



Effect of micro-vacuum storage on active oxygen metabolism, internal browning and related enzyme activities in Laiyang pear (*Pyrus bretschneideri* Reld)



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ARTICLE INFO

Article history:

Received 16 October 2015

Received in revised form

4 May 2016

Accepted 5 May 2016

Available online 7 May 2016

Keywords:

Laiyang pear

Micro-vacuum storage

Reactive oxygen species (ROS)

Antioxidant enzymes

Internal browning

ABSTRACT

To investigate the effects of micro-vacuum (MV) storage on Laiyang pear fruit senescence, freshly harvested Laiyang pears (*Pyrus bretschneideri* Reld) were subjected to MV storage (70 ± 5 kPa) and atmospheric pressure storage at 3 ± 1 °C for 120 days. MV storage was found to be more effective in maintaining post-harvest Laiyang pear quality, preventing increased respiration and decreased firmness and inhibiting internal browning. Furthermore, MV storage reduced the rate of $O_2^{\cdot -}$ production and the accumulation of H_2O_2 and malondialdehyde (MDA), and maintained significantly higher activities of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD), although the activities of lipoxygenase (LOX) and polyphenol oxidase (PPO) were inhibited. These results indicate that the delay in senescence and ripening of Laiyang pears stored under MV is conditions due to inhibition of respiration and maintenance of higher antioxidant enzymes activity (SOD, CAT, POD), leading to reduced reactive oxygen species (ROS) accumulation, lower PPO activity and better cell membrane integrity.

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1. Introduction

Laiyang pear (*Pyrus bretschneideri* Reld) is a well-known cultivar, which has been grown widely in Shandong Province for many years. This variety has excellent taste qualities with high nutritional value and is conducive to curing phlegm and relieving coughs (Liu, Lai, Xu, & Tian, 2013). As a climacteric fruit, the ripening of Laiyang pear is dependent on post-harvest storage conditions, which should not exceed one month in air at room temperature (Li et al., 2013). The post-harvest shelf-life of pears is limited because of their rapid senescence once ripening has been triggered, which is a serious problem for the marketing of this fruit.

Disruption of the integrity of the cell membrane, resulting in the loss of semi-permeability, is one of the preliminary and main features of senescence (Paliyath & Droillard, 1992). During fruit ripening and senescence, cell homeostasis and a disproportionate increase in reactive oxygen species (ROS) production, cause oxidative stress (Mittler, 2002). Plants have different enzymatic

systems to deal with this oxidative stress (Jiménez et al., 2002). Among the enzymatic systems that remove ROS, catalases (CAT), peroxidases (POD) and superoxide dismutases (SOD) play key roles in ROS removal. Some studies show that oxidative stress is also induced by a rise in lipid peroxidation, a process which is catalyzed mainly by lipoxygenase (LOX) and results in ROS generation (Marangoni, Palma, & Stanley, 1996; Gardner, 1995). Moreover, the internal browning associated with pear fruit senescence occurs due to the action of polyphenol oxidase (PPO), which catalyzes the oxidation of phenolic compounds (Tomás-Barberán & Espín, 2001). The balance between ROS production and their removal by the antioxidant defense systems determines the speed of ripening and senescence and, therefore, the fruit shelf-life (Mondal, Sharma, Malhotra, Dhawan, & Singhet, 2004). Various storage and treatment methods have been tested to extend the shelf-life and improve the post-harvest quality of different pear cultivars. These approaches include refrigeration storage (Li et al., 2013), modified atmosphere packaging (MAP) (Wang & Sugar, 2013), and controlled atmosphere storage (CA) (Liu et al., 2013; Lara et al., 2003) as well as treatment with plant oil (Ju, Duan, & Ju, 2000), 1-methylcyclopropene (1-MCP) (Li et al., 2013), and chitosan (Ochoa-Velasco & Guerrero-Beltrán, 2014).

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Although the senescence and decay of pears can be controlled by means of storage and treatment methods, some technical drawbacks limit the application of these technologies for extending the shelf-life of pears. For example, MAP methods in which the fruit are packaged under high CO₂ or low O₂ increase physiological damage to the fruit and accumulation of acetaldehyde leading to flavor changes (Lange, 2000). CA storage has detrimental effects on fruit qualities such as water content, ascorbic acid levels (Veltman, Kho, Schaik, Sanders, & Oosterhaven, 2000) and aroma (Lara et al., 2003).

