African Journal of Biotechnology Vol. 12(7), pp. 689-694, 13 February, 2013
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.1446
ISSN 1684–5315 ©2013 Academic Journals

Full Length Research Paper

Effect of 1-methylcyclopropene (1- MCP) treatment on antioxidant enzymes of postharvest Japanese apricot

Ting Shi[1], Zhiqiang Li[2], Zhen Zhang[1], Chunchun Zhang[1] and Zhihong Gao[1]*

[1]College of Horticulture, Nanjing Agricultural University, Nanjing 210095, P. R China.
[2]Jiangsu Academy of Agricultural Sciences, Nanjing 210014, P. R China.

Accepted 27 July, 2012

'Longyan Mei', a Japanese apricot species, was used to investigate the effect of 1-methylcyclopropene treatment on the browning index, soluble solid content (SSC), titratable acidity (TA) and enzyme activity of active oxygen-related metabolism of post-harvest Japanese apricot. The results show that 1-MCP inhibited the browning rate, maintained a high content of SSC and TA, controlled the increase of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activity at prophase of storage, and increased the enzyme activities of SOD, POD and CAT at the end of the storage.

Key words: Japanese apricot, 1-MCP, superoxide dismutase (SOD), peroxidase (POD), catalase (CAT).

INTRODUCTION

1-methylcyclopropene (1-MCP), an ethylene inhibitor, combined with ethylene receptors inhibited the role of ethylene in plants, increased the post-harvest life of fruits and vegetables and provided more flexibility during storage, distribution and retail. Several studies have shown the usefulness of 1-MCP in maintaining the quality of and delaying the ripening of fruits (Blankenship and Dole, 2003; Kahl et al., 2000). When 1-MCP was applied to *Pyrus communis* prior to cold storage, the synergistic interaction of cold temperatures and 1-MCP resulted in an extended post-harvest life after transfer to room temperature. In contrast, the application of 1-MCP after cold storage did not affect most indices of ripening (Trinchero et al., 2004). The browning rates of peach (*Prunus persica*) flesh treated by 1-MCP were largely depressed (Yin et al., 2002). The respiratory peak values of 1-MCP-treated persimmon (*Diospyros kaki*) were lower than that of controls, while the treated group was more effective than the control group at retarding the decrease in hardness and slowing down the increase in membrane permeability. In addition, the 1-MCP-treated fruit retained a high content of vitamin C (ascorbic acid) and retarding the decrease in pectin levels (Zhuang et al., 2007). 1-MCP has also been applied to the storage of *Actinidia* (Boquete et al., 2004). *Fragaria ananassa* (Li et al., 2006; Tian et al., 2000) *Prunus avium* (Gong et al., 2002; Liu et al., 2005) and *Citrullus lanatus* (Mao et al., 2004). Recently, the research fields of 1-MCP have been extended to cover molecular biology (Cools et al., 2011; Huber et al., 2010; Kita et al., 2007).

Superoxide dismutases (SODs), including copper-zinc superoxide dismutase (Cu/ZnSOD), manganese super-oxide dismutase, and extracellular superoxide dismutase, play a crucial role in scavenging O_2^- (Miao et al., 2009). The peroxidase (POD) enzyme is a major chemical component that causes changes during the maturation of fruits influencing the final quality of vegetable products (Vanini et al., 2010). Plant peroxidases (PODs) have been ascribed a variety of biological functions, including hydrogen peroxide detoxification, lignin biosynthesis, hormonal signaling, and stress response (Gao et al., 2010). Catalase (CAT) as the important biological substance; its main function is to participate in active oxygen metabolism process and play significant roles

*Corresponding author. E-mail: gaozhihong@njau.edu.cn.

Abbreviations: PODs, Peroxidases; **SODs,** superoxide dismutases; **CAT,** catalase; **SSC,** soluble solids content; **TA,** titratable acidity; **NBT,** nitroblue tetrazolium.

in getting rid of the oxygen free radicals and H_2O_2 and preventing hydroxyl radicals forming (Spanou et al., 2011).

