvest Biology and Technology 12: 21-34.

Abeles, F.B., Morgan P.W. and Saltveit, M.E. 1992. Ethylene in plant biology. San Eiego, California: Academic Press.

Adam-Philips, L., Barry C. and Giovannoni, J. 2004. Signal transduction systems regulating fruits ripening. Trends Plant Science 9: 331-338.

Blankenship, S.M. and Dole, J.D. 2003.1-MCP: a review. Postharvest Biology and Technology 28(1): 14-19.

Givannoni, J. 2001. Molecular biology of fruits maturation and ripening. Annual Review of Plant Physiology and Plant Molecular Biology 52: 725-749.

Guill' en, F., Castillo, S., Zapata, P.J., Mart'inez-Romero, D., Serrano, M. and Valero. 2006. Efficacy of 1-MCP treatment in tomato fruit. 2. Effect of cultivar and ripening stage at harvest. Postharvest Biology and Technology 42: 235-37.

Guill' en, F., Castillo, S., Zapata, P.J., Mart'inez-Romero, D., Serrano, M. and Valero, D. 2007. Efficacy of 1-MCP treatment in tomato fruit: 1.duration and concentration of 1-MCP treatment to gain an effective delay of post harvest ripening. Postharvest Biology and Technology 43: 23-27.

Hoeberichts, F.A., Van der Plas, L.H.W. and Woltering, E.J. 2002. Ethylene perception is required for the expression of tomato ripening-related genes associated physiological changes even at advanced stages of ripening. Postharvest Biology and Technology 26: 125–133.

Inge B., Bram, V. D. P., Maarten. L. H., Maurice. P. D. P., Annemie. H. G. and Bart. M. N. 2011. An updated integrated methodology for analysis of metabolites and enzyme activities of ethylene biosynthesis. Plant Methods 7(17): 5-7.

Jiang, Y. and Joyce, D. 2002. 1-MCP treatment effects on intact and fresh cut apple. Journal of Horticultural Science and Biotechnology 77: 19-21.

Leliver J.M., Latche A., Jones B., Bouzayen M. and Pech J.C. 1998. Ethylene and fruit ripening. Plant Physiology102: 336-60.

Lohani, S., Trivedi, P.K. and Nath, P. 2004. Changes in activities of cell wall hydrolases during ethylene-induced ripening in banana: Effect of 1-MCP, ABA and IAA. Postharvest Biology and Technology 31:119-126.

Mostofi, Y., Toivonen, P.M.A., Lessani, H., Babalar, M. and Lu, C. 2003. Effects of 1-MCP on ripening of greenhouse tomatoes at three storage temperatures. Postharvest Biology and Technology 27: 285–292.

Mwaniki, M.W., Mathooko, F.M., Matsuzaki, M., Hiwasa, K., Tateishi, A. and Ushijima, k. 2005. Expression characteristic of seven members of the beta-galactosidase gene family in La france pear (*Pyrus communis* L.) fruits during their growth and their regulation by 1-Methylcyclopropene during postharvest ripening. Postharvest Biology and Technology 36: 253-263.

Opiyo, A.M. and Ying, T.J. 2005. The effects of 1-methylcyclopropene treatment on the shelf life and quality of tomato (*Lycopersicon esculentum* var. cerasiforme) fruit. International Journal of Science and Technology 40: 665–673.

Rongcai, Y. and Jiangou, L. 2008. Effect of sprayable 1-MCP, NAA and AVG on ethylene biosynthesis, preharvest drop, fruit maturity, and quality of delicious apple. Horticultural Science 43(5): 1454-1460.

Rosa, S. R. 2005. Postharvest management of fruits and vegetables in the Asia-Pacific Region. India. Seminar on Reduction of Postharvest Losses of Fruits and Vegetables. Report of APO (Asian Productivity Organization) and Food and Agriculture Organization (FAO).

Saltveit M.E.1999. Effect of ethylene on quality of fresh fruits and vegetables. Postharvest Biology and Technology 15: 279-292.

Sisler E.C. and Blankenship S.M. US Patent No.5,518,988. 1996. Methods of counteracting an ethylene response in plants, United States Patent.

Sisler EC. And Serek, M. 1997. Inhibitors of ethylene responses in plants at the receptor level: recent developments. Plant Physiology 100:577-582.

Sisler EC. and Serek, M. 2003. Compound interacting with the ethylene receptors in the plant. Plant Biology 5: 473-80.

Valero, D., Martinez-Romero, D., Valverde, J.M., Guillen, F. and Serrano, M. (2003). Quality improvement and extension of shelf life by 1-methylcyclopropene in plum as affected by ripening stage at harvest. Innovative Food Science and Emerging Technologies 4: 339-348.

Valero, D. and Serrano, M. 2010. Postharvest Biology and Technology for Preserving Fruit Quality. In1-Methylcyclopropene treatments. 8:163. New York: CRS Press.

Watkins, C.B. 2002. Ethylene synthesis, mode of action, consequences and control. In: Knee M, editor. Fruits quality and its biological basis. p.180-224. Boca Raton, Florida: Sheffield Academic Press.

Watkins, C.B. 2006. The use of 1-Methylecyclopropene on fruits and vegetables. Biotechnology Advances 24: 389-409.

Wills, R.B.H. and Ku, V.V.V. 2002. Use of 1-MCP to extend the time to ripen of green tomatoes and postharvest life of ripe tomatoes. Postharvest Biology and Technology 26: 85-90.

Yin, Y.G., Tominaga, T., Iijima, Y., Aoki, K., Shibata, D., Ashihara, H., Nishimura, S., Ezura, H. and Matsukura, C. (2010). Metabolic Alterations in Organic Acids and gamma-Aminobutyric Acid in Developing Tomato (*Solanum lycopersicum* L.) Fruits. Plant and Cell Physiology 51(8):1300-1314.

Yuan, R. and Carbaugh D.H. 2007. Effects of NAA, AVG, and 1-MCP on ethylene biosynthesis, preharvest fruit drop, fruit maturity, and quality of 'Golden Supreme' and 'Golden Delicious' apples. Horticultural Science 42: 101–105.