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Effect of combined heat and 1-MCP treatment on the quality and antioxidant level of peach fruit during storage

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ABSTRACT

Peach fruit undergo a fast ripening and senescence after harvest, which results in fruit quality deterioration. In this study, a combined treatment with hot water and 1-MCP (HM) was applied to peach fruit to investigate its effect on the quality and antioxidant level during room temperature (RT) storage and low temperature (LT) storage. HM treatment is effective in slowing fruit softening, increasing total soluble solids (TSS) concentration and delaying fruit senescence during RT storage. However, HM treatment has no positive effect on delaying fruit senescence during LT storage, and even decreases fruit sweetness during the late stage of LT storage. Moreover, HM treatment can induce a short-term oxidative stress on the first day of both storages. However, during the late stage of RT storage, HM treatment reduces electrolyte leakage (EL) and suppresses reactive oxygen species (ROS) accumulation by enhancing the antioxidant ability at enzymatic and transcriptional levels in peach fruit. In contrast, HM treatment causes higher EL and ROS level and induces antioxidant activity only at the enzymatic level during the late stage of LT storage. In conclusion, HM treatment is more effective at improving fruit quality and suppressing oxidative stress for fruit at RT, compared with fruit stored at LT.

1. Introduction

Peach (*Prunus persica* L.) is a worldwide stone fruit with a desirable flavor and high marketing value. However, peach deteriorates rapidly after harvest, leading to significant changes of fruit quality traits (flavor, weight, total soluble solids, titratable acidity and firmness) as well as physiological traits (respiration rate and ethylene production) and antioxidant activity (Ramina et al., 2008). Cold storage is a conventional and useful method for slowing fruit ripening and prolonging the storage life of peach. However, peach fruit are sensitive to low temperature and exhibit physiological disorders when stored for a long period. Therefore, developing an effective method to reduce postharvest loss and extent shelf life of peach fruit has been a long-standing goal for producers and traders.

As a climacteric fruit, peach is characterized by a peak in ethylene production in concert with a burst of respiration during the ripening stage. Commercial ethylene inhibitor, 1-MCP, is widely used to improve storage potential and maintain fruit quality in apple (Watkins, 2008). In peach fruit, it has been reported that application of 1-MCP can delay fruit softening, inhibit respiratory rate decrease, maintain titratable acid concentration and alleviate chilling injury (Jin et al., 2011; Watkins, 2006; Yang et al., 2014). However, the effect of 1-MCP on peach fruit is temporary and limited (Dal Cin et al., 2006). In contrast to single 1-MCP treatment, combined treatments such as 1-MCP combined heat or controlled atmosphere may be more effective on maintaining fruit quality and limiting fruit disorders (Leverentz et al., 2003; Lum et al., 2017). Heat treatment is another safe and environmentally friendly postharvest technology. It has been reported that heat treatment can inhibit fruit softening and TA loss, while maintaining high TSS, slowing respiration rate and ethylene production, and alleviating chilling injury in peach fruit (Zhou et al., 2015). Our preliminary study has shown that the application of 1-MCP, in combination with heat treatment, can have a synergistic effect that enhances the antioxidant potential and maintain fruit quality of peach fruit (Huan et al., 2016; Jiang et al., 2014). However, the underlying mechanism involved in the regulation of the antioxidant system, under the combination of 1-MCP and heat, remains largely unknown.

Fruit ripening and senescence is an oxidative phenomenon accompanied by a pronounced increase in ROS, particularly H_2O_2 and O_2– accumulation (Mondal et al., 2004; Tian et al., 2013). In normal conditions, ROS are rapidly scavenged by various cellular enzymatic and non-enzymatic mechanisms. Enzymatic antioxidant defense in plants

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includes enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione peroxidase (GPX). The balance between ROS and antioxidant enzymes is closely related to the ripening and senescence of fruit, since an imbalance can cause oxidative stress, leading to cell death (Perotti et al., 2014). For example, Singh et al. (2012) reported that a faster decline in the enzymatic antioxidant system correlates with a faster rate of ripening and senescence in plums {Singh, 2012 #11}. Induction of antioxidant-related gene expression was reported to enhance stress tolerance in a range of fruits such as banana (Wu et al., 2014), kiwifruit (Xia et al., 2016) and peach (Spadoni et al., 2014). Recently, there is an increasing interest in understanding regulation of antioxidant genes in fruit ripening process (Manganaris et al., 2017; Mou et al., 2015).

To date, little information exists regarding the effect of applying HM treatment in combination with cold storage on fruit quality and the antioxidant system of peach. The objective of this work is not only to investigate the efficiency of HM treatment on fruit quality stored under different temperatures, but also to further understand the mechanism of the antioxidant system in regulating oxidative stress in peach fruit. Our results may provide a guide for developing a new postharvest treatment to extend shelf life of peach fruit.

