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Research Article

Exploring the Effects of Different 1-MCP Concentration Treatment on Chilling Injury of Postharvest Peach Fruit

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Peach (*Prunus persica* (L.) *Batsch*) fruit are susceptible to chilling injury that is often manifested as browning of the internal flesh. As the ethylene antagonist, 1-methylcyclopropene (1-MCP) is an important contributor to resist chilling stress during peach (*Prunus persica* (L.) *Batsch*) storage at low temperatures, but the molecular mechanism of it is still elusive. The study examined in detail the effects of two concentrations (1 and 2 $\mu L \cdot L^{-1}$) of 1-MCP on alleviating chilling injury (CI) of postharvest peach fruit stored at $4 \pm 0.5^{\circ}C$. The results showed that although all treatments relieved CI, treatment with 1 $\mu L \cdot L^{-1}$ more effectively alleviated CI symptoms of postharvest peach fruit than 2 $\mu L \cdot L^{-1}$. The internal browning (IB) and weight loss were significantly inhibited by 1 $\mu L \cdot L^{-1}$ 1-MCP. Lower concentration of 1-MCP maintained normal softening by decreasing the gene expression of expansin (Exp) 1,3, polygalacturonase (PG) 3, and pectate lyase (PLY) 1, 2, 3 and increased the content of soluble sugar during the whole storage period. Meanwhile, the activities of polygalacturonase, pectinmethylesterase (PME), and endo-1,4-β-glucanase (EGase) were reduced by lower concentration of 1-MCP. Our results demonstrate that 1-MCP inhibited CI by suppressing the degradation of cell wall. This study provides a theoretical basis for revealing that 1-MCP alleviates chilling injury in peach fruit.

1. Introduction

Maintaining the quality of peach fruit during cold storage has been a major problem that has restricted the development of peach preservation and deep processing. Peach is a climacteric fruit and susceptible to CI under unsuitable low temperature conditions, accompanied by changes in IB, abnormal softening, and loss the ability of after-ripening, which decreases the commercial value of peach fruit. Chilling injury is the phenomenon of a series of physiological disorders caused by the disruption of cell membrane lipid permeability in fruit under low temperature stress [1]. Thus, suppressing CI of postharvest peach fruit is a major problem to be solved.

As a means to alleviate CI during cold storage, treatments with the 1-MCP have been widely studied [2–4], and it is an effective method for maintaining the quality and extending the shelf life in various species, including banana and figs [3, 4]. However, the effectiveness of 1-MCP varies widely, ranging from almost compromising the ability of the fruit to ripen properly (e.g., European pear, avocado), to relatively small and transitory effects (e.g., peach and nectarine) [5–7]. This necessitates the careful optimization of treatment strategy to gain the greatest benefit. In the case of appropriate concentration, 1-MCP treatment is highly effective, alleviating peach fruit CI during storage. Zhang et al. [8] found that multiple 1 $\mu L \cdot L^{-1}$ 1-MCP treatment more effectively alleviated postharvest nectarine chilling injury than conventional one-time 1-MCP treatment by regulating ROS and energy metabolism. However, Jin et al. [9] sorted out the 0.5 $\mu L \cdot L^{-1}$ concentration of 1-MCP from concentration gradient, which alleviated CI by promoting the activities of antioxidant enzyme. 1-MCP has been reported to alleviate the browning symptoms and effectively extend the shelf life of plum [10–12]. The authors in [13] found that 1-MCP treatment increased the activity of antioxidant and the content of phenol to high level of peach fruit. In addition, the quality of postharvest fruit could be maintained by 1-MCP application, which because of the 1-MCP regulated the metabolism of energy, including the genes expression and

enzyme activity of energy [14–16]. Nevertheless, even in the fruit industry where 1-MCP treatment has entered wide commercial use, variability in response between cultivars is evident, and requires optimization of treatment regime regarding fruit maturity, 1-MCP concentration, time between harvest and treatment, and other factors [6].

