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Melatonin treatment reduces chilling injury in peach fruit through its regulation of membrane fatty acid contents and phenolic metabolism

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ABSTRACT

Effects of 0.1 mM melatonin (MT) on chilling injury (CI), membrane fatty acid content and phenolic metabolism in peach fruit were studied during storage at 1°C for 28 days. MT treatment delayed the development of CI in peach fruit, as was illustrated by MT-treated fruit showing lower CI incidence, CI index and firmness loss than the control. MT treatment prevented membrane lipid peroxidation and contributed to maintaining a higher ratio of unsaturated to saturated fatty acids in peach fruit. MT treatment also stimulated the activities of glucose-6-phosphate dehydrogenase, shikimate dehydrogenase and phenylalanine ammonia lyase, but inhibited the activities of polyphenol oxidase and peroxidase. This would help in activating the accumulation of total phenolic and endogenous salicylic acid that might have a direct function in alleviation of CI. These results indicate that MT treatment can be an effective technique to reduce postharvest CI during low temperature storage of peach fruit.

1. Introduction

Chilling injury (CI) is a physiological disorder commonly occurring in a large collection of peach cultivars during low temperature storage, especially when storing at a potential risk zone of temperature between 2.2 and 7.6 °C (Lurie & Crisosto, 2005). The disorder is characterized mainly by flesh browning and/or mealiness, abnormal ripening and higher sensitivity to decay (Lurie & Crisosto, 2005), and is considered as a primary limitation of application of low temperature to preserve peach fruit. Many hypotheses have been put forward for a CI mechanism in plants, such as involvement of imbalances in metabolism, accumulation of toxic compounds, decreased water dynamic state and increased permeability (Lyons, 1973; Naruke et al., 2003; Purwanto et al., 2013). To deal with this problem, researchers have been devoting a great deal of attention to finding economical, convenient and highly effective techniques to reduce CI in peach fruit.

Melatonin (MT) is an indoleamine hormone ubiquitous in nature. In plants, MT has been identified in nearly all organs and tissues, and is shown to be a signaling molecule involved in numerous physiological processes such as differentiation, growth, ripening and senescence of plant and the protective effect against various forms of environmental stress (Reiter et al., 2015; Zhang Huber et al., 2015; Zhang Sun et al., 2015). Exogenous MT has been demonstrated to be effective in alleviating chilling stress-induced damage in plants through different mechanisms. For example, Posmyk, Balabusta, Wieczorek, Sliwinska, and Janas (2009) revealed that MT treatment significantly counteracts the adverse effect of chilling stress on cucumber seeds by influencing the structure and function of cellular membrane. Likewise, in another study, researchers found that application of MT contributes to reducing the risk of ultrastructural damage in meristematic cells of *Vigna radiata* roots due to chilling exposure (Szafrańska, Glińska, & Janas, 2013). Their data also documented that MT-medicated *Vigna radiata* root acclimation to chilling stress is associated with its ability to activate the pathway of phenolic (Szafrańska, Szewczyk, & Janas, 2014). It was also shown that MT has a positive regulation in the expression of C-repeat-binding factors and reactive oxygen species (ROS)-related antioxidant genes, which helps Arabidopsis thaliana induce resistance to chilling stress (Bajwa, Shukla, Sherif, Murch, & Saxena, 2014). However, more recently, attention has been partially directed toward the effect of exogenous MT application on oxidative stress-induced senescence and CI in postharvest fruit. Sun et al. (2016) found that MT plays a crucial role in the regulation of senescence in tomato fruit. Gao, Zhang, & Lv et al. (2016) putted forward a similar result that MT at a concentration of 0.1 mM leads to a clear delay of senescence in peach fruit during ambient storage, through antioxidative mechanism. Aghdam and Fard (2017) reported that MT is conductive to attenuating postharvest decay in strawberry fruit by mechanisms that trigger the accumulation of hydrogen peroxide and phenolic compounds and induces the activity of

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γ-aminobutyric acid shunt. Cao et al. (2016) proved that MT ensures better prevention of CI in peach fruit during low temperature storage, and such effect has been attributed in part to MT-induced promotion of polyamine, γ-aminobutyric acid and proline. These findings undoubtedly reveal new insights into the physiological roles of MT in plants. However, further work along these lines would be valuable, since there is only a limited understanding at present.

The purpose of this paper is to advance our understanding of the possible underlying mechanisms of how MT induces a defence response of peach fruit to avoid CI. In peach fruit, previous studies have reported that both biosynthesis of phenolic compounds (Gao, Zhang, & Lv et al., 2016; Gao, Zhang, & Chai et al., 2016; Jin, Zheng, Tang, Rui, & Wang, 2009) and maintenance of a higher ratio of unsaturated to saturated fatty acids (Jin, Zhu, Wang, Shan, & Zheng, 2014) may account for the enhancement of chilling tolerance. Therefore, the focus of the present study was on whether the MT-induced changes in membrane fatty acid contents and phenolic metabolism are linked to the enhanced tolerance to CI in peach fruit during low temperature storage.

