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Effects of 1-methylcyclopropene on the physiological response of Yali pears to bruise damage

Jian Li[a], Jiaqi Yan[b], Mark A. Ritenour[b], Jingfa Wang[c], Jiankang Cao[c], Weibo Jiang[c,*]

[a] Beijing Engineering and Technology Research Center of Food Additives, Beijing Technology and Business University, Beijing 100048, PR China
[b] Indian River Research and Education Center (IRREC), University of Florida, Fort Pierce, FL 34945, USA
[c] College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, PR China

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ABSTRACT

Yali pear (*Pyrus bretschneideri* Rehd.) is a fragile fruit and very susceptible to bruise damage. In this study, effects of 1-methylcyclopropene (1-MCP) on the physiological response of bruised Yali pears were investigated. Pears were treated with 10 ppm 1-MCP before or after bruise damage, respectively, and then stored at 16 °C. Results showed that 1-MCP treatments both before and after delayed the decrease of firmness, total soluble solids (TSS) contents and titratable acid (TA) contents in damage fruit. Bruise volume and L^* value were also reduced by 1-MCP treatments. In addition, 1-MCP treated fruit had lower levels of hydrogen peroxide (H_2O_2) and superoxide radical production rate, associated with increased activities of catalase (CAT), superoxide dismutase (SOD) and ascorbate peroxidases (APX), as compared to the control. The protective effects of 1-MCP treatments on bruised fruits may be related to their ability to reduce and scavenge reactive oxygen species (ROS) by enhancing the antioxidant enzymes activity.

1. Introduction

Most fruits are susceptible to mechanical damage during harvest, transportation, handling and packing (Aliasgarian et al., 2013). Bruise, defined as damage to fruits caused by external forces (Boydas et al., 2014), is a common type of mechanical damage, which has become one of the most important factors limiting fruit shelf life. Bruise can lead to cellular alterations resulting in tissue browning, which reduces visual quality (Opara and Pathare, 2014). Bruise damage can also increase fruit senescence. Held et al. (2015) reported that tomato firmness declined rapidly after bruising. In addition, wound ethylene is induced by bruise injury (Yu and Yang, 1980). The high concentrations of ethylene not only accelerate deterioration of the bruised fruit, but also affect nearby non-bruised fruit. Furthermore, bruised fruit are easily infected by pathogens, which in turn serves as inoculum to infect nearby healthy fruit (Li et al., 2012). Therefore, there is an urgent need to develop an effective method to reduce loss caused by bruise damage.

Multiple studies suggested that appropriate postharvest treatments could prevent fruit mechanical damage (Lee et al., 2005; Martínez-Romero et al., 2002). Calcium and heat treatments applied before storage have been used to protect plum fruit against stress (Serrano et al., 2004). Pérez-Vicente et al. (2002) reported that treatment with polyamines inhibited tissue softening caused by mechanical damage in plum fruit. Putrescine has also been used to reduce the bruising response of apricot (Martínez-Romero et al., 2002) and lemon (Martínez-Romero et al., 1999) fruit.

1-Methylcyclopropene (1-MCP) is a synthetic fruit ripening regulator and has been used to maintain quality of bruised fruit. A 1-MCP treatment was able to improve the storage life and resistance to mechanical bruising of 'Blackamber' plums (Candan et al., 2006). In addition, 1-MCP treatments extended shelf life of bruised plums (Lippert and Blanke, 2004) and apricots (De Martino et al., 2006). However, there is a limited information on the effect of 1-MCP on the physiological response of bruised Yali pear, which is very susceptible to bruise damage.

The response of fruit to bruising may be reduced by the increase of reactive oxygen species (ROS) scanning activity (Ruuhola and Yang, 2006; Li et al., 2010). In fact, our previous findings showed that 1-MCP could enhance the antioxidant potential in non-bruised Yali pear (Fu et al., 2007). Therefore, in this study, we wonder whether 1-MCP treatments could reduce the physiological response to bruise damage. Fruit quality and bruise volume were determined. In order to explore the involvement of oxidative stress in the bruise injury, H_2O_2 contents, superoxide radical production rate and antioxidant enzymes activities were also determined.

* Corresponding author. Fax: +86 1062736565.
E-mail address: jwb@cau.edu.cn (W. Jiang).

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2. Materials and methods

2.1. Fruit

Yali pears (*Pyrus bretschneideri* Rehd.) were obtained from an experimental orchard at commercial maturity stage. Pears were packed individually in net bags and transported to the laboratory within 3 h of harvest. The fruit of uniform size and absence of mechanical injury or diseases were selected for the experiment.

2.2. Treatments

The selected fruits were randomly divided into 3 lots, each containing 160 fruits. In the 1st lot, pears were dropped from a height of 15 cm onto a smooth flat to simulate the bruise damage, and considered as control. Fruits from the 2nd lot were bruised as described above and then immediately treated with 10 ppm 1-methylcyclopropene (1-MCP) (EthylBloc, Rohm and Haas China, Inc.) for 24 h at 16 °C. Pears from the 3rd lot were treated with 10 ppm 1-MCP for 24 h at 16 °C and then bruised as described above. After treated, all fruits were stored at 16 °C. Fruits were sampled at the 15th, 30th, 45th and 60th day of storage. Samples were directly frozen in liquid nitrogen, and then stored at -80 °C until analyzed.

