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Food Chemistry 91 (2005) 99–104
Food Chemistry
www.elsevier.com/locate/foodchem

# Effects of high oxygen concentration on pro- and anti-oxidant enzymes in peach fruits during postharvest periods

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Received 23 May 2003; revised 27 May 2004; accepted 27 May 2004

**Abstract**

Peach fruits (*Amygdalus persica* cv. Okubao) were stored in air, controlled atmospheres (CA) of 5% $O_2$ plus 5% $CO_2$, or CA with high $O_2$ concentration (70% $O_2$ plus 0% $CO_2$ for 15 days, then in CA with 5% $O_2$ plus 5% $CO_2$) at 0 °C, to determine the effects of different $O_2$ and $CO_2$ atmospheres on the activities of lipoxygenase (LOX), peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT), as well as malondialdehyde (MDA) content and membrane integrity during storage periods with or without 3 days of post-storage ripening at 20 °C. SOD, CAT and POD activities of the fruits stored in air markedly decreased during the first 30 days of storage, while LOX activity increased after 15 days. CA (5% $O_2$ plus 5% $CO_2$) reduced chilling injury, and delayed the reduction of SOD, CAT and POD activities compared to the control. CA with high $O_2$ treatment induced SOD and CAT activities and maintained membrane integrity, but no significant effect on alleviating chilling injury was found compared to CA storage. The results indicated that the decrease of SOD and CAT might contribute to the development of chilling injury in peach fruits.
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*Keywords:* Antioxidant enzymes; Chilling injury; High oxygen treatment; Peach fruits

## 1. Introduction

Due to rapid ripening after harvest and thus a short shelf-life at room temperature, refrigeration is usually used to store peach fruits for its beneficial effect of extending storage life both by maintaining fruit quality and by reducing storage decay. However, peach fruits easily develop a serious physiological disorder known as chilling injury (CI) during low temperature storage (Anderson & Penney, 1975; Brovelli, Becht, Sherman, & Sims, 1998; Crisosto, Mitcham, & Kader, 1996). Some strategies, such as delayed storage (DS), intermittent warming (IW), and controlled atmosphere (CA), have been applied to prevent or alleviate chilling injury (Fernández-Trujillo & Arté, 1997; Lill, O'Donoghue, & King, 1989; Lurie, 1993; Perkins-Veazie, Roe, Lasswell, & McFarland, 1999). However, DS has produced conflicting results, IW is cumbersome during long-term or large-scale storage, CA storage has been identified as a good method for alleviating CI during storage, the specific gas concentrations depending upon cultivars (Tonini, Brigati, & Caccioni, 1989; Zhou et al., 2000).

Meanwhile, atmospheres with high $O_2$ levels have recently been suggested as an innovation of modified atmosphere packaging (MAP) for postharvest fruits and vegetables, to inhibit physiological metabolism, maintain quality and safety, and prolong shelf time (Jacxsens, Devlieghere, Van der Steen, & Debevere, 2001; Tian, Xu, Jiang, & Gong, 2002). Nevertheless, sensitivity to $O_2$ toxicity varies among species (Jiang, Tian, & Xu,

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doi:10.1016/j.foodchem.2004.05.053

2002; Kader & Ben-Yehoshua, 2000). Increased $O_2$ concentrations around and within the commodity may also result in higher levels of reactive oxygen species (ROS), such as superoxide, hydrogen peroxide and the hydroxyl radical, that can damage plant tissues (Fridovich, 1986). But it is still not clear whether high $O_2$ atmospheres ameliorate or aggravate chilling injury, and there is little information about the effects of high $O_2$, combined with CA storage, on pro- and anti-oxidant enzymes in peach fruits. The objective of this experiment was to investigate the effects of different $O_2$ and $CO_2$ atmospheres on the activity of some pro- and anti-oxidant enzymes, such as LOX, SOD, CAT and POD, as well as MDA content in peach fruits during storage with or without 3 days of post-storage ripening at 20 °C.