Abnormal softening is a key phenotype of CI and quality deterioration of fruit during cold storage. The change of fruit firmness is a complicated process, and it is generally believed that the change in pectin caused by the degradation of the cell wall is the main reason for softening [17]. Transcript abundance of cell wall degradation-related genes were increased during fruit softening, including polygalacturonase (PG), β-1,4-endoglucanase (EGase), pectate lyase (PLY), expansin (Exp), xylosidase (Xyl), and xyloglucan endotransglucosylase/hydrolase (XTH2) [18–21]. In the previous study, the increase in the sugar levels was correlated to the promoting ability of CI resistance. Sucrose, as the member of soluble solids, alleviated the CI of postharvest peach fruit [22].

Although many studies have researched the effect of 1-MCP on alleviating CI, the effectiveness varies widely. Also, the exact mechanisms of 1-MCP on ameliorating chilling resistance are still poorly understood. In this experiment, certain concentrations of 1-MCP treatment were selected from two different concentrations. The effect of certain concentration of 1-MCP on alleviating CI of postharvest peach fruit was explored. The study was expected to provide a theoretical basis for revealing the mechanism of alleviating CI during cold storage peach fruit, and we expect to provide theoretical guidance for the commercial use of 1-MCP.

2. Materials and Methods

2.1. Plant Material and Treatments. Fruits of peach (*Prunus persica* (L.) *Batsch*, cv "Lv Hua 9") were harvested at commercial maturity, which were eighty percent mature, from an orchard in Pinggu, Beijing, China, in 2019, and transported to our laboratory in Beijing by truck within 2 h. Fruits with uniform size and ripeness and without blemish or mechanical damage were divided into three batches of 80 fruits each. Fruits were treated in sealed jars for 2 h at 20°C with low concentration (1 μL·L^{-1}) and high concentration (2 μL·L^{-1}) of 1-MCP released from a commercial tablet formulation (Xianyang Xiqin Biological Technology Co. Ltd., Freshdoctor, Shanxi, China) by following the manufacturer's instructions. The control fruit was kept in a similar sealed condition, without 1-MCP, for 2 h at 20°C. Treatment times were kept short, to avoid the problems of excessive accumulation of CO_2 for peach fruit stored under sealed conditions for 12 or 24 h [23]. After treatments, fruits of control and treated groups were stored in air at 4 ± 0.5°C, which was the temperature prone to chilling injury, with 90–95% relative humidity for up to 28 d.

Fruit weight, firmness, rate of ethylene production, and soluble sugar content were measured at harvest and at intervals during cold storage. After measurement, slices of mesocarp were immediately frozen in liquid nitrogen and stored at −80°C for biochemical and molecular analysis. Based on the observed differences in fruit quality and physiology indices during the experiment, we selected 21 d after treatment for real-time quantitative PCR analysis. The treatments of low-1-MCP concentration, high-1-MCP concentration, and control were designated as L-1-MCP, H-1-MCP, and control, respectively.

2.2. Physiological Measurements. The method from Zhao et al. [24] was used to quantify the IB index. Fruit weight was determined by reweighing the same 10 fruits at each time point, and data are expressed as weight loss as a percentage of the initial weight.

For the measurement of flesh firmness, small areas of skin were removed on opposite sides of the fruit equator, and firmness was measured on both sides using a GY-4 fruit firmness tester (Zhejiang TOP Instrument Co., Ltd., China) fitted with 11.1 mm flat probe that was inserted 10 mm into the flesh. The peak force was recorded in Newtons (N) and used to indicate fruit flesh firmness.

For ethylene measurements, two fruits were placed in each of three 1.5 L-capacity sealed containers for 4 h. Aliquots of headspace gas were measured for ethylene content using an F950 ethylene analyzer (Felix Instruments Camas, WA, USA). The peach volume of the fruit was measured by determining the amount of water displaced by the fruit. The calculation of ethylene evolution was as follows:

$$X = C \times (V_1 - V_2)/(W \times H), \quad (1)$$

where X = ethylene production, nL·kg^{-1}·s^{-1}; C = ethylene concentration released from the sample, nL·L^{-1}; V_1 = container volume, L; V_2 = fruit volume, L; W = fruit weight, kg; H = incubation time, s [22].

Soluble sugar content was determined using a soluble sugar content tester (SYS-PAL1, Atago, Japan). A droplet of juice was expressed from the fruit and was placed on the prism glass of the tester to analyze the sugar content. Results are expressed as percentages (%).

