

Changes in physiology and quality of Laiyang pear in long time storage

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ARTICLE INFO

Article history:

Received 6 August 2012

Received in revised form 16 October 2012

Accepted 19 October 2012

Keywords:

Pear

1-MCP

Storage quality

Anti-oxidant enzymes

ABSTRACT

Physiological disorders easily occur in Laiyang pear (*Pyrus bretschneideri* cv. Laiyang) fruit after harvest and result in quality deterioration and short postharvest life. In this paper, we mainly investigated the effects of 1-methylcyclopropene (1-MCP) treatment and controlled atmosphere (CA) with 2% O₂ plus 2% CO₂ on quality of Laiyang pear and storage time. The results indicated that 1-MCP treatment and CA were effective in maintaining quality and prolonging storage time of Laiyang pear fruit, because 1-MCP and CA could significantly delay fruit senescence via limiting ethylene production, reducing fruit respiration rate, regulating anti-oxidant enzymes and membrane permeability. We consider that the major action modes of 1-MCP and CA, that can maintain harvested quality of Laiyang pear fruit, may be greatly contributed to inhibiting ethylene biosynthesis and regulating anti-oxidant pathways.

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1. Introduction

Laiyang pear, as a known cultivar in China, has been widely planted in Shandong province with a long cultivation history over 300 years. The fruit is favorable for special taste and function for curing phlegm and relieving coughs after processed as Chinese drug (Laiyang pear syrup) with a high market value. However, the fruit shows to be a short storage time no more than 1 month in air at room temperature, due to easily occur physiological disorders, leading to great economic losses in postharvest storage periods. Therefore, how to effectively control physiological disorders and extend postharvest storage time becomes a crucial issue for affecting development of Laiyang pear.

Controlled atmosphere (CA) has been widely used to store climacteric fruits, because of effectively extending storage life via controlling O₂ and CO₂ concentration (Jiang et al., 2002; Lin et al., 2004; Wu et al., 2009). Decreased O₂ and/or elevated CO₂ levels over long-term storage can reduce respiration, delay ripening and senescence, as well as slow down firmness loss and color changes, thereby maintaining postharvest quality and extending shelf-life of pear (Ke et al., 1990), longan (Tian et al., 2002) and sweet cherry (Tian et al., 2004). But the specific gas concentrations depend upon different fruit cultivars, for instance, using atmospheres of 1.5% O₂ and 3% CO₂ for long time storage reduced loss of firmness, acidity, and scald severity in 'Anjou' pears (Drake, 1994).

1-Methylcyclopropene (1-MCP) is an inhibitor of ethylene action for extending shelf life of fruit and vegetables by blocking

ethylene receptors (Sisler and Serek, 1997; Blankenship and Dole, 2003). It has been widely used to extend postharvest storage life in a wide range of fruit, such as apples (Baritelle et al., 2001), bananas (Harris et al., 2000), avocado (Jeong et al., 2002), 'Bartlett' pears (Trincherio et al., 2004) and jujube fruit (Zhang et al., 2012). Calvo and Sozzi (2004) reported that application of 1-MCP slowed loss of firmness, titratable acidity, and color change of 'William's' pears. Ekman et al. (2004) ascertained that 1-MCP could reduce the incidence of superficial scald and other physiological disorders in 'Bartlett' pear fruit.

Although application of CA storage and 1-MCP treatment has been well documented in various fruits, it is empty of the effects of them on quality of Laiyang pear fruit in long storage periods. Besides, the modes of actions, by which CA storage and 1-MCP regulate physiological properties of the pear fruit, still need to be further explored. The objectives of our study were to investigate the effects of 1-MCP treatment and CA storage on quality of Laiyang pear fruit during long time storage, and the involved mechanisms related to ethylene production, respiration rate, membrane damage and anti-oxidant enzymes.

2. Materials and methods

2.1. Fruit and treatments

Pear (*Pyrus bretschneideri* cv. Laiyang) fruit were harvested at commercial maturity in Laiyang city, Shandong province of China, and immediately transported to Beijing on the day of harvest. Fruit with similar size and without physical injuries or infections were selected.

