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Postharvest 1-methylcyclopropene treatments maintain the quality of *Rosa sterilis* D. shi during storage

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Abstract

Effects of different concentrations of aqueous 1-methylcyclopropene (1-MCP) on *Rosa sterilis* D. shi fruits were investigated. The fruits were harvested at commercial maturity stage and treated with 1-MCP treatment at 0.5, 1.0, and 1.5 μL L^{-1} for 20 h at 25 °C in an air tight chamber along with control sample to evaluate the effect of 1-MCP concentration on its quality during storage. The result showed that postharvest treatment with 1.0 μL L^{-1} 1-MCP suppressed the respiration rate, ethylene production rate and POD activity in *Rosa sterilis* D. shi fruits, inhibited the transport of reducing sugar and the increase of cellulose, delayed the increase in PPO activity, and retarded the decrease in ascorbic acid. The regulation of fibrosis process with postharvest 1.0 μL L^{-1}1-MCP treatment could be used to maintain the quality of *Rosa sterilis* D. shi fruits.

Keywords: *Rosa sterilis* D. shi; 1-MCP; quality; storage.

Practical Application: Evaluating the effectiveness of 1-MCP in maintaining the quality of *Rosa sterilis* D. shi during storage.

1 Introduction

Rosa sterilis D. shi is also known as seedless chestnut *Rosa*, fruit is an excellent source of protein, sugar, vitamins (Li et al., 2016; Zhang et al., 2016), amino acids (Lu et al., 2015), phenolic compounds (Xie et al., 2017), trace elements, SOD, functional components (Zhang et al., 2016; Xie et al., 2018b), yellow pigment (Xie et al., 2018a), and rich volatile aroma compounds (Fu et al., 2012; Jiang et al., 2013; Zhang et al., 2016), and which has high antioxidant activities (Xie et al., 2017, 2018b), it is a third generation wild fruit tree with great development and utilization value.

Rosa sterilis D. shi fruit is a climacteric (Lin et al., 2016; Xie et al., 2017), water loss, wilting, fibrosis, rot and disease of *Rosa sterilis* D. fruit resulted in the loss of eating quality and commercial value during storage. There is litter literature about postharvest deterioration of quality in *Rosa sterilis* D. fruit, and reported that polyethylene bags combined with low temperature (1 °C) could better maintain its the quality (Xu et al., 2016; Lin et al., 2016; Xie et al., 2017). The tissue became rough and fibrous, and the total dietary fiber, soluble dietary fiber and insoluble dietary fiber were changed (Rios et al., 2003; Prolla et al., 2010). Cellulose is an important component of cell wall. As the cell lengthens and the secondary wall thickens, a large amount of cellulose is synthesized and formed fiber bundles. The lignin was synthesized and deposited in the fiber bundle grid (Miao et al., 2012). In the senescence process of fruits and vegetables, the content of cellulose increases continuously, which promotes fibrosis and texture aging, which affect the commodity value and the edible value (Wang et al., 2013). The synthesis of cellulose in common bean is commonly related to the activities of POD and PPO (Miao et al., 2012), and is generally an increase in activities of POD and PPO, and a decrease in reducing sugar (Miao et al., 2012; Wang et al., 2013).

1-Methylcyclopropene (1-MCP) is an inhibitor of ethylene action by binding at the receptor site, and has been widely used in postharvest technology to prolong the storage life of many fruits and vegetables. Postharvest 1-MCP treatment, which could retard lignin and cellulose accumulation, and delay senescence, have been largely reported in bamboo shoot (Luo et al., 2007; Song et al., 2011), loquat fruit (Cao et al., 2009), Tsai Tai (Zhang et al., 2010), pears (Chen et al., 2017), kiwifruit (Li et al., 2018), and plum (Lin et al., 2018). In postharvest application of 1-MCP, increasing evidence has indicated that 1-MCP treatment can inhibit the expression of ETR2, EIL1, Exp, CAD, and glutathione peroxidase gene, reduce the activity of ACO, PAL, PPO, CAD, POD and LAC enzyme, suppress the increase of cellulose content, and thus inhibit the fibrosis of fruits and vegetables (Luo et al., 2007; Choi & Huber, 2009; Song et al., 2010; Zhang et al., 2010; Li et al., 2015, 2017; Huan et al., 2016; Yihui et al., 2018; Thongkum et al., 2018). No works have been published that analyse the influence of 1-MCP concentration on postharvest quality of *Rosa sterilis* D. shi fruits. The purpose of this study was to investigate the effects of 1-MCP on quality of *Rosa sterilis* D. shi fruits during storage at 1 ± 0.5 °C.