## 2. Materials and methods

### *2.1. Plant material and experimental design*

Peach fruits (*Amygdalus persica* cv. Okubao) were harvested from an orchard at Pinggu district, Beijing, and quickly transported to our laboratory on the day of harvest. The fruits were selected for uniform size, maturity and appearance and freedom from defects. Sound fruits were divided into three groups. Group 1 (Control); fruits were kept in air. Group 2 (Controlled atmosphere, CA); fruits were placed in a chamber with 5% $O_2$ + 5% $CO_2$. Group 3 (CA with high $O_2$ treatment); the fruits were treated in 70% $O_2$ atmosphere for 15 days, then stored in CA with 5% $O_2$ + 5% $CO_2$ atmosphere. Fruit firmness, soluble solids content (SSC) and pH value were 56.6 Newtons (N), 9.4% and 3.74, respectively, at the beginning of the experiment. There were about 120 kg of fruits in each controlled atmosphere chamber (FC-701, made in Italy) with about 95% relative humidity. The concentrations of $O_2$ and $CO_2$ were automatically controlled and recorded by a computer. All the treatments were kept at 0 °C. At regular intervals, fruits were removed from cold storage and subsequently held at 20 °C for 3 days of ripening. On the day of removal and on the third day of ripening, three replicates of nine fruits were sampled from each treatment. The experiment was conducted twice.

### *2.2. Evaluation of flesh browning and fruit decay*

Flesh browning was assessed by measuring the extent of browned area on each fruit, using 60 fruits for storage and 30 fruits for post-storage, on the following scale: 0 = no browning; 1 = less than 1/4 browning; 2 = 1/4–1/2 browning; 3 = 1/2–3/4 browning; 4 = more than 3/4 browning. The browning index was calculated using the following formula: $[(1 \times N_1 + 2 \times N_2 + 3 \times N_3 + 4 \times N_4) \times 100/(4 \times N)]$, where $N$ = total number of fruits measured and $N_1$, $N_2$, $N_3$ and $N_4$ were the number of fruits showing the different degrees of browning. The degree of surface decay was measured using the same scale of browning judgment.

### *2.3. Extraction, enzymes assays and protein analysis*

Flesh (10 g) was collected from nine fruits in each treatment and homogenized in 25 ml of ice-cold extraction buffer and 0.5 g polyvinyl polypyrrolidone (PVPP) with a Kinematica tissue grinder (Crl-6010, Kriens-LU, Switzerland). For LOX assay, the buffer was Tris–HCl buffer (100 mM, pH 8.0). For CAT analysis, 50 mM sodium phosphate (pH 7.0) was used as extraction buffer. For SOD, POD and MDA content, 100 mM sodium phosphate buffer (pH 6.4) was used. The homogenate was centrifuged at 27,000*g* for 50 min at 4 °C and the resulting supernatants were used directly for assay.

LOX activity was determined spectrophotometrically by measuring the formation of conjugated dienes at 234 nm and 30 °C, according to Pérez, Sanz, Olías, and Olías (1999) with the following modifications: the reaction mixture contained 2.85 ml sodium phosphate buffer (100 mM, pH 6.0), 50 µl of sodium linoleic acid solution (10 mM) and 0.1 ml sample protein. The blank contained 2.95 ml of sodium phosphate buffer (100 mM, pH 6.0) and 50 µl of sodium linoleic acid. The increase in absorbance at 234 nm was measured using a UV-160 Spectrophotometer (Shimadzu, Japan). The LOX specific activity was expressed as U/mg protein, where one unit was expressed as 1 µmol hydroperoxide formed per min at 30 °C.

Determination of SOD was performed by the method of Constantine and Stanley (1977) with slight modifications. The reaction mixture (3 ml) contained 50 mM sodium phosphate buffer (pH 7.8), 13 mM methionine, 75 µM nitroblue tetrazolium (NBT), 10 µM EDTA, 2 µM riboflavin and 0.1 ml of the enzyme extract. The mixtures were illuminated by light (60 $\mu mol/m^2/s$) for 10 min and the absorbance was then determined at 560 nm. Identical solutions held in the dark served as blanks. One unit of SOD was defined as the amount of enzyme that caused a 50% decrease of the SOD-inhibitable NBT reduction. The specific activity was expressed as U/mg protein.

CAT activity was measured according to Beers and Sizer (1952) with slight modifications. The reaction mixture consisted of 2 ml sodium phosphate buffer (50 mM, pH 7.0), 0.5 ml $H_2O_2$ (40 mM) and 0.5 ml enzyme. The decomposition of $H_2O_2$ was measured by the decline in absorbance at 240 nm. The specific activity was expressed as U/mg protein, where one unit of catalase converts one µmol of $H_2O_2$ per min.

