Effect of 1-methylcyclopropene on Ripening and Superficial Scald of Japanese Pear (*Pyrus pyrifolia* Nakai, cv. Akemizu) Fruit at Two Temperatures

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In this paper, effect of 1-methylcyclopropene (1-MCP) on ‘Akemizu’ pear fruit was investigated to evaluate fruit quality, respiration rate, ethylene release rate, membrane lipid peroxidation and superficial scald at room temperature (25±2°C) for 20 d and at low temperature (4±1°C) for 72 d. The results showed that at two temperatures, 1-MCP treatment inhibited obviously the declines of fruit quality during storage, suppressed strongly respiration rates and delayed the ethylene peaks. Furthermore, compared with the control, lipoxidase (LOX) activities and malondialdehyde (MDA) contents of 1-MCP-treated fruits appeared lower at two temperatures. The development of fruit superficial scald was alleviated to a great extent by 1-MCP or low temperature, and superficial scald could be completely inhibited under 1-MCP plus cold condition. The contents of α -farnesene and conjugated trienols in 1-MCP-treated or cold-treated fruits were significantly lower than in untreated fruits.

Keywords: Japanese pear (*Pyrus pyrifolia* Nakai), 1-methylcyclopropene, ripening, superficial scald

Introduction

Pear (*Pyrus pyrifolia* Nakai) is one of the most important fruit in China. ‘Akemizu’ pear is a precocious Japanese pear cultivar with golden yellow appearance and high quality, which is introduced to China from Japan in recent years. Just as the other early ripening Japanese cultivars, ‘Akemizu’ pear has a short storage period at room temperature. Softening and superficial scald during storage are serious commercial problems. Because of the delicate nature of the fruit, poor handling practices, and inadequate transportation and storage facilities in China (Gong *et al.*, 2007), proper handling techniques and control of the ripening process are crucial for the development of a good pear industry in China.

The ripening process of pear fruit is regulated by ethylene (Itai *et al.*, 1999, 2003). Therefore, methods for preventing ethylene accumulation or inhibiting ethylene action in pear fruit would be of considerable commercial interest. It has been presumed that 1-methylcyclopropene (1-MCP), an inhibitor of ethylene receptors, can tightly bind to ethylene binding sites of ethylene receptors in plant tissues (Serek and

Sisler, 2001), thus preventing ethylene action after treatment. 1-MCP has been extensively applied on fruits at the experimental level to reveal the mechanisms controlling the physiology and biochemistry of the ripening processes (Blankenship and Dole, 2003). These data indicated that 1-MCP has a potentially commercial value in maintaining quality and extending storage life of fruit.

Previous studies have reported that 1-MCP could effectively delay many pear fruits ripening (Calvo and Sozzi, 2004; Ekman *et al.*, 2004; Isidoro and Almeida, 2006; Szczerbanik *et al.*, 2005; Itai and Tanahashi, 2008). However, effect of 1-MCP on cell membrane lipid peroxidation and superficial scald has not been elucidated in pear (*Pyrus pyrifolia* Nakai) fruit in detail.

The aim of this study was to assess the potential role of 1-MCP in controlling ripening of pear fruit at room and low temperatures, and to clarify the physiological mechanism concerning 1-MCP how to delay cell membrane lipid peroxidation and the occurrence of superficial scald of pear fruit.

Materials and Methods

Fruit harvest and 1-MCP fumigation ‘Akemizu’ fruit was harvested on day 112 after bloom from an orchard in

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Nanjing City, Jiangsu Province, China in 2006 and 2007, and transferred instantly to the lab after harvest. One thousand fruits with uniform size and free from defects were selected and divided randomly into two groups, which were sealed in two containers of the same specification ($1\text{ m} \times 1\text{ m} \times 1\text{ m}$), respectively. One group was treated with $1.0\text{ }\mu\text{l l}^{-1}$ 1-MCP for 12 h at room temperature ($25 \pm 2^\circ\text{C}$). The other group wasn't treated with 1-MCP as control. Then, a half of each group was stored at room temperature ($25 \pm 2^\circ\text{C}$), the other half at low temperature ($4 \pm 1^\circ\text{C}$) in two constant temperature storerooms ($10\text{ m} \times 10\text{ m} \times 6\text{ m}$) for later analysis, respectively. To eliminate the effect of atmospheric changes on fruit storage, the contents of CO_2 and O_2 in two rooms were determined by an air analyzer (Model O_2/T Telaire 700, SpectrumTM Technologies Inc, USA) once every day during fruit storage and the two gases contents kept consistent in the two rooms.

