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Physiological and biochemical responses in peach fruit to oxalic acid treatment during storage at room temperature

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Abstract

Physiological and biochemical responses in peach fruit (*Prunus persica* L.) cv. 'Bayuecui' to two concentrations (1 and 5 mM) of oxalic acid treatment were studied during storage at room temperature (25 °C). Slower relative leakage rate, higher flesh firmness, lower respiration, increased activities of antioxidant enzymes (superoxide dismutase, SOD; peroxidase, POD; catalase, CAT; ascorbate peroxidase, APX) and polyphenol oxidase (PPO), and a decreased lipoxygenase (LOX) activity in treated fruit were observed as compared with the control. Moreover, significant decreases in the production of active oxygen species (AOS) (superoxide, $O_2^{\cdot-}$ hydrogen peroxide, H_2O_2) and lipid peroxidation in treated fruit were found at the later time of storage. The effects of oxalic acid could therefore contribute to maintaining the membrane integrity and delaying the fruit ripening process. Increased activities of POD, SOD, and PPO might also possibly be of benefit to disease resistance during storage.

Keywords: Antioxidant enzymes; Oxalic acid; Peach fruit; PPO; Ripening; Storage

1. Introduction

Oxalic acid is an organic acid ubiquitously occurring in plants, fungi, and animals, and plays several different roles in different living organisms (Shimada, Akamatsu, Tokimatsu, Mii, & Hattori, 1997). Recently, oxalic acid application has received much attention in relation to systemic resistance and antioxidant systems (Malenčić, Vasić, Popović, & Dević, 2004; Mucharroman & Kuc, 1991; Tian, Wan, Qin, & Xu, 2006; Zhang, Peng, Jiang, Xu, & Li, 1998; Zheng, Zhao, & Peng, 1999). For example, Malenčić et al. (2004) have found that antioxidant systems in different genotypes of sunflower are affected by different concentrations of oxalic acid. In addition, Kayashima and Katayama (2002) considered that oxalic acid is available as a natural antioxidant and may play an important role in the natural and artificial preservation of oxidized materials. To date, oxalic acid has been shown to be an antibrowning agent for harvested vegetables (Castaner, Gil, & Artes, 1997), banana slices (Yoruk, Balaban, Marshal, & Yoruk, 2002) and litchi fruit (Zheng & Tian, 2006), but little information is available about oxalic acid application in fresh fruits during postharvest storage.

Although fruit antioxidative activity decreases with progress of senescence (Srilaong & Tatsumi, 2003), the efficient antioxidant system contributes to delaying the senescence process in harvested fruit (Lacan & Baccou, 1998; Mondal, Sharma, Malhotra, Dhawan, & Singh, 2004). In plants, increases in activity of antioxidant enzymes which are caused by chemical treatment with methyl jasmonate (Wang, 1999) and fungicides (azoxystrobin and epoxiconazole) (Wu & Tiedemann, 2002) also delay the senescence process.

Peach fruit is very perishable during storage at room temperature due to rapid ripening and high susceptibility

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to pathogens (Bonghi, Ramina, Ruperti, Vidrih, & Tonutti, 1999). The search for alternative treatments that would delay fruit ripening led us to investigate the possible effects of oxalic acid on physiological and biochemical behavior of peach fruits during storage at room temperature.

2. Materials and methods

2.1. Material and treatment

Peach fruit of late season cv. 'Bayuecui' (*Prunus persica* L.) were harvested from a commercial orchard in Beijing, China. About 10 kg harvested fruit were placed in a carton, and then all the harvested fruit were immediately transported 80–100 km by a ventilated car to a laboratory. The fruit were selected for uniformity of size and appearance, and blemished and diseased fruit were discarded. Sixty selected fruit each were placed in a clean plastic box with fruit touching, and then three plastic boxes each were dipped in water (as control), 1 and 5 mM oxalic acid solutions at 25 °C for 10 min, respectively. After air drying, each box was wrapped in a polyethylene bag to maintain relative humidity and was held in a room at 25 ± 1 °C with 95% RH. Fruit were 238 ± 19.8 g with 8.10 ± 0.35 cm axial diameter and 7.82 ± 0.35 cm longitudinal diameter. All the parameters were daily measured using the flesh of fruit.