2. Material and methods

2.1. Plant material and treatments

Mature pre-climacteric fruit of peach (*Prunus persica* Batsch cv 'Chuanzhongdao') were hand-collected from a well-managed commercial orchard in Xi'an, China. Fruit were chosen for uniformity without any defects, and then separated into 2 lots at random. Fruit of the first lot were dipped in a solution of 0.1 mM MT for 10 min in the low light to prevent light-induced MT degradation (Gao, Zhang, & Lv et al., 2016; Gao, Zhang, & Chai et al., 2016). Under the same condition, fruit of the second lot were soaked in distilled water and designed as the control. Afterwards, all fruit were dried in the air and stored at 1 °C with a relative humidity of 85–90% for 28 days. Fruit were removed from each lot after 0, 7, 14, 21 and 28 d of low temperature storage to evaluate CI incidence, CI index and firmness loss. Meanwhile, flesh tissue samples derived from 10 fruit were collected and stored at −80 °C for subsequent measurements. For each lot, three replicates were performed.

2.2. CI incidence, CI index and firmness

CI incidence was defined as the ratio of CI-fruit to total fruit and expressed as%.

CI index was assessed visually based on the percentage of the cut surface of peach slices that exhibited browning with a scale from 0 to 4: 0 (none), 1 (< 25%), 2 (25–50%), 3 (50–75%), 4 (> 75%), according to Wang, Chen, Kong, Li, and Archbold (2006). CI index was obtained from the formula: CI index = Σ (CI scale × number of fruit in each scale)/(4 × total number of fruit).

Firmness was determined with a fruit firmness tester (GY-3, Aidebao Instrument Co., Ltd, Leqing, China) fitted with an 8 mm diameter probe and expressed as N.

2.3. Malondialdehyde content

Malondialdehyde (MDA) content was tested using a modified method described by Dhindsa, Pulmb-Dhindsa, and Thorpe (1981). Flesh tissue (2 g) was homogenized with 10 ml of 10% trichloroacetic acid containing 0.5% (w/v) thiobarbituric acid. The mixture was boiled at 100 °C for 10 min, cooled quickly thereafter, and centrifuged at 5000*g* for 15 min. Absorbance of the supernatant was measured at 450, 532 and 600 nm. MDA content was expressed on a fresh weight basis as μmol g^{-1}.

2.4. Measurement of composition of fatty acid

The extraction and quantification of fatty acids was performed according to the method described by Jin et al. (2014). Flesh tissue (20 g) was homogenized with chloroform/methanol (2:1) solution and the mixture was acidified with 0.1 N HCl. After centrifugation at 4000*g* for 10 min, the organic layer was collected and taken to dryness. Total fatty acids were methylated by adding trifluoride 14% methanolic solution and methylated fatty acids were extracted with hexane. After that, the solvent was dried and the methylated fatty acids were redissolved in 0.2 ml chloroform and analyzed for fatty acid composition by a Hitachi 663–30 Gas Chromatograph with a flame ionization detector. Fatty acids were identified based on the retention time of the internal standard free fatty acid C17:0. The ratio of unsaturated to saturated fatty acid was calculated from the formula: [oleic acid (18:1) + linoleic acid (18:2) + linolenic acid (18:3)]/[palmitic acid (16:0) + stearic acid (18:0)].

2.5. Total phenolic and endogenous salicylic acid contents

Flesh tissue (2 g) was homogenized in 5 ml of methanol. After centrifugation at 12,000 × *g* for 15 min, the supernatant was collected and used to measure the content of total phenolic. In brief, 0.5 ml of supernatant was gently mixed with 1.0 ml of Folin-Ciocalteu reagent and 3 ml of 1 M sodium carbonate and the total volume of the mixture were adjusted to 10 ml with distilled water. After the mixture had been kept at room temperature for 1 h, the absorbance was read at 760 nm (Hinneburg, Dorman, & Hiltunen, 2006). Results were expressed as the mass of gallic acid equivalents on a fresh weight basis in mg g^{-1}.

Endogenous salicylic acid content was measured by an enzyme-linked immunosorbent assay (ELISA). A plant salicylic acid ELISA kit was used following the instructions of the manufacturer (Nanjing Senbeijia Biological Technology Co., Ltd, Nanjing, China). In brief, flesh tissue (1 g) was homogenized in 5 ml of methanol and the supernatant collected. To test the supernatant 10 μl was diluted with 50 μl sample dilution in a 96-well microplate. The mixtures were incubated in the water bath at 37 °C for 30 min. After washing 5 repeats, HRP-Conjugate reagent and chromogen solution were added and incubated for 15 min at 37 °C in the dark. The reaction was stopped by sulfuric acid and the changes in colour were noted at 450 nm. The content of endogenous salicylic acid was calculated following the standard curve.