Japanese apricot (*Prunus mume* Sieb. et Zucc) originated from China and belongs to the *Rosaceae* family of fruits. The fruit are mainly used in the preparation of preserved fruit and wine, but can also be used as a diet ingredient which has high nutritional and economic value (Chu, 1999; Xia et al., 2011). Meanwhile, there is a traditional custom of eating the Japanese apricot in Japan and Korea. Therefore, the Japanese apricot is a significant exported commercial fruit (Wang et al., 2008). Temperature is usually high in the ripening and picking seasons of the Japanese apricot, which easily causes the browning and rotting of fruits and the lack of raw material processing. It is a climacteric fruit that produces large amounts of ethylene as it ripens (Mita et al., 1999, 2006). Consequently, the storage of the Japanese apricot has become a serious problem (Xia et al., 2010). The hot water treatment could delay the ripening of post-harvest Japanese apricot fruit and the onset of the climacteric peaks of CO_2 and ethylene production (Luo, 2006). Only few researches have been published that analyses the influence of 1-MCP treatment on apricot fruit (Botondi et al., 2003; Cao et al., 2009; Dong et al., 2002; Kita et al., 2007; Luo et al., 2006) but scarcely involved in the activity changes of antioxidant enzymes. In this study, the effect of 1-MCP treatment on the quality changes and other antioxidant enzymes of Japanese apricot fruit during refrigerated storage were evaluated.

MATERIALS AND METHODS

'Longyan mei' were picked from the Genebank Field of Japanese apricot in the Nanjing Agricultural University, China on June 19th 2010 and 2011. The selected fruit was neat, of average size and shape, no mechanical injury or plant diseases and insect pests, and was of medium to increased maturity. Fruits were divided into two groups with one group being the control while the other underwent treatment.

1-MCP treatment

A few improvements were made to Egea's methods (Egea et al., 2010). Fruit was treated with 500 $\mu l \cdot L^{-1}$ 1-MCP after one day harvested, and processed for 24 h at 10°C. After treatment with 90 to 95% RH, fruit was stored at 10°C and the relevant index determination was performed regularly. The group without 1-MCP treatment was the control. 1-MCP was supplied by the Jiangsu Academy of Agricultural Sciences.

Investigation of browning index

The browning situation of fruit was evaluated once in every two days. Samples were cut along the surface near the Japanese apricot core according to the browning proportion of the longitudinal section. Browning levels were divided into the following four levels; level 0 was with no browning; level 1 was 0 to 30%; level 2 was 30 to 60%; and level 3 was more than 60%. The formula of browning index depended on the description as published by Yin et al. (2002).

Soluble solids content (SSC) and titratable acidity (TA)

The content of SSC and TA was investigated once in every six days. Juice was squeezed from fruit pulp, centrifuged for 10 min at 12,000 and the supernatant was retrieved in order to investigate the content of SSC and TA. The PR-201 handheld brix metre was used to determine the content of SSC. An acid-base titration method was utilized to investigate the value of TA (Guo et al., 2010).

Enzyme activity related to active oxygen

The enzyme activity related to active oxygen was investigated once in every six days. First, the SOD activity assay was based on the method described by Beauchamp et al. (1971) which measured the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) spectrophotometrically at 560 nm. One unit of enzyme activity was defined as the quantity of SOD required to produce a 50% inhibition of the reduction of NBT, and the specific enzyme activity was expressed as U g^{-1}. Secondly, the POD activity was based upon the method described by Herzog et al. (1973), which measured the increase in absorbance at 470 nm by the rate of formation of the oxidized 3,3'-diaminobenzidine (DAB) in 0.15 M of sodium phosphate citrate buffer. The reaction mixture contained DAB solution and 0.6% H_2O_2. The increase in A_{470} was followed for 2 min. One enzyme unit was defined as U g^{-1} that destroyed H_2O_2 per min and thirdly, the CAT activity was performed according to the method described by Bergmeyer (1965), which measured the decline of the extinction of H_2O_2 at the maximum absorption of 240 nm. The decrease in the absorption was followed for 3 min and U g^{-1} H_2O_2 destroyed per min was defined as one unit of CAT.