2 Materials and methods

2.1 Plant material and treatment with 1-MCP

Rosa sterilis D. shi fruits were hand-harvested when the peel turns yellow from green on September 28, 2015 (160 days after anthesis) from an orchard in Puding County, Guizhou

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Province, in China, and transported to the laboratory of Guizhou engineering research center for fruit processing within three hours. Fifty kg similar fruits without physical injuries were selected for uniformity in shape, colour, and size. The fruits were divided into an polythene plastic boxes (500 ± 25 g), and randomly divided into four groups for 1-MCP treatment. The four groups of *Rosa sterilis* D. shi fruits were sealed in four 220 L container, exposed to air and 0.5, 1.0, and 1.5 μL L^{-1} 1-MCP (SmartFreshTM, AgroFresh Inc., USA), and air circulation was maintained with mini fan. After treatment for 20 h at 25 °C, each treatment of *Rosa sterilis* D. shi fruits were placed into nine commercial polyethylene bags, and the bags were sealed after pre-cooling at 1.0 ± 0.5 °C for 20 h. All *Rosa sterilis* D. shi were stored at 1.0 ± 0.5 °C for 90 d.

2.2 Evaluation of the marketable fruits rate

The marketable fruits rate was assessed using a scale developed by Xie et al. (2014). The marketable fruits rate was scored on a 5-0 scale where 5 = excellent, 4 = very good, 3 = good (marketable), 2 = poor (unmarketable), 1 = very poor (unmarketable). The marketable fruits rate was calculated as 100 ×∑ (decay score × fruits within each class) / (total fruits × 5).

2.3 Respiration rate and ethylene production rate

The respiration rate and ethylene production rate were measured by incubating approximately 0.5 kg fruit per replicate in a 3.4 L sealed desiccator for 1 h at 25 °C. The concentrations of CO_2 and ethylene were measured using a headspace CO_2 analyser (Model: 6600, Illinois Tool Works Inc., Peoria, USA) and ethylene analyzer (Model: PGD3-C-C_2H_4, Shenzhen Xinss Technology Development Co., Ltd., Shenzhen, China). The respiration rate was expressed as mg CO_2 kg^{-1} h^{-1}. Ethylene production rate was expressed as mg C_2H_4 kg^{-1} h^{-1}.

2.4 Cellulose, reducing sugar, total ascorbic acid, and glutathione content

The cellulose was determinate by using the acid detergent method of Miao et al. (2012). The 1.0 g fruits samples were placed into a reflux device and 50 mL acid detergent was added with boiling for 5 to 10 minutes and boiling back for 50 minutes. Samples were transferred to the filter, vacuum filtration, and hot water at 90-100 °C soak for 15 to 30 s. The soaked samples were drained and rinsed until washed with detergent. The filter was washed two or three times with acetone until the filtrate was colorless. The acetone extracted from the filter residue was dried for 3 h at 100 °C, weighed after cooling to 25 °C. The results were reported as % (FW).

The reducing sugar content was measured according to the 3,5-dichlorosalicylic acid (DNS) method (Xie et al., 2016), and the reaction mixtures contained 0.5 mL of the relevant reducing sugar solution and 1.5 mL of either DNS reagent. The reaction mixture was boiled in a water bath for 5 min and cooled to room temperature in a water-ice bath. Subsequently, the mixture was diluted to 18.0 mL with distilled water. The absorbance was measured at 540 nm. Glucose was used as the standard and the results were reported as % (F_W).