The needed amount of 1-MCP powder (0.14% 1-MCP; Rohm and Hass Company, PA, USA) was put into a bottle sealed with a septum cap at room temperature. After warm water (40°C) was injected therein to dissolve the powder, a fraction of the headspace was injected with a syringe into the fumigating container according to its void volume. The amount of product, water and the fraction of headspace needed to give a concentration of $1.0\text{ }\mu\text{l l}^{-1}$ 1-MCP were calculated according to the 1-MCP technical bulletin of the company.

Quality assays Fruit quality, including firmness, soluble solid content (SSC) and titratable acidity (TA), was measured once every 2 d in 15 fruits stored at room temperature and once every 8 d at low temperature. Flesh firmness was determined by using a Force Five pressure tester (Model FDV-30, Wagner Instruments, Greenwich, CT) with an 3.0 mm diameter flathead probe on opposite sides of peeled fruit. Then, SSC and TA were measured. Thirty slices from 15 fruits were squeezed for juice, SSC was determined by a hand refractometer and TA content was determined by titration of 2 ml juice with 0.1 N NaOH, expressed as percentage of malic acid.

Respiration rate and ethylene release rate Respiration rate and ethylene release rate were measured once every 2 d in 6 fruits stored at room temperature and once every 8 d at low temperature. Respiration rate was measured using an air analyzer (Model O_2/T Telaire 700, SpectrumTM Technologies Inc, USA) at room temperature after the fruits were sealed in a 9.6 L container for 15 min. CO_2 concentration in the container was recorded once every 5 min, totally for 45 min. The result was expressed as $\text{nmolCO}_2\text{ g}^{-1}\text{ FW min}^{-1}$. Then, the fruits were sealed in the above container for 2 h at room temperature to measure ethylene release rate. Samples of 600 μl

headspace gas were drawn from the container to measure the ethylene release rate by GC-9A gas chromatography (Shimadzu Seisakusho Co., Kyoto, Japan), which was equipped with a FID detector. The detector operated isothermally at 120°C through a Porapak N column ($3.2\text{ mm} \times 2\text{ m}$; 80/100 mesh) maintained at 70°C with N_2 as the carrier gas of which flow was 20 ml min^{-1} .

Membrane lipid peroxidation assessment Membrane lipid peroxidation of fruit was estimated by lipoxygenase (LOX, EC 1. 13. 11. 12) activity and the level of malondialdehyde (MDA) production. LOX activity was measured once every 2 d in 15 fruits stored at room temperature and once every 8 d at low temperature by the method of Axelrod *et al.* (1981). Two grams of flesh tissue added with 5 ml ice-cold 50 mM pH7.0 phosphate buffer were ground in mortar with a pestle. The homogenate was centrifuged at 4,000 rpm at 4°C for 20 min, and the supernatant was used as crude enzyme extraction. The activity of LOX was measured spectrophotometrically at 234 nm using sodium linoleate as substrate. The substrate was prepared as follows: 70 mg of linoleic acid and 70 ml Tween-20 were mixed in 4 ml of oxygen-free water and 0.5 M NaOH was added to clear the solution. Finally, the volume of the solution was increased to 25 ml with distilled water and then stored at -70°C for later use. The absorbance of the mixture containing 2.775 ml 0.2 M pH 6.5 phosphate buffer, 25 μl substrate solution and 0.2 ml enzyme extraction was recorded at 234 nm. LOX activity was expressed as $\Delta A_{234}\text{ min}^{-1}\text{ g}^{-1}\text{ FW}$. The MDA content was measured by thiobarbituric acid (TBA) method (Hodges *et al.*, 1999).

Superficial scald assessment Superficial scald of fruits was evaluated visually. 15 Fruits of each treatment were individually ranked according to an index system, that is no scald = 0, < 25% = 1, 25-50% = 2, and > 50% = 3, based on the fruit surface area affected (Zanella, 2003). The scald index was normalized to 100 by multiplying the value by 100/3.

α -Farnesene and conjugated trienols analysis 15 Fruits of each treatment were removed from storage at regular intervals over storage periods. Extraction and analysis of α -farnesene and conjugated trienols were performed as described by Anet (1972) with a little modification. Peel was taken off from the equatorial region on opposite sides of 5 fruits, and disks about 10 cm^2 were excised from the peel and then immersed in 10 ml of hexane and incubated for 10 min at room temperature with agitation. After incubation the solvent was filtered through a Florisil column and the final volume was adjusted to 5 ml. After filtration, the absorbance of the extract was immediately measured at 232 nm and at the range of 281-290 nm with a UV-visible spectrophotometer (model UV-Vis Lambda 176, Perkin-Elmer Instruments,

USA). Concentrations of α -farnesene and conjugated trienols were calculated using the molar extinction coefficients $\epsilon_{232} \text{ nm} = 27,740$ for α -farnesene and $\epsilon_{281-290} \text{ nm} = 25,000$ for the conjugated trienols, and expressed as nmol cm^{-2} .