2.3. RNA Extraction and Real-Time Quantitative PCR Analysis. RNA was isolated from frozen tissue samples using a cetyltrimethylammonium bromide (CTAB) method [25]. Samples consisted of three independent biological replicates, each composed of pooled flesh tissue from three fruits, for a total of four treatment/time points (2 treatments × 2 time points × 3 biological replicates), resulting in 12 analyzed samples. RNA quality was verified by RNase-free agarose-gel electrophoresis, and the total RNA concentration of each sample was quantified using a DNA/Protein Analyzer (Merinton Technology Co., Ltd., Beijing, China) at 260 and 280 nm. Preparation of RNA-Seq libraries and sequencing were carried out by OE Biotech Co. Ltd. (Shanghai, China) using an Illumina HiSeqTM2500 platform with a 100 bp single read sequencing flow cell.

A SYBR Green Q-PCR Kit (Takara, RR420A, Japan) was used as described in the manufacturer's instructions, and reactions were performed on a 7500 Fast Real-Time PCR System (Thermo Fisher Scientific, USA). Primer sequences of the genes analyzed are shown in Table S1.

2.4. Statistical Analysis. All experiments were performed in triplicate ($n = 3$), and an ANOVA test (using SPSS 24.0 statistical software, SPSS Inc., Chicago, USA) followed by using the LSD test at the significance level of $p \leq 0.05$ was used to compare the mean values of each treatment. Statistical comparisons between treatment and controls were made using Duncan's multiple range test. The results represented mean ± standard error (SE) of three replicated determinations. Origin 8.6 (Microcal Software, Northampton, MA) was used to plot figures.

3. Results

3.1. Effect of Different Concentration 1-MCP on CI Index of Postharvest Peach Fruit. The effect of 1-MCP treatment on IB index of postharvest peach fruit was dependent on the concentration of 1-MCP. The results showed that the low concentration of 1 $\mu L \cdot L^{-1}$ significantly decreased the IB index of peach fruit during the last 21 days of storage (Figure 1). The internal browning appeared at the 14 days of storage in control and 2 $\mu L \cdot L^{-1}$ 1-MCP treatment, which were earlier than 1 $\mu L \cdot L^{-1}$ 1-MCP treatment at about 7 days.

3.2. Effect of Different Concentration 1-MCP on Physiological Index. The quality of postharvest peach can be reflected by the firmness, respiration rate, sugar content, and weight loss rate during the storage. The firmness of all fruit decreased during the whole storage period. The firmness of the fruit for 1-MCP treatment was higher than that of the control, while that of L-1-MCP treatment was significantly higher than that of H-1-MCP treatment from the days of 7 to 28 (Figure 2(a)). The content of sugar in all peach fruit increased during the first 7 days and decreased from the 7th day. Interestingly, compared with the control, the L-1-MCP concentration treatment maintained the sugar content at a high level, while the H-1-MCP concentration treatment reduced the sugar content (Figure 2(b)).

Ethylene production remained at a low level during the first 7 days and increased from the 14th day. Meanwhile, 1-MCP treatment suppressed the ethylene level during cold storage, and there were no significant difference between the different concentration treatments of 1-MCP (Figure 2(c)). The weight loss rate of all peach fruit increased throughout the whole cold storage period. The 1-MCP treatment declined the weight loss rate of peach fruit compared with the control, but there were no significant difference between the L-1-MCP treatment and H-1-MCP treatment (Figure 2(d)).

3.3. Expression of Genes Related to Cell Wall Metabolism. According to the above physiological results, the mechanism of low concentration 1-MCP (1-MCP) regulating the chilling injury of postharvest peach fruit in the cold storage for 21 days was discussed subsequently.

The expression abundance of enzymes related to cell wall metabolism were analyzed, including β-1,4-endoglucanase (EGase), expansin 1 (Exp 1), galactosidase (GAL), polygalacturonase 1 (PG1), PG2, PG3, pectate lyase 1 (PLY1), PLY2, and pectin methylesterase (PME). The transcript level of cell wall-related genes declined under the effect of 1-MCP