Total ascorbic acid content was determined according to the method of Xie & Tan (2015), and 2.5 g of fruit was ground with 5 mL 50 g L^{-1} trichloroacetic acid (TCA). After grinding, the sample was added to a 25 mL brown volum bottle and fixed to the scale with 50 g L^{-1} TCA solution, it was then extracted for 10 minutes at low temperature and filtered to collect the filtrate for use. The 1.0 mL extracts were added to 1.0 mL 50 g L^{-1} TCA and 1.0 mL ethanol, mixed and shaken, followed by 0.5 mL 0.4% phosphoric acid - ethanol, 1.0 mL g L^{-1} bathophenanthroline - ethanol and 1.0 mL g L^{-1} $FeCI_3$ – ethanol. The mixture was incubated at 30 °C for 1 h, and the absorbance was measured at 534 nm. The results were reported as mg Ascorbic Acid Equivalents (AAE) in 100 g fresh weight of fruit.

Glutathione content were quantified according to the method of Wang & Zhu (2017) and appropriate modification. Briefly, 1 mL supernatant was added to the reaction system, including 1 mL 0.1 mol L^{-1} pH 7 PBS, 0.5 mL 4 mmol L^{-1} DTNB (prepared by 0.1 mol L^{-1} pH 7 PBS). The mixture was incubated at 25 °C for 10 min, and the absorbance was measured at 412 nm. Reduced glutathione were used as the standard and results were expressed as μmol GSH g^{-1}(F_W).

2.5 Activities of POD and PPO

Peroxidase (POD) activities were estimated by using the method (Xie et al., 2014). Each 2.0 g fruit sample was weighed and grounded with a pestle in an ice-cold mortar with 3 mL extracting solution (1 mmol PEG, 4% PVPP and 1% Triton X-100). The homogenates were centrifuged at 12,000 × g for 30 min at 4 °C. The resulting supernatants were used for a POD assay of enzymatic activities. The change in absorbance at 470 nm were followed every minute by spectrophotometry (model UV-2550; Shimadzu, Japan) for POD. The specific activity was expressed as U g^{-1} fruits, where 1 unit was defined as increase 0.001 ΔOD_{470} min^{-1} F_W.

Polyphenoloxidase (PPO) activities were estimated according the method of Tian et al. (2014). Each 3.0 g fruits sample wase weighed and ground with a pestle in an ice-cold mortar with 5 mL of sodium acetate buffer (100 mM, pH 5.5). The homogenates were centrifuged at 12,000×g for 30 min at 4 °C. The supernatants were used for a PPO assay of enzymatic activities. The changes in absorbance at 420 nm were followed every minute by spectrophotometry (model UV-2550; Shimadzu, Japan) for PPO. The specific activity was expressed as U g^{-1} (Fw), where one unit was defined as increase 0.01 ΔOD_{470} min^{-1}.

2.6 Statistical analysis

All sample characterization results were reported as the mean ± standard error. Statistical tests were performed using the SPSS® computer program, version 22.0 (SPSS Statistical Software, Inc., Chicago, IL, USA) to determine differences at the 5% level between different maturity stage using a one-way analysis of variance (ANOVA).

3 Results

3.1 Marketable fruits rate

The *Rosa sterilis D.* shi fruits began to decay at 30 d, the marketable fruits rate of control *Rosa sterilis D.* shi fruits significantly decreased during storage (Figure 1). The decrease of marketable fruits rate in *Rosa sterilis D.* shi was significantly suppressed by treatment of 0.5 or 1.0 μL L^{-1} 1-MCP ($P < 0.05$).

3.2 Respiration rate and ethylene production rate

The respiration rate of *Rosa sterilis* D. shi fruits decreased with further storage, the respiration rate was significantly suppressed by treatment of 1-MCP (Figure 2A).