Statistical analysis Each assay was at least designed 3 replicate samples. Data were subjected to analysis of variance (ANOVA) according to treatment. All ANOVAS were analyzed using treatments as a statistical parameter at a significant level of $P < 0.05$, with SPSS11.0 statistical software.

Results

Fruit quality Figure 1 A, B and C illustrated that 1-MCP could maintain quality of fruits at room temperature.

During the prolonged storage, 1-MCP delayed significantly the reduction of flesh firmness, SSC and TA ($P < 0.05$). Flesh firmness of 1-MCP-treated fruit was always higher than that of the control during storage (Figure 1 A). The highest SSC was found in 1-MCP-treated fruit (Figure 1 B). TA content of the control decreased linearly after harvest, whereas that of 1-MCP-treated fruit did not decrease rapidly until day 12 (Figure 1 C). At low temperature (Figure 1 D, E and F), the fruits treated with 1-MCP could maintain good quality for a long time. After 48 d, the firmness of 1-MCP-treated fruit decreased by 34.9 N only, whereas that of control fruit decreased by 54.7 N (Figure 1 D). Moreover, SSC and TA were also significantly higher in 1-MCP-treated than in the

Fig. 1. Effect of 1-MCP on firmness (Panel A and D), soluble solids contents (SSC; Panel B and E) and titratable acidity (TA; Panel C and F) of pear fruits. The fruits of Panel A, B and C are stored at $25 \pm 2^\circ\text{C}$, those of Panel D, E and F are stored at $4 \pm 1^\circ\text{C}$. Each data point is the average of three independent samples. Vertical bars represent ($\pm$) standard deviations of the mean.

control at later stage ($P < 0.05$; Figure 1 E and F).

Respiration rate and ethylene release rate No obvious respiration peaks were observed during the whole storage stage of pear fruits at two temperatures, and respiration rates were significantly inhibited by 1-MCP (Figure 2 A and C). At room temperature, a rapid increase of respiration of the control fruits was found to begin on day 4 after harvest, and maintained at high levels until the end of experiment, whereas respiration rate of the 1-MCP treatments kept at a rather low level in the early 12 d. And then, it increased gradually. Even this, it was still much lower than that of the control ($P < 0.05$). At low temperature (Figure 2 C), 1-MCP inhibited respiration rate of fruit for a longer time than at room temperature (Figure 2 A).

Compared with the control, 1-MCP treatment postponed ethylene release peak for 4 d at room temperature (Figure 2 B). At low temperature (Figure 2 D), the ethylene peak of the control appeared on day 40 after harvest, whereas 1-MCP treatment delayed ethylene peak for 16 d. However, the value of the peak in 1-MCP-treatment fruits was higher than that of the control whether at room temperature or at low temperature (Figure 2 B and D).

Membrane lipid peroxidation As shown in Figure 3 A and C, the LOX activities in the control fruits increased linearly within 6 d of storage at room temperature or during days 8-48 of storage at low temperature, and then gradually decreased. In 1-MCP-treated fruits, the LOX activities kept generally lower levels during storage at two temperatures. The contents of MDA in the control fruits were always rapidly increasing during the early and middle periods of storage at figure 3 B and D. The contents of MDA in 1-MCP-treated fruits increased slowly and significant differences were statistically found between the control and 1-MCP treatment ($P < 0.05$).

Superficial scald Evaluation of scald incidence and severity was combined in a scald index as described. After fruits were stored at room temperature for 14 d, the scald index of 1-MCP-treated fruits was 3.7%, while the scald index of the control had been up to 64.8%, as 17.5 times high as that of 1-MCP treatment. $1.0 \mu\text{l l}^{-1}$ 1-MCP reduced substantially the severity of scald injury (Figure 4 A). Moreover, cold condition significantly reduced fruit scald index, compared with that at room temperature (Figure 4 A and B), 1-MCP plus cold condition prevented completely the occur-

Fig. 2. Effect of 1-MCP on respiration rate (Panel A and C) and ethylene release rate (Panel B and D) of pear fruits. The fruits of Panel A and B are stored at $25 \pm 2^\circ\text{C}$, those of Panel C and D are stored at $4 \pm 1^\circ\text{C}$. Each data point is the average of three independent samples. Vertical bars represent ($\pm$) standard deviations of the mean.

rence of scald incidence (Figure 4 B).