Our previous study which data have not yet been published revealed that the use of MV storage equipment (70 ± 5 kPa) decreased Laiyang pear respiratory intensity, ethylene production and inhibited the reduction of fruit weight and firmness. This indicated the ability of MV storage systems to prolong the shelf-life and to maintain the quality of Laiyang pears. In the present study, we performed an evaluation of the capacity of micro-vacuum (MV) storage involving modified hypobaric storage equipment (based on Wang's (2004) invention patent CN1530290A) to delay senescence and extend the post-harvest life of Laiyang pears during cold storage, without negative effects on the final quality. A special emphasis was placed on determining the effects of this storage equipment on ROS production and the capacity of enzymatic systems to maintain the equilibrium between oxidation and antioxidant activity.

2. Materials and methods

2.1. Experimental apparatus

The experimental MV storage equipment (Fig. 1) was designed and manufactured by the Food Science and Engineering College of Qingdao Agriculture University (China). This equipment consisted of a vacuum pump, compressor, moistener, soft airbag, storage chamber (120 cm × 75 cm × 95 cm), and a control box. This equipment was placed in refrigerated housing (3 ± 1 °C, 85–95% relative humidity [RH]).

2.2. Plant materials and treatments

Laiyang pears were harvested from plantations in Laiyang and transported to the laboratory of Qingdao Agricultural University, within 3 h of harvesting. Pears of uniform size, and free of scars and insects were selected and pre-cooled at 3 °C for 12 h. The pears were packaged using low-density polyethylene bags. Laiyang pears were divided into two groups (30 kg each). In the experimental group, pears were placed in the MV experimental equipment storage chamber, and stored for 120 days at 70 ± 5 kPa (3 ± 1 °C; 85–95% RH). In the control group, pears were stored under atmospheric pressure in a cold storeroom under the same conditions of temperature and RH. Freshly harvested pears were used as the 0 day sample. Eight pears were selected randomly to determine the fruit characteristics; this process was repeated three times.

2.3. Determination index

2.3.1. Determination of respiration rate

For each group, eight pears were sealed in 5 L gas-tight jars at 25 °C. After 3 h, a 50 mL gas sample was collected from each jar using a syringe. Respiration rates (expressed in mg CO₂ kg⁻¹ h⁻¹ FW) were determined by gas chromatography (Agilent, 7890A, USA) using a thermal conductivity detector and a PLOT capillary column (15 m × 320 μm × 15 μm). Three replicates were analyzed for eight pears per time-point.

2.3.2. Determination of firmness

Firmness (N) was determined by using a TA-XT plus texture analyzer (Stable Micro Systems, Godalming, UK) fitted with a 5 mm-diameter probe. The penetration rate was 1 mm/s, with a final penetration depth of 10 mm. The fruit was cut into two halves and placed the sample on the plate for the measurement. The fruit flesh firmness was measured on opposing sides of the each fruit equator. The maximum force of the first peak for extrusion were used to determine firmness of the fruits. Three replicates were analyzed for three pears per time-point.

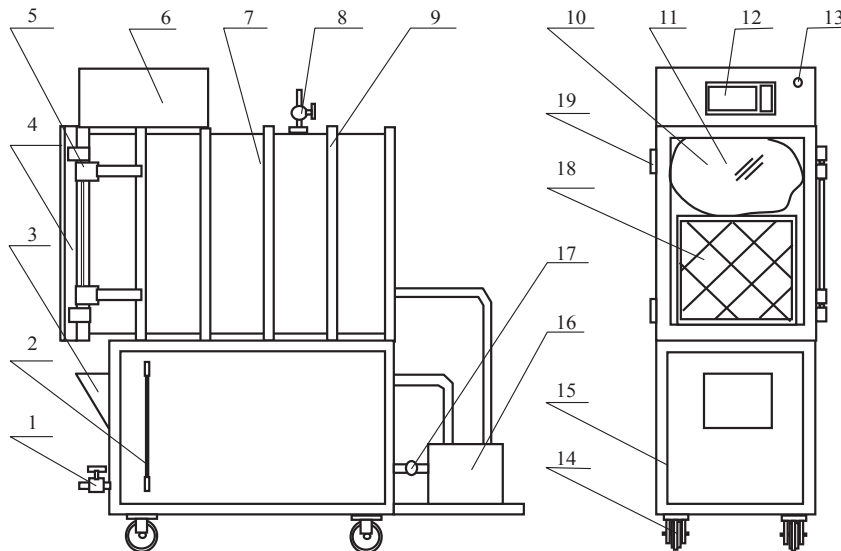


Fig. 1. Structure schematic diagram of Micro-vacuum storage experimental equipment 1 blow-down valve, 2 water level gauge, 3 filling pipe end, 4 air-tight door, 5 hinge, 6 control box, 7 storage chamber, 8 inlet or outlet air valve, 9 stiffener, 10 air bag, 11 glass, 12 PLC display screen, 13 indicator lamp, 14 return pulley, 15 water tank, 16 vacuum pump, 17 electromagnetic valve, 18 turnover box, 19 fastening handle.

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