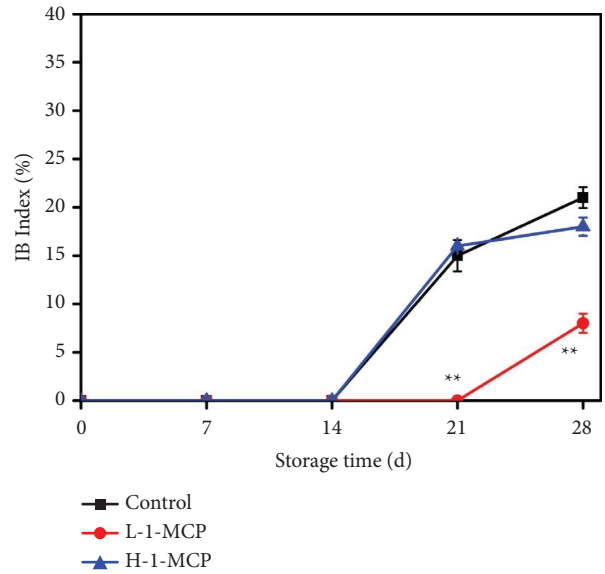


Figure 1: Effect of different concentration 1-MCP treatment on the IB index. Data are expressed as means of triplicate samples (each consisting of three fruits) ± standard error ($n = 9$). Asterisks indicate that mean values are significantly different between jasmonic acid treatment and control ($p < 0.05$) according to Duncan's multiple range test.

(Figure 3). Among them, PG1, 2, 3, and PpEGase decreased significantly. Meanwhile, the transcript level of PpPG4, PpPLY1, 2, PpPME, and PpGAL were inhibited by 1-MCP treatment during the cold storage. The data showed that the effect of L-1-MCP on maintaining the firmness of peach fruit was related to these genes.

3.4. Activities of Cell Wall-Degrading Enzymes. Since the activities of enzymes related to cell wall disassembly are an important component of fruit softening, we analyzed the activities of enzymes related to cell wall disassembly, including polygalacturonase, pectinesterase, β-galactosidase, and cellulase (Figure 4). Activities of pectinesterase and β-galactosidase in controls were approximately similar throughout the storage period, which were increased during the first 21 d of storage then gradually declining (Figures 4(a and d)). The patterns of activity for polygalacturonase and cellulase were similar in controls, increasing during the first 14 d of storage then gradually declining (Figures 4(b and c)). Treatment with 1-MCP decreased the activity of pectinesterase, polygalacturonase, β-galactosidase, and cellulase.

4. Discussion

The study found that the low concentration 1-MCP treatment reduced CI index of 4°C-stored peach fruit. Many studies have researched the effect of 1-MCP on postharvest peach fruit, but the results were not consistent. Jin et al. [9] found that 0.5 $\mu L \cdot L^{-1}$ was the optimum concentration for alleviating CI of peach fruit. However, Zhang et al. [8] showed that 1 $\mu L \cdot L^{-1}$ 1-MCP treatment alleviated postharvest nectarine CI by regulating ROS and energy