α-Farnesene and conjugated trienols When the control fruits were stored at room temperature, α -farnesene accumulated rapidly from $0.15 \text{ nmol cm}^{-2}$ at harvest to a peak of $51.14 \text{ nmol cm}^{-2}$ on day 10 after harvest, and then declined thereafter. α -Farnesene content in 1-MCP-treated fruits also

increased but was much lower rate than the control (Figure 5 A). After fruits were stored for 20 d, the maximum content of α -farnesene in 1-MCP-treated fruits was $31.06 \text{ nmol cm}^{-2}$, 60.7% of that in the control. At low temperature, the α -farnesene content was much lower than that of fruits at room temperature, and the maximum content of α -farnesene

Fig. 3. Effect of 1-MCP on LOX activity (Panel A and C) and MDA content (Panel B and D) of pear fruits. The fruits of Panel A and B are stored at $25 \pm 2^\circ \text{C}$, those of Panel C and D are stored at $4 \pm 1^\circ \text{C}$. Each data point is the average of three independent samples. Vertical bars represent ($\pm$) standard deviations of the mean.

Fig. 4. Changes of scald index in pear fruits treated with 1-MCP at $25 \pm 2^\circ \text{C}$ (Panel A) and $4 \pm 1^\circ \text{C}$ (Panel B). Each data point is the average of three independent samples. Vertical bars represent ($\pm$) standard deviations of the mean.

Fig. 5. Effect of 1-MCP on α -farnesene content (Panel A and C) and conjugated trienols content (Panel B and D) of pear fruits. The fruits of Panel A and B are stored at $25\pm 2^{\circ}\text{C}$, those of Panel C and D are stored at $4\pm 1^{\circ}\text{C}$. Each data point is the average of three independent samples. Vertical bars represent ($\pm$) standard deviations of the mean.

in control was only $18.56 \text{ nmol cm}^{-2}$ on day 32 after harvest, as about 3 times as that at room temperature, whereas content of α -farnesene in 1-MCP-treated fruit ranged from 0 to $6.24 \text{ nmol cm}^{-2}$ (Figure 5 C). Therefore, 1-MCP and low temperature could effectively inhibit α -farnesene accumulation in the fruit peel throughout the storage period.

No conjugated trienols could be detected in the first 4 d of the control fruits stored at room temperature. However, they increased rapidly afterward, which formed a peak about 20 nmol cm^{-2} on day 14 of storage (Figure 5 B). It was coincident with superficial scald index. 1-MCP treatment and cold storage inhibited significantly accumulation of conjugated trienols in pear peel ($P < 0.05$; Figure 5 B and D). During days 6–18 at room temperature and days 32–72 at low temperature, conjugated trienols contents of 1-MCP-treated fruits were much lower than those of the controls. Compared with the control, the levels of conjugated trienols in 1-MCP-treated fruit were reduced by 84.5% and 88.8% on day 14 at room temperature and day 48 at low temperature, respectively.

Discussion

Japanese pear (*Pyrus pyrifolia* Nakai) fruits are often attractive to consumers because of their aesthetic qualities of flavor and texture. However, Japanese pear is a soft fruit that suffers a rapid loss of quality during ripening, which contributes greatly to its short postharvest life and susceptibility to physiological disorder. The general objective in fruit storage is to slow down the ripening processes related to qualities of flavor and texture. Cold condition and 1-MCP are widely applied for this purpose in pear fruits (Ekman *et al.*, 2004; Bai *et al.*, 2006; Spotts *et al.*, 2007; Itai and Tanahashi, 2008). Fruit firmness is affected by cell turgidity and the structure and composition of the cell wall polysaccharides (Lohani *et al.*, 2004; Hayami *et al.*, 2004). The data indicated that maintenance of 1-MCP to fruit firmness (Figure 1) might also inhibit the activities of pectin degrading enzymes, which were related to fruit softening during senescence (Zhou *et al.*, 2007). Soluble solids and organic acids of fruits are substrates that are consumed by respiration during storage (Luo and Shen, 2006). In this study, Cold condition and 1-MCP treatment inhibited significantly respiration rate of fruit (P

< 0.05; Figure 2 A and C), the consumptions of SSC and TA reduced also evidently in treated fruits ($P < 0.05$; Figure 1).

