Melatonin and 1-Methylcyclopropene Improve the Postharvest Quality and Antioxidant Capacity of ‘Youhou’ Sweet Persimmons during Cold Storage

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ABSTRACT
The objective of this study was to identify the effects of 100 μM melatonin (MT), 1 μL/L 1-methylcyclopropene (1-MCP), and their combination treatments on the postharvest characteristics, nutritional quality, and antioxidant capacity of ‘Youhou’ persimmons during cold storage at 0°C. Compared with the control fruit, all three treatments suppressed ethylene production and respiration rate, leading to delayed softening, reduced peel discoloration, and soluble solid content loss. These treatments increased plasma membrane stability by inhibiting lipoxygenase activity and decreasing H₂O₂, electrolyte leakage, and malondialdehyde concentrations, resulting in higher tolerance to chilling injury (CI). At the end of storage, the CI index of fruit treated with MT, 1-MCP, and MT+1-MCP respectively exhibited 54.67%, 14.57%, and 5.54%, which were significantly lower than the control ones (78.36%). In addition, the phenolics, flavonoids, and ascorbic acid contents were also improved by the above treatments, which contributed to higher antioxidant activity than the control fruit at the late stage of storage. The combination of MT and 1-MCP treatment was more effective in improving the postharvest quality and preserving high antioxidant ability levels than either treatment alone, indicating a promising measure to preserve sweet persimmons up to 70 d.

KEYWORDS
Sweet persimmon; melatonin; 1-methylcyclopropene; postharvest quality; antioxidants

INTRODUCTION
Persimmons (Diospyros kaki Thunb.) are cultivated widely around the world because of their unique flavor and high nutrient content, such as polyphenols, flavonoids, and vitamins that can improve human immunity and health by scavenging free radicals (Ding et al., 2017; Kim et al., 2020; Zhang et al., 2018). The pollination-constant non-astringent (PCNA) persimmon, which has improved taste without any postharvest de-astringent treatments, has become the most desirable type and is the main goal of persimmon breeding programs (Sato and Yamada, 2016). Owing to its excellent taste and grateful appearance, the ‘Youhou’ sweet persimmon is one of the main PCNA cultivars in China (Zhao et al., 2020). However, this cultivar is a typical climacteric fruit with poor storability, because it ripens rapidly and softens easily when stored at ambient temperature (Jung et al., 2017; Zhang et al., 2010). Low-temperature (stored below 15°C) can effectively mitigate the softening process of sweet persimmon, but may lead to chilling injury (CI) of the fruit because of its sensibility to chilling (Orihuel-Iranzo et al., 2010; Woolf et al., 1997). During cold storage, the chilling stress will generally result in the accumulation of reactive oxygen species (ROS) in persimmon and ultimately induce numerous physiological disorders, including flesh softening, browning, peel color change, membrane damage,
and antioxidant compounds loss (Arnal et al., 2008; Besada et al., 2014; Kim et al., 2020; Lyons, 1973; Saleem et al., 2020; Valenzuela et al., 2017). Peel and flesh browning is the main CI symptoms in sweet persimmon, which normally occur after transferring the fruit to ambient temperature from cold storage (Besada et al., 2015; Li et al., 2018). Due to the aforementioned postharvest problems, researchers have focused on discovering highly effective techniques to improve quality of sweet persimmon during cold storage.

Melatonin (N-acetyl-5-methoxytryptamine, MT) is a well-known free-radical scavenger hormone in plants that has been proven to play important roles in regulating plant growth, development and stress responses (Cao et al., 2016; Zhang et al., 2015). In recent years, exogenous MT has shown promise as a postharvest treatment used to prolong storage period and maintain nutritional quality of postharvest fruits by enhancing their antioxidant capacity (Rastegar et al., 2020). It has also been used to increase the chilling tolerance of peaches by reducing H2O2 accumulation and activating the expression of antioxidative genes (Cao et al., 2018), delay senescence and attenuate the postharvest decay of strawberries (Aghdam and Fard, 2017), improve the postharvest quality of grapes (Xu et al., 2018), and promote fruit ripening and anthocyanin accumulation in tomatoes (Sun et al., 2016). However, few study has been conducted to research the effect of MT on the postharvest quality of sweet persimmons.

Ethylene is a gaseous plant hormone that has been identified to be correlated with the development of plant. In addition, either exogenous and endogenous ethylene can affect postharvest fruit negatively by causing senescence, quality deterioration, nutrient composition loss, and physiological disorders. The competitive combination of 1-methylcyclopropene (1-MCP) with ethylene receptor can remarkably delay postharvest senescence induced by ethylene (Watkins, 2006). The fumigation of 1-MCP can extend the storage life of ‘Qiandaowu’ persimmons, delay the softening and color evolution of ‘RojoBrillante’ persimmons, and alleviate the chilling injuries of ‘Youhou’ and ‘Karaj’ persimmons (Luo, 2007; Rasouli and Khadem, 2017; Salvador et al., 2004; Zhao et al., 2020).