RESULTS

Effect of 1-MCP treatment on the browning index

The browning phenomenon in the control and treated groups occurred after 24 and 30 days of storage, respectively (Figures 1c and d). This shows that the browning of the control group occurred much earlier than that of the treated group, and the browning index was significantly higher than that of the treated group. The final browning index was 75.7 and 31% in the control and treated groups, respectively (Figure 2).

Effect of 1-MCP treatment on the content of SSC and TA

Figure 3 indicates that the content of SSC in the control group appeared to decrease from the 12th day after storage, while the treated group almost maintained the same for 18 days and appeared to show a rising trend from the 18th day. In general, the SSC of the control group was slightly less than that of the treated group. These results suggested that 1-MCP treatment could repress the decrease in the content of SSC in the

Figure 1. The browning feature of Japanese apricot treated with 1-MCP; a-d: Longyan Mei; a: first day treatment; b: 12 days after treatment; c: 24 days after treatment; d: 30 days after treatment; left is control and right is treated in every photo.

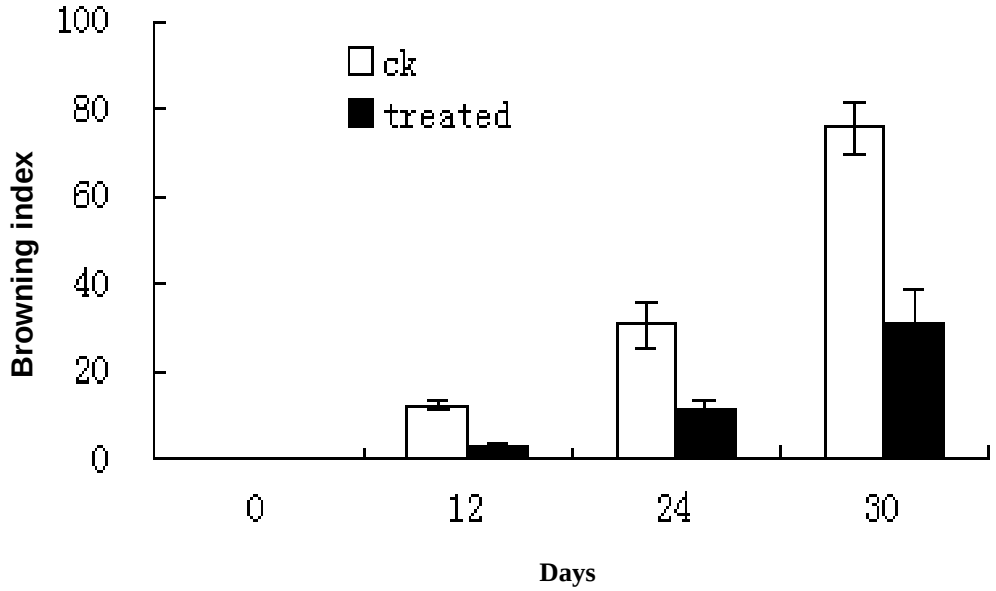

Figure 2. The browning index of Japanese apricot stored after 36 days. The ck is the control group.