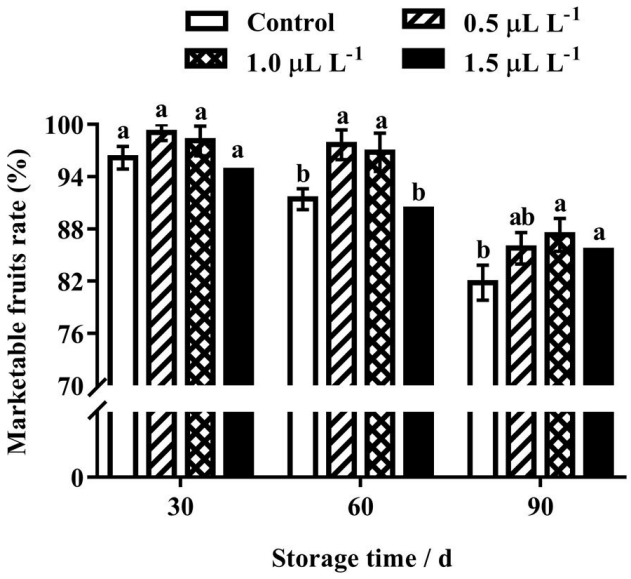

Figure 1. Effects of 1-MCP on marketable fruits rate of *Rosa sterilis* D. shi during storage. Vertical bars represent standard error of the means. Means with same superscript do not differ significantly.

The ethylene production rate of *Rosa sterilis* D. shi fruits increased firstly, and then decreased with storage, the ethylene production rate was significantly suppressed by 1-MCP treatment. The 1-MCP treatment delayed the peak time of the ethylene production rate, and the peak value of 0.5, 1.0 and 1.5 μL L^{-1} 1-MCP treated Rosa sterilis D. shi fruits were respectively 40.27%, 38.8% and 46.3% lower than that of control ($P < 0.05$).

3.3 Cellulose, reducing sugar, ascorbic acid and glutathione

The cellulose of control *Rosa sterilis* D. shi fruits increased during storage. However, the cellulose of 1-MCP treated *Rosa sterilis* D. shi fruits decreased, then increased with storage. 1.0 and 1.5 μL L^{-1} 1-MCP treatment significantly decreased the of cellulose in *Ros*a sterilis D. shi fruits from the 30 to 60 day ($P < 0.05$) (Figure 3A).

The reducing sugar of *Rosa sterilis* D. shi fruits increased firstly and then decreased during storage, the increase of reducing sugar in *Rosa sterilis* D. shi fruits was significantly suppressed by 1-MCP treatment ($P < 0.05$), the reducing sugar in 0.5 or 1.0 μL L^{-1} 1-MCP treated *Rosa sterilis* D. shi fruits were lower than that of control during storage (Figure 3B).

The ascorbic acid in control and 1.5 μL L^{-1} 1-MCP treated *Rosa sterilis* D. shi fruits increased firstly, and then decreased during storage. The 1.5 μL L^{-1} 1-MCP treatment was significantly suppressed the decrease of ascorbic acid in *Rosa sterilis* D. shi fruits during storage. The ascorbic acid in *Rosa sterilis* D. shi fruits was significantly maintained by 0.5 μL L^{-1} 1-MCP treatment at the 60 day ($P < 0.05$). However, the ascorbic acid of 1.0 μL L^{-1} 1-MCP treated *Rosa sterilis* D. shi fruits significantly decreased at the 30 day, then the changes in ascorbic acid were significantly suppressed after the 30 day (Figure 3C).

The glutathione in *Rosa sterilis* D. shi fruits decreased in the first period of storage and then increased, with remarkable lowest value. The decrease in glutathione in *Rosa sterilis* D. shi fruits was suppressed by 1.0 or 1.5 μL L^{-1} 1-MCP treatments during storage. The 1.0 or 1.5 μL L^{-1} 1-MCP treatments delayed the