In addition, according to the climacteric status, pears are divided into climacteric cultivars and non-climacteric cultivars (Downs *et al.*, 1991; Itai *et al.*, 1999). In climacteric cultivars, the fruit displays a high respiration rate in early storage stage and declined as fruit ripening. However, in non-climacteric cultivars, the respiration rate of fruit hasn't a great change during storage. As a early ripening Japanese cultivar, the respiration rate increased obviously in early storage stage after harvest (Figure 2 A) and the ethylene release rate had obvious peaks (Figure 2 B) in the control fruits of 'Akemizu' pear at room temperature. Furthermore, the ethylene release rate in control fruit ranged about from 6.85 to 19.22 nl g⁻¹ h⁻¹ at room temperature (Figure 2 B), belonging to middle level in Japanese pear (Itai *et al.*, 1999, 2003). Cultivars with middle or high ethylene release rate produced usually climacteric types (Downs *et al.*, 1991; Itai *et al.*, 1999). Therefore, 'Akemizu' pear was a climacteric cultivars.

At two temperatures, 1-MCP treatment not only inhibited obviously the respiration rates ($P < 0.05$; Figure 2 A and C), but also delayed effectively ethylene peaks ($P < 0.05$; Figure 2 B and D), which meant that 1-MCP treatment inhibited pear fruit ripening by regulating ethylene action and inhibiting ethylene biosynthesis (Cin *et al.*, 2006). In apple fruit, 1-MCP treatment caused an inhibition of ethylene production but also ACC synthase (ACS) activity, ACC oxidase (ACO) activity and ACC level, and ACC was partially transformed into malonyl-ACC (MACC) through the action of malonyl transferase (Vilaplana *et al.*, 2007). When plum fruits were stored at 0°C, 1-MCP treatment activated the conversion of ACC into MACC for 7 d, then MACC level kept stable, and the accumulation of ACC had a significant increase (Larigaudière *et al.*, 2009). In our study, ethylene peak values were higher in 1-MCP-treated fruits than in the control (Figure 2 B and D), which likely was the result that after middle storage stage, the regulating ability of 1-MCP to MACC accumulation decreased, thus the conversion of ACC into MACC stopped or slowed down, and ACC was accumulated rapidly in a short time, resulting in more ethylene production and higher ethylene peak values.

In the process of fruit ripening, reactive oxygen species are largely involved to destroy membrane integrity, causing lipid peroxidation (Jimenez *et al.*, 2002). As good indicators of lipid degradation, higher LOX activity and MDA content are closely correlated with cell lipid peroxidation during fruit ripening (Rogiers *et al.*, 1998). Data here showed that LOX activities in control fruits markedly increased at the early stage after harvest, whereas in 1-MCP-treated fruit, they kept low levels during storage at two temperatures (Figure

3 A and C). In general, MDA contents in control fruits were significantly higher than that in 1-MCP-treated fruit during storage at two temperatures ($P < 0.05$; Figure 3 B and D). Therefore, it suggested that 1-MCP treatment could inhibited cell lipid peroxidation, protecting membrane integrity in pear fruit.

Furthermore, 1-MCP inhibition to superficial scald has been reported recently in European pear cultivars (Ekman *et al.*, 2004; Isidoro and Almeida, 2006; Spotts *et al.*, 2007). However, there is no report on effect of 1-MCP on superficial scald of *Pyrus pyrifolia* Nakai. The mechanism of superficial scald development has not yet been completely understood. From the viewpoint of postharvest practice, α -farnesene metabolism may be a manipulating point to control superficial scald, a major physiological disorder in apples and pears (Ju and Curry, 2000; Isidoro and Almeida, 2006). The reactive conjugated trienols resulting from the *in vivo* oxidation of α -farnesene are believed to be directly responsible for primary events leading to development of superficial scald (Rowan *et al.*, 1995; Whitaker *et al.*, 1997). In our experiment, the accumulations of α -farnesene and conjugated trienols in control fruits had accumulating peaks during storage, and the peaks of conjugated trienols were always later than that of α -farnesene (Figure 5), which meant that α -farnesene fluctuation was followed by the accumulation of conjugated trienols. In cold-treated and 1-MCP-treated fruits, the accumulations of α -farnesene and conjugated trienols had much lower levels than that of the controls during storage periods, and 1-MCP plus cold condition had additional effects (Figure 5). As showed in Figure 4, cold condition or 1-MCP was evidently effective in inhibiting superficial scald in 'Akemizu' pear, and 1-MCP plus cold could completely inhibit occurrence of superficial scald. Above data indicated that cold condition and 1-MCP were effective ways to inhibit superficial scald, the accumulations of α -farnesene and conjugated trienols, and α -farnesene and conjugated trienols had crucial roles in development of superficial scald in oriental pears.

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