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For 1-MCP treatment, pear fruit were enclosed in a sealed airtight plastic tent (internal volume 1 m³) on the day of harvest. 1-MCP was applied as gas released from commercial powder (Rohm and Haas Inc., Philadelphia, USA) dissolved in distilled water. The final gas concentration of 1-MCP was 1 $\mu\text{L L}^{-1}$, and the equal volume sterile water served as control. The fruit were treated for 24 h at 25 °C, and then put into polypropylene bags to maintain a high relative humidity (95%) and stored at 0 °C in air, with untreated fruit in the air as the control. For CA storage, pear fruit were stored in CA cabinets (105 cm × 55 cm × 100 cm), in which gas composition and concentration (2% O₂ plus 2% CO₂) can be monitored by an atmosphere analyzer (FC-701, Italy). Three replications for each treatment were performed, and each replicate contained at least 200 fruits. All fruit were stored up to 8 months. Parameters of fruit quality and physiology in each treatment were analyzed each month.

2.2. Determination of firmness, soluble solids content, titratable acidity

Firmness was determined on opposite peeled cheeks of the fruit using a hand-held fruit firmness tester (FT-327, Italy) with an 8 mm diameter plunger. Soluble solids content (SSC) was determined using a hand-held pocket refractometer (PAL-1, Japan). Twenty grams of frozen tissue from six fruits was homogenized with 10 mL distilled water and then centrifuged. Titratable acidity (TA) of the supernatant was determined by titration with 0.01 mol L⁻¹ NaOH to pH 8.2 and calculated as percent malic acid. There were 3 replications with 10 fruits in each treatment.

2.3. Determination of ethylene production and respiration rate of fruit

For each treatment, eight fruits were sealed in 5 L gas-tight jars at 25 °C. After 3 h, a 50 mL gas sample was collected from each jar using a syringe. Ethylene concentration were determined using a gas chromatography (Agilent, 7890A, USA) equipped with a FID detector and a PLOT capillary column (15 m × 320 μm × 15 μm). Respiration rate (mg CO₂/kg/h) was measured by the same gas chromatograph fitted with a thermal conductivity detector. There were 3 replications with 10 fruits in each treatment.

2.4. Determination of membrane permeability

Flesh (10 g) was collected from eight fruits in each treatment and incubated in 50 ml of distilled water for 3 h, and then the initial electric conductivity was measured with a conductivity meter (Model EC 215, Italy). Disks were boiled for 30 min, cooled in room temperature, and then the total conductivity was measured. Membrane permeability was expressed as relative electric conductivity percentage of the initial electric conductivity. There were 3 replications with 10 fruits in each treatment.

2.5. Determination of lipid peroxidation

Lipid peroxidation was determined by measuring malondialdehyde (MDA) content according to the method of Heath and Packer (1968) with slight modifications. About 20 g of frozen tissue was homogenized in 10 ml of 10% TCA. The homogenate was centrifuged at 12,000 × g for 30 min, and then 1.5 ml supernatant of sample was mixed with 2.5 ml of 0.5% 2-thiobarbituric acid (TBA). The mixture was heated at 100 °C for 30 min, and quickly cooled, then centrifuged at 12,000 × g for 10 min. The absorbance was measured at 600 and 532 nm respectively using a UV-160 spectrophotometer (Shimadzu, Kyoto, Japan). The concentration of MDA was calculated

using an extinction coefficient of 155 m M⁻¹ cm⁻¹. There were 3 replications with 10 fruits in each treatment.

2.6. Measurement of anti-oxidant enzyme

About 20 g of frozen tissue was ground with 25 ml of ice-cold extraction buffer containing 0.5 g of polyvinyl pyrrolidone (PVPP) and homogenized using a Kinematica tissue grinder (Cr1-6010, Kriens-LU, Switzerland). The homogenate was centrifuged for 30 min at 12,000 × g at 4 °C, and the supernatants were immediately assayed for enzyme activities using a UV-160 spectrophotometer (Shimadzu, Kyoto, Japan). All extract procedures were carried out at 4 °C. Potassium phosphate buffer (100 mM, pH 7.0) was used as the extraction buffer for CAT, POD, and LOX determination. For SOD analysis, the buffer was potassium phosphate buffer (10 mM, pH 7.8). Activities of SOD, POD, LOX, and CAT were measured at 560, 460, 234, and 240 nm, respectively, according to our previous method (Wang et al., 2005).