Recent studies have shown that applying combined treatments of multiple technologies is more effective in maintaining the postharvest quality of persimmons, which may result from combining their respective physiological regulatory effects. For example, Li et al. (2018) confirmed that the combination of 1-MCP and oxalic acid is more effective in alleviating chilling injuries and maintaining the firmness of ‘Youhou’ sweet persimmons than the control treatment and single-use of either oxalic acid or 1-MCP. In addition, the combination of 1-MCP and 5% CO2 can prolong storage period and preserve high nutrient levels and the antioxidant ability of persimmon fruit (Zhang et al., 2018). Both MT and 1-MCP have been proven effective postharvest treatments to prolong storage period, enhance chilling tolerance and improve quality of cold-stored fruit. 1-MCP can notably inhibit quality deterioration and physiological disorder induced by ethylene in sweet persimmons, while MT is well known for its distinct regulatory ability to improve the antioxidant capacity of fruit (Watkins, 2006; Zhang et al., 2010; Aghdam and Fard, 2017; Ding et al., 2017). Therefore, there is a hypothesis in this study that the postharvest quality and antioxidant capacity of fruit will both be effectively improved, if combining the MT and 1-MCP treatments on sweet persimmons. However, to our knowledge, such studies have not been reported so far.

The aim of this study was to investigate the effects of MT, 1-MCP, and a combination of MT and 1-MCP on the postharvest quality, nutrient content, and antioxidant capacity of sweet persimmons stored at 0°C for 70 days.

**Materials and Methods**

**Plant Materials and Postharvest Treatments**

‘Youhou’ sweet persimmons (Diospyros kaki L. cv. Youhou) were harvested at the commercial maturity stage from an experimental orchard in Yuncheng, Shanxi Province, China. We selected
fruit of uniform size (80 ~ 90 mm) and color and with no visible diseases, insects, or mechanical injuries, and then transported them to the laboratory immediately.

The fruit were randomly divided into group A (CK, 400 fruits with no further treatment), group B (MT, 400 fruits treated with MT), group C (1-MCP, 400 fruits treated with 1-MCP) and group D (MT+1-MCP, 400 fruits treated with MT and 1-MCP), and three replicates were used for each treatment group. For the control treatment, sweet persimmons were immersed in distilled water for 10 min and then air-dried. For the MT treatment, sweet persimmons were immersed in 100 μM of MT solution for 10 min and then air-dried (Gao et al., 2016). For the 1-MCP treatment, sweet persimmons were immersed in the distilled water for 10 min, and then fumigated with 1 μL/L 1-MCP in a 1 m³ container at ambient temperature (25°C) for 24 hours. The control and MT treatments were performed in a similar manner, but without the inclusion of the 1-MCP. For the MT+1-MCP treatment, sweet persimmons were immersed in 100 μM of MT solution for 10 min, and then fumigated with 1 μL/L 1-MCP in a 1 m³ container at ambient temperature (25°C) for 24 hours. Subsequently, every 10 kg of fruit were placed in a plastic turnaround box lined with polyethylene package that was 0.02 mm thick, 50 cm wide, and 70 cm long. After 12 hours of precooling, the packages were tied up and stored at 0 ± 0.5°C and a relative humidity of 90–95% for 70 days.

In the total storage period of 70 days, 30 pieces of fruit from each treatment were randomly selected every 10 days to evaluate their ethylene production, respiration rate, firmness, soluble solid content (SSC), ethylene production, color parameters, and electrolyte leakage. Meanwhile, flesh tissues were frozen in liquid nitrogen, and stored at −80°C for subsequent analysis. Additionally, other 30 fruit per treatment were transferred every 10 days to ambient temperature (25°C) for 5 days to evaluate CI index during cold storage.

**Postharvest Quality of Sweet Persimmons during Storage**

**Determination of CI Index**
The CI index was assessed on the proportion of visual flesh and peel browning (Li et al., 2018). CI severity grade was counted with a scale of 0 ~ 4: 0 (no CI symptoms), 1 (slight CI, 1–25%), 2(moderate CI, 25–50%), 3 (heavy CI, 50–75%), and 4 (serious CI, 75–100%). The CI index was calculated via the following formula: [Σ (CI grade) × (number of fruits at the CI grade)] ÷ (total CI scales) × (total number of fruit).

