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RESEARCH ARTICLE

Impact of 1-MCP on postharvest quality of sweet cherry during cold storage

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Abstract **Sweet cherry (*Prunus avium* L.) is a kind of fruit with short postharvest life. Postharvest treatment of 1-methylcyclopropene (1-MCP: 1.0, 1.5 and 2.0 μL/L) was applied to cherry fruits, and its effects on postharvest fruit quality during 60-day-storage at cold temperature was investigated. Sweet cherry fruits were harvested at commercial fruit maturity and exposed to 1-MCP at 20±1°C for 24 h. Following 1-MCP treatments, the fruits were stored for 60 d at 90±5% RH and, 0±1°C, in three concentrations of 1.0, 1.5, and 2.0 μL/L 1-MCP, of which 1.5 μL/L 1-MCP had the most positive effect on fruit quality. Postharvest treatments of 1-MCP significantly reduced endogenous ethylene production, malondialdehyde (MDA) content, and polyphenol oxidase (PPO) activity in cherry fruits 60 d after cold storage when compared to untreated fruits. 1-MCP treatment was associated with lower respiration rate and relative electric conductivity (REC) and maintained cell membrane integrity. The results indicated that the treatment 1-MCP was effective in inhibition of the declining of POD activity and CAT activity. In conclusion, 1-MCP treatment could be a good candidate for maintaining postharvest quality of cherry, and 1-MCP could prolong the storage life of sweet cherry.**

Keywords sweet cherry, 1-methylcyclopropene (1-MCP), postharvest quality, cold storage

Introduction

Sweet cherry (*Prunus avium* L.) is a profitable fruit with a high commercial value (Anonymous, 2004). However, the storage period and market life of fresh cherries are always limited by postharvest senescence (Mathooko et al., 1993). The physiologic and biochemical changes are related to fruit ripening (Choi et al., 2002), such as skin color, sugar, organic acid metabolism, and fruit softening (Valero et al., 2005). The weather of its harvest season is usually hot and rainy hence unfavorable for storage and transportation. Consequently, significant postharvest losses occur with a short storage life and even at low temperature (Kupferman et al., 1995); the physiologic and biochemical changes are related to fruit ripening, therefore, searching adaptive technologies of preserving and keeping fresh cherries is rather important and urgent.

It is well known that fruit is partially controlled by ethylene (Abdi et al., 1997). Sweet cherry, as a nonclimacteric fruit, reveals that ethylene is considered to have an apparent effect on its ripening and senescence. During storage, the respiratory rate and ethylene production of the fruit can be significantly enhanced by exogenous ethylene (Hartmann 1989). 1-MCP (1-methylcyclopropene), an ethylene action inhibitor, can significantly delay the ripening of the cherries (Sisler et al., 1996). Application of 1-MCP at low concentrations, prior to the climacteric increase, is effective and delays the onset of the climacteric peaks of CO_2 and ethylene production (Ku and Wills, 1999). The safety, toxicity, and environmental profiles of 1-MCP in regard to humans and animals are extremely favorable. At present, the effects of 1-MCP in delaying ripening have been studied on several fruits including apple (Marin et al., 2009), loquat (Cai et al., 2006), pear (Lu et al., 2009), plum (Luo et al., 2009), and strawberry (Jiang et al., 2001), which indicates that 1-MCP has potential for commercially controlling the ripening of harvested fruits.

The aims of our study were to investigate the role of exogenous application of 1-MCP in the regulation of ethylene production, MDA content, and activities of POD, PPO, and CAT and simultaneously determine the fruit respiration rate and REC in pulp tissues of cherries during cold storage.

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Materials and methods

Plant materials

Experiments were performed on sweet cherries (*Prunus avium* L.) by hand-harvesting fruits at commercial maturity in Baoding, Hebei Province, China, in 2010 growing season, which were transported to the laboratory on the intraday of sampling. Fruit harvest should be conducted 2–3 d after raining to prevent decay development during storage and shelf-life and test the efficacy of treatments on sensitive fruit. Harvested fruits with uniformed size and color together without defects were selected according to the exporting criteria of packinghouses. The selected fruits were placed in plastic bins of 60 cm × 30 cm × 40 cm and ventilated from all sides via small holes.