Protein content was measured by the method of Bradford (1976), using BSA as the standard protein. The enzymes activity was expressed as U mg⁻¹ protein. Fruit samples were obtained from 10 fruits every month during the storage. Three replications were carried out in each treatment.

2.7. Statistical analysis

Statistical analyses were performed with SPSS. All data were analyzed for significant differences by one-way analysis of variance (ANOVA). Differences at $p < 0.05$ were considered as significant.

3. Results

3.1. Effects of CA storage and 1-MCP treatment on fruit physiology

The results showed that 1-MCP treatment and CA storage delayed fruit ripening and affected the changes of flavor, aroma and color during long time storage (Fig. 1C). The increase in ethylene production associated with the respiration climacteric was observed in all treatments. As compared to the control, fruit treated with 1-MCP and stored in CA condition showed a distinctly lower level of ethylene in the first 4 months. The ethylene production of pear fruit reached the maximum value after 5 months in both 1-MCP and CA treatment, while 4 months in the control (Fig. 1A). The maximum peak of respiration in the fruit treated with 1-MCP and stored in CA condition occurred later than that of the control (Fig. 1B). These results indicate that 1-MCP-treated and CA storage could significantly reduce respiration rate, leading to delay in the process of physiology and metabolism of Laiyang pear fruit.

3.2. Effects of CA and 1-MCP treatment on fruit quality

Fruit firmness showed a declining trend during storage time in all treatments. However, both 1-MCP treatment and CA storage inhibited the decline of fruit firmness (Fig. 2A). SSC content increased at the early stage of storage, and then dropped thereafter (Fig. 2B). The contents in 1-MCP treatment and CA were lower than that in the control at the first 4 months, but higher at the last 3 months. TA content in both 1-MCP- and CA-treated fruit decreased slowly compared to that in the control (Fig. 2C).

3.3. Effects of CA storage and 1-MCP treatment on membrane permeability, lipid peroxidation and LOX activity

The membrane permeability was expressed as the relative electrolyte leakage. There was a continually increase in relative leakage