**Determination of Ethylene Production, Respiration Rate, Firmness, and SSC**
Five fruit per replication were enclosed in 3 L airtight glass containers at 0°C for 4 hours to evaluate the ethylene production and respiration rate. For ethylene production determination, gas samples (10 mL) were collected and 1 mL sample was quantified for ethylene concentration by means of gas chromatograph (GC-14C; Shimadzu, Kyoto, Japan). The results were expressed in μL/kg·h. For respiration rate determination, the CO₂ concentration released by fruit in the container was detected using a F-950 fruit and vegetable gas analyzer (Felix, Camas, WA., USA).

Firmness was determined by measuring opposite peeled sides at the equatorial region of six fruit per replication, using a TA-XT-plus Texture Analyzer with a P/5 cylindrical probe. The testing parameters covered in this section including pretest speed, test speed, posttest speed, and test distance were according to the standards of manufacture. The firmness values were recorded as the maximum compression force and expressed as g.

After extracting and mixing one drop of juice from each sample, the SSC of the fruit was determined using a digital portable refractometer (PAL-1; Atago Co., Ltd., Tokyo, Japan) and expressed as a percentage.

**Color Evaluation of Fruit**
Peel color was measured at two opposite points of the fruit using a Chroma Meter CR-400. The mean values of three fruit per replication were recorded and expressed as L*, a*, b*, and hue angle (H* = tan⁻¹b*/a*).
**Determination of Electrolyte Leakage, MDA Content, H$_2$O$_2$ Concentration, and LOX Activity**

Electrolyte leakage was measured using the method described by Zhao et al. (2020). Cubes of 1 cm$^3$ round were cut from the equatorial flesh of three fruit per replication using a hole punch, and then cut into 2 mm thick slices. Thirty randomly selected slices were placed in 40 mL of distilled water and incubated at 25°C for 2 hours. The conductivity of the incubated solution (EM$_0$) was measured using a DDS-11A conductivity meter (Leici Co., Ltd., Shanghai, China). Subsequently, the solution was boiled for 15 min and incubated at 25°C for 30 min. The volume of the solution was replenished to 40 mL to determine the conductivity (EM$_t$). Electrolyte leakage was calculated using the formula (EM$_0$ /EM$_t$) × 100%.

The MDA content was determined according to the method described by Dhindsa et al. (1981). Frozen tissue samples (1 g) from each replicate were homogenized in 4 mL of 5% (w/v) trichloroacetic acid on ice and centrifuged at 12,000 × g for 20 min at 4°C. Subsequently, 2 mL of the supernatant were mixed with 3 mL of 0.67% thiobarbituric acid. The mixture was boiled for 15 min and immediately cooled on ice. After a centrifugation session at 12,000 × g for 10 min, the supernatant was collected and its absorbance was measured at 450 nm, 532 nm, and 600 nm using an Ultrospec 2000 spectrophotometer (AmershamBiosciences, Buckinghamshire, UK). The MDA content of the fruit was calculated using the formula: 6.45× (A532-A600)-0.56 × A450. The results were expressed as µmol/g.

The H$_2$O$_2$ concentration was measured using a commercially available H$_2$O$_2$ assay kit (A064-1-1; Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer’s instructions.

LOX activity was evaluated using the method described by Liu et al. (2011). Frozen tissue samples (1 g) from each replicate were homogenized in 2 mL of 0.1 M phosphate buffer (pH 6.8) containing 1% (w/v) polyvinylpyrrolidone and 1% (w/v) Triton X-100. After a centrifugation session at 12,000 × g for 30 min at 4°C, the supernatant was used as the crude enzyme extract to measure LOX activity. Meanwhile, a mixture containing 2.7 mL phosphate buffer and 100 µL 0.5% (v/v) linoleic acid sodium solution was prepared, incubated at 30°C for 30 min, and used as the reaction solution. Then, 200 µL of crude enzyme extract were added to the mixture solution and the change in absorbance was measured at 234 nm every 30s. One unit of LOX was defined as the amount of enzyme that increased the absorbance of 0.01 unit per min at 234 nm, and the result was expressed as U/kg.

**Nonenzymatic Antioxidant Content and Antioxidant Capacity**

**Total Phenolics, Total Flavonoids, and Ascorbic Acid Measurements**

The total phenolics content in the persimmons was determined using the method described by Youryon and Supapvanich (2016). Frozen tissue samples (1 g) from each replicate were homogenized in 50 mL of distilled water and heated to 100°C for 30 min. After the solution was cooled and filtered, the filtrate was collected as an extract and readjusted to a volume of 50 mL. Next, 10 mL of extract were admixed with 2.5 mL of Folin-Ciocalteu regent and 7.5 mL of 20% (w/v) Na$_2$CO$_3$ solution. The mixture was incubated at 75°C for 10 min and its absorbance was recorded at 760 nm. The results were expressed as milligrams of gallic acid equivalents per gram of fresh weight (mg/g). The concentration of gallic acid stock solution was 10 mg/mL, which was used to construct the standard curve with a concentration range of 0, 0.2, 0.4, 0.6, 0.8, 1.0 mg/mL.