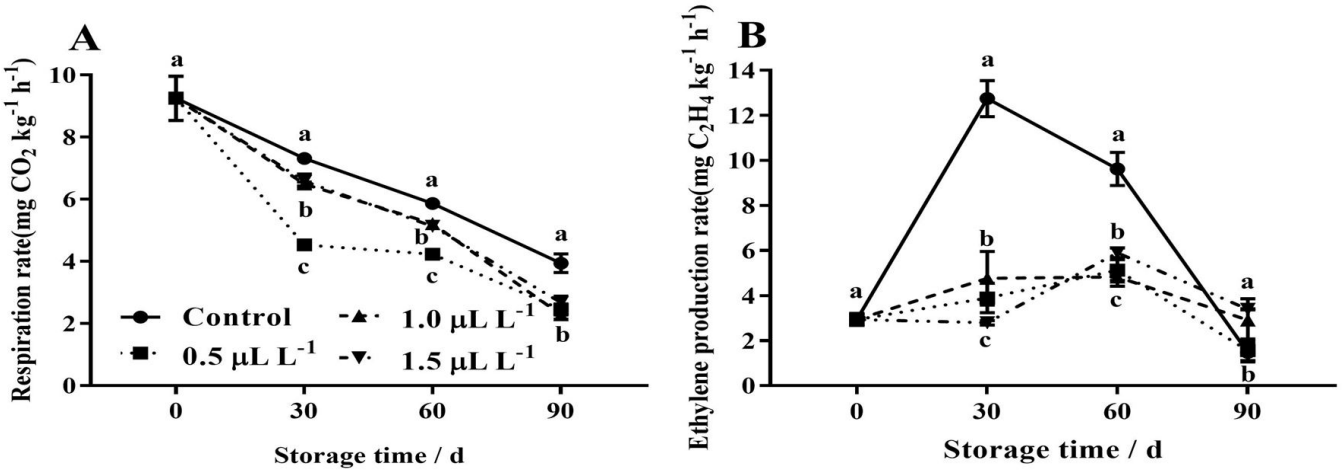

Figure 2. Effects of 1-MCP on respiration rate (A) and ethylene production rate (B) of *Rosa sterilis* D. shi during storage. Different letters are significantly different at P ≤ 0.05 level by Duncan's multiple range test.

lowest time of glutathione to the 60 day. The glutathione lowest values for the 1.0 or 1.5 μL L^{-1} 1-MCP treated *Rosa sterilis* D. shi fruits were respectively 12.6% or 112.7% higher ($P < 0.05$) than that of control (Figure 3D).

3.4 POD and PPO

The POD activity of control, *Rosa sterilis* D. shi fruits increased at the 60 day, and then decreased with storage. The POD activity increase in *Rosa sterilis* D. shi fruits was significantly enhanced by 1-MCP treatment at the 30 day ($P < 0.05$), and significantly maintained the POD activity at the 90 day, but, there was no significant difference among the 1-MCP treatment and control at the 60 day (Figure 4A).

The PPO activity of *Rosa sterilis* D. shi fruits increased in the first period of storage and then decreased, with a remarkable highest value. The increase in PPO activity in *Rosa sterilis* D. shi fruits was suppressed by 1-MCP treatment, and the PPO activity peak of 1-MCP treatments were significantly lower than that of control ($P < 0.05$). While the 0.5 μL L^{-1} 1-MCP treatment delayed the peak time of PPO activity from the 30 day to the 60 day (Figure 4B).