1-MCP application and experimental design

Fruits were divided into four lots of 360 fruits each, with three replicates of 120, which were treated with 1.0 μL/L, 1.5 μL/L, and 2.0 μL/L 1-MCP, respectively, for 24 h at 20±1°C in an airtight 72 L container, and fruit treated with 0 μL/L 1-MCP as control. The treated fruits were then stored at 90±5% relative humidity at 0±1°C for 60 d. The 1-MCP treatments were carried out, as described by Jiang et al. (2001). 1000 μL/L 1-MCP stock gas was made by dissolving 1.6 g EthylBlock™ powder (active ingredient 0.43% 1-MCP, BioTechnologies for Horticulture Inc., Waterboro, SC, USA) with water (at 35–40°C) in a sealed 1000-mL glass flask following the method of Lalel et al. (2003). The appropriate volume of 1-MCP stock gas determined to be necessary to attain the desired 1-MCP concentrations (1.0, 1.5 and 2.0 μL/L) in each container (72 L) was injected using a syringe into the sealed containers containing 120 fruits (at 20 ±1°C) each, with injection holes immediately sealed by using adhesive tape, and the containers were left to stand for 24 h at 20±1°C.

Determination of respiration rate

Individual fruits (six fruits per treatment) were sealed in airtight jars and held at 0±1°C and 90±5% RH for 2 h. Headspace gas samples were withdrawn with a 10-mL syringe. The carbon dioxide concentration in the gas sample was determined with a gas chromatograph (Agilent Technologies, 7890A Network GC system, Palo Alto, CA, USA) fitted with a GS-GASPRO 113-4332 column and a thermal conductivity detector (Pesis et al., 1994).

Determination of ethylene production

To measure ethylene, 1 kg fruit as a replicate were sealed in an airtight jar (10 L) fitted with a rubber septum for 2 h at 0±1°C and 90±5% RH. One mL gas sample was collected by syringe from each jar 2 h after sealing, and the ethylene concentration was measured using a gas chromatograph (Agilent Technologies, 7890A Network GC system, Palo Alto, CA, USA) fitted with a GS-GASPRO 113-4332 column, held at 60°C, and detection temperature of 220°C. The N_2 carrier gas was used at a flow pressure 0.18 MPa, and the air and H_2 pressures used in the flame ionization detector (FID) system were controlled at 0.08 and 0.05 MPa, respectively.

Malondialdehyde (MDA) content

MDA (malondialdehyde) content was determined with thiobarbituric acid reaction (Zhao et al., 1994). Briefly, 0.25 g of tissue was homogenized in 5 mL of 0.1% (w/v) trichloroacetic acid. The homogenate was centrifuged at 10000 × *g* for 5 min. Four milliliter of 20% (w/v) trichloroacetic acid containing 0.5% (w/v) thiobarbituric acid was added to a 1-mL aliquot of the supernatant. The mixture was heated at 95°C for 15 min and cooled immediately, and the absorption of the supernatant was read at 450, 532, and 600 nm, respectively.

$$\text{MDA content (μmol/g FW)} = 6.45\,(OD_{532}–OD_{600}) – 0.56\,OD_{450}.$$

Determination of catalase (CAT) activity

Catalase (CAT) activity was determined by monitoring the decomposition of H_2O_2 at 240 nm following the method of Aebi (1984). The reaction mixture contained 1.7 mL 50 mmol/L sodium phosphate buffer (pH 7.0), 1 mL double distilled water, and 0.2 mL tissue extract. The reaction was initiated by adding 0.1 mL 100 mmol/L H_2O_2. One unit of catalase was defined as the amount of enzyme that caused a 0.1 decrease of A_{240} per min at 25°C.