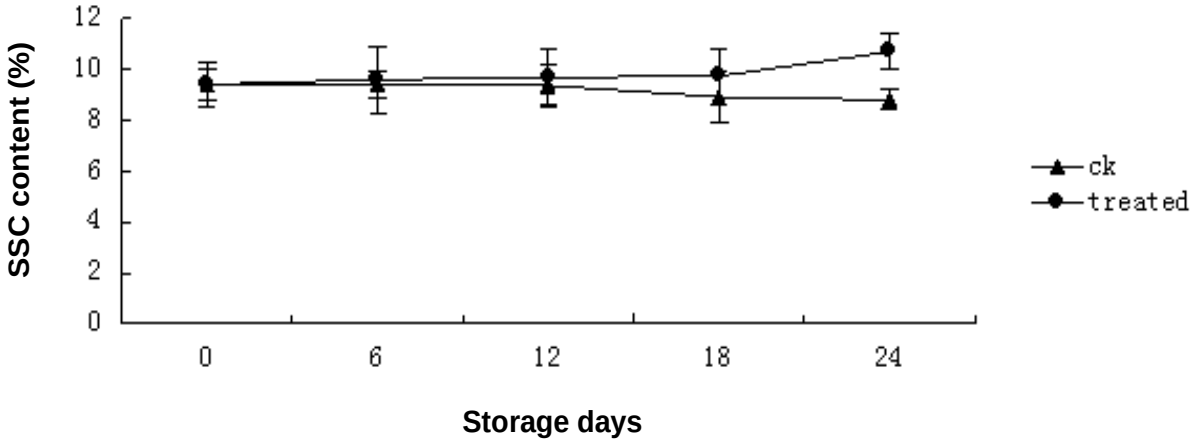

Figure 3. Changes in the SSC content of Japanese apricot treated with 1-MCP. The ck is the control group.

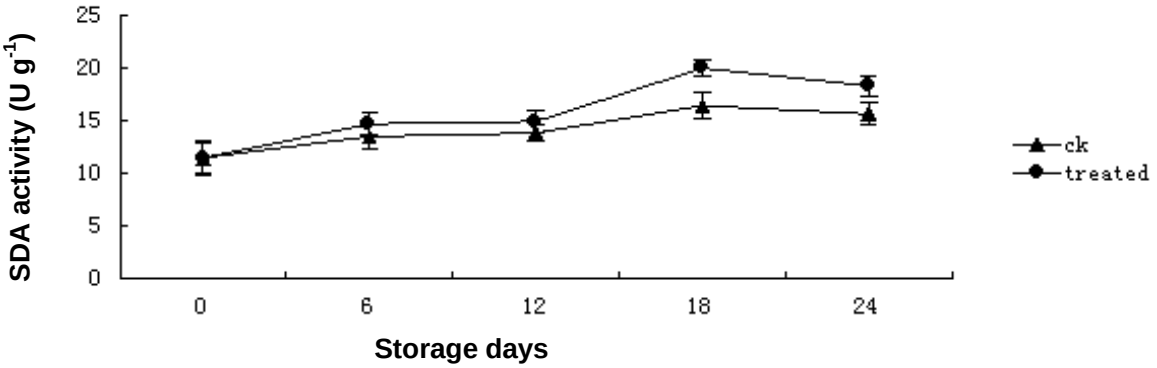

Figure 4. Changes in the SOD activity of Japanese apricots treated with 1-MCP. The ck is the control group.

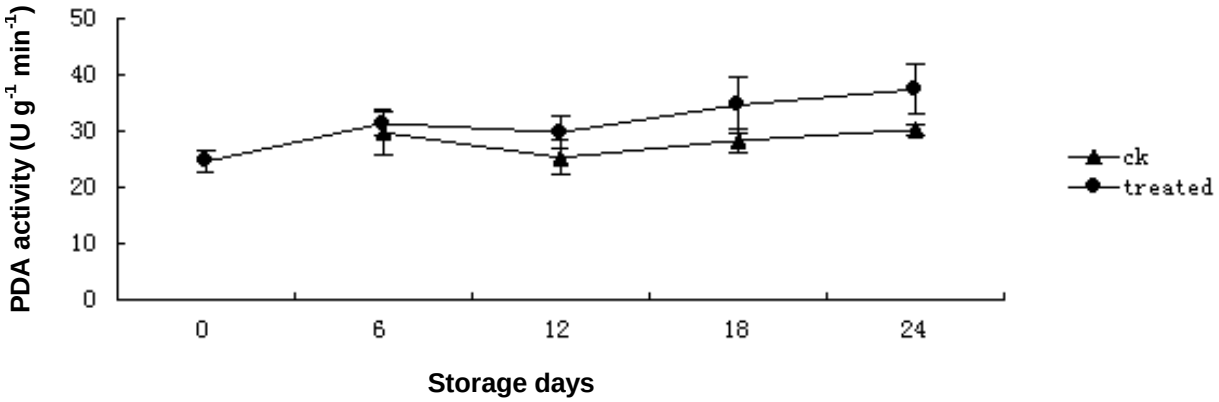

Figure 5. Changes in the POD activity of Japanese apricots treated with 1-MCP. The ck is the control group.