The total flavonoids content was determined according to the method described by MohdFadzelly et al. (2009). First, ultrasonic extraction was carried out by homogenizing 1 g of frozen tissue samples from each replicate with 5 mL of 70% ethanol solution. The assay was conducted by mixing 1.5 mL of extract, 0.3 mL of 5% (w/v) NaNO$_2$ solution, 0.3 mL of 10% (w/v) Al(NO$_3$)$_3$ solution, and 1.4 mL of 10% (w/v) NaOH solution. After the mixture was reacted for 15 min in a water bath at 25°C, the absorbance was measured at 510 nm and the results were expressed as milligrams of rutin equivalents per gram of fresh weight (mg/g). The concentration of rutin stock solution was 1 mg/mL, which was used to construct the standard curve with a concentration range of 0, 0.02, 0.04, 0.06, 0.08, 0.10 mg/mL.
To measure the ascorbic acid content, we employed a method reported by Lu et al. (2012). The results were expressed as milligrams of ascorbic acid per 100 g of fresh weight (mg/100 g).

Assessment of Antioxidant Capacity

1,1-diphenyl-2-trinitrophenylhydrazine (DPPH), ferric reducing antioxidant power (FRAP) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging capacity were used to evaluate the antioxidant properties of the persimmons. Frozen tissue samples (10 g) from each replication were extracted with 100 mL of 80% (v/v) methanol solution for 30 min using an ultrasonic wave. After a centrifugation session at 12,000 × g for 20 min at 4°C, the supernatant was collected and used for the antioxidant capacity analysis.

The DPPH radical scavenging capacity was determined using the method described by Brand-Williams et al. (1995) with slight modifications. In this method, 1 mL of persimmon extract was mixed well with 4 mL of 25 mg/L DPPH solution (dissolved in methanol). After the mixture reacted in the dark for 30 min, its absorbance change was measured at 517 nm. To measure the radical scavenging capacity of ABTS, we used the method reported by Hsu et al. (2011). The ABTS radical solution containing 5 mL of 7 mM ABTS solution and 88 µL of 140 mM K2S2O8 solution was prepared and then incubated in a dark room for 12 hours. Subsequently, the mixture was diluted with methanol to obtain an absorbance of 0.70 ± 0.02 at 732 nm. We mixed 100 µL of extract with 3.9 mL of ABTS radical solution and the mixture reacted for 6 min in the dark. Before and after the reaction, the absorbance of the mixture was measured at 732 nm. The radical scavenging capacity was expressed as the percentage of DPPH and ABTS scavenging (%).

FRAP was determined using the method described by Benzie and Strain (1996) with slight modifications. The extract (200 µL) was mixed with 1 mL of distilled water and 1.8 mL of FRAP reagent, which consisted of tripyridyl triazine (TPTZ), FeCl3, and acetate buffer. After the mixture was incubated at 37°C for 10 min, the absorbance was measured at 593 nm. FRAP was calculated from a standard curve using FeSO4 and expressed as micromoles of FeSO4 per gram of fresh weight (µmol/g).

Statistical Analysis

All experiments were performed in triplicate, and the results were presented as mean ± standard deviation (SD). The values were analyzed using ANOVA and significant differences were determined using Duncan’s new multiple range test at P < .05 in IBM SPSS Statistics for Windows, version 23.0 (IBM Co., Armonk, NY, USA).

Results

Changes in the Quality Attributes of Sweet Persimmons during Storage

Chilling Injury Index

CI symptoms of sweet persimmon fruit featured with flesh or peel browning occurred after 30 days of cold storage in the untreated fruit, which developed rapidly with the storage period (Figure 1A, B). All three treatments significantly reduced the CI index (Figure 1A; p<.05) and showed evidently lower browning grades than the control group (Figure 1B). Furthermore, 1-MCP and MT+1-MCP treatments respectively delayed CI occurrence time to 60th and 70th day of storage, with a visibly better effect than MT group. There were few CI symptoms in the fruit treated with MT+1-MCP (Figure 1B), indicating an effective measure to improve the cold storage safety of persimmons fruit.

Ethylene Production, Respiration Rate, Firmness, and SSC

Fruit of untreated and treated had clear differences in their ethylene production (Figure 2A). MT, 1-MCP, and combined MT and 1-MCP treatments had delayed the climacteric ethylene peaks and caused a decrease in the peak values compared with the control group. The ethylene production in
Untreated fruit peaked on day 20, with the highest value being 0.071 μL/kg·h. The MT and 1-MCP treatments delayed the ethylene production peaks by 10 and 20 days, with reductions in the ethylene production values to 0.059 and 0.047 μL/kg·h, respectively. The ethylene production of the combined MT and 1-MCP treatment was significantly inhibited, it peaked after a 20 days delay, and reached the lowest value of 0.040 μL/kg·h when compared to other treatments.