Determination of peroxidase (POD) activity

Peroxidase (POD) activity was determined by measuring the increase in absorption at 470 nm according to modification. The reaction initiated by the addition of 20 μL H_2O_2 was carried out at 25°C for 20 min in 3-mL reaction mixture containing 0.02 mL tissue extract, 50 mmol/L sodium phosphate buffer (pH 7.0), and 20 mmol/L guaiacol. One unit of POD was defined as the amount of enzyme that caused a 0.01 increase of A_{470} per min under the assay condition. Each experiment was repeated three times.

Determination of polyphenoloxidase (PPO) activity

For PPO, 5.0 g of frozen tissue powder was homogenized in 20 mL of 0.1 mol/L sodium phosphate buffer, pH 6.0, together with 1.0 g of polyvinylpolypyrrolidone (PVP), which was centrifuged at 20000 × *g* for 15 min. The supernatant was used for the PPO activity. The assay was

performed using 0.5 mL of 100 mmol/L 4-methylcatechol, 1.0 mL of 0.1 mol/L sodium phosphate buffer (pH 6.0), and 1.5 mL of the supernatant. The increase in absorbance at 410 nm at 25°C was recorded for 2 min. One unit of enzyme activity was defined as the amount of the enzyme, which caused a change of 0.01 in absorbance in 1 min.

Determination of relative electric conductivity (REC)

Plugs of flesh tissue excised from the four fruit per replicate with a 6-mm diameter stainless steel cork borer were cut into 2-mm thick flesh disks with a stainless steel razor blade. Each set of replicate fruit provided 20 disks, with 3 × 20 disks per treatment. The flesh samples were placed in 30 mL of 0.8 mmol/L mannitol and incubated for 2 h. The conductivity of the solution (EC_0) was measured using a DJS-1C conductivity meter (Shanghai Analytical Instrument Co., Shanghai, China). The solution was then boiled for 5 min and readjusted to a volume of 30 mL before the total conductivity of the solution (EC_T) was measured. The relative electric conductivity was calculated as follows:

Relative electric conductivity (%) = $EC_0/EC_T \times 100\%$.

Statistical analysis

Three replicates per treatment and 120 fruits per replicate were used in all treatments. Standard errors (S.E.) were calculated by Origin (Microcal Software Inc., Northampton, MA, USA), and differences indicated in the following figures were based on Duncan's new multiple range method test (DPS version 3.11) at the 5% level. LSDs (P = 0.05) were calculated for mean separations.

Results

Effect of 1-MCP on respiration rate

The respiration rate of fruit stored at 0±1°C showed a trend of nonclimacteric fruits. Fruits treated with 1-MCP exhibited a suppressed respiration rate compared with untreated fruits. On the 30th day of ripening, the respiration rate was 28.7, 24.8, 17.4, and 23.2 mg/kg fresh weight/h in the control and 1-MCP treatments (1.0, 1.5, and 2.0 μL/L), respectively. No significant difference ($P > 0.05$) between the two 1-MCP treatments (1.0 and 2.0 μL/L) was found. 1-MCP (1.5 μL/L) decreased in terms of respiration rate ($P < 0.05$) than any one of the other 1-MCP treatments (1.0 and 2.0 μL/L) and the control (Fig. 1).

Effect of 1-MCP on ethylene production

The ethylene production of cherries during storage at 0±1°C after harvest fluctuated from 0.064 to 0.185 μL/(kg·h). Fruits treated with 1-MCP exhibited inhibited ethylene production

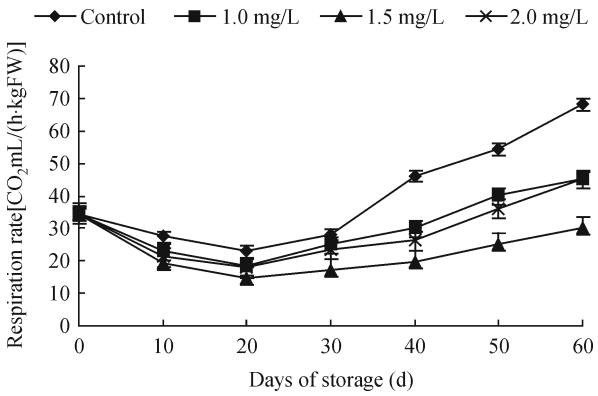

Figure 1 Effects of 1-MCP treatments on respiration rate of cherries. Bars indicate ± S.E.