Japanese apricot fruit pulp. The value of TA in the control group remained stable during all stages of cold storage. However, the value for the treated group was slightly increased. In summary, the value of TA in the control group was less than that of the treated group. Therefore, 1-MCP treatment could promote the TA value in the Japanese apricot fruit pulp, but the effect of this repression was not significant (Data not shown).

Effect of 1-MCP treatment on the activity of SOD

With the extension of the storage period, there was an upward trend of SOD activity. From the 12th day after storage, the SOD activity was significantly higher in the treated group. On the 18th day of storage, the activity in the control group showed the highest value of 16.5U·g^{-1}, and then began to show a slow downward trend. Similarly, the peak of SOD activity of the treated group also emerged on the 18th day, which was remarkably higher than that of the control group. The SOD activity of the treated group was relatively higher than that of the control group for the entire storage period (Figure 4).

Effect of 1-MCP treatment on the activity of POD

As is shown in Figure 5, there was a rise in the POD activity in the six days from the start of storage. However, the activity declined from the 6th to the 12th days. There was a dramatic increasing trend from the 12th day, and the highest value was seen on the 24th day in both groups. The POD activity in the treated group was higher than that of the control group, which indicates that 1-MCP might contribute to the increase in POD activity.

Effect of 1-MCP treatment on the activity of CAT

Figure 6 shows that the CAT activity of the control group remained relatively constant throughout the entire storage period. Interestingly, the activity of the treated group rose

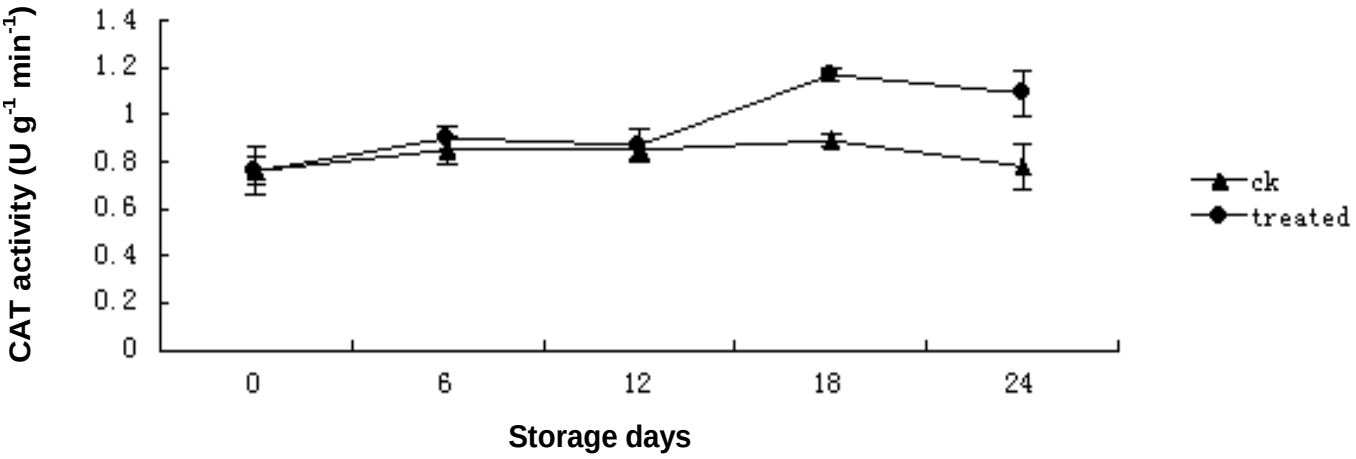

Figure 6. Changes in the CAT activity of Japanese apricot treated with 1-MCP. The ck is the control group.

sharply from the 12th to the 18th day and showed the highest value on the 18th day, with a gradual decrease from the 18th day. The CAT activity of the treated group was higher than that of the control group, which indicates that 1-MCP could stimulate the CAT activity.