POD activity was assayed according to the method used by Jiang et al. (2002); 0.5 ml of enzyme was incubated in 2 ml buffered substrate (100 mM sodium phos-

phate, pH 6.4 and 8 mM guaiacol) for 5 min at 30 °C and the increasing absorbance measured at 460 nm for 120 s after adding 1 ml of $H_2O_2$ (24 mM). Specific activity was expressed $\Delta A_{460}$/min/mg protein.

Protein content was measured according to the method of Bradford (1976), using bovine serum albumin (BSA) as the standard protein.

### 2.4. Determination of MDA content

The assay of MDA was performed as described by Jiang et al. (2002). The MDA content was determined by adding 2 ml of 0.5% TBA in 15% trichloroacetic acid to 1 ml sample. The solution was heated at 95 °C for 20 min, quickly cooled in an ice-bath for 5 min, and then centrifuged at 12,000*g* for 10 min to clarify the solution. Absorbance at 532 nm was measured and subtracted from the absorbance at 600 nm. The amount of MDA was calculated with an extinction coefficient of 155 mM/cm.

### 2.5. Measurement of membrane integrity

Nine discs, 10 mm diameter and 4 mm thick, from nine fruits were washed three times in deionized water for about 1 min each time, dried with filter paper, and put together into 50 ml conical flasks containing 40 ml of deionized water. Initial electrolyte leakage was determined using a conductivity meter (Model EC 215, Italy), following incubation of the flask at 25 °C for 180 min. The solution was then placed in a water bath (95 °C) for 30 min before the final conductivity (total electrolyte leakage) was measured. The membrane integrity percentage was calculated as: % membrane integrity = [1 − (electrolyte leakage after 180 min of submersion/total electrolyte leakage)] × 100.

### 2.6. Data analysis

All analyses were done in three replications and the results were statistically evaluated, using Tukey's test at $P = 0.05$.

## 3. Results

### 3.1. Fruit decay and flesh browning

In all treatments, fruit decay was not observed until 60 days of storage, but control fruits showed disease incidences of 42.8% and 100% with 22.2% and 33.3% decay indices after 45 and 60 days of storage at 0 °C plus 3 days at 20 °C, respectively (Fig. 1(a) and (b)).

A flesh browning rate of 67% was found in the control fruits after 45 days of storage, but CA and CA with high $O_2$ treatment significantly delayed the occurrence of browning (Fig. 2(b)). No browning was found in the fruits stored in CA conditions after 60 days at 0 °C. But, when the fruits were transferred to room

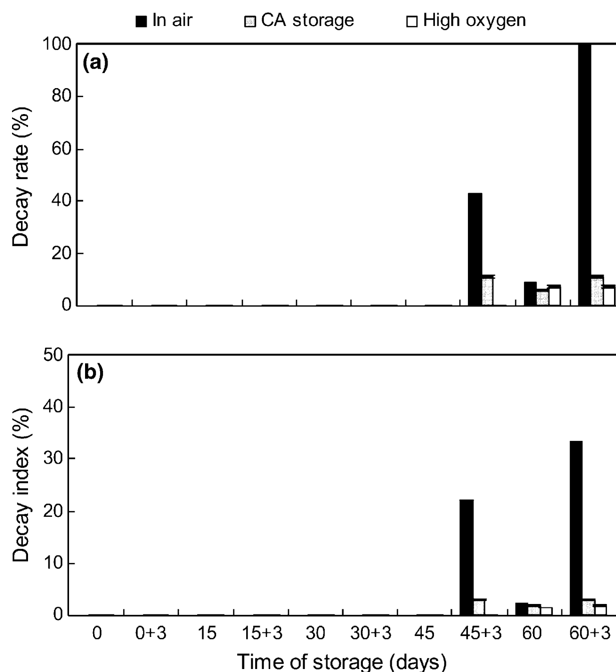


Fig. 1. Decay rate (a) and decay index (b) in peach fruits stored in air, in controlled atmospheres of 5% $O_2$ plus 5% $CO_2$, or in 70% $O_2$ atmosphere for 15 days, then in CA with 5% $O_2$ plus 5% $CO_2$ at 0 °C, with or without 3 days of post-storage ripening at 20 °C. Data are means ± SE of three replicates.