2.2. Measurement of membrane leakage

Twelve discs (3–4 mm thickness × 13 mm diameter) from 6 fruit at the equatorial region (two discs per fruit on opposite region) were rinsed and incubated in 50 ml of distilled water for 4 h, and then an initial electrolyte leakage was monitored with a conductivity meter (DDS-11A, Shanghai, China). Each sample was continued rinsing for 2 h after being boiled for 5 min, and then a final electrolyte leakage (total electrolyte) was monitored again. Relative leakage rate was defined as percent of initial electrolyte.

2.3. Determination of fruit fresh firmness

Following the method of Fernãndez-Trujillo and Artés (1998), firmness measurements of the fruits were made using a Bertuzzi Penetrometer with a 8 mm probe tip (FT327, Italy) on opposite pared cheeks at the equatorial region of 6 fruits. The mean of two measurements of each fruit was expressed in kilogram.

2.4. pH measurement

Ten-gram of flesh tissues from 6 fruit was homogenized with 25 ml distilled water and filtered, and then pH of the solutions was measured with a pH meter (Hanna pH 211, Italy) at 25 °C.

2.5. Measurement of respiration

CO_2 measurement was made on 6 fruit of each treatment and sealed in 5 l gas-tight jar for 3 h at 25 °C prior to gas sampling. CO_2 was monitored with a SQ-Z06 model gas chromatograph (Beifen, Beijing, China) equipped with EFZ × 110 model TCD (Shanghai, China; oven at 300 °C, injector and detector both at 150 °C).

2.6. Assay of enzymic activities

Five-gram of flesh samples (about 1–5 mm deep under peel at the equatorial region) from 6 fruit in each treatment were ground in different buffer containing 0.3 g of polyvinyl polypyrrolidone (PVPP) (Sigma, USA) to measure different enzymes: 30 ml sodium phosphate buffer (100 mM, pH 7.8) for SOD, POD, CAT and PPO; and 20 ml sodium phosphate buffer (100 mM, pH 7.0, containing 1 mM EDTA) for APX and lipoxygenase (LOX). Each sample was homogenized with a Kinematica tissue grinder (Crl-6010, Kriens-LU, Switzerland) and centrifuged at 20 000*g* for 30 min. The supernatants were as enzyme extracts for assaying enzymatic activities. All steps in the preparation of extracts were carried out at 4 °C.

Total SOD (EC 1.15.1.1) activity was determined by using "SOD Detection Kit" based on the manufacturer instruction (NJBI, Nanjing, China). The absorbance was monitored at 550 nm (Shimadzu UV-160, Japan). One unit of SOD is the amount of extracts that gives 50% inhibition of reduction of xanthine.

POD (EC 1.11.1.7) activity was based on the determination of guaiacol oxidation at 470 nm by H_2O_2. The change in absorbance at 470 nm was followed every 20 s by a spectrophotometer (Lacan & Baccou, 1998). One unit of POD defined as the amount of enzyme caused 0.01 absorbance increase per min under the conditions of assay.

CAT (EC 1.11.1.6) activity determination was performed according to the method of Beers and Sizer (1952) with slight modifications. The reaction mixture consisted of 2 ml of sodium phosphate buffer (50 mM, pH 7.0), 0.5 ml H_2O_2 (40 mM) and 0.5 ml enzyme extract in a total volume of 3.0 ml. The decomposition of H_2O_2 was measured by decline in absorbance at 240 nm. One unit was of 0.01 absorbance change per minute.

APX (EC 1.11.1.11) activity was assayed according to the method of Nakano and Asada (1981). Three milliliters of the reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbate, 0.1 mM H_2O_2 and 0.2 ml enzyme extract. The hydrogen peroxide-dependent oxidation of ascorbate was followed by a decrease in the absorbance at 290 nm [coefficient 2.8 (mM^{-1} cm^{-1})].