compared with untreated ones. On the 30th day of ripening, 0.153, 0.147, 0.071, and 0.142 μL/(kg·h) ethylene were produced in the control and 1-MCP-treated fruits (1.0, 1.5, and 2.0 μL/L), respectively, without significant difference ($P > 0.05$) from 1.0 and 2.0 μL/L 1-MCP treatments and the control. However, 1.5 μL/L 1-MCP treatment lowered the ethylene production ($P < 0.05$) more than anyone of the other two treatments (1.0 and 2.0 μL/L) and the control (Fig. 2).

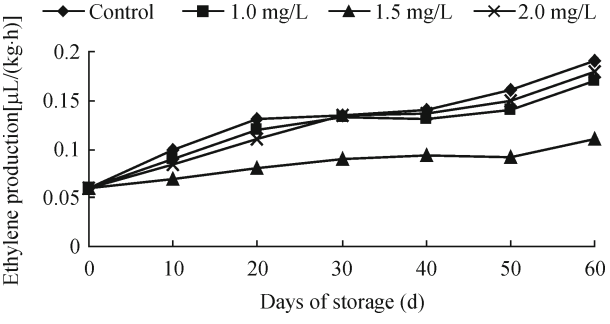

Figure 2 Effects of 1-MCP treatments on ethylene production of cherries. Bars indicate ± S.E.

Effect of 1-MCP on MDA content

MDA content was significantly reduced in 1-MCP-treated fruit pulp tissues during 60 days of ripening compared with the control, increasing as ripening progressed in 30 days of ripening, with the highest MDA content (22.4 nmol/g) ($P > 0.05$), followed by 1.0, 2.0, and 1.5 μL/L 1-MCP treatments, respectively (17.5, 16.5, and 14.3 nmol/g; Fig. 3).

Effect of 1-MCP on CAT activity

Changes in CAT activity of cherries are shown in Fig. 4. CAT activity rose in the initial and then declined steadily with time during low temperature storage. In 10 days of storage, CAT activity of the control reached its peak value [7.19 μmol H_2O_2/(min·g FW)]. Compared with the control, the CAT

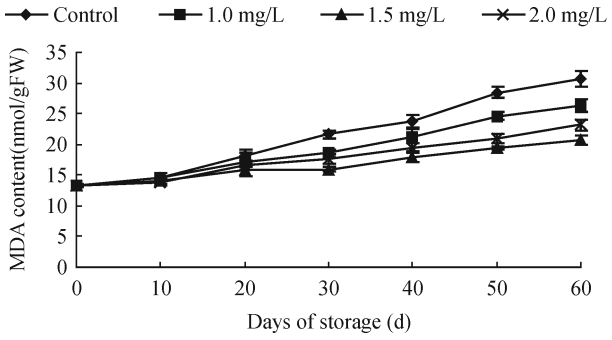

Figure 3 Effects of 1-MCP treatments on MDA content of cherries. Bars indicate ± S.E.

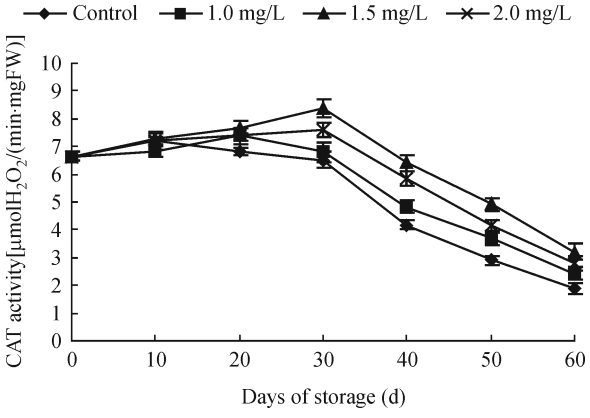

Figure 4 Effects of 1-MCP treatments on CAT of cherries. Bars indicate ± S.E.