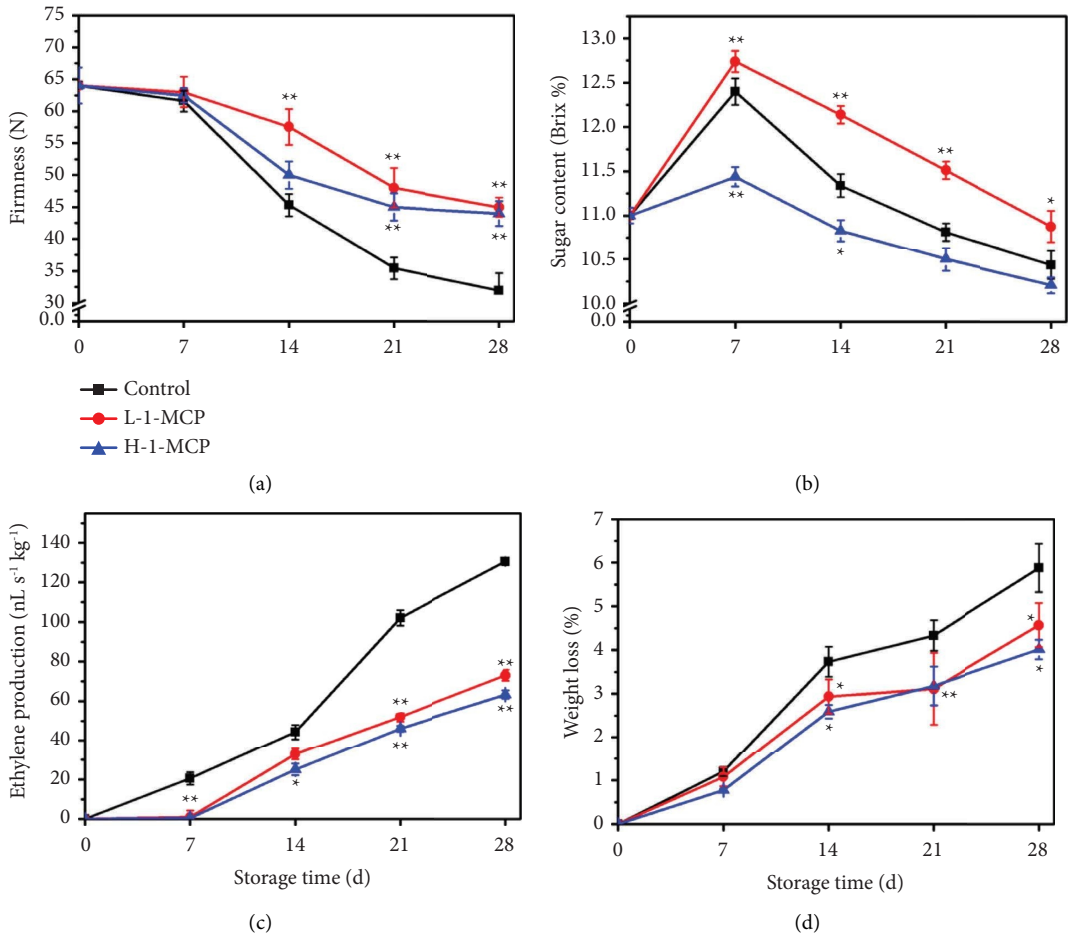


Figure 2: Effect of a prestorage 1-MCP treatment on physiological index. (a) Firmness of fruit after storage at 4°C for the indicated time. (b) Sugar content of fruit after storage at 4°C for the indicated time. (c) Ethylene production of fruit after storage at 4°C for the indicated time. (d) Weight loss rate of fruit after storage at 4°C for the indicated time. Data are expressed as means of triplicate samples (each consisting of three fruits) ± standard error ($n = 9$). Asterisks indicate that mean values are significantly different between 1-MCP treatment and control ($p < 0.05$) according to Duncan's multiple range test.

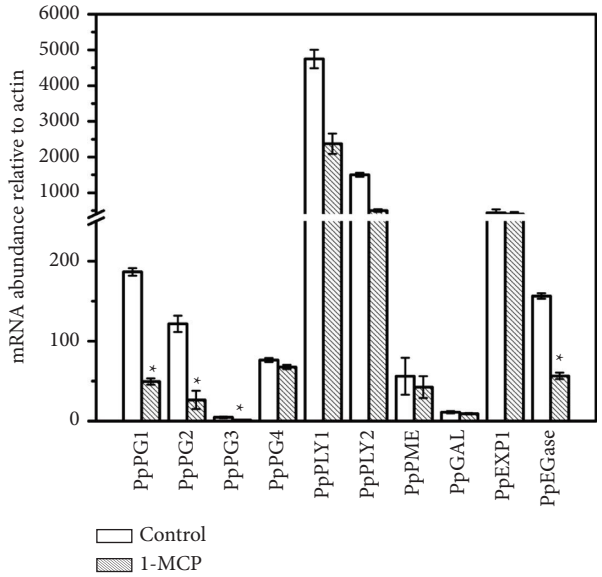


Figure 3: Effect of 1-MCP treatment on the expression of genes related to cell wall metabolism in peach fruit during storage at 4°C. Data are presented as means of three biological replicates ± standard error. Asterisks indicate that mean values are significantly different between 1-MCP treatment and control ($p < 0.05$) according to Duncan's multiple range test, with a minimum of a 2-fold difference in transcript abundance.