PPO (EC 1.10.3.2) activity was measured by incubating 0.5 ml of enzyme extract to 2.5 ml of buffered substrate (100 mM sodium phosphate, pH 6.4 and 50 mM Catechol), and then monitoring the change of absorbance at 398 nm (Wang, Tian, Xu, Qin, & Yao, 2004). One unit of activity of PPO was defined as the amount of enzyme causing 0.01

absorbance increase per minute under the conditions of assay.

LOX activity was assayed at 30 °C by monitoring the formation of conjugated dienes from linoleic acid at 234 nm, according to the method of Wang et al. (2004). Three milliliters of reaction mixture contained 2.75 ml sodium phosphate buffer (100 mM, pH 7.0), 50 μl sodium linoleic acid solution (10 mM) and 0.2 ml enzyme. The blank contained 2.95 ml sodium phosphate buffer (100 mM, pH 7.0) and 50 μl sodium linoleic acid. One unit of LOX was expressed as 1 μmol hydroperoxide formed per minute at 30 °C.

Protein content of enzyme extracts was measured according to the method of Bradford (1976), using bovine serum albumin (BSA) as the standard protein. APX activity was expressed as μmol ascorbate oxidized mg^{-1} protein h^{-1}, and the other enzymes as U mg^{-1} protein.

2.7. Determination of $O_2^{\cdot-}$ and H_2O_2 production

$O_2^{\cdot-}$ production was according to the method of Wang and Lou (1990). One milliliter of enzyme extracts as aforementioned for SOD was mixed with 1 ml of 1 mM hydroxylammonium chlroride, and then incubated for 30 min at 30 °C. One milliliter of incubated solution was then added to 1 ml of 17 mM 3-aminobenzenesulfonic acid (Sigma, USA) and 1 ml of 7 mM 1-naphthylamine (Sigma, USA), and then further incubated for 20 min at 30 °C. The absorbance of the solution was monitored at 530 nm. $O_2^{\cdot-}$ production was expressed as nmol mg^{-1} protein h^{-1}.

H_2O_2 determination followed the method described by Mukherjec and Choudhuri (1983). H_2O_2 was extracted by homogenizing 2.0 g flesh tissue from 6 fruit (about 1–5 mm deep under peel at the equatorial region) with 10 ml of acetone (0 °C), and then centrifuged at 15000*g* for 15 min at 4 °C. One milliliter of extracted solution was mixed with 0.1 ml of 5% titanium sulphate and 0.2 ml ammonia, and then centrifuged at 15000*g* for 10 min at 4 °C again. The pellets were dissovled in 3 ml 10% H_2SO_4 (v/v), and then centrifuged at 15000*g* for 10 min. Absorbance of the supernatant was measured at 415 nm.

2.8. Determination of lipid peroxidation

Lipid peroxidation was determined by estimating malondialdehyde (MDA) content in flesh according to Heath and Packer (1968). Two milliliters of enzyme extracts as aforementioned for SOD was reacted with 2 ml of 0.6% 2-thiobarbituric acid (TBA) in 20% trichloroacetic acid. The solution was heated at 95 °C for 30 min, and quickly cooled in an ice-bath for 5 min, and then centrifuged at 12000*g* for 10 min to clarify the solution. Absorbances were monitored at 532 and 600 nm, respectively. Calculation of MDA was used an extinction coefficient of 155 mM cm^{-1}.

2.9. Statistical analysis

All treatments were done with three replicates. Data represent the means ± SD, and they were analyzed by one way analysis of variance (ANOVA) using SPSS version 11.5 (SPSS Inc., Chicago, IL, USA). Significance of difference among means of control and treatments using Duncan's multiple range tests at 5% level. Difference at $p < 0.05$ was considered as significant.