2.6. Measurement of enzymes activities associated with phenolic metabolism

For determination of lipoxygenase (LOX), shikimate dehydrogenase (SKDH), polyphenol oxidase (PPO) and peroxidase (POD), flesh tissue (2 g) was homogenized in 5 ml of 50 mM potassium-phosphate buffer, pH 6.8. And then sample was centrifuged at 12,000*g* for 15 min at 4 °C. For determination of glucose-6-phosphate dehydrogenase (G6PDH), flesh tissue (2*g*) was homogenized in 10 ml of potassium-phosphate buffer, pH 7, containing 1 mM EDTA, 3 mM of magnesium sulfate and 1 mM polyvinylpyrrolidone. The sample was then centrifuged at 12,000*g* for 15 min at 4 °C. For determination of phenylalanine ammonia-lyase (PAL), flesh tissue (2*g*) was homogenized with 5 ml of 0.2 M borate buffer, pH 8.8, containing 6*g* of polyvinylpyrrolidone. The sample was then centrifuged at 12,000*g* for 15 min at 4 °C. The supernatants were then used for the enzyme activity.

LOX activity was assayed according to the method of Surrey (1963). Reaction mixture consisted 2.775 ml 100 mM sodium acetate buffer, pH 5.5, 25 μl linoleic acid and 0.2 ml of supernatant. The absorbance was measured by an increase in absorbance at 234 nm. LOX activity was expressed on a fresh weight basis as U g^{-1}, where U = 0.01 $\Delta A_{234\ nm}$ per min.

G6PDH activity was determined using the method described in Debnam and Emes (1999). 0.2 ml of supernatant was mixed with 1.8 ml of 50 mM Hepes-NaOH phosphate buffer, pH 8, 5 mM glucose-6-phosphate disodium salt, 0.8 mM $NADP^+$ and 2 mM magnesium chloride. The absorbance was measured by an increase in absorbance at 340 nm.

G6PDH activity was expressed on a fresh weight basis as U g^{-1}, where U = 0.01 $\Delta A_{340\ nm}$ per min.

SKDH activity was measured in a mixture consisting of 1.9 ml of 100 mM Tris-HCl, pH 9.0, 1.45 ml of 2 mM shikimic acid and 1.45 ml of 0.5 mM NADP and 0.2 ml of supernatant (Sánchez-Rodríguez, Moreno, Ferreres, Rubio-Wilhelmi, & Ruiz, 2011). The absorbance was read by the reduction of NADP at 340 nm. SKDH activity was expressed on a fresh weight basis as U g^{-1}, where U = 0.01 $\Delta A_{340\ nm}$ per min.

PAL activity was determined using the method described by Sánchez-Rodríguez et al. (2011). The reaction mixture consisted of 2 ml of 0.2 M borate buffer, pH 8.8, 1.0 ml of 0.6 mM L-phenylalanine and 0.2 ml of supernatant for 1 h at 37 °C. An increase in absorbance at 290 nm caused by the formation of *trans*-cinnamate was recorded. PAL activity was expressed on a fresh weight basis as U g^{-1}, where U = 0.01 $\Delta A_{290\ nm}$ per min.

PPO activity was assayed in a mixture consisting of 2.8 ml of 100 mM catechol and 0.2 ml of supernatant (Murr and Morris1974). The increase in absorbance at 420 nm was recorded. PPO activity was expressed on a fresh weight basis as U g^{-1}, where U = 0.01 $\Delta A_{420\ nm}$ per min.

POD activity was measured according to the method of Kochba, Lavee, and Spiege (1997). 0.4 ml of the supernatant was mixed with 2.1 ml of 50 mM phosphate buffer, pH 6.8, 0.25 ml of 0.16 M guaiacol and 0.25 ml of 0.88 M H_2O_2. The absorbance was measured by an increase in absorbance at 470 nm. POD activity was expressed on a fresh weight basis as U g^{-1}, where U = 0.01 $\Delta A_{470\ nm}$ per min.

2.7. Statistical analysis

The experiments were performed in a completely randomized design. All experiments were conducted in triplicate and average values with standard errors are reported. Analysis of variance was carried out, and means were compared using Tukey's multiple range tests at a significance level of 0.05 with Origin (Version 7.5).

3. Results

3.1. Effects of MT treatment on chilling injury and fruit firmness

Flesh browning is a notable CI symptom in this study, and it was first observed in control fruit after 7 day storage at low temperature, and more intensive incidence and severity of flesh browning were exhibited thereafter. MT treatment has a good control effect for chilling-induced flesh browning as reflected by reduced CI incidence and CI index, as shown in Fig. 1A and B. By the end of storage, 46% and 63% decreases in CI incidence and CI index were respectively recorded in comparison to the control fruit.

Accelerated loss of firmness is indicative of chilling-induced injury. Firmness loss in control fruit occurred mostly in the first 7 days of storage, and declined by 88% over the initial value. MT treatment inhibited the loss of firmness. After 7 days of storage, firmness in MT-treated fruit was about 3 times higher than that in control fruit (Fig. 1C).

3.2. Effects of MT treatment on MDA content and LOX activity

A similar change pattern was obtained for MDA content where MT-treated fruit displayed an obvious decrease. MT treatment caused 31% and 46% deceases of MDA content on day 14 and day 28, respectively (Fig. 2A).

LOX activity in control fruit increased at first, and arrived at the maximum value of 209.92 U g^{-1} on day 21, and then decreased slightly. MT treatment inhibited the activity of LOX. On day 21, LOX activity in MT-treated fruit was 88.29 U g^{-1}, which was about 2.4 times lower than that in control fruit (Fig. 2B).