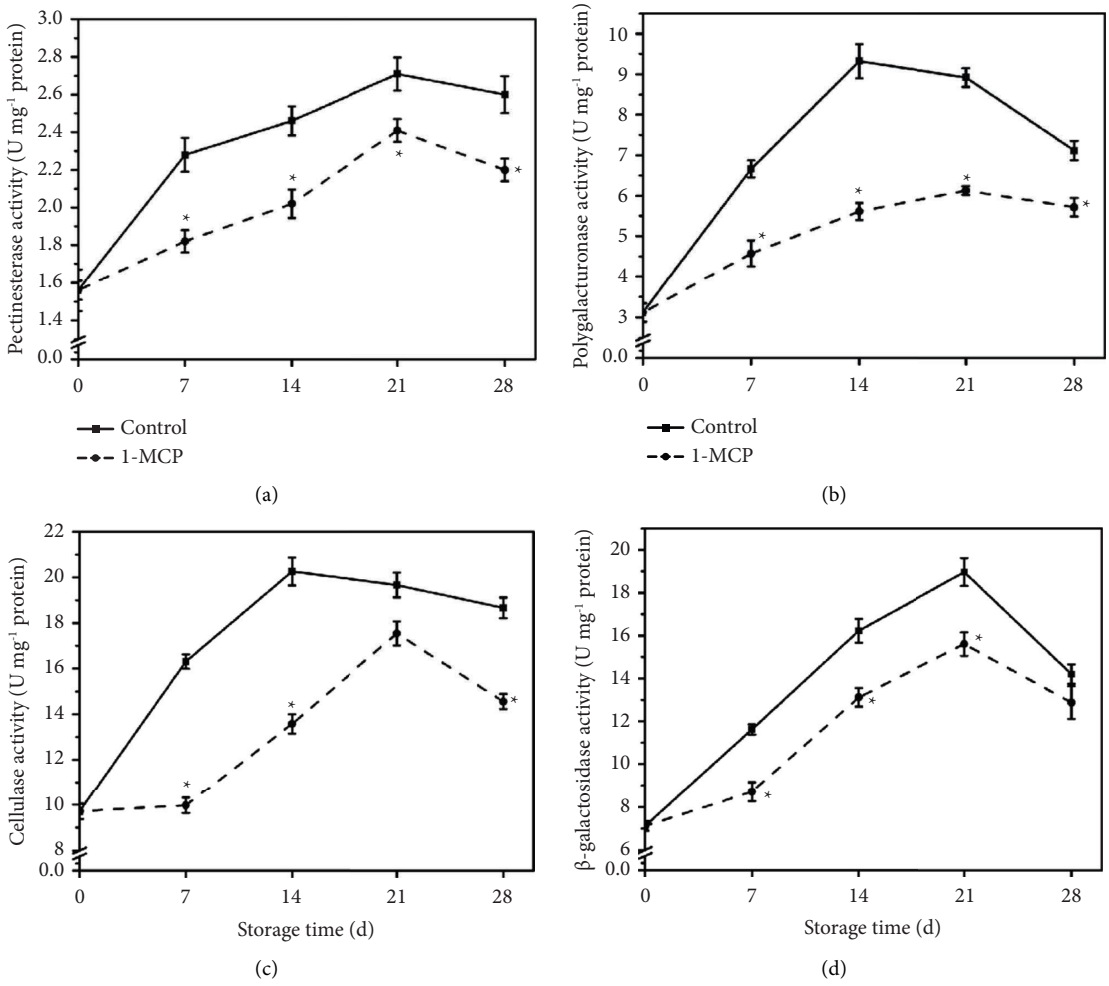


Figure 4: Effect of 1-MCP treatment on the activity of enzymes related to cell wall metabolism in peach fruit during storage at 4°C. Data are presented as means of three biological replicates ± standard error. Asterisks indicate that mean values are significantly different between 1-MCP treatment and control ($p < 0.05$) according to Duncan's multiple range test.

metabolism. In our research, 1 μL·L^{-1} 1-MCP treatment was the optimum concentration for alleviating CI of postharvest peach fruit. These different results may be due to the different responses of different peach varieties to 1-MCP. The 1-MCP treatment reduced ethylene production by hindering the functioning of ethylene receptors during the whole storage period. The delayed and reduced ethylene production was presumably the main cause of reductions in softening over the storage period [26]. In "Elberta" peaches, a 4 h treatment with 1-MCP delayed the climacteric by 4 d and resulted in firmer fruit if stored at 0 or 5°C [27]. Increasing research studies show that 1-MCP has potential contribution to retard CI of horticultural products such as blood orange [28], tomato fruit [29], and banana [30]. The previous study showed that 1000 ppb 1-MCP maintained better pear storage quality than 750 and 500 ppb [31]. In the current study, exogenous 1-MCP retarded chilling injury of peach fruit, delayed the increase of IB index, ion leakage, and MDA accumulation in peaches, indicating that 1-MCP treatment had a positive role in facilitating chilling resistance during cold storage.