3. Results

3.1. Changes in membrane permeability, flesh firmness, respiration, and pH

The membrane permeability was expressed as relative leakage rate. Although the relative leakage rate in all fruit gradually increased with storage time increase, lower relative leakage in oxalic acid treated fruit was observed with respect to the control during storage ($p < 0.05$) (Fig. 1(a)). Fruit softening rate was slowed down by oxalic

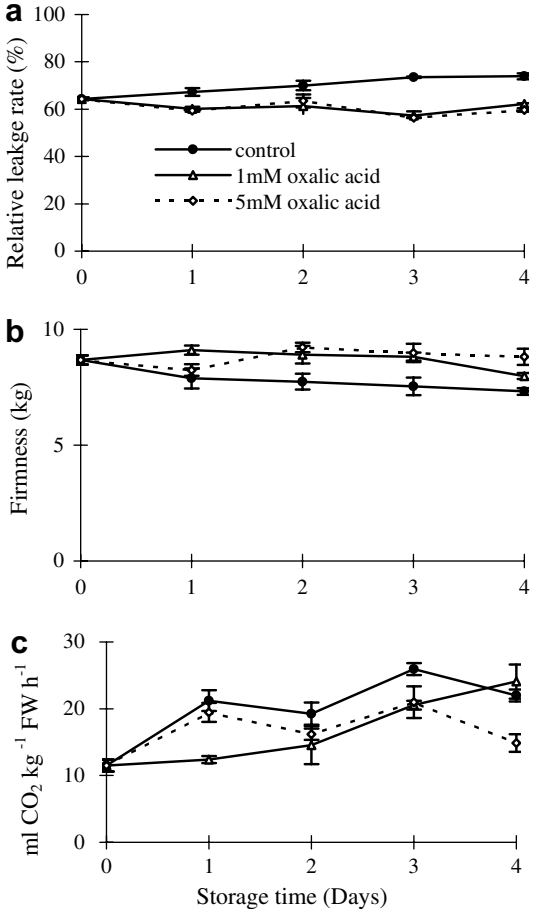

Fig. 1. Changes in relative leakage rate (a), flesh firmness (b), and respiration rate (c) in untreated (control) and oxalic acid-treated peach fruit during storage. Data are means of three replicates ± SE.

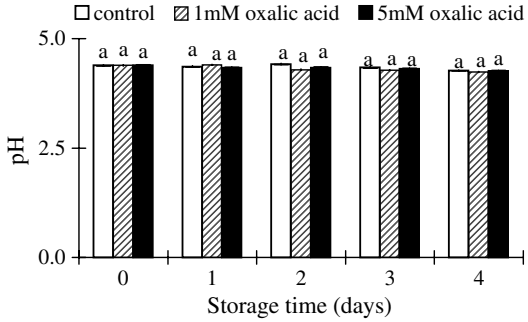

Fig. 2. pH change in untreated (control) and oxalic acid-treated peach fruit during storage. Data are means of three replicates ± SE. Different letter indicates significant differences among treatments according to Duncan's multiple range tests ($p < 0.05$).

acid, as the fruit flesh firmness in treated fruit maintained a higher level in contrast to the control during storage ($p < 0.05$) (Fig. 1(b)). The respiration in fruit treated with 1 mM oxalic acid gradually increased and presented significantly lower levels during the first three days, but not on the fourth day as compared with control; while when treated with 5 mM oxalic acid, a pattern of change in respiration was similar to the control, but with lower respiration in treated fruit observed after one day ($p < 0.05$) (Fig. 1(c)). There was no significant difference in pH between treated fruit and control, and pH was in the range of 4.24–4.42 during storage (Fig. 2).

3.2. Effect of oxalic acid on activity of antioxidant enzymes

SOD activity in treated fruit increased to a peak on the first day after oxalic acid treatment and then decreased during the rest days whereas, by contrast, in control fruit SOD decreased steadily during this time. SOD activity in fruit treated with 1 and 5 mM oxalic acid was higher than that in the control at the beginning of the second and third days, respectively ($p < 0.05$) (Fig. 3(a)). POD activity increased steadily in treated and control fruit during storage, and significantly higher POD activity in treated fruit was found except for 1 mM treatments on the fourth day ($p < 0.05$) (Fig. 3(b)). CAT activity in all fruit maintained high levels during storage as compared to the initial value. After 2 days, CAT activity in 1 and 5 mM treatments showed significantly high levels for 1 and 2 days, respectively, in contrast to the control ($p < 0.05$) (Fig. 3(c)). The pattern of APX activity change in all fruit was similar during storage, but APX activity was significantly higher in treated fruit than in the control for the first 3 days ($p < 0.05$) (Fig. 3(d)).