2.3. Determination of bruise damage

For bruise volume determination, thirty fruits were cut through the center of bruise. The depth (h) and diameter (d) of the brown area were evaluated by vernier caliper. Bruise volume (cm^3) was calculated based on the formula given by Saltveit (1984):

$$BV(cm^3) = \frac{\pi d}{24}(3w^2 + 4d^2)$$

in which d is the full depth of bruise and w is the bruising width across the major axes.

L^* value was also used as a measure for fruit bruise. L^* value of the damage zone was measured by a colorimeter (WCS-S, China).

2.4. Measurement of fruit quality

Firmness was determined at two freshly pared sites on opposite sides of the equator of each fruit with a firmness tester (FHM-5, Japan) (12 mm diameter probe). Ten fruits were used at each experiment.

Pulp (20 g) from 10 fruit was homogenized to extract the juice for testing total soluble solids (TSS) and titratable acid (TA). TSS content was determined by a refractometer (PAL-1, Japan). TA content was measured by titrating with 0.01 M NaOH.

2.5. Reactive oxygen species (ROS) assessment

Hydrogen peroxide (H_2O_2) was extracted by homogenizing 10.0 g fruit pulp with 10 ml acetone and determined according to the protocol of Prochazkova et al. (2001). H_2O_2 content was expressed as $\mu mol\ g^{-1}$ fresh weight (FW).

Superoxide radical was extracted by homogenizing 5.0 g fruit pulp with 5 ml of 50 mM phosphate buffer (pH 7.8). Superoxide radical production rate was measured as described by Elstner (1976) and expressed as $nmol\ min^{-1}\ g^{-1}$ FW.

2.6. Enzymatic activity analysis

10.0 g fruit pulp was homogenized with 10 ml of 0.1 M phosphate buffer (pH 7.8, containing 0.2 g polyvinypolypyrolidone), and then centrifuged at 10,000 × g at 4 °C for 20 min. The supernatant was collected for enzymatic activities assay.

Catalase (CAT) activity was measured as described by Klapheck et al. (1990). One unit was defined as $0.01 \triangle OD240\ min^{-1}$. CAT activity was expressed as $U\ g^{-1}$ FW.

Ascorbate peroxidase (APX) activity was determined according to the method of Nakano and Asada (1981) by monitoring the decline in absorbance at 290 nm. APX activity was expressed as $U\ g^{-1}$ FW, where one unit was defined as $0.01 \triangle OD290\ min^{-1}$.

Superoxide dismutase (SOD) activity was determined using the method of Gay and Tuzun (2000). SOD activity was expressed as $U\ g^{-1}$ FW. One SOD unit was defined as the amount of enzyme that inhibit the nitroblue tetrazolium (NBT) photoreduction by 50%.

For peroxidases (POD), enzyme was extracted by 100 mM acetate buffer (pH 5.5). POD activity was determined according to the method of Hammerschmidt and Kuc (1982) by the oxidation of guaiacol, which was measured the absorbance at 470 nm. POD activity was expressed as $U\ g^{-1}$ FW, where one unite was defined as $0.01 \triangle OD470\ min^{-1}$.

2.7. Statistical analysis

The experiments were carried out at two years with similar results. For each harvest date, the experiment was conducted as a completely randomized design with three treatments. Significant differences were compared using Duncan's multiple range test or T-test with the statistical software SPSS 17.0 (SPSS Inc., Chicago, IL, USA). Differences were considered to be significant at the 95% confidence level. Data were summarized in figures as means ± standard errors.

3. Results

3.1. Effects of 1-methylcyclopropene (1-MCP) treatments on fruit quality in bruised Yali pears

As shown in Fig. 1a, 1-MCP treatments delayed the decrease of firmness. On the 60th day of storage, the fruit treated with 1-MCP before or after bruised were 14.3% or 9.8% firmer than the untreated fruit.

1-MCP treatments also maintained total soluble solids (TSS) content, which was 7.5% or 1.8% higher in the fruit treated with 1-MCP before or after bruised respectively than control fruit on the 60th day of storage.

Titratable acid (TA) content in the bruised fruit reached a peak on the 15th day of storage and then remarkably decreased, while TA content in 1-MCP treated fruit maintained at a stable level throughout of storage. At the end of storage, TA content in 1-MCP + bruised or bruised + 1-MCP fruit was 36.1% or 30.4% higher than the control.

3.2. Effects of 1-MCP treatments on bruise recovery in bruised Yali pears

For investigating the bruise recovery of bruised fruit, bruise volume was determined at the end of storage. As shown in Fig. 2a, both 1-MCP + bruised and bruised + 1-MCP treatments reduced bruise volume, which indicated that 1-MCP treatment could enhanced the recovery in bruised fruit. Bruise volume in the fruit treated with 1-MCP before or after bruised was 38.5% or 83.1% lower than the control.

L^* value had a similar tendency. L^* value in those fruit treated with 1-MCP before and after significantly higher ($P < 0.05$) than that in the fruit without 1-MCP treatment, which showed that 1-MCP could reduce fruit brown after bruised.