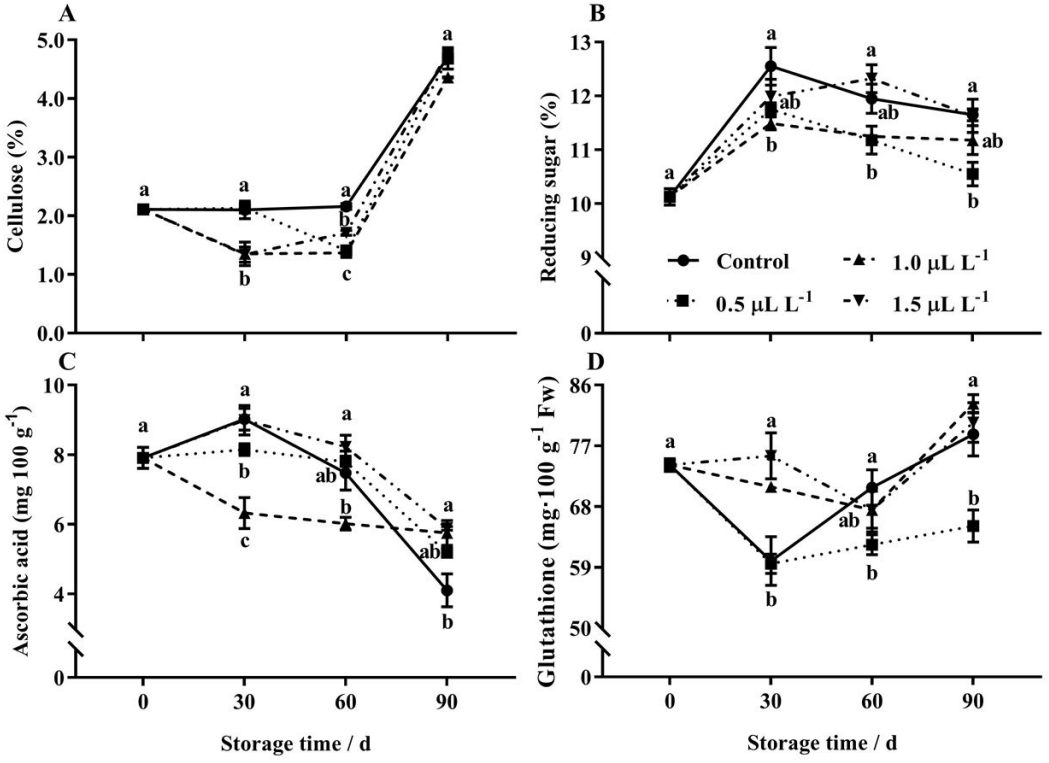

Figure 3. Effects of 1-MCP on cellulose (A), reducing sugar (B), ascorbic acid (C), and glutathione (D) of *Rosa sterilis* D. shi during storage. Different letters are significantly different at P ≤ 0.05 level by Duncan's multiple range test.

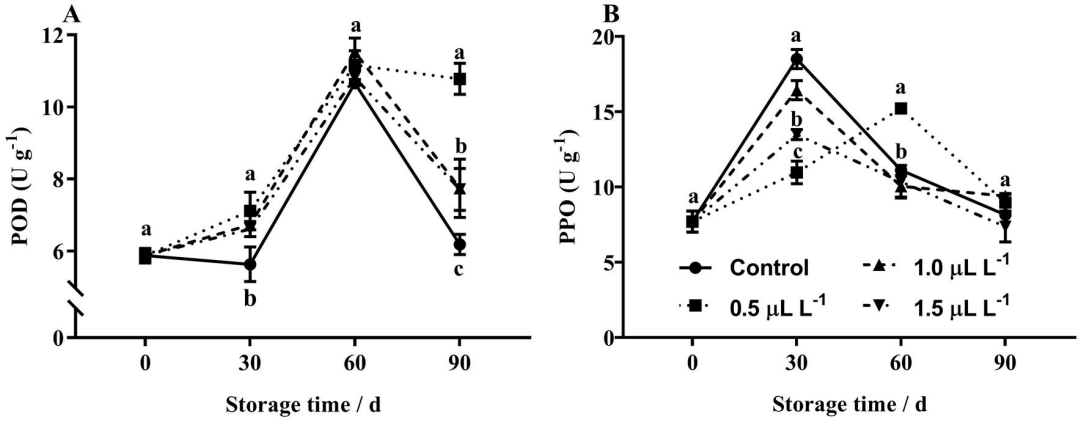

Figure 4. Effect of 1-MCP on POD (A) and PPO (B) of *Rosa sterilis* D. shi during storage. Different letters are significantly different at P ≤ 0.05 level by Duncan's multiple range test.

