Scientia Horticulturae 225 (2017) 547–552

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Research Paper

Hypobaric storage reduced core browning of Yali pear fruits

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ARTICLE INFO

Keywords:
Pears fruit
Respiratory enzyme
Browning
Quality
Electron transport chain

ABSTRACT

Core browning is a major issue of pear fruits. The primary factor leading to development of core browning is high levels of CO_2 in storage. Hypobaric storage is a new postharvest technique to extend the shelf life of fruits and can reduce CO_2 partial pressure. In this study, the effect of hypobaric storage on core browning of Yali pear fruits (*Pyrus bretschneideri* Rehd.) was evaluated. Results showed that fruits after 0.025 MPa hypobaric treatment had 37.3% lower rate of core browning development and higher acidity and soluble solid content than control fruits. In addition, 0.025 MPa hypobaric treatment increased activities of cytochrome oxidase (CCO) and peroxidase (POD), and 2,2-diphenylpicrylhydrazyl (DPPH) radicals and superoxide anion scavenging capabilities. In comparison with the control, hypobaric treated fruits had lower activity of polyphenol oxidase (PPO) and higher level of total polyphenols. Hypobaric treatment also reduced an increase of succinate dehydrogenase (SDH) and glucose-6-phosphate dehydrogenase (G-6-PDH) activities. Results indicated that hypobaric treatment could control core browning of pear fruits by regulating reactive oxygen species (ROS) generation and elimination enzymes to maintain cell membrane integrity and reduce enzymatic browning.

1. Introduction

Core (Heart) browning is one of physiological disorders of pears during storage (Yan et al., 2013; Cheng et al., 2015). Brown discoloration often initiates from the core tissue of fruits and then extends to the whole pulp. Since pears with serious core browning are unacceptable to consumers, it is an urgent need to find an effective method to decrease the development of core browning. High levels of CO_2 is considered as the major cause inducing the core browning (Franck et al., 2007; Cheng et al., 2015). Previous studies showed that core browning was prevented by reducing the concentration of CO_2 in storage (Pintó et al., 2001; Veltman et al., 2003).

Hypobaric storage is a kind of storing system, in which air is ventilated at less than atmospheric pressure (Wang and Dilley, 2000) and the partial pressure of CO_2 is reduced (Hashmi et al., 2013b). Thus, internal CO_2 of fruits is escaped from fruit tissues to maintain the gas equilibrium (Hashmi et al., 2016). In addition, respiration rate of fruits is slowed down under hypobaric condition so as to reduce CO_2 production (Wang and Dilley, 2000). Hence, hypobaric storage may be used to remedy the physiology disorders caused by CO_2. However, there is little information on using hypobaric method to prevent pears from the development of core browning.

It is known that polyphenoloxidase (PPO) catalyzes oxidation of polyphenols, which leads to core browning of pears (Veltman et al., 1999). Since PPO and polyphenol are mostly located in different cells, oxidation-caused browning would occur until the intracellular membrane is broken down. Our previous studies showed that intracellular membrane can be oxidized by excessive reactive oxygen species (ROS) (Li et al., 2010), which can be produced from the electronic transport chain and scavenged off by antioxidant enzymes. Thus, control on activities of key enzymes of the electron transport chain and antioxidant system would be a potential postharvest treatment for prevention of enzymatic browning (Pintó et al., 2001). However, the influence of hypobaric storage on ROS generation and elimination systems remains unknown. In this work, we measured the core browning rate under hypobaric storage of pear fruits. In addition, fruit quality, enzymatic activity, and cellular ultra-structure were evaluated under optimal hypobaric condition.

2. Materials and methods

2.1. Fruits

Yali pears (*Pyrus bretschneideri* Rehd.) were collected from an orchard in Beijing, China. After harvest, fruits were immediately transported to the laboratory within 2 h. The fruits with similar color and maturity and without mechanical damage, pests and diseases were selected for the experiments.

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http://dx.doi.org/10.1016/j.scienta.2017.07.031
Received 24 May 2017; Received in revised form 14 July 2017; Accepted 20 July 2017
Available online 07 August 2017
0304-4238/

2.2. Fruit treatments

Experiment 1: For hypobaric treatment, fruits were stored in sealed tanks with 0.025 MPa, 0.05 MPa, and 0.075 MPa of vacuum degree, respectively, while fruits stored at atmosphere condition were used as control. For each treatment, three tanks were used as three repeats. All fruits were stored in 0.5 ± 0.5 °C cold room. 120 fruits were used for each treatment. The rate of core browning development was evaluated after 120 days of storage.

Experiment 2: Pears (n = 200) were stored under 0.025 MPa conditions. Fruits stored at atmosphere conditions were used as control. All fruits were stored in 0.5 ± 0.5 °C cold room. Fruits (n = 200) were sampled after 0, 30, 60, 90 and 120 days of storage, respectively. Fruit quality and enzymatic activity were evaluated.

2.3. Determination of core browning rate

As described by Lin et al. (2007), pear fruits were cut in half through the fruit transverse-section and core or tissue of fruits with any browning area was considered as core browning fruits. The rate of core browning development was statistically analyzed.

2.4. Fruit quality measurement

Soluble solid content (SSC) was measured by an Atago digital refractometer (PAL-1, Japan). Three replications of 10 fruit each were used.