3.3. Effect of oxalic acid on LOX activity

LOX activity in control fruit markedly increased as compared to the initial value during storage whereas, in treated fruit it was maintained at a relatively constant level for the first 3 days and then sharply increased to the similar

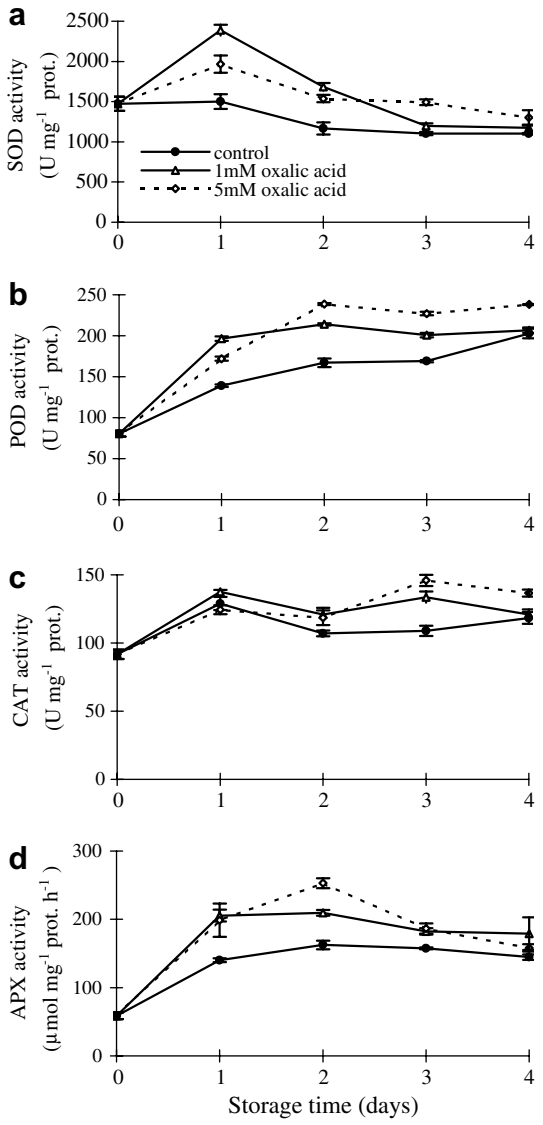

Fig. 3. Changes in activities of SOD (a), POD (b), CAT (c), and APX (d) in both untreated (control) and oxalic acid-treated peach fruits during storage. Data are means of three replicates ± SE.

level as control fruit on the fourth day. A significantly lower LOX activity in treated fruit was found at 2 and 3 days with respect to control ($p < 0.05$) (Fig. 4).

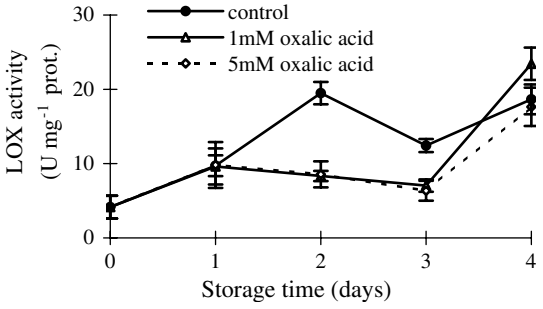

Fig. 4. Change in LOX activity in both untreated (control) and oxalic acid-treated peach fruits during storage. Data are means of three replicates ± SE.