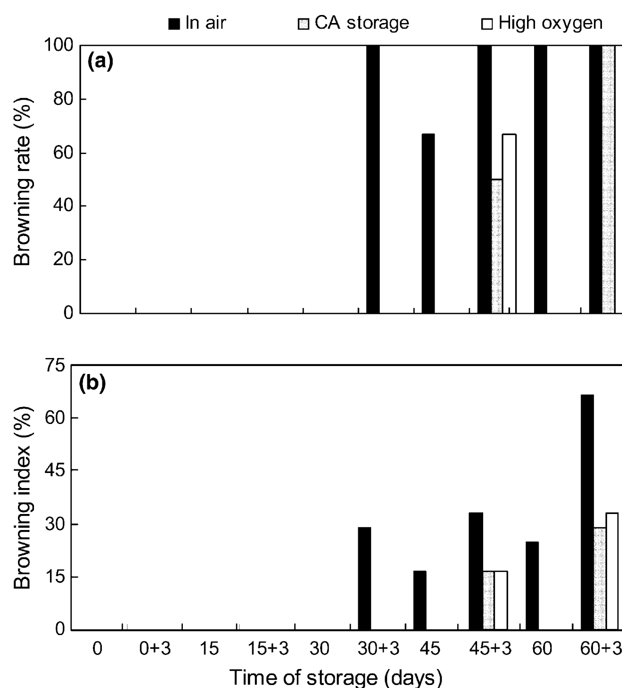


Fig. 2. Changes of browning rate (a) and browning index (b) in peach fruits stored in air, controlled atmospheres of 5% $O_2$ plus 5% $CO_2$, or 70% $O_2$ atmosphere for 15 days, then in CA with 5% $O_2$ plus 5% $CO_2$ at 0 °C, with or without 3 days of post-storage ripening at 20 °C. Data are means ± SE of three replicates.

temperature for ripening, both browning rate and index significantly increased (Fig. 2(a) and (b)).

### 3.2. MDA content, membrane integrity and lipoxygenase activity

The tendency of MDA content proved to be similar in all treated fruits, but marked increase in MDA content of the fruits stored in air was found when the fruits were kept at 20 °C for 3 days of ripening. There was no significant difference in MDA content of the fruits stored in CA conditions (Fig. 3(a)).

The membrane integrity decreased in control fruits after 15 days of storage. In contrast, the fruits stored in CA and CA with high $O_2$ treatment showed relatively higher levels of membrane integrity than the control during storage periods but, when the fruits were removed to 20 °C for 3 days of ripening, the membrane integrity significantly decreased (Fig. 3(b)).

Changes in LOX activities of the fruits kept in different storage conditions were similar during storage periods (Fig. 3(c)). The maximum levels of LOX activity of the fruits in post-storage occurred earlier than those during storage. CA conditions could obviously delay the maximum peak of LOX activity both during storage and post-storage, but the fruits kept in CA with high $O_2$ treatment showed higher LOX activity, after 60 days at 0 °C plus 3 days at 20 °C for ripening, than did fruits in CA storage.

### 3.3. Superoxide dismutase, catalase, and peroxidase activities

SOD activity in the control fruits continually decreased in 30 days of storage, then increased and declined rapidly at the end of the storage (Fig. 4(a)). Although SOD activities in the fruits stored in CA conditions also decreased rapidly after 30 days, relatively higher levels of SOD activity were found during the first 30 days of storage.

During the 30 days of storage, CAT activity in the control fruits decreased quickly, but CAT activity of the fruits stored in CA conditions increased within 15 days, then declined gradually with storage time (Fig. 4(b)). No significant difference of CAT activity was