4 Discussion

Rosa sterilis D. shi fruits are more appreciated by consumers due to their aroma compounds, sensorial and nutritional characteristics. However, untimely consumption or improper preservation after harvesting, it is very easy to water lose, fibrosis, and even decay, with a loss of nutritional and commercial value (Lin et al., 2016; Xie et al., 2017). Cellulose is an important component of cell walls. In the process of mature and senescence of fruits and vegetables, the content of cellulose increases continuously, which promotes fibrosis and texture aging, which affects the commodity value and the edible value (Wang et al., 2013). In the present study, we found that the cellulose was accumulated in *Rosa sterilis* D. shi fruits, which was consistent with previous report (Xie et al., 2017). As is reported, the synthesis of cellulose in fruits and vegetables is commonly related to the activities of POD and PPO, there is generally an increase in activities of POD and PPO, and a decrease in reducing sugar (Miao et al., 2012; Wang et al., 2013). Our results showed that the degradation of polysaccharide in *Rosa sterilis* D. shi fruits leads to increase of reducing sugar and decline of cellulose during early storage. However, the cellulose of the *Rosa sterilis* D. shi fruits gradually accumulated with the decrease of reducing sugar content and cellulose activity during late storage. The content of reducing sugar decreased, and the content of cellulose increased during storage in our experiment, indicating the gradual transformation of reducing sugar into cellulose. In agreement with other reports in bamboo shoot (Luo et al., 2007, 2008; Song et al., 2010) and common bean (Dai et al., 2010; Wang et al., 2013), our result showed that *Rosa sterilis* D. shi fruits treated with 1.0 μL L^{-1} 1-MCP effectively inhibited the transportation of reducing sugar and the increase of cellulose during storage. 1-MCP treatment can effectively inhibit the expression of ethylene synthesis and action including ACO, ACS, ETR, CTR, EIN, and EIL1, and suppress respiration and ethylene production rates (Yang et al., 2013; Escribano et al., 2017; Li et al., 2017; Mata et al., 2018; Thongkum et al., 2018). Our research revealed that postharvest 1-MCP treatment suppressed the respiration rate, ethylene production rate of *Rosa sterilis* D. shi fruits. 1-MCP have been confirmed to inhibit the increase of cellulose content in fruit and vegetable, which inhibit the expression of ethylene synthesis and action including Exp, CAD, and glutathione peroxidase gene, reduce the activity of PAL, PPO, CAD, POD and LAC enzyme, suppress the increase of cellulose content, and thus inhibit the fibrosis of fruits and vegetables (Luo et al., 2007, 2008; Choi & Huber, 2009; Cao et al., 2010; Song et al., 2011; Zhang et al., 2010; Li et al., 2015, 2017; Tian et al., 2014; Huan et al., 2016; Yihui et al., 2018). Our data showed 1-MCP markedly accelerated POD activities, consistent with the report by Li et al. (2017), postharvest treatment with 1-MCP suppressed PPO activity in *Rosa sterilis* D. shi fruits, in contrary with the inhibition of cellulose content during cold storage (Figure 3A and Figure 4B), consistent with research on Tsai Tai and lettuce leaves with 1-MCP treatment (Zhang et al., 2010; Tian et al., 2014), postharvest 1.0 μL L^{-1} 1-MCP treatment retarded the decrease of ascorbic acid, and maintained the marketable fruits rate of *Rosa sterilis* D. shi fruits.

In conclusion, postharvest treatment with 1.0 μL L^{-1} 1-MCP significantly suppressed the respiration rate, ethylene production rate and POD activity, delayed the increase of PPO activity, and retarded the decrease in ascorbic acid, maintained the marketable fruits rate of *Rosa sterilis* D. shi fruits. Accumulation of cellulose related enzymes was also delayed in response to 1-MCP treatment. Fibrosis was slightly suppressed at the 1.0 μL L^{-1} 1-MCP. However, fibrosis was enhanced at the higher 1-MCP concentration, which may reflect senescence-associated cell dysfunction. Therefore, postharvest 1.0 μL L^{-1} 1-MCP treatment could maintain the quality of *Rosa sterilis* D. shi fruits.

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