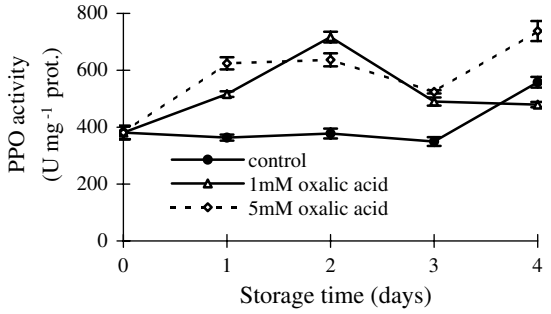

Fig. 5. Change in PPO activity in both untreated (control) and oxalic acid-treated peach fruits during storage. Data are means of three replicates ± SE.

3.4. Effect of oxalic acid on PPO activity

PPO activity in treated fruits increased during storage time and reached a maximum level on the second and fourth day in 1 and 5 mM oxalic acid respectively, while in the control it remained almost constant for the first 3 days but in contrast markedly increased to the similar level as the 1 mM treatment on the fourth day. Thus, a significant increase in PPO activity was apparent in treated fruit ($p < 0.05$) (Fig. 5).

3.5. Effect of oxalic acid on AOS production and lipid peroxidation

$O_2^{\cdot-}$ production in fruit with 1 mM oxalic acid treatment was significantly lower at 3 days, while with 5 mM, was significantly lower at 2 and 3 days in contrast to the control (Fig. 6(a)). H_2O_2 content in all fruit sharply increased after 1 day and showed less change in the control during the rest time whereas, a significant decrease in 1 and 5 mM oxalic acid treated fruit at 3 days, and 3 and 4 days, respectively (Fig. 6(b)).

AOS induce lipid peroxidation of fatty acids that results in formation of several byproducts such as MDA. Fig. 6(c) showed that lipid oxidation was inhibited by oxalic acid at the later time of storage, as the MDA content was significantly lower in treated fruit than in control after 2 days.

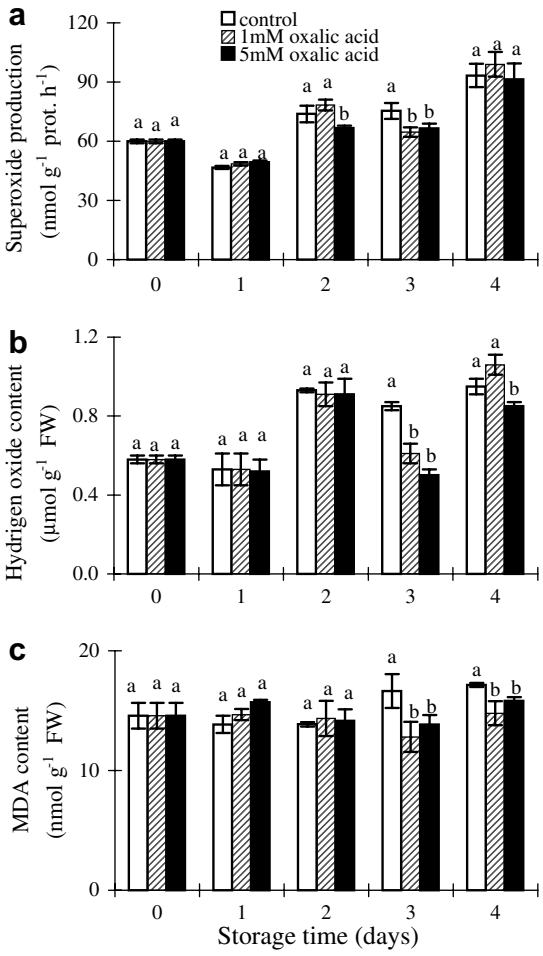

Fig. 6. Changes in superoxide production (a), hydrogen peroxide (b) and MDA content (c) in untreated (control) and oxalic acid-treated peach fruit during storage. Data are means of three replicates ± SE. Different letter indicates significant differences among treatments according to Duncan's multiple range tests ($p < 0.05$).