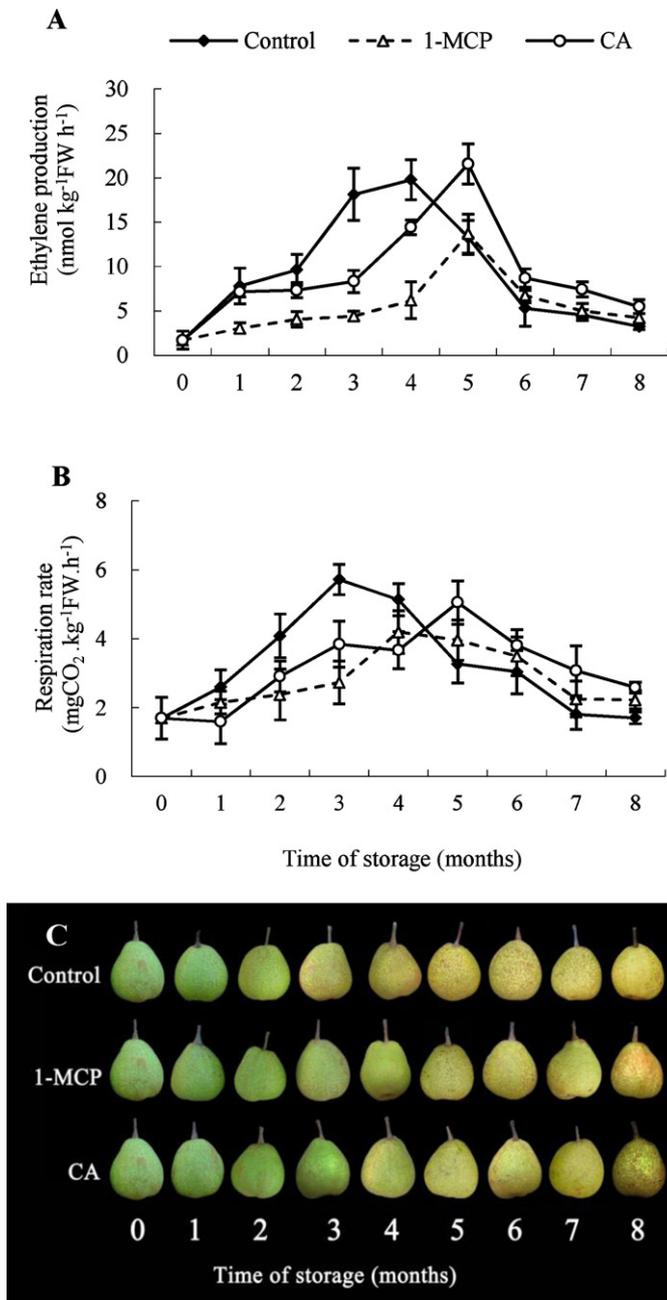


Fig. 1. Changes of ethylene production (A) and respiration rate (B) and peel color (C) in control, 1-MCP treated, and CA storage pear fruit during long time storage at 0°C. Data are means ± SE of three replicates. Vertical bars represent standard error of means. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

rate after fruit harvest, suggesting a gradual loss of cell membrane integrity. However, the fruit stored in CA condition and treated with 1-MCP showed relatively lower level of membrane permeability than control fruit. Furthermore, the effect of CA storage on maintaining cell membrane integrity was better as compared to 1-MCP treatment (Fig. 3A). Malondialdehyde (MDA) is one of the intermediate products of lipid peroxidation and the content of MDA often is used as an indicator of lipid peroxidation resulting from oxidative stress (Dhindsa et al., 1981). The changes of MDA content support the evidence of membrane deterioration. MDA content in control fruit increased quickly with prolonged storage time, and was obviously higher than that of other treatments. At the 6th month, the MDA content of 1-MCP treated and CA stored fruit

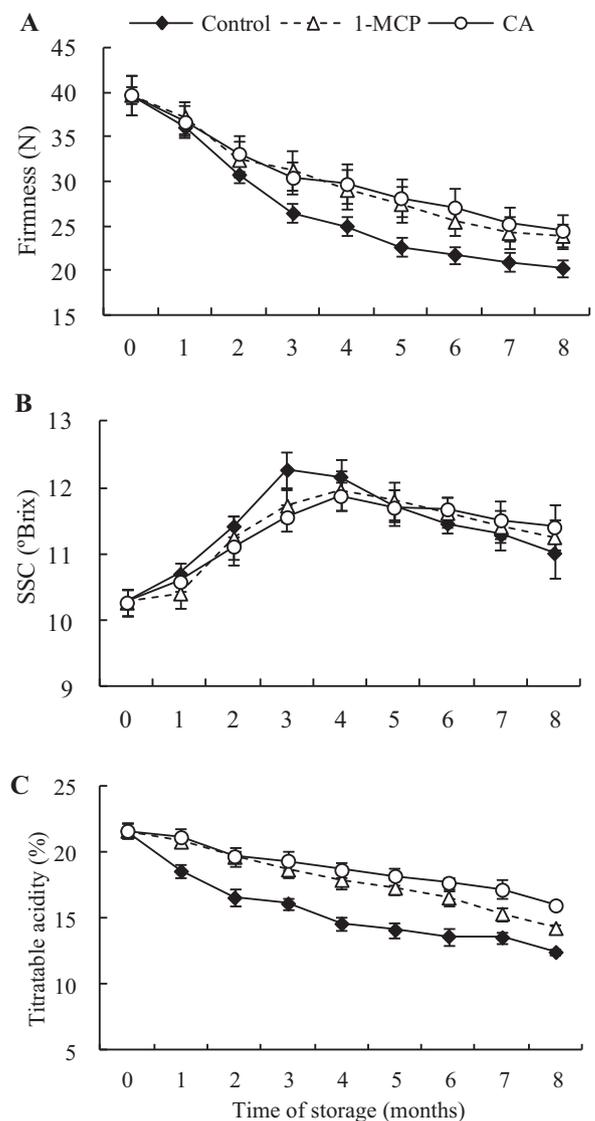


Fig. 2. Changes of firmness (A), SSC (B), and titratable acidity (C) of pear fruit in control, 1-MCP treated, and CA storage pear fruit during long time storage at 0°C. Data are means ± SE of three replicates. Vertical bars represent standard error of means.