As a typically climacteric fruit, sweet persimmon showed two respiratory peaks during cold storage (Figure 2B). Same to the ethylene production, all three treatments inhibited respiration intensity and decreased the peak values of fruit, while the combined MT and 1-MCP treatment showed better effect.

As is shown in Figure 2C, the flesh firmness of all treatments declined with prolonged storage time. It is obvious that the firmness of the treated fruit was significantly higher than that of the control ones throughout the storage period (p < .05). In addition, 1-MCP was more effective in maintaining fruit firmness than MT. The fruit treated with MT + 1-MCP had better firmness, but the difference was not significant compared with 1-MCP treatment (p > .05).

Figure 1. CI index (A) and photograph (B) of ‘Youhou’ sweet persimmons in different treatments during storage at 0°C plus 5 d at 25°C. The values are expressed as means ± standard deviations (SD).
As is shown in Figure 2D, the SSC of persimmons initially increased gradually and then decreased in the late period of storage. The SSC of the control fruit peaked first, while its subsequent decline rate was the fastest, which resulted in a lower SSC than that of the treated fruit at 70 days. The fruit treated with MT+1-MCP showed higher SSC than that of other groups after 50 days of storage and this was significantly different from the SSC that resulted from the MT treatment (p < .05), but not from the SSC that resulted from the 1-MCP treatment (p > .05).

**Fruit Color Development**

The color change of the peel is closely related to the storage quality of the persimmons. As is shown in Figure 3A, the L* value in the control fruit decreased rapidly and was significantly lower (p < .05) than that in treated fruit after the 40th day of storage. There were no significant differences between the MT, 1-MCP, and MT+1-MCP treatments at the end of storage (p > .05), while the combination of MT and 1-MCP treatment exhibited higher L* values of fruits. During cold storage, the a* value increased by a slower rate in the treated fruit compared to the control ones, while the 1-MCP and MT+1-MCP treatments resulted in the slowest increase rates (Figure 3B). The H* value of the untreated fruit decreased rapidly during storage (Figure 3C), which was evidently slowed down by all three treatments. MT+1-MCP treatment were able to maintain H* values better than other treatments, which resulted in a significant difference in results with the MT treatment (p < .05), but not with 1-MCP treatment (p > .05).

**Electrolyte Leakage, MDA Content, \(H_2O_2\) Concentration, and LOX Activity**

The electrolyte leakage and MDA content in all treatments displayed continuously increasing trends during storage (Figure 4A, B). The increase in these two indicators was significantly inhibited by all
three treatments in comparison with the control group. The fruit treated with 1-MCP and MT +1-MCP maintained lower levels of MDA and electrolyte leakage than those treated with MT; the difference was significant from 30 to 70 days of storage (p < .05). On the other side, no significant difference was observed between the 1-MCP and MT+1-MCP-treated fruit during most of the storage period, except for day 70 of storage, on which both the MDA concentration and electrolyte leakage were significantly different, and day 40, on which only the MDA concentration was significantly different.

Compared with the control, all other treatments markedly reduced the accumulation of H₂O₂ in fruit (Figure 4C), with the differences starting to be significant on the 20th day of storage (p < .05). In the last 30 days, the MT treatment resulted in a lower H₂O₂ concentration than the 1-MCP treatment; however, this concentration was still higher than the one that resulted from the MT+1-MCP treatment. Meanwhile, the differences were significant among the three treatments (p < .05).

Initially, LOX activity gradually increased and then declined in all treatments (Figure 4D). The increase in LOX activity was inhibited by the three treatments compared with the control treatment, with the MT+1-MCP resulting in lower values during the entire storage period. The LOX activity of MT-treated fruit peaked on day 20 of storage, which was also the case for the control fruit, but the peak value was 27% lower than that of the control, which represented a significant difference (p < .05). The 1-MCP and MT+1-MCP treatments delayed the peak of LOX activity by 20 days and maintained a lower level than the control and MT groups.

Figure 3. L* (A), a* (B), and hue angle H*(C) of ‘Youhou’ sweet persimmons during storage at 0°C. The data represent the mean of three replications ± standard deviations (SD).
Changes in Antioxidant Attributes during the Storage of Sweet Persimmons

Total Phenolics, Total Flavonoids, and Ascorbic Acid

As is shown in Figure 5A, the total phenolics content of the untreated and MT-treated fruit initially increased and then decreased from day 30 to day 70 of storage; however, the phenolics content fluctuated more smoothly under the 1-MCP and MT+1-MCP treatments. Before and after 40 days of storage, the fruit treated with MT and MT+1-MCP, respectively, had higher total phenolics contents. At day 70, the total phenolics content in the control fruit decreased by 29.12%, which was 10.68%, 12.9%, and 22.19% higher than that in the MT, 1-MCP, and MT+1-MCP treated fruit, respectively, with the differences being significant (p < .05).