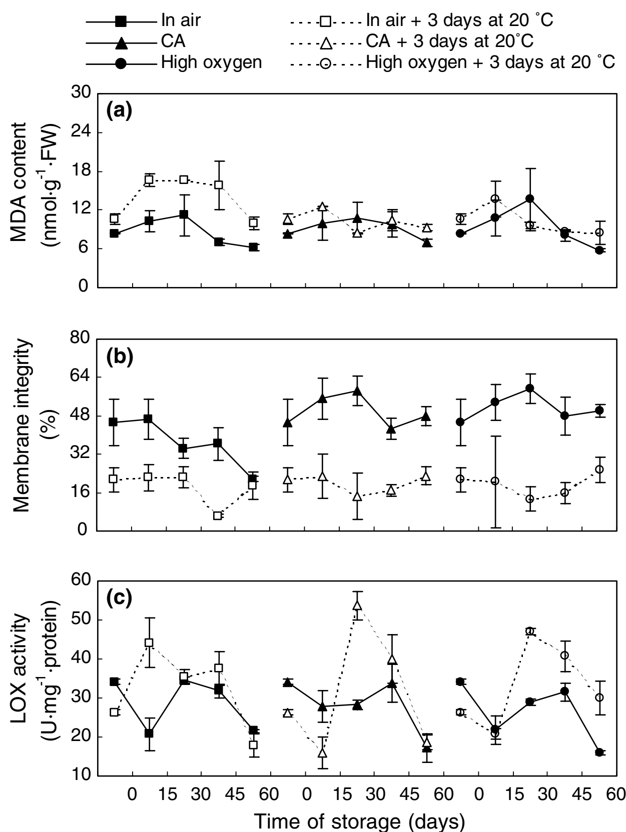


Fig. 3. Changes of MDA content (a), membrane integrity (b) and LOX activity (c) of peach fruits stored in air, in controlled atmospheres of 5% $O_2$ plus 5% $CO_2$, or in 70% $O_2$ atmosphere for 15 days, then in CA with 5% $O_2$ plus 5% $CO_2$ at 0 °C, with or without 3 days of post-storage ripening at 20 °C. Data are means ± SE of three replicates.

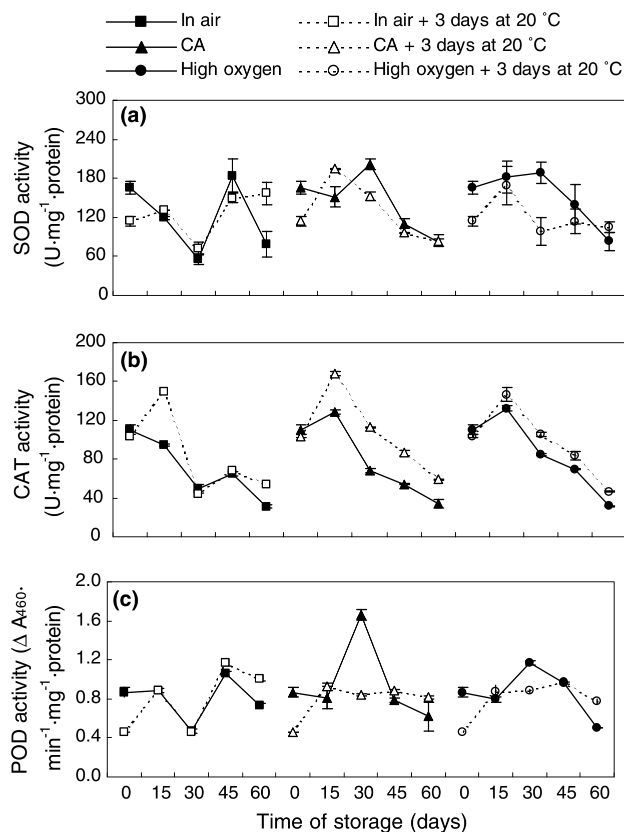


Fig. 4. Changes of activities of SOD (a), CAT (b) and POD (c) of peach fruits stored in air, in controlled atmospheres of 5% $O_2$ plus 5% $CO_2$, or in 70% $O_2$ atmosphere for 15 days, then in CA with 5% $O_2$ plus 5% $CO_2$ at 0 °C, with or without 3 days of post-storage ripening at 20 °C. Data are means ± SE of three replicates.

found in the fruits kept in CA and CA with high $O_2$ treatment.

POD activity in the control samples showed a marked decrease, especially within 30 days, while markedly higher levels of POD activity were found in the fruits stored in CA conditions at the same time (Fig. 4(c)). At the end of storage, POD activity proved to be low in all treated fruits.

## 4. Discussion

Chilling injury, as a physiological disorder of peach fruits, easily occurs in low temperature storage, and directly affects storage life and marketing quality of peaches (Crisosto et al., 1996). Chilling injury is related to the accumulation of ROS (Hariyadi & Parkin, 1991; Sala, 1998). The accumulation of ROS resulted from altered balance between ROS production and capacity of scavenging for them (Mittler, 2002). LOX was considered to be partly response for the formation of lipid peroxidation products, as well as superoxide ($O_2^{\bullet-}$) and singlet oxygen (Fobel, Lynch, & Thompson, 1987; Gardner, 1995; Siedow, 1991), while SOD, CAT and POD are important active free-radical scavenging enzymes (Lee & Lee, 2000; Scandalios, 1993).