is 1.6 and 1.8 times lower than that of the control, respectively (Fig. 3B). LOX activity of the control fruit increased rapidly in the initial period, reaching its peak value at the 4th month, and then declined steadily. The LOX activity in the fruit treated by 1-MCP and stored in CA condition was distinctly lower compared to the control fruit in the first 4 months, and reached the peak value after 5 and 6 months, respectively (Fig. 3C).

3.4. Effects of CA storage and 1-MCP treatment on CAT, POD, and SOD activities

CAT activity firstly gradually increased in the early stage of storage, and then declined thereafter in both treated and control fruit. In the whole storage period, fruit treated by 1-MCP or stored in CA showed markedly higher levels of CAT activity (Fig. 4A). The tendency of POD activity in control fruit showed to be similar in other treatments. POD activity of pear fruit decreased from 57 to 26 U mg⁻¹ protein after 8 months storage in air. Both 1-MCP treatment and CA storage could maintain relative high activity of the enzyme, and the performance of CA storage was better than that of

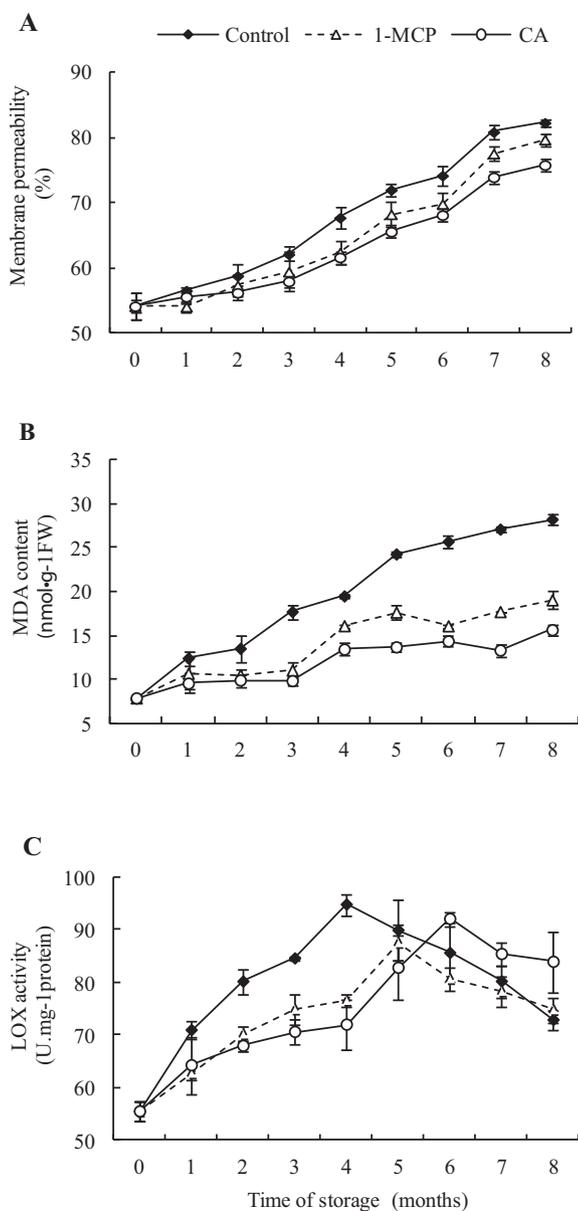


Fig. 3. Changes of membrane permeability (A), MDA content (B), and LOX activity (C) in control, 1-MCP treated, and CA storage pear fruit during long time storage at 0°C. Data are means \pm SE of three replicates. Vertical bars represent standard error of means.

1-MCP treatment (Fig. 4B). Compared with above two anti-oxidant enzymes, the change of SOD activity in pear fruit was smaller, ranging from 0.2 to 0.4 U mg⁻¹, during all storage periods. CA storage and 1-MCP treatment showed to be more effective in maintaining high activity of SOD as compared to control, especially in the 3rd and 4th months (Fig. 4C).