DISCUSSION

According to the analysis of physiological and organoleptic indices of Japanese apricots with 1-MCP treatment, 1-MCP delayed the change of the content of SSC and TA, retarded the rotting process in Japanese apricot fruit, enhanced the effect of cold storage and increased the storage value. 1-MCP could decrease the glycol-metabolism through repressing the respiration while the polysaccharides hydrolysed to soluble sugar. Therefore, the SSC content in treated fruit increased during the storage (Nuñez-Elisea et al., 1999). At the same time, 1-MCP was found to control the activities of some enzymes related to active oxygen, such as SOD, POD and CAT, in the earlier periods of storage, and to increase their activities in the later periods. 1-MCP was effective at decreasing the rate of active oxygen production and delaying the senescence of the fruit. In addition, 1-MCP delayed the process of fruit browning. Therefore, the effect of prolonging the acceptable time of appearance was obvious.

Egea et al. (2010) treated apricots (*Prunus armeniaca* L. cv. B´ ulida) with a concentration of 1 $mL \cdot L^{-1}$ of 1-MCP, which was significantly higher than that in previous reports of other species. An example of this is eggplant (*Solanum melongena* L.), which was treated with a concentration of 1 $\mu L \cdot L^{-1}$ of 1-MCP (Massolo et al., 2011). In addition, different concentration treatments were tested and 500 $\mu L \cdot L^{-1}$ was identified as the most suitable concentration for Japanese apricots.

There was a close relationship between the style of antioxidant enzymes and the ripening and senescence of the fruits. Reactive oxygen could lead to membrane lipid peroxidation, which caused severe injury to the cell membrane and resulted in the senescence, but 1-MCP had the ability to influence the activities of the enzymes which eliminated active oxygen (Li et al., 2007). There were many protective enzymes that eliminated active oxygen in plant tissues, such as SOD, POD and CAT, which played a significant role in keeping the balance of oxygen metabolism (Jiao et al., 2008; Xie et al., 2004). It was also reported that 1-MCP repressed the increase of SOD and POD activities in the early stages and the decrease of the same in late stage (Aryanpooya et al., 2010), which was shown to be similar by the results of this study. However, 1-MCP was not shown to affect SOD and POD activities in *Litchi chinensis* (Pang et al., 2001). 1-MCP also had no effect on CAT and POD in the strawberry plant (Li et al., 2006), but distinctly influenced SOD activity. These results indicated that 1-MCP had different effects on eliminating active oxygen in the different species. On one hand, POD was one of the main enzymes eliminated by active oxygen in the plant cell, which hydrolyzed H_2O_2 to obtain detoxification. On the other hand, POD was also a key enzyme involved in plant tissue browning, and an increase of POD activity easily caused fruit browning. The browning phenomenon is usually attributed to enzymatic browning. POD is responsible for enzymatic browning by oxidizing some phenolic substrates (Lee et al., 1995). POD has many roles in plant growth and development. It was reported that POD was associated with chlorophyll degradation and lipid peroxidation in senescent plant tissues (Campa, 1991). This might be the reason for large-scale browning in both of the groups after the 24th day of storage.

In general, 1-MCP was a highly effective anti-browning agent for horticultural produce (Watkins, 2006). It has a definite effect in the application of storing Japanese apricots and the prolongation of the storage time by

enhancing the activities of SOD, POD and CAT.

REFERENCES

Chang YL, John RW (1995). Enzymatic browning and its prevention. American Chemical Society. pp 2-22.

Beauchamp C, Fridovich I (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal. Biochem. 44:276-287.

Bergmeyer HU (1965). Methoden der enzymatischen Analyse. Verlag Chemie, Weinheim, Germany.

Blankenship SM, Dole JM (2003). 1-Methylcyclopropene: a review. Postharv. Biol. Technol. 28:1-25.

Boquete EJ, Trinchero GD, Fraschina AA, Vilella F, Sozzi GO (2004). Ripening of 'Hayward' kiwifruit treated with 1-methylcyclopropene after cold storage. Postharv. Biol. Technol. 32:57-65.