4. Discussion

4.1. Effects of oxalic acid on antioxidant enzymes and LOX in relation to delay of ripening in peach fruits during storage

In higher plants, senescence is characterized by the breakdown of cell wall components and membrane disruption resulting in cellular decompartmentation and the loss of tissue structure (Paliyath & Droillard, 1992). It is demonstrated that active oxygen species (AOS) are largely involved in the process of ripening, particularly in membrane deterioration (Rogiers, Kumar, & Knowles, 1998), since increased AOS levels in fruit during the ripening process not only are to alter membrane integrity, but also react with unsaturated fatty acids causing lipid peroxidation (Jimenez et al., 2002; Lacan & Baccou, 1998). However, the antioxidant enzymes such as SOD, POD, CAT and APX play crucial roles in antioxidant defense during the fruit ripening process, as SOD converts $O_2^{\cdot-}$ to H_2O_2 while H_2O_2 removal by CAT, POD and APX (Mondal et al., 2004; Schantz, Schreiber, Guillemaut, & Schantz, 1995) and the cooperation between H_2O_2-generating SOD and H_2O_2-scavenging enzymes rather than the individual antioxidant enzyme confers oxidant resistance/tolerance (Lacan & Baccou, 1998; Schantz et al., 1995; Ye & Gressel, 2000). For instance, Lacan and Baccou (1998) have reported that the higher activities of SOD and CAT with high SOD/CAT ratio contribute to delay in the senescence process in muskmelon fruit variety-Clipper during storage. Data here showed that significantly increased activities of antioxidant enzyme including SOD, CAT, POD and APX in peach fruit were induced by oxalic acid (Fig. 3), which apparently increased the antioxidative ability against oxidative damage. In addition, LOX is partly response for

the imitation of the lipid degradation process, as well as $O_2^{\cdot-}$ and singlet oxygen (Gardner, 1995). Researchers have identified that increased lipid peroxidation leading to membrane deterioration is correlated with higher LOX activity during fruit ripening (Kausch & Handa, 1997; Rogiers et al., 1998). In our work, we observed that LOX activity in control fruit markedly increased after harvested whereas, in oxalic acid treated fruit, significantly decreased at 2 and 3 days during storage (Fig. 4). Therefore, it suggested that such effects of oxalic acid application on these antioxidant enzymes and LOX in peach fruit could result in decrease in the AOS level as well as inhibition of the lipid peroxidation (Fig. 6), and in turn could contribute to maintaining the membrane integrity and delaying the ripening process in peach fruit during storage at room temperature. This would explain why the significant decrease in the relative leakage rate, softening rate and respiration were observed in peach fruit with oxalic acid treatment during storage (Fig. 1). Jin, Xiaoyu, Honglin, Jiaxun, and Yaguang (1989) have concluded that fruit senescence is delayed on tomatoes and mandarins exposed to ozone and negative ions, as the respiratory intensity is lowered.

4.2. Contribution of increased activities of SOD, POD and PPO by oxalic acid to disease resistance in peach fruits during storage

In higher plants, POD is considered to be associated with disease resistance as POD could generate phenolic cross-links connecting neighboring bipolymer chains (Devi & Prasad, 1996). PPO acts as a defensive enzyme and plays a key role in the defense system in fruit (Mayer & Harel, 1991; Wang et al., 2004). Exogenous oxalic acid is an effective agent for inducing disease resistance in plants against *Colletotrichum gloeosporioides* and watermelon mosaic virus-2 (WMV-2) combined with increasing the activities of different forms of POD and inducing a synthesis of new POD isoforms (Zhang et al., 1998; Zheng et al., 1999). Recently, Tian et al. (2006) have reported that oxalic acid inhibited the progress of *Alternaria rot* in harvested pear fruit due to inducing an increase in defense-related enzyme such as POD, PPO, and PAL. Interestingly, salicylic acid has been showed to induce disease resistance in plant associated with increased SOD activity (Foder, Gullner, & Adam, 1997). Our present work did not show data to point out whether oxalic acid treatment had a direct effect on induction of disease resistance in peach fruit. However, these facts together with the evidence in our work suggested that the increased activities of SOD (Fig. 3(a)), POD (Fig. 3(b)), and PPO (Fig. 5) in fruit induced by oxalic acid might possibly be of benefit to disease resistance during storage as well. Further research that oxalic acid indirectly or/and directly effects on mechanism of disease resistance in fruit during storage needs to be addressed.

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