4. Discussion

Fruit ripening and senescence are characterized by various physiological and biochemical changes, including an increase in respiration and ethylene production. Based on the respiratory behavior during ripening, fruit can be divided into the two categories of climacteric and non-climacteric (McMurchie et al., 1972). In the present study, we found that significant peak of respiration rate and ethylene production of Laiyang pear fruit were detected at the 3rd and 4th months of storage at 0°C, respectively,

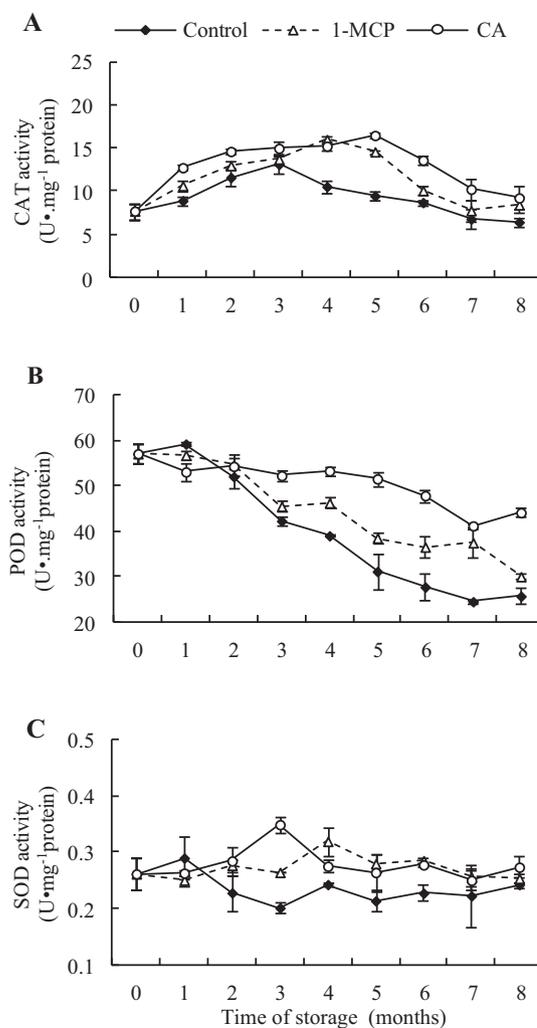


Fig. 4. Changes of activities of CAT (A), POD (B), and SOD (C) in control, 1-MCP treated, and CA storage pear fruit during long time storage at 0°C. Data are means \pm SE of three replicates. Vertical bars represent standard error of means.

accompanying a persistent decrease in firmness and TA during storage periods (Fig. 2A and C). In general, the ripening of fleshy fruits represents the unique coordination of developmental and biochemical pathways leading to changes in color, texture, aroma, and nutritional quality of mature seed-bearing plant organs, and ethylene plays a key regulatory role in ripening of climacteric fruit (Lelievre et al., 1998). Many researches indicated that using exogenous factors to inhibit ethylene production could significantly delay fruit ripening (Tian et al., 2004; Zheng et al., 2007; Wang et al., 2009). 1-MCP, as an inhibitor of ethylene perception, can interact with ethylene receptors and thereby prevent ethylene-dependent responses (Sisler and Serek, 1997). An exciting new strategy for controlling ethylene production and thus ripening and senescence of fruit, especially climacteric ones, as well as senescence of vegetative tissues, has emerged with the discovery and commercialization of 1-MCP (Blankenship and Dole, 2003; Watkins, 2006). The effective concentrations of 1-MCP generally ranged from 0.5 to 4 μ L L⁻¹ in pear fruit (Baritelle et al., 2001; Mahajan et al., 2010). In this study, Laiyang pear fruit treated by 1-MCP at the concentration of 1 μ L L⁻¹ or stored in CA storage (2% O₂ plus 2% CO₂) showed an 1 month delay in peaks of respiration rate and ethylene production as compared to control fruit (Fig. 1A and B), indicating both 1-MCP or CA storage can effectively retard fruit senescence and maintain quality of Laiyang pear fruit via regulating the ethylene

pathway. However, the mode of the actions of 1-MCP or CA storage may be different, because 1-MCP effects ethylene-dependent changes by competing with ethylene receptors for the binding site (Sisler and Serek, 1997), and CA storage may act by delaying ethylene-dependent processes by inhibiting the conversion of ACC to ethylene (De Wild et al., 2005).