Botondi R, DeSantis D, Bellincontro A, Vizovitis K, Mencarelli F (2003). Influence of ethylene inhibition by 1-methylcyclopropene on apricot quality, volatile production, and glycosidase activity of low-and high-aroma varieties of apricots. J. Agric. Food Chem. 51:1189-1200.

Campa A (1991). Biological roles of plant peroxidases: known and potential function. Peroxidases in Chem. Biol. 2:25-50.

Cao J, Zhao Y, Wang M, Lü H, Jiang W (2009). Effects of 1-methylcyclopropene on apricot fruit quality, decay, and on physiological and biochemical metabolism during shelf-life following long-term cold storage. J. Hort. Sci. Biotechnol. 84:672-676.

Chu MY (1999). China fruit records-Mei (in Chinese). China Forestry, Beijing.

Cools K, Chope GA, Hammond JP, Thompson AJ, Terry LA (2011). Ethylene and 1-methylcyclopropene differentially regulate gene expression during onion sprout suppression. Plant Physiol. 156:1639.

Dong L, Lurie S, Zhou HW (2002). Effect of 1-methylcyclopropene on ripening of 'Canino' apricots and 'Royal Zee' plums. Postharvest Biol. Technol. 24:135-145.

Egea I, Flores FB, Martínez-Madrid MC, Romojaro F, Sánchez-Bel P (2010). 1-Methylcyclopropene affects the antioxidant system of apricots (*Prunus armeniaca* L. cv. Búlida) during storage at low temperature. J. Sci. Food Agric. 90:549-555.

Gao C, Wang Y, Liu G, Wang C, Jiang J, Yang C (2010). Cloning of ten peroxidase (POD) genes from *Tamarix hispida* and characterization of their responses to abiotic stress. Plant. Mol. Biol. Rep. 28:77-89.

Gong Y, Fan X, Mattheis JP (2002). Responses of `Bing' and `Rainier' sweet cherries to ethylene and 1-methylcyclopropene. J. Am. Soc. Hort. Sci. 127:8.

Guo X, An L, Wang Y, Li L, Wang G, Chen X (2010). Principal components analysis of the nfluence of 1-MCP and salicylic acid treatment on fruit quality of Angeleno plum. Food Sci. 31:416-422.

Herzog V, Fahimi H (1973). Determination of the activity of peroxidase. Anal. Biochem. 55:554-562.

Huber DJ, Hurr BM, Lee JS, Lee JH (2010). 1-Methylcyclopropene sorption by tissues and cell-free extracts from fruits and vegetables: evidence for enzymic 1-MCP metabolism. Postharvest Biol. Technol. 56:123-130.

Jiao Y, Yin Y, Yan M, Zhao J, Han Q (2008). Daily change of anti-ageing enzymes activities of different peanut cultivars. Chin. Agric. Sci. Bull. 24:220-224.

Kahl J, Siemens DH, Aerts RJ, GaÈbler R, KuÈhnemann F, Preston CA, et al. (2000). Herbivore-induced ethylene suppresses a direct defense but not a putative indirect defense against an adapted herbivore. Planta. 210:336-342.

Kita M, Kato M, Ban Y, Honda C, Yaegaki H, Ikoma Y, Moriguchi T. (2007). Carotenoid accumulation in japanese apricot (Prunus mume Siebold & Zucc.): Molecular analysis of carotenogenic gene expression and ethylene regulation. J. Agric. Food Chem. 55:3414-3420.

Li Z, Wang L, Gong W, Wang Z (2006). Effect of 1- MCP on senescence and quality of strawberry fruit. J. Fruit Sci. 23:125-128.

Li Z, Wang L, Wang W, Zhu Y (2007). Physiological effect and application of 1-MCP on delaying fruit senescence. Plant Physiol. J. 43:201-206.

Liu Z, Zen M, Dong S, Song Y, Zhang Y (2005). Effects of 1-methylcyclopropene on fruit browning of sweet cherry. J. Fruit Sci. 22: 488-491.

Luo Z (2006). Hot water treatment of postharvest mei fruit to delay ripening. Hort. Sci. 41:737-740.