2. Materials and methods

2.1. Fruit materials and storage condition

Assays were conducted with peach fruit (*Prunus persica* L. cv. Xiahui 6) grown in the orchard of Jiangsu Academy of Agricultural Sciences in Nanjing, Jiangsu Province, China. “Xiahui 6” peach fruit is a typical melting flesh (MF) peach cultivar. Our previous study has revealed that ripe fruit is more sensitive to HM treatment compared with unripe fruit or half-ripe fruit (Huan et al., 2016). Therefore, in this study, approximately one thousand peach fruit, uniform in color, size and firmness were harvested at the ripe stage (110 days after full bloom; flesh firmness: 16–20 N). After 2 h of removing the field heat, selected fruit (day 0) were randomly divided into four groups (Table 1). At each time point for each group, 30 fruit samples in three biological replicates of 10 fruit each were taken for analysis of fruit quality, ROS level, and membrane condition. After the above determination, the pulp from the same fruit sample was collected, immediately frozen in liquid nitrogen and stored at −80 °C for further analysis.

2.2. Determination of fruit firmness, total soluble solids and titratable acid

Flesh firmness was measured twice using a fruit hardness tester (FHM-1, Japan) on the opposite sides of each fruit equator after removal of a 1 mm-thick slice of skin. The results were expressed as Newton (N).

TSS and TA of fruit flesh were measured from the pressed juice of each fruit sample using a Pocket Brix-Acidity Meter (PAL-BX|ACID 5, Atago, Japan). TSS and TA were expressed as percentage of Brix and malic acid, respectively.

Table 1
Treatments and storage conditions of peach fruit in four groups.

Group	Treatment	Storage condition	Sample collection time
RT	No	RT[b]	Day 1, 3, 5 and 7
LT	No	LT[c]	Day 1, 3, 5, 7, 14, 21, 28 and 35
HMR	Heat + 1-MCP[a]	RT[b]	Day 1, 3, 5 and 7
HML	Heat + 1-MCP[a]	LT[c]	Day 1, 3, 5, 7, 14, 21, 28 and 35

[a] Fruit were treated with hot water at 48 °C for 10 min and then with 10 μL L^{-1} 1-MCP for 12 h.
[b] Fruit were stored at 25 ± 1 °C with 85–90 % humidity.
[c] Fruit were stored at 4 ± 0.5 °C with 85–90 % humidity.

2.3. Determination of respiration rate and ethylene production

At each time point for each group, five fresh fruit were randomly selected from each biological replicate and enclosed in 4.7 L glass jars at 25 °C or 4 °C for 1 h. Respiration rate was directly measured by a CO_2 gas analyzer (CheckMate 3, Dansensor, Denmark). For ethylene production analysis, 1 mL headspace gas was injected into a gas chromatograph (Agilent GC7890 A) equipped with a 2-m stainless-steel packed column (1.8 m × 2 mm) and a flame ionization detector (FID). The temperatures of injector, column, and detector were 50 °C, 50 °C and 150 °C, respectively. Respiration rate was expressed as mg kg^{-1} h^{-1} of CO_2 and ethylene production was expressed as μg kg^{-1} h^{-1}, respectively.

2.4. Determination of superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2) concentrations

Each fresh sample (2.0 g) was homogenized in 6 mL of 65 mM sodium phosphate buffer (pH 7.8) and centrifuged at 10,000 × *g* at 4 °C for 15 min. The resulting supernatant was collected for O_2^- assays using the method of Huan et al. (2017). A standard curve with $NaNO_2$ was used to calculate the O_2^- concentration. O_2^- concentration was expressed as micromoles per gram of fresh weight.

To determine H_2O_2 concentration, flesh sample (2.0 g) was homogenized with 5 mL of chilled 100% acetone and then centrifuged at 10,000 × *g* for 20 min at 4 °C. The supernatant was collected immediately for H_2O_2 analysis according to the method of Patterson et al. (1984). The H_2O_2 concentration was expressed as micromoles per kilogram of fresh weight.

2.5. Determination of electrolyte leakage (EL) and malondialdehyde (MDA)

EL was determined using 20 disks (5 mm diameter) of flesh tissue of 10 fruit, in three replicates. The disks were immersed in 50 mL of doubly distilled water in glass vials at 25 °C. One hour later, the conductivity was measured (C1) and the disks were boiled for 30 min to achieve 100% electrolyte leakage (C2). Relative electrolyte leakage was calculated as (C1/C2) × 100.

MDA was measured according to the method of Shah et al. (2001) and expressed as micromoles per gram of fresh weight.

2.6. Enzyme analysis

Superoxide dismutase (SOD) activity was determined according to the method of Luo et al. (2012) and expressed as units (U) per milligram of protein. One unit of SOD activity was defined as the amount of enzyme that caused a 50% inhibition of nitro blue tetrazolium (NBT) at A_{560}.

Catalase (CAT) activity was determined according to the method of Change and Maehly (1955) and expressed as units (U) per milligram of protein. One unit was defined as a decrease at A_{240} in one minute.

Ascorbate peroxidase (APX) activity was determined according to the method of Nakano and Asada (1981) and expressed as units (U) per milligram of protein. One unit was defined as a decrease at A_{290} in one minute.

Glutathione peroxidase (GPX) activity was determined according to the method of Huan et al. (2017) and expressed as units (U) per kilogram of protein. One unit was defined as a decrease at A_{340} in one minute.

The total soluble proteins of the enzyme extract were determined by the method of Bradford (1976) using bovine serum albumin as a standard.