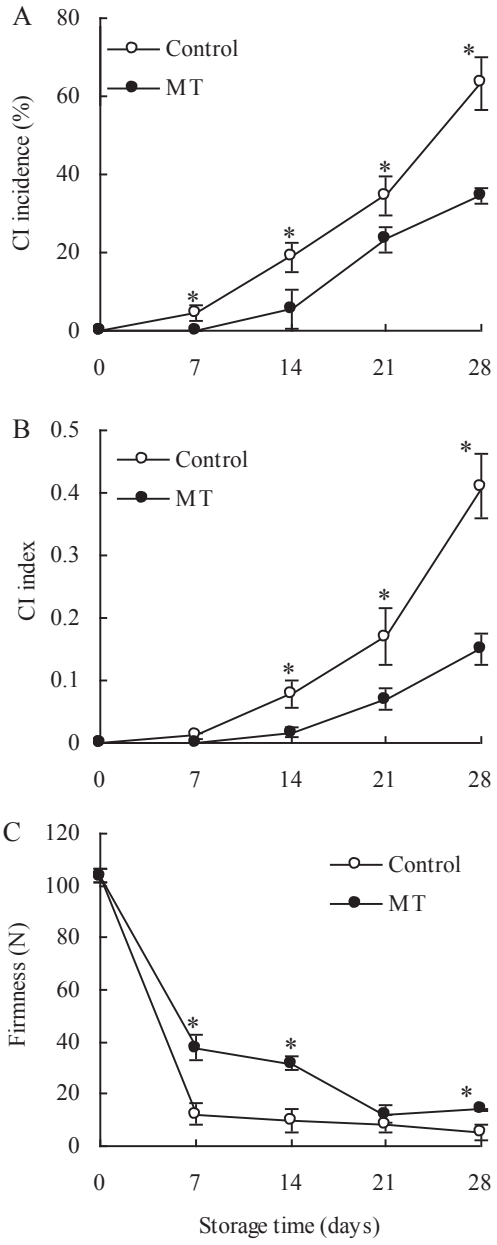

Fig. 1. Effects of MT treatment on CI occurrence (A), CI index (B) and firmness (C) of peach fruit during low temperature storage. Vertical bars represent the standard errors of the means of triplicate assays. Values with different letters for each day were significantly different at $P < .05$.

3.3. Effects of MT treatment on fatty acid composition

Five fatty acids were analyzed in flesh tissue of peach fruit. Among the quantified fatty acids, palmitic and stearic acids are saturated fatty acid, whereas oleic, linoleic and linolenic acids belong to unsaturated fatty acid. During the whole low temperature storage, an increasing trend was found for the contents of palmitic, stearic and oleic acids (Fig. 3A, B and C); however, contents of linoleic and linolenic acids and the ratio of unsaturated to saturated fatty acids presented an opposite change pattern, namely, decreased continuously (Fig. 3D, E and F). MT treatment has a good control effect for the enhancement of palmitic, stearic and oleic acids and the reduction of linoleic and linolenic acids, which resulted in a higher ratio of unsaturated to saturated fatty acids in MT-treated fruit.

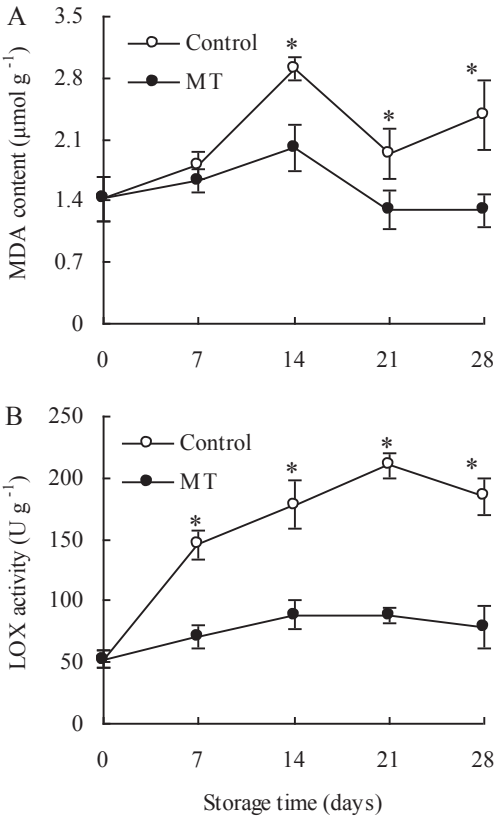

Fig. 2. Effects of MT treatment on MDA content (A) and LOX activity (B) of peach fruit during low temperature storage. Vertical bars represent the standard errors of the means of triplicate assays. Values with different letters for each day were significantly different at $P < .05$.

3.4. Effects of MT treatment on total phenolic and endogenous salicylic acid contents

Control fruit showed a gradual decrease in total phenolic content from 0.34 mg g^{-1} (at initial) to 0.15 mg g^{-1} (at the end of storage). However, MT treatment suppressed the decline (Fig. 4A). During the whole low temperature storage, MT treatment helped to reduce the loss of total phenolic in peach fruit by up to 82% as compared to the control.