The total flavonoids content in all samples exhibited a fluctuating downward trend during cold storage (Figure 5B). Compared with the control treatment, all three treatments inhibited this decrease. As was the case in the change of total phenolics, the MT treatment maintained a higher content of total flavonoids during early storage, while the MT+1-MCP treatment was more beneficial for the maintenance of total flavonoids in the later stages of storage. At the end of the storage period, the total flavonoids content of MT-treated fruit was 9.00% higher than that of the control fruit, while those of the 1-MCP and MT+1-MCP-treated fruit were 14.95% and 25.95% higher, respectively.

The ascorbic acid content initially increased and then decreased during cold storage. The ascorbic acid content of the control fruit decreased rapidly from day 40 to day 70, while that of the MT, 1-MCP, and MT+1-MCP-treated fruit decreased in a delayed manner and were significantly higher than that of the control fruit (p < .05). At day 70, the ascorbic acid content of the MT+1-MCP-treated fruit was approximately 2-fold higher than that of the control fruit and also higher than that of the 1-MCP and MT-treated fruit (Figure 5C).
**Antioxidant Capacity Evaluation**

The antioxidant capacity of persimmon extracts was evaluated by DPPH, ABTS radical scavenging, and FRAP assays. A similar trend was observed for both radicals (Figure 6A, B). The DPPH and ABTS radical scavenging capacity of the treated fruit was significantly higher than that of control fruit from day 50 to day 70 of storage (p < .05). The radical scavenging capacity of MT-treated fruit improved noticeably during the early storage period, however, it subsequently decreased more rapidly than with the other treatments. At the end of storage, the MT+1-MCP treatment exhibited the highest radical-scavenging capacity.

An obvious decline in FRAP in untreated fruit was observed after 20 days of storage (Figure 6C). Fruits of all three treatments showed slower decline rates and exhibited significantly higher FRAP levels than the control treatment at the end of storage (p < .05). Additionally, the MT+1-MCP treatment was significantly more effective in maintaining the FRAP of persimmons than the 1-MCP and MT treatments (p < .05).

**Discussion**

Cold storage is a usual method of prolonging the storage time of fruit. However, quality deterioration induced by chilling, especially CI, is a main limiting factor for application of refrigeration to preserve sweet persimmons. It is a topic of interest to researchers for developing effective technologies to improve quality of sweet persimmons stored at low temperature. In particular, sweet persimmon fruit is famous and popular due to its prominent physiological and pharmacological effects on human health, therefore the maintenance of the bioactive antioxidant compounds during cold storage has been paid great attention recently (Pu et al., 2013; Zhang et al., 2018). Previous
studies have proved that 1-MCP can alleviate the chilling injury and extend the storage time of 'Youhou' sweet persimmons (Li et al., 2018; Zhang et al., 2010; Zhao et al., 2020). However, these studies focused more on the inhibitory effect of chilling injury, without the preservation of the antioxidants. In addition, it was reported that 1-MCP may cause adverse effects on the antioxidant quality of fruit after cold storage, because of its excessive inhibition to the fruit ripening (Fabi et al., 2007). As a strong free-radical scavenger against chilling stress, MT has been confirmed to positively regulate the antioxidant capacity by increasing antioxidant enzyme activity and antioxidant ingredients content in peach, strawberry, and tomato fruit (Cao et al., 2018; Aghdam and Fard, 2017; Ding et al., 2017a). Based on this, we tried to combine the characteristic physiological functions of MT and 1-MCP on sweet persimmons, with a purpose of improving the chilling tolerance to extend storage life, and both the nutritional value from the perspective of fresh-eating quality. Interestingly, this hypothesis was confirmed in our study that combined treatment obtained the lowest CI index and the highest antioxidant ability levels. These results documented a possible response mechanism against chilling stress mediated by combined MT and 1-MCP treatment, contributing to a reduction of the quality and antioxidant capacity loss in sweet persimmons.