activity kept a higher level in 1-MCP treatments during storage life and delayed the peak value, with 1.0 μL/L 1-MCP treatment reaching its peak value [7.43 μmol H_2O_2/ (min·g FW)] in 20 days of storage and 1.5 and 2.0 μL/L 1-MCP treatments reaching their peak values [8.35 and 7.60 μmol H_2O_2/(min·g FW], respectively) in 30 days of storage, which were significantly higher ($P < 0.05$) than that in the control.

Effect of 1-MCP on POD activity

Variations in POD activity of cherries during storage are shown in Fig. 5. POD activity rose rapidly in the initial period, reaching its peak value 30 days after treatment and then declined steadily. In 20 days of storage, the POD activity of the control reached its peak value [0.417/(min·g FW)]. The POD activities of 1-MCP treatments (1.0, 1.5, and 2.0 μL/L) reached their peak values [0.489, 0.604, and 0.541/(min·g FW), respectively] in 30 days of storage. The 1.5 μL/L 1-MCP treatment significantly maintained the highest POD activity ($P < 0.05$) among all the 1-MCP treatments and the control.

Effect of 1-MCP on PPO activity

The PPO activity in 1-MCP-treated fruit pulp tissues was

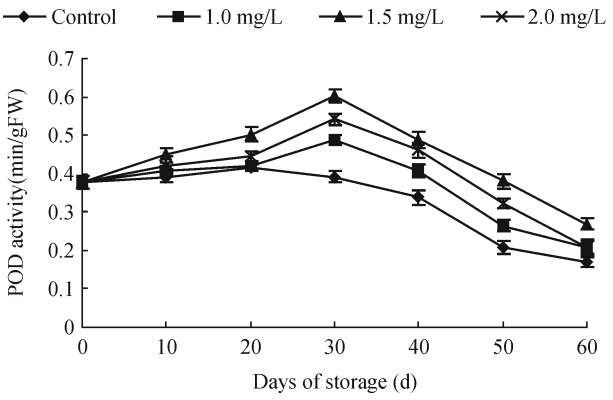

Figure 5 Effects of 1-MCP treatments on POD of cherries. Bars indicate ± S.E.

significantly suppressed compared with the control during the 60 days of ripening (Fig. 6). In the 30 days of ripening, the PPO activity in the control and 1.0, 1.5, and 2.0 μL/L 1-MCP-treated fruits was 9.12, 8.01, 6.45, and 7.91 0.01OD/(min·g FW), respectively. No significant difference ($P > 0.05$) between the two 1-MCP treatments (1.0 and 2.0 μL/L) was found, with the highest PPO activity ($P < 0.05$) reduced in the 1.5 μL/L 1-MCP treatment among all the treatments and control.

Effect of 1-MCP on relative electric conductivity

The relative electrical conductivity of cherries continually increased after harvest (Fig. 7), suggesting a gradual loss of cell membrane integrity. In 50 days of storage, relative electrical conductivity was 20.1, 13.0, and 18.2% in 1.0, 1.5, and 2.0 μL/L 1-MCP-treated fruits, respectively, lower ($P < 0.05$) than that of the control (30.2%).

Discussion

The reduction in ethylene production during cold temperature storage with 1-MCP application may be ascribed to the ability

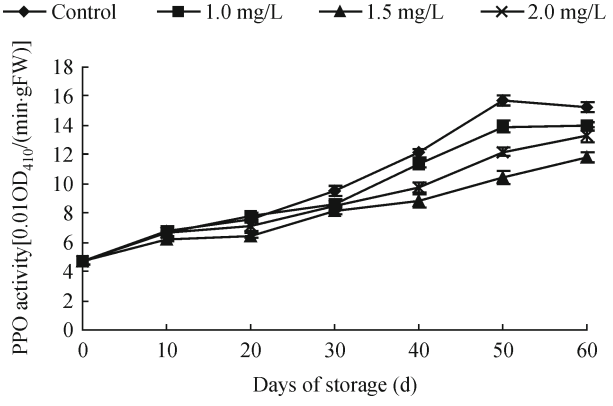

Figure 6 Effects of 1-MCP treatments on PPO of cherries. Bars indicate ± S.E.