Chilling injury is considered to be primary and secondary. The primary injury is readily reversible if the temperature is raised to non-chilling conditions. Secondary injuries occur as a consequence of the primary injury and may not be reversible, which contributes to visual and cellular symptoms (Wang, 1982). Moreover, chilling injury symptom may not be evident until rewarming (Parkin, Marangoni, Jackman, Yada, & Stanley, 1989; Shewfelt & Erickson, 1991). The present results show that, in control fruits, no flesh browning occurs during the first 15 days of storage, but the drastic increase of LOX activity during subsequent ripening suggests that reversible chilling injury might occur. The irreversible chilling injury (flesh browning) in control fruits occurred after 30 days at 0 °C plus 3 days at 20 °C. In contrast, fruits stored in CA did not show irreversible chilling injury until after 45 days of storage plus 3 days at 20 °C.

During the development of irreversible chilling injury (flesh browning) in control peach fruits, SOD, CAT and POD activities as well as membrane integrity decreased significantly, the lipid peroxidation (MDA content) increased gradually, while LOX activity increased quickly after 15 days of storage. In contrast, CA storage delayed the reduction of SOD, CAT and POD activities, maintained membrane integrity, and retarded occurrence of the maximum peak of LOX activity during post-storage. Thus, the decrease of SOD, CAT and POD, combined with the increase of LOX, may contribute to the development of chilling injury in peach fruits. The effectiveness of CA in delaying the occurrence of chilling injury may result from delaying the reduction of antioxidant enzymes during the first 30 days of storage. Similar results were also reported by Wang (1995), who found that both SOD and CAT activities decreased greatly in zucchini squash when exposed to chilling stress. CAT, as a major antioxidant enzyme, has been reported to be involved in the defence mechanism against chilling injury (Sala & Lafuente, 2000; Saruyama & Tanida, 1995).

High $O_2$ levels have been reported to be effective for inhibiting enzymic discoloration, preventing anaerobic fermentation reactions, and influencing microbial growth (Day, 1996). Our previous study also showed that CA with high $O_2$ significantly decreased ethanol production in the flesh, maintained a lower pH value in the peel and maintained peel green colour of longan fruits (Tian et al., 2002). But a detrimental effect of high $O_2$ concentrations was also shown on the fruit quality of strawberry (Wszelaki & Mitcham, 2000) and sweet cherry (Jiang et al., 2002). This study indicates that, although the reduction of SOD and CAT activities was delayed significantly, no aggravated chilling injury was found in peach fruits after stored in CA with 70% $O_2$ treatment for 15 days, and the membrane integrity also remained satisfactory. However, CA with high $O_2$ treatment was not significantly effective in preventing chilling injury and maintaining quality, during the experiment compared to CA storage.

Conflicting results concerning the relationship between ion leakage and chilling stress have been reported (Marangoni, Palma, & Stanley, 1996). Our results showed that ion leakage in control fruits did not change significantly during the first 15 days, but increased greatly after the fruits were transferred to 20 °C. These results were consistent with those reported by Sharom, Willemot, and Thompson (1994), who found that ion leakage did not increase in tomato chilled for 20 days at 5 °C and increased only after transfer to higher temperature. But we also found that the ion leakage increased significantly after 15 and 30 days, respectively, in control and CA fruits. The inconsistent results may be attributed to the duration of chilling injury, which depends on fruit species, storage temperature and time. Meanwhile, the ion leakage might reflect the occurrence of severe chilling injury rather than increased immediately upon exposure to chilling temperatures.

In conclusion, the present data show that the decrease of SOD and CAT, combined with the increase of LOX, might contribute to the development of chilling injury in peach fruits. The effectiveness of CA storage in delaying the occurrence of chilling injury may be due to delaying the reduction of antioxidant enzymes during the first 30 days of storage. There was no significant difference in alleviating chilling symptoms of peach fruits between CA and CA with high $O_2$ treatment.

## Acknowledgement

This work was financed by the National Science Fund for Distinguished Young Scholars of China (30225030) and the National Natural Science Foundation of China (30170663).


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