Firmness, SSC, and visual color are important postharvest quality aspects of sweet persimmons for consumers, because it reflects the ripening stage and taste of fruit. During cold storage, the adverse changes of the above quality indexes are closely related to fruit ripening and senescence process, which
is mainly regulated by ethylene (Nakano et al., 2001). In this process, ethylene could respectively increase hydrolysis enzymes activity of cell wall, enhance respiration intensity of fruit, and induce the degradation of chlorophyll and carotenoid pigments, result in accelerated fruit softening, SSC loss and peel color change (Kittur et al., 2001; Nakano et al., 2001; Rasouli and Khadem, 2017). Moreover, previous studies have proved that chilling stress can induce the increase of ethylene production, and which was positively correlated with the development of CI symptom in some cold-sensitive fruit (Vera-Guzman et al., 2017; Zhang et al., 2007). Sweet persimmons is a typical climacteric and cold-sensitive type of fruit, therefore the inhibition of ethylene production might be a vital protective mechanism against chilling stress, and could be an explanation for slowing down the quality deterioration process. The results of this study indicated that all three treatments exhibited delayed ethylene production peak and reduced peak values in sweet persimmons compared with the control, while the combined MT and 1-MCP treatment showed the best inhibition effect on the ethylene release rate. Thus, the respiration rate of fruit was greatly reduced, and the ripening and senescence process under chilling stress was effectively postponed. Consequently, combined treatment maintained the highest quality with the lowest levels of CI index, firmness and SSC loss, and color variation. These results are consistent with the reports of Zhai et al. (2018) who found limited ethylene production and lower loss of firmness in pears treated with MT, and Orihuel-Iranzo et al. (2010) who noted a similar effect in ‘Rojo Brillante’ persimmons treated with 1-MCP. In all treatments, only MT treatment had no significant effect on attenuating the loss of SSC compared to the control at the end of storage, which might be due to its weaker ability to reduce ethylene production and delay fruit senescence process than 1-MCP. Similar results were reported by Rastegar et al. (2020) in mango fruit treated with MT.

Peel color is an apparent manifestation of sweet persimmon ripeness, which changes from green-orange to yellow-orange to red-orange during ripening and senescence process. Hence, the increase in \( a^* \) value and the decrease in \( H^* \) values in the peel represents the advanced stages of fruit ripening. The better freshness quality evidenced by the lower \( a^* \) and higher \( H^* \) values was observed in all treatments, while the results of the 1-MCP and MT+1-MCP treatments were optimal, which was due to their strong inhibition on ethylene release. Besides, decrease of \( L^* \) value indicates the blackens or browns of persimmons pericarp during cold storage, that may be induced by CI (Arnal and Del Rio, 2004). In this study, all three treatments slowed down the decrease rates of \( L^* \) values in fruit compared with the control treatment, while the MT+1-MCP treatments resulted in the lowest values. This result could be inferred that all three treatments reduced peel browning via improving the fruit tolerance to chilling. Similarly, Liu et al. (2018) reported that slower changes in \( L^* \) values and higher \( H^* \) values were observed in strawberry fruit treated with MT than in control fruit after storage. Rasouli and Khadem (2017) stated that 1-MCP was effective on retarding the reduction of \( L^* \) and \( H^* \) values in persimmons fruit. To sum up, our results indicated that combined treatment could alleviate CI and preserve higher firmness, SSC, and visual quality by inhibiting ethylene production and respiration rate of fruit.

It is well known that cold temperature is an oxidative stress for fruit and accelerates the generation of ROS, which may cause physiological disorders, and stimulates quality deterioration (Zhai et al., 2018). The over-accumulation of \( H_2O_2 \), an important ROS type in fruit during postharvest storage, may destroy the integrity of the cell membrane by causing lipid peroxidation, increasing its permeability, and decreasing fluidity (Bhattacharjee, 2005). This cytomembrane disruption is considered as a main cause of CI in refrigerated fruit (Lyons, 1973). In general, relative electrolyte leakage is a reliable indicator that is used to reflect cell membrane permeability. MDA, a product of membrane lipid peroxidation, is closely related to the severity of cell membrane damage. Furthermore, lipid peroxidation is considered to be triggered by the LOX enzyme, which catalyzes the oxidation of polyunsaturated fatty acids to produce hydroperoxyl fatty acids (Gao et al., 2018). The results of our study showed that all three treatments significantly reduced \( H_2O_2 \) accumulation and inhibited LOX activity, resulting in lower electrolyte leakage and MDA concentration in sweet persimmons than the control treatment during cold storage. These treatments effectively alleviated the damage of cell membrane, and thus enhanced the chilling resistance in fruit. Interestingly, MT exhibited higher \( H_2O_2 \) scavenging capacity than 1-MCP at later storage, while 1-MCP resulted in lower LOX activity than MT, which
embodying their different physiological regulation effect. Cao et al. (2018) found that the MT treatment significantly reduces the \( \text{H}_2\text{O}_2 \) and MDA contents, resulting in lower lipid peroxide, oxidative damage and CI index in peach fruit. Zhao et al. (2020) reported that 1-MCP-treated sweet persimmons showed lighter CI symptoms resulting from maintaining better membrane status and lower LOX activity, electrolyte leakage, and MDA content. In this study, the MT+1-MCP treatment apparently combined the positive effects of the two respective treatments in alleviating membrane damage, which led to the lowest electrolyte leakage and MDA values. Thus, combined treatment could protect the plasma membrane system by improving the resistance to ROS oxidative stress and reducing the process of lipid peroxidation, and then resulted in slight CI symptoms.