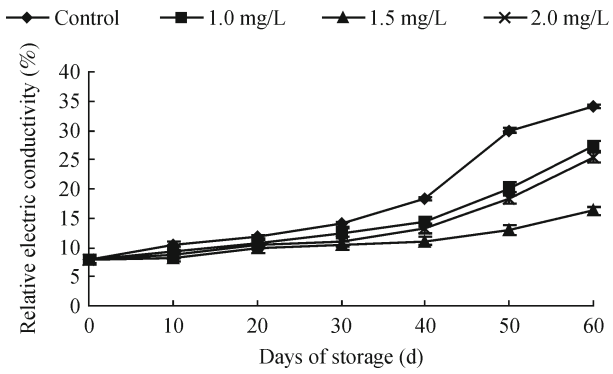

Figure 7 Effects of 1-MCP treatments on relative electric conductivity of cherries. Bars indicate ± S.E.

of 1-MCP to interact with ethylene receptors and its competition with ethylene for binding sites or to the ability of 1-MCP to interfere with autocatalytic ethylene production (Lalel et al., 2003), as ethylene binding sites are irreversibly blocked by 1-MCP. Reduction in ethylene production in 1-MCP-treaten fruit during storage may also be attributed to the reduced activities of ethylene biosynthesis enzymes and ACC content during cold storage. Reduction in endogenous ethylene production by 1-MCP has also been reported in plum stored at low temperature.

Our results showed a series of consistent changes that occurred as sweet cherry fruit passed through ripening and senescence after harvest. It is a short-lived fruit and so undergoes ripening and senescence relatively quickly. For identifying the controls of ripening and senescence in a fruit with such a relatively short postharvest life, the fruit responded to ethylene, in a similar manner to that of other nonclimacteric fruit, such as strawberry (Tian et al., 2000), by enhancing all of the ripening and senescence-associated changes, such as PPO, POD, CAT, MDA, and electrical conductivity.

The present study showed that in 1-MCP-treated sweet cherry fruit, the ethylene production, and PPO activities were significantly suppressed (Fig. 2 and Fig. 5). By inhibiting the ethylene action, 1-MCP probably reduced the disruption of the cell membranes and hence maintained the cell compartments intact, so that the PPO enzyme could not interact with its phenolic substrates, and there was less pulp browning.

The reason for exponential behavior of PPO is probably because at high chilling injury (CI) levels; when the flesh is brown, there is more damage to membranes, which enables more substrate interaction with the enzyme PPO. The initial level of PPO activity in sweet cherry fruit in non-1-MCP treatments was higher than that in 1-MCP treatments, but still, there existed an exponential correlation with the increased CI and PPO activity. It is possible that differences in total activity of PPO were accountable for the differences in browning of sweet cherry.

The increase in electrical conductivity of the tissue is a further indication of increasing senescence of the tissue, and the pattern of MDA content supports the evidence of membrane deterioration. MDA is believed to be a major contributor to senescence-related membrane deterioration in a number of plant tissues (Paliyath and Droillard, 1992), Zhang et al. (2002) observed similar results in kiwifruit, and the current data suggest that MDA plays a role in loquat senescence as well.

The response to 1-MCP also suggests that ethylene receptors are active in the fruit in this postharvest period. Our study as well as those on other nonclimacteric fruits, such as oranges (Porat et al., 1999), pineapples (Selvarajah et al., 2001), and clementine mandarins (Laamim et al., 2005), showed that 1-MCP effectively inhibited ethylene responses, as indicated by the delay in senescence. Effects of 1-MCP were concentration-dependent, and we found that 1.5 μL/L 1-MCP provided the best effect.

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