Titratable acid (TA) content was determined by titration with 0.01 mol/L sodium hydroxide (NaOH). TA content was calculated as malic acid. Each measurement was repeated three times.

The LFRA texture analyzer (FHM-5, Japan) (12 mm diameter probe) was used to measure firmness. After removed the peel, three measuring points were taken around the middle of the fruit. The moving speed of the probe was 1 mm/s and the puncture distance was 10 mm. Maximum forces was recorded as fruit firmness. Ten fruits were used for each treatment.

2.5. Enzymatic activity analysis

Succinate dehydrogenase (SDH) activity was assayed according to the method of Li et al. (2016) with minor modifications. One unit of SDH activity was defined as a change of 0.01 at 600 nm absorbance per minute under the assay conditions.

Cytochrome oxidase (CCO) activity was determined according to the method of Jin et al. (2013). One unit of CCO activity was defined as an increase of 0.01 in absorbance at 510 nm per minute under the assay conditions.

Glucose-6-phosphate dehydrogenase (G-6-PDH) activity was measured according to the method of Corpas et al. (1998) with slight modifications. The activity of G-6-PDH was assayed spectrophotometrically by recording the reduction of NADP+ at 340 nm, where one unit of activity was defined as amount of the enzyme required to reduce 1 nmol of NADP+ per minute.

Alcohol dehydrogenase (ADH) activity was conducted by following the method of Wang et al. (2009). Absorbance was measured at 340 nm. One unit was defined as a decrease of 0.001 in absorbance per minute at 340 nm. ADH activity is expressed as U/g fresh weight (FW).

Peroxidase (POD) activity was assayed according to the method described by Li et al. (2016). 0.3 mL of enzyme solution was homogenized with 2 mL of 8 mmol/L guaiacol (pH = 6.4) and 1 mL of 24 mmol/L H_2O_2. Absorbance at 470 nm was measured. One unit of POD activity was defined as a change of 0.001 in absorbance per minute at 470 nm. The enzyme activity was expressed as U/g FW.

Polyphenol oxidase (PPO) activity was measured using the method of Jiang et al. (2002). 0.3 mL enzyme solution was mixed with 2.5 mL of catechol. Absorbance at 398 nm was measured. The enzyme activity was expressed as U/g FW, where one unit was defined as a change of 0.001 in absorbance per minute at 398 nm.

2.6. Pro- and anti-oxidant substances

The content of malondialdehyde (MDA) was determined according to the method of Wang et al. (2004). The absorbance of supernatant was measured at 532 nm and 600 nm, respectively, and the lipid peroxidation product content was calculated. MDA content was expressed as nmol/g FW.

The radical-scavenging activity of 2,2-diphenylpicrylhydrazyl (DPPH) was measured following the method of Larrauri et al. (1998). 50% of DPPH radical-scavenging activity was defined as an activity unit and DPPH radical-scavenging activity was expressed as U/g FW.

Superoxide anion scavenging activity was measured by scavenging the superoxide radicals generated in a riboflavin-light-nitro blue tetrazolium (NBT) system (Saeed et al., 2012). The changes of absorbance at 560 nm were recorded. One unit was defined as 50% of superoxide anion radical-scavenging activity.

Total polyphenol content was assayed using a modified Folin-Ciocalteu colorimetric method (Cao et al., 2011). The content of total polyphenol was expressed as micrograms of gallic acid equivalent per gram of fresh weight.

2.7. Cellular ultra-structure of core tissue

At the end of storage, cellular ultra-structure of core tissue was measured according to the method of Dong et al. (2015). The samples of core tissue were fixed in 2% (v/v) glutaraldehyde and 4% (w/v) paraformaldehyde in 0.1 mol/L phosphate buffer (pH 7.2) for 24 h at 4 °C, and then fixed in 1%(w/v) OsO_4 at 4 °Cfor 3 h and dehydrated by passing through a 30–100% gradient of ethanol-water solution. The samples were embedded in the resin. Ultra-thin sections (75 nm thick) were cut with a diamond knife on an ultra-microtome. Ultra-thin sections were counterstained with 5% (w/v) uranyl acetate for 20 mins and then exposed to a 2% (w/v) lead citrate solution for 3 mins. A transmission electron microscope (H-7650, Hitachi, Tokyo, Japan), at 80 kV, was used to measure cellular ultra-structure of core tissue.

2.8. Statistical analysis

The experiments were carried out in two years with similar results. For each harvest date, the experiment was conducted as a completely randomized design with three repeats. Analysis of variance was performed by ANOVA or T-test and $P < 0.05$ was considered as significance difference. Data were summarized as means ± standard errors in figures.

3. Results

3.1. Impact of hypobaric treatment on core browning

Results showed that all the rate of core browning development of the pear fruits stored under 0.025 MPa, 0.05 MPa, 0.075 MPa pressures were significantly lower ($P < 0.05$) than the control, in a dose dependent pattern (Fig. 1). 0.025 MPa hypobaric treatment significantly reduced the core browning rate by 37.3% in comparison with the control.

3.2. Impact of hypobaric treatment on fruit quality

As shown in Fig. 2A, no significant changes of soluble solid content (SSC) were observed in both control and 0.025 MPa treated fruit in the first 30 days of storage. After 30 days of storage, SSC in control decreased dramatically, whereas that in fruits of hypobaric treatment kept at stable level. At the end of storage, SSC in fruits treated with