Change in endogenous salicylic acid content was similar to that of total phenolic. As shown in Fig. 4B, MT treatment delayed the loss of endogenous salicylic acid as well, showing about 29% decline over the initial value by the end of storage.

3.5. Effects of MT treatment on G6PDH, SKDH and PAL activity

As shown in Fig. 5A, G6PDH activity in control fruit decreased at first, and then increased and declined again by the end of storage. MT treatment enhanced G6PDH activity, and the activity of G6PDH was more than 2.3 times higher in MT-treated fruit than in control on day 28. SKDH activity in MT-treated fruit increased before 7 day storage under low temperature and fluctuated thereafter between 0.96 and 0.76 U g^{-1}, and was higher than that in control fruit during the whole storage (Fig. 5B). A similar trend was observed for PAL activity where MT-treated fruit displayed an obvious increase. MT treatment resulted in 36% enhancement of PAL activity after 28 day storage under low temperature, as shown in Fig. 5C.

3.6. Effects of MT treatment on PPO and POD activity

PPO activity in MT-treated fruit increased before 15 days and decreased thereafter. Higher activity of PPO was observed in control fruit than MT-treated fruit during the whole low temperature storage (Fig. 6A). POD activity in control fruit decreased between day 0 and day 7, and then increased thereafter (Fig. 6B). MT treatment inactivated POD activity and the activity of POD in MT-treated fruit was 1.31 U g^{-1}, which was about 2.1 times lower than that in control fruit.

4. Discussion

CI is the principal limiting factor for application of low temperature to preserve peach fruit. Therefore, seeking effective techniques for alleviating CI in peach fruit has always been a centre of attention. Previous studies have demonstrated that MT fulfills a crucial role in protection against CI in plants (Bajwa et al., 2014; Ding, Liu, & Zhang, 2017; Posmyk et al., 2009; Szafrańska et al., 2013; Szafrańska et al., 2014; Turk & Erdal, 2015; Turk et al., 2014); however, there are few studies on the effect of MT on CI in postharvest fruit. In a recent study, Cao et al. (2016) concluded that exogenous MT application leads to a decreased incidence of CI in 'Jinghu' peach cultivar, providing the first evidence that MT treatment contributes to enhancing chilling resistance in postharvest fruit. In this study it was found that MT also alleviates CI in 'Chuanzhongdao' peach cultivar, as was illustrated by which MT-treated fruit showed lower CI incidence, CI index and firmness loss (an indicative of CI) than that control (Fig. 1), consistent with the findings of Cao et al. (2016).

It is well known that destabilization of the cell membrane is the primary cause of CI in plants (Lyons, 1973). Increase of membrane lipid desaturation during chilling acclimatisation promotes the stability of cell membrane, and as a result, acts as an important determinant of plant tolerance to chilling stress (Graham & Patterson, 1982). In accordance with this, previous studies have shown that membrane fatty acids from chilling-sensitive plants presented a lower proportion of unsaturated fatty acids than did chilling-resistant plants (Boonsiri, Ketsa, & van Doom, 2007; Wongsheree, Ketsa, & van Doom, 2009). Maintenance of a high level of membrane lipid desaturation has also been shown to be propitious to control CI in postharvest fruit. For example, 'Nanguo' pear fruit fumigated with 1-methylcyclopropene resulted in a higher ratio of unsaturated to saturated fatty acids, which attenuated the impact of CI (Cheng, Wei, Zhou, Tan, & Ji, 2015). Treatment of peach fruit with oxalic acid improved resistance to CI and was accompanied by an elevated ratio of unsaturated to saturated fatty acids (Jin et al., 2014). The results presented in our study showed that the development of CI in peach fruit was associated with the increase in contents of palmitic and stearic acids and the decrease in contents of linoleic and linolenic acids. However, MT treatment maintained higher contents of the two unsaturated fatty acids, which inclined the MT-treated fruit to exhibit an advanced ratio of unsaturated to saturated fatty acids (Fig. 3). These results suggested that MT may help the cell membrane avoid destabilization by desaturating fatty acids of membrane lipids, giving rise to the chilling resistance in peach fruit during low temperature storage. Aghdam and Fard (2017) have come to a similar conclusion focusing on the effect of MT on membrane lipid desaturation in strawberry fruit during ambient temperature storage.

On the other hand, under chilling stress, cell membranes can become destabilized because of lipid peroxidation. The peroxidation of membrane lipids can be triggered by the enzyme LOX which catalyzes the reaction of molecular oxygen with polyunsaturated fatty acids containing cis, *cis*-1, 4-pentadiene structures to produce hydroperoxy fatty acids (Shewfelt & del Rosario, 2000). Eventually, MDA is formed and has been utilized very often as a reliable estimator for lipid peroxidation (Dhindsa, Pulmb-Dhindsa, & Thorpe, 1981). Higher LOX activity and MDA content have been found to contribute to the development of CI in peach fruit during low temperature storage (Cao, Hu, Zheng, & Lu, 2010; Gao, Zhang, & Lv et al., 2016, Gao, Zhang, & Chai et al., 2016). In the current study, the activation of LOX and the formation of MDA in peach fruit due to chilling stress were inhibited by MT treatment (Fig. 2). This indicated that MT treatment has facilitated