Persimmons are potential sources of natural antioxidants, such as phenols, flavonoids, and ascorbic acid, which can improve human health and prevent chronic diseases by eliminating the negative effects of ROS (Pu et al., 2013). Therefore, the nutrient content and antioxidant capacity of fruit are important indicators of storage quality. During cold storage, these non-enzymatic bioactive compounds can help fruit overcome oxidative stress and prevent damage to the membrane and intracellular structures through their antioxidant activity against ROS (Ghasemnezhad et al., 2008; Rastegar et al., 2020). In this study, all the treated fruit maintained a higher content of total phenolics, total flavonoids, and ascorbic acid at the later stage of storage, compared with the control fruit. The total phenolics and ascorbic acid contents initially increased and then decreased during cold storage, which corroborates the results obtained in ‘Fangshan’ persimmons and nectarines (Zhang et al., 2018; Zhao et al., 2018). However, the change in total flavonoids showed a gradual downward trend, which was inconsistent with the results of the two aforementioned studies. The differences may be due to the different types of flavonoids in the various fruit types used, as well as the different harvest maturity times and storage conditions. Additionally, the increases in the total phenolics and ascorbic acid contents at the early stage of storage resulted from fruit ripening process, while the decline in the three antioxidants was associated with their consumption that helped neutralize free radicals under stress conditions (Mphahlele et al., 2014; Naser et al., 2018). Notably, the MT treatment resulted in the highest total phenolics and total flavonoids contents compared to the other treatments during the early storage period. This result may be related to the fact that MT may favor the synthesis of phenolics and flavonoids compounds, thereby promoting tolerance of persimmons against the chilling stress. Similar to our results, Gao et al. (2018) found that MT could activate the biosynthesis of phenolics compounds in peaches by elevating the essential enzymes activities for phenolic metabolism involved in the pentose phosphate, shikimate, and phenylpropanoid pathways. However, MT did not exert an obvious effect on the promotion of the accumulation of ascorbic acid. On one hand, 1-MCP was not conducive to maintaining high contents of the three active components at the early stage of storage, which was due to its strong inhibition effect on the fruit ripening. Similar observations have been reported in other fruit. Liu et al. (2015) stated that 1-MCP exerted negative effects on phenolics biosynthesis and antioxidant activity in peaches, and Larrigaudière et al. (2004) noted that 1-MCP was associated with lower ascorbic acid levels in ‘Blanquilla’ pears during storage. On the other hand, the content of these antioxidants in 1-MCP-treated fruit changed mildly and reduced at a slower rate than in MT-treated fruit during the late period of storage. This could be explained by the fact that 1-MCP strongly delayed fruit senescence process and improved the chilling tolerance, thereby reducing phenols, flavonoids, and ascorbic acid loss in scavenging ROS. The MT+1-MCP treatment seemed to combine the advantages of the two respective treatments in maintaining the antioxidant levels, which led to the retention of the highest nutritional value of persimmons throughout the storage period.

DPPH, ABTS radical scavenging capacity, and FRAP are commonly used to evaluate the antioxidant properties of fruits in vitro. Cantin et al. (2009) found that antioxidant capacity is closely related to the content of antioxidant components, including phenols, flavonoids, and ascorbic acid in fruit. Zhang et al. (2018) also argued that total phenolics and total flavonoids may be the major contributor to the DPPH and ABTS radical scavenging capacity of persimmons, respectively. In this study, all
treated fruit retained higher total phenolics, total flavonoids, and ascorbic acid contents than the control fruit at the later stage of storage, thereby exhibiting more advanced DPPH, ABTS radical scavenging, and FRAP antioxidant capacity. Moreover, the MT+1-MCP treatment could be the most effective way to enhance the antioxidant potential of persimmons, owing to its ability to achieve the maximum retention of the aforementioned active compounds.

**Conclusion**

Both the MT and 1-MCP treatments had positive effects on preserving the quality and improving the antioxidant properties of ‘Youhou’ sweet persimmons during cold storage. However, the combined effect of these two treatments was higher than either treatment alone. To our knowledge, this is the first report on the application of MT in postharvest persimmons. Compared with 1-MCP, MT was less effective in inhibiting ethylene production and maintaining quality during long-term storage, but showed a higher capacity to elevate chilling tolerance by promoting the accumulation of antioxidants. In addition, the effect of MT can be enhanced if it was combined with 1-MCP, which helped maintain higher levels of firmness, SSC, phenolics, flavonoids, ascorbic acid, and antioxidant capacity, and lower levels of ethylene production, respiration rate, color change, LOX activity, electrolyte leakage, MDA, and H₂O₂ concentrations. These results suggest that the MT+1-MCP treatment may be a promising strategy to improve the postharvest quality and nutritional value of sweet persimmons.

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