Luo Z, Shen J (2006). Involvement of 1-methylcyclopropene in ripening of harvested mei (Prunus mume) fruit. J. Hortic. Sci. Biotechnol. 81:813-818.

Mao L, Karakurt Y, Huber DJ (2004). Incidence of water-soaking and phospholipid catabolism in ripe watermelon (Citrullus lanatus) fruit: induction by ethylene and prophylactic effects of 1-methylcyclopropene. Postharvest Biol. Technol. 33:1-9.

Massolo JF, Concellón A, Chaves AR, Vicente AR (2011). 1-Methylcyclopropene (1-MCP) delays senescence, maintains quality and reduces browning of non-climacteric eggplant (Solanum melongena L.) fruit. Postharvest Biol. Technol. 59:10-15.

Miao L, St Clair DK (2009). Regulation of superoxide dismutase genes: implications in disease. Free Radical Biol. Med. 47:344-356.

Mita S, Kirita C, Kato M, Hyodo H (1999). Expression of ACC synthase is enhanced earlier than that of ACC oxidase during fruit ripening of mume (Prunus mume). Physiol. Plant 107:319-328.

Mita S, Nagai Y, Asai T (2006). Isolation of cDNA clones corresponding to genes differentially expressed in pericarp of mume (Prunus mume) in response to ripening, ethylene and wounding signals. Physiol. Plant. 128:531-545.

Nuñez-Elisea R, Crane JH (1999). 582 Off-season carambola (Averrhoa carambola L. cv. Arkin) production under glass. HortScience 34:547-547.

Pang X, Zhang S, Duan X, Ji Z (2001). Effects of ethylene and 1-methylcyclopropene on fruit peel browning of Litchi chinesis. J. S. China Agric. Univ. 22:11-14.

Spanou CI, Veskoukis AS, Stagos D, Liadaki K, Aligiannis N, Angelis A, Skaltsounis AL, Anastasiadi M, Haroutounian SA, Kouretas D (2011). Effects of Greek legume plant extracts on xanthine oxidase, catalase and superoxide dismutase activities. J. Physiol. Bioch. 68:37-45.

Tian M, Prakash S, Elgar H, Young H, Burmeister D, Ross G (2000). Responses of strawberry fruit to 1-methylcyclopropene (1-MCP) and ethylene. Plant Growth Reg. 32:83-90.

Trinchero G, Sozzi G, Covatta F, Fraschina A (2004). Inhibition of ethylene action by 1-methylcyclopropene extends postharvest life of "Bartlett" pears. Postharvest Biol. Technol. 32:193-204.

Vanini LS, Kwiatkowski A, Clemente E (2010). Polyphenoloxidase and peroxidase in avocado pulp (Persea americana Mill.). Ciência Tecnol. Alime. 30:525-531.

Wang S, Cai B, Zhang Z, Hou J, Xu J, Gao Z (2008). Analysis of differential proteins in perfect and imperfect flowers of Japanese apricot (Prunus mume Sieb. et Zucc.). J. Plant Gen. Res. 9:465-468.

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

Xia D, Shi J, Gong J, Wu X, Yang Q, Zhang Y (2010). Antioxidant activity of Chinese mei (Prunus mume) and its active phytochemicals. J. Med. Plants Res. 4:1156-1160.

Xia D, Wu X, Shi J, Yang Q, Zhang Y (2011). Phenolic compounds from the edible seeds extract of Chinese Mei (Prunus mume Sieb. et Zucc) and their antimicrobial activity. LWT-Food Sci. Technol. 44:347-349.

Xie M, Zhang J, Xie J (2004). Relationships between some physio-biochemical changes and senescence during storage in bitter gourd. Acta Bot. Boreali-Occidential Sinica. 24:716-719.

Yin Y, Feng S, Zhao Y (2002). Effect of 1-MCP on the storage effect of peach fruit. Food Sci. 23:105-108.

Zhuang Y, Guo C, Wu B, Rao J, Mu G (2007). Effect of 1-MCP on physiological changes of persimmon fruits during storage period. J. Northwest Sci-Tech Univ. of A & F. 35:91-96.