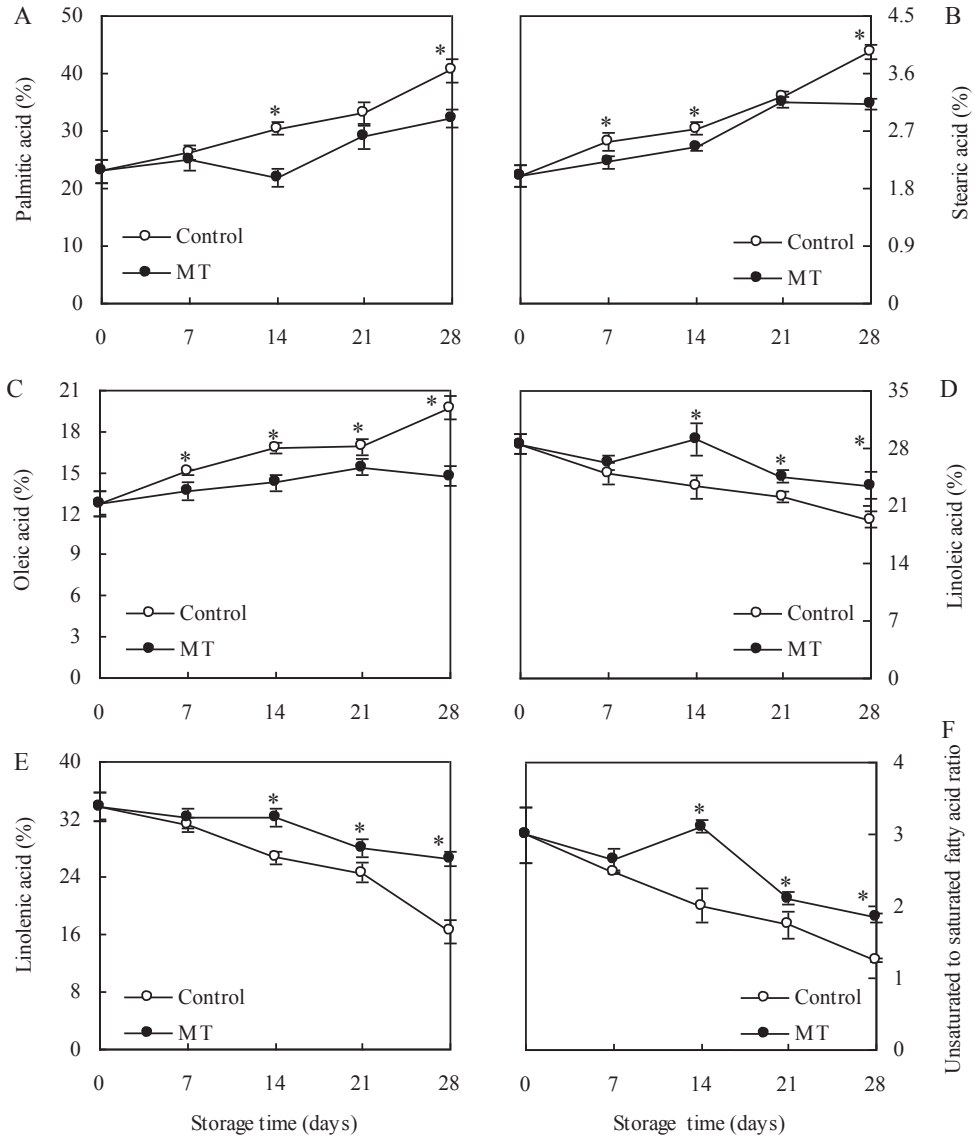

Fig. 3. Effects of MT treatment on palmitic acid (A), stearic acid (B), oleic acid (C), linoleic acid (D) and linolenic acid (E) contents and the ratio of unsaturated to saturated fatty acids (F) of peach fruit during low temperature storage.

the reduction of membrane lipid peroxidation and cell membrane destabilization, which could, in turn, promote the ability of peach fruit to resist CI. Similar alleviation of CI by suppression of membrane lipid peroxidation due to MT treatment has been reported in cucumber seeds (Posmyk et al., 2009), *Vigna radiata* root (Szafrańska et al., 2013; Szafrańska et al., 2014), wheat and maize seedlings (Turk et al., 2014; Turk & Erdal 2015) and tomato plants (Ding et al., 2017).

During low temperature storage of postharvest peach fruit, the increased accumulation of total phenolics appears to confer increased resistance to CI (Gao, Zhang, & Lv et al., 2016, Gao, Zhang, & Chai et al., 2016; Jin et al., 2009). Undoubtedly that is partially because phenolic compounds can protect membrane lipid against peroxidation by preventing the initiation and propagation of oxidizing chain reactions (Pennycooke, Cox, & Stushnoff, 2005). In this study, the result of the correlation analysis showed that total phenolic content was negatively related to LOX activity ($r = -0.94$) in control fruit, while the loss in the content of total phenolic was delayed by MT treatment during the whole low temperature storage (Fig. 4A), which indicated that MT might lead to accumulation of phenolic compounds and thus provide peach fruit with direct and sufficient chain-breaking activity. This observation is further supported by two types of observations, namely, endogenous salicylic acid content is higher in MT-treated fruit (Fig. 4B), and there is a strong inverse relationship between endogenous salicylic acid content and LOX ($r = -0.95$). Salicylic acid has been reported to be effective in reducing lipid peroxidation in peach fruit during low temperature storage (Wang et al., 2006), and as a consequence, contributing to the activation of the chilling tolerance. Taken together, it is therefore believed that phenolic compounds are implicated in the MT-induced chilling tolerance in peach fruit, and it can improve the antioxidant potential of peach fruit under chilling stress.