Reductions in autocatalytic ethylene production resulting from 1-MCP treatment can be expected to reduce the transcript abundance of several genes involved in cell wall modification. Some of cell wall metabolism-related genes are directly ethylene regulated, while there is evidence that others are more developmentally regulated and so indirectly respond to ethylene via its effects on developmental advancement [32]. In peach, some genes encoding ripening-related endopolygalacturonase and expansin are ethylene regulated, whereas others are not [33]. After 1-MCP treatment, reductions in transcript abundance were observed at 21 d for PpPGs, PpPLYs, PpPME, PpGAL, and PpEGase, among which the regulation of 1-MCP on PpPG1, 2, 3, and PpEGase were significant. Studies measuring cell wall-related enzyme activities (which are often encoded by multiple genes) found that 1-MCP treatment decreased the activities of pectinesterase, polygalacturonase, β-galactosidase, and cellulase during the whole cold storage period. Previous study showed that 1-MCP treatment reduced the activities of polygalacturonase, but not β-galactosidase, when assayed poststorage during shelf life at

20°C [34]. These effects are likely mediated via reduced autocatalytic ethylene production.

Suppression of ethylene production and responses by 1-MCP can have effects on several other aspects of ripening metabolism, but effects are dependent upon cultivar, treatment, and storage regime. 1-MCP treatment can increase the sucrose content of fruit (Figure 2(b)), which enhances resistance to chilling injury [35]. The content of sugar in all peach fruit increased during the first 7 days and decreased from the 7th day. Also, compared with the control, the L-1-MCP concentration treatment maintained the sugar content at a high level. Our study has investigated some of the physiological and gene expression changes caused by 1-MCP treatment. We have shown that in the short-term, storage life can be increased by 1-MCP treatment, delaying the climacteric during the first few days after treatment and reducing softening over a few weeks. This is consistent with previous studies that found 1-MCP to be suitable only for short extensions of storage life [27, 33, 36]. Reductions in expression of genes related to cell wall disassembly were observed at 21 d of storage, probably due to reduced CI index, and some physiological effects were observable during the storage period. Other researchers have attempted to make the effects of 1-MCP more enduring by applying multiple successive 1-MCP treatments [37], or by combining 1-MCP treatment with other postharvest treatments such as aminoethoxyvinylglycine (AVG), hot water dips, or intermittent warming [13, 33, 38], and this may be the way to obtain commercially useful benefits from 1-MCP for the storage of peach.

5. Conclusion

There is a commercial need to identify treatments that maintain fruit quality using nontoxic chemicals. The low concentration ($1\,\mu L{\cdot}L^{-1}$) of 1-MCP was shown to be more effective than the high concentration in alleviating CI of peach fruit. 1-MCP reduced the mRNA abundances of genes involved in cell wall metabolism, particularly PpPG1, 2, 3, and PpEGase, which regulated the softening of postharvest peach fruit. Meanwhile, the enzyme activities of cell wall metabolism were decreased corresponding. 1-MCP increased the content of soluble sugar, which may help the fruit withstand the stress of cold storage. The study provides new insights into the regulation mechanism of 1-MCP on cell wall and sugar metabolism in peach fruit during low-temperature storage, revealing a high-value target for breeding fruit with improved tolerance to cold stress.

Data Availability

The data used to support the findings of this study are included within the article and supplementary information files.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors' Contributions

Yaoyao Zhao performed the conceptualization, investigation, formal analysis, and visualization and wrote the original draft. Congcong Song and Qiong Lin reviewed and edited the manuscript. Yuquan Duan conceptualized the study.

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Supplementary Materials

Table S1: primers used for analyzing the expression of genes related to cell wall metabolism. (*Supplementary Materials*)

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