The breakdown of cell wall component and membrane disruption are another physiological phenomena of fruit senescence, resulting in increased membrane permeability as a common feature accompanying senescence (Paliyath and Droillard, 1992). Functional membranes are fluid since phospholipids can move rather freely in lateral dimensions, but the presence of proteins and sterols influence membrane fluidity. A common feature accompanying senescence is increased membrane permeability, expressed as increasing leakage of ions (Marangoni et al., 1996). Peroxidation of fatty acids with resulting free radical formation has been described as one of the major deteriorative processes of membranes. Lipoxygenase (LOX) was reported to be response for lipid degradation processes and contribute to senescence-related membrane deterioration (Paliyath and Droillard, 1992). Unsaturated fatty acids are prone to attack by LOX and increased free radical production has been observed in a variety of senescent tissues (Marangoni et al., 1996). Data here showed that LOX activity increased in the initial period and then declined steadily, which accounted for the rise in membrane permeability and lipid peroxidation (MDA content) during storage. Both 1-MCP treatment and CA storage could significantly inhibit or delay the increase of MDA content and LOX activity (Fig. 3B and C), which effectively suppressed the lipid peroxidation in pear fruit. Wang et al. (2005) reported that CA storage maintained better membrane integrity of peach fruit compared to the control. Larrigaudiere et al. (1998) indicated the similar effects of 1-MCP treatment on Blanquilla pears. Increased membrane peroxidation leads to increased gel-phase formation and losses in membrane functionality, resulting in browning which is a consequence of membrane damage caused by a combination of oxygen free radical action and a lack of maintenance energy (Veltman et al., 2003).

Membrane deterioration caused by oxidative damage has been proved to be related to reactive oxygen species (ROS). Generally, an appropriate intracellular balance between the production of ROS and scavenging exists in all cells (Stadtman, 1992). In the senescent tissue, the balance usually is broken, and superfluous ROS are produced and accumulated (Jimenez et al., 2002). ROS at higher concentration can cause protein degradation, membrane deterioration, and consequently, resulting in various disorders in fruit (Franck et al., 2007). In the previous study, we proved that ROS could cause oxidative damage of mitochondria and accelerate senescence processes in peach fruit (Qin et al., 2009). Oxidative damage of specific mitochondrial proteins is greatly responsible for impairment of mitochondrial function, thus, leading to fruit senescence. As ROS such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH) are inevitably generated in plant cells as a consequence of normal metabolism, anti-oxidant enzymes such as SOD, POD and CAT play important roles in scavenging of ROS during the process of fruit ripening, because SOD converts O_2^- to H_2O_2 while H_2O_2 is removed by CAT and POD (Jimenez et al., 2002; Mondal et al., 2004). In this experiment, we found that 1-MCP treatment and CA storage could induce the activity of SOD, CAT and POD in Laiyang pear fruit during storage (Fig. 4), which enhanced anti-oxidant capacity to scavenge ROS, maintained membrane integrity and retarded fruit senescence. The results was consistent with the previous report by Rupinder and Dwivedi (2008), who found that 1-MCP induced anti-oxidant enzymes of mango during fruit ripening. However, our results firstly report the effect of 1-MCP on the anti-oxidant enzyme activity responses in pear.

In conclusion, both 1-MCP treatment and CA storage were effective in delaying fruit ripening and senescence, via inhibiting fruit

respiration rate and ethylene production, and regulating activity of the antioxidant enzymes, which is beneficial for maintaining quality and prolonging storage time of Laiyang pear fruit. Regulating ethylene and anti-oxidant pathways may act as the major action modes of 1-MCP treatment and CA storage to control physiological properties and harvested quality of Laiyang pear fruit.

Acknowledgements

This work was supported by the National Basic Research Program of China (973 Program, 2011CB100604), the National Natural Science Foundation of China (31030051) and the Scientific and Technological Development project of Laiyang (201013).

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