G6PDH, SKDH and PAL are three essential enzymes for phenolic anabolism. Among them, G6PDH is the first rate-limiting enzyme of the pentose phosphate pathway, and its main function is to supply the substrate erythrose-4-phosphate for shikimate pathway (Debnam & Emes, 1999). The reaction that SKDH catalyzes is the third and fourth steps of the shikimate pathway, converting 3-

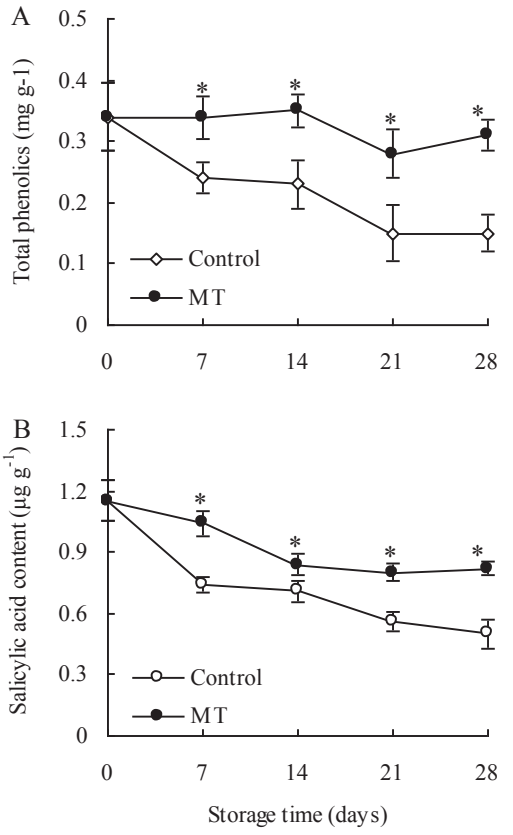

Fig. 4. Effects of MT treatment on total phenolic (A) and endogenous salicylic acid (B) contents of peach fruit during low temperature storage. Vertical bars represent the standard errors of the means of triplicate assays. Values with different letters for each day were significantly different at $P < .05$.

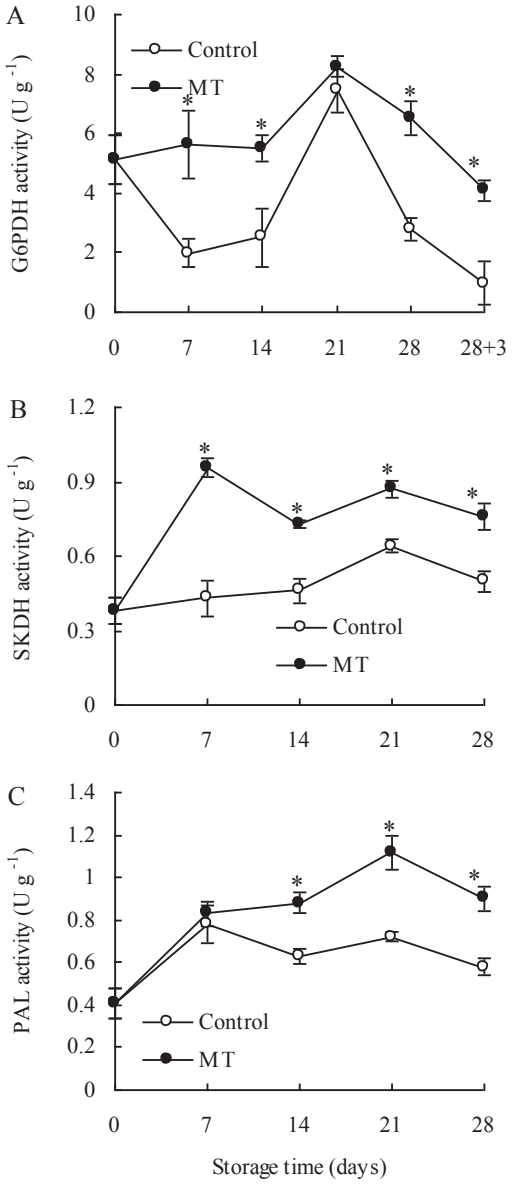

Fig. 5. Effects of MT treatment on G6PDH (A), SKDH (B) and PAL (C) activities of peach fruit during low temperature storage. Vertical bars represent the standard errors of the means of triplicate assays. Values with different letters for each day were significantly different at $P < .05$.

dehydroquinate into shikimate. Shikimate itself is a precursor to those aromatic amino acids like L-phenylalanine (Díaz & Merino 1997). PAL is the principal enzyme responsible for transformation of L-phenyalanine into trans-cinnamic acid at the entry point of the phenylpropanoid pathway driving towards the generation of phenolic compounds (Sánchez-Rodríguez et al., 2011). There is empirical evidence that enzymes involved in phenolic compounds mobilization have been relevant to plant tolerance to chilling stress. In cucumber seedlings, for example, *Arbuscular mycorrhizal* fungi inoculation resulted in higher activities of G6PDH, SKDH and PAL as well as their corresponding gene expression, along with the induction of chilling adaptive response (Chen et al., 2014). It was also reported that 24-epibrassinolide immersion induced tolerance of peach fruit to chilling stress, which has been partially attributed to the elevated activities of SKDH and PAL (Gao, Zhang, & Lv et al., 2016, Gao, Zhang, & Chai et al., 2016). We reported herein that MT induced increases in activities of G6PDH, SKDH and PAL, and stimulated the accumulation of total phenolic as well as endogenous salicylic acid. Furthermore, MT-treated fruit showed a lower occurrence of CI than control fruit during the whole low temperature storage. These findings revealed that the ability of MT treatment protects peach fruit from CI may be due to activation of phenolic compounds biosynthesis to some extent. This is in agreement with a formerly reported result by Szafrańska et al. (2014), who proposed that one of the functional routes of MT activity may be to prevent *Vigna radiate* root from CI, by stimulating the biosynthesis of phenolic compounds. Molecular evidence also indicates that MT can up-regulate the expression of genes pertaining to salicylic acid pathway in Arabidopsis plants (Weeda et al., 2014). In strawberry fruit, Aghdam and Fard (2017) proposed that the phenolic anabolism due to MT treatment is beneficial to attenuating fungal decay.

In the case of CI, PPO and POD are deemed to have an antagonistic effect on enzymes affecting phenolic anabolism. These two enzymes work together to catalyze the oxidation of phenolic compounds to quinines and result in flesh browning (Zhang Huber et al., 2015; Zhang Sun et al., 2015), which has been reported to be a notable CI symptom in peach fruit (Lurie & Crisosto, 2005). Therefore, for example, inhibited activities of PPO and POD were observed in peach fruit treated by hot air in combination with methyl jasmonate vapour, this being less sensitive to CI after treatment (Jin et al., 2009). Also, Gao, Zhang, & Lv et al. (2016), Gao, Zhang, & Chai et al. (2016) found that 24-epibrassinolide alleviated CI of peach fruit, and this process was accompanied by lower activities of PPO and POD. The results presented in our study showed that the application of MT treatment led to a decrease in activities of PPO and POD during the whole low temperature storage compared to control fruit, for which increase of these two enzymes was observed (Fig. 6). In this sense, the decrease in activities of PPO and POD could be a defence mechanism against chilling stress, and could be

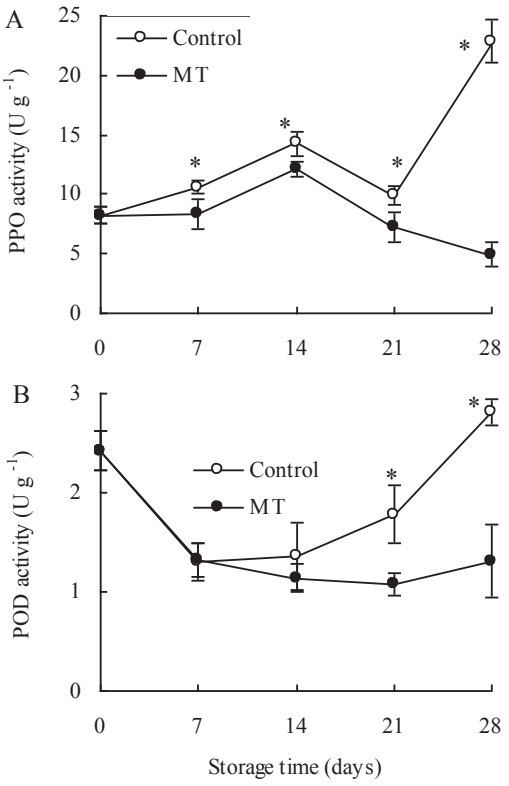

Fig. 6. Effects of MT treatment on PPO (A) and POD (B) activities of peach fruit during low temperature storage. Vertical bars represent the standard errors of the means of triplicate assays. Values with different letters for each day were significantly different at $P < .05$.

account for the lower flesh browning found in MT-treated peach fruit.

In conclusion, treatment with 0.1 mM MT alleviated CI in peach fruit and the enhancement of resistance against CI by MT treatment may be derived from its capacity to maintain a higher ratio of unsaturated to saturated fatty acids, inhibit membrane lipid peroxidation as well as stimulate phenolic mobilization. Given that MT has minimal toxicity to human beings over a very wide dose range (Galano, Tan, & Reiter, 2011), these results suggest that the application of MT treatment to peach fruit could be considered as an effective postharvest technique with good results in terms of reducing CI damage.

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Conflict of interest

We declare that we have no conflict of interest.

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