

Effects of 1-MCP on Softening, Yellowing and H₂O₂ Content in Post-harvest ‘Jingbaili’ Pear Fruit during and after Cold Storage

Yu Dong^{1,2}, Liqin Liu^{1,2}, Yingying Zhang^{1,2}, and Junfeng Guan^{1,2*}

¹Institute of Genetics and Physiology, Hebei Academy of Agriculture and Forestry Sciences, Shijiazhuang 050051, China

²Plant Genetic Engineering Center of Hebei Province, Shijiazhuang 050051, China

*Corresponding author: junfeng-guan@263.net

Received December 23, 2013 / Revised May 5, 2014 / Accepted June 23, 2014

© Korean Society for Horticultural Science and Springer 2014

Abstract. ‘Jingbaili’ pear (*Pyrus ussuriensis* Maxim.) fruit shows rapid softening, yellowing and short shelf-life at ambient temperature storage. In this study, ‘Jingbaili’ pear fruits were treated with 1.0 $\mu\text{L}\cdot\text{L}^{-1}$ 1-methylcyclopropene (1-MCP) for 24 hours at $25 \pm 2^\circ\text{C}$ and then stored at 0°C . After 60 and 120 days of cold storage at 0°C , pear fruits were removed and stored at $20 \pm 2^\circ\text{C}$ to assess their shelf-life. The results indicated that the 1-MCP treatment delayed the decrease in firmness and chlorophyll (*a*, *b*, and *a+b*) content of peel, reduced the rates of respiration and ethylene production, and inhibited the accumulation of hydrogen peroxide (H₂O₂), which was observed in the untreated fruits during and after cold storage. Less difference was found in soluble solids content (SSC) between the control and 1-MCP-treated fruits during storage. The correlation analysis showed that flesh H₂O₂ content was negatively related to firmness and also peel H₂O₂ content to peel chlorophyll *a+b* content. These results suggested that the 1-MCP treatment could delay the fruit softening and chlorophyll degradation by suppressing the accumulation of H₂O₂ content during and after cold storage in ‘Jingbaili’ pear.

Additional key words: 1-methylcyclopropene, chlorophyll content, hydrogen peroxide, *Pyrus ussuriensis* Maxim., softening

Introduction

The ‘Jingbaili’ pear (*Pyrus ussuriensis* Maxim.) fruit shows rapid softening and short shelf-life after harvest when stored at room temperature (Wei et al., 2009). In practice, pear fruits are harvested at the mature stage, and afterwards stored at low temperature to prolong their storage life. Strategies such as 1-methylcyclopropene (1-MCP) (Trincherio et al., 2004) and modified atmosphere packaging (MAP) (Wang and Sugar, 2013) have been applied to delay pear fruit softening. However, the mechanisms involved in the softening process are still not clear.

The 1-MCP, an inhibitor of ethylene action, has been shown to reduce ethylene production and delay fruit softening, and extensively used in many kinds of fruits, such as apple, apricot, plum, avocado, peach, nectarine and pear (Blankenship and Dole, 2003; Watkins, 2006; Watkins et al., 2000). It has been shown that ethylene could accelerate chlorophyll degradation in fruit peel (Jeong et al., 2002; Porat et al., 1999; Purvis and Barmore, 1981; Trebitsh et al., 1993). Recently, it was

proved that 1-MCP could effectively delay the chlorophyll degradation and suppress the expression of chlorophyll degradation-associated genes of peel in pear fruit (Cheng et al., 2012).

Oxidative stress from excess reactive oxygen species (ROS), such as hydrogen peroxide (H₂O₂), superoxide anion (O₂⁻), hydroxyl radical ([•]OH) and singlet oxygen (¹O₂), has been proved to promote the fruit ripening and senescence (Brennan and Frenkel, 1977; Brennan et al., 1979; Cheng et al., 2008). Fruits are protected against ROS damage by a complex antioxidant system. This involves lipid-soluble antioxidants (α -tocopherol and carotenoids), water-soluble reductants (glutathione and ascorbate) and enzymes such as catalase (CAT), glutathione reductase (GR), ascorbate peroxidase (APX) and superoxide dismutase (SOD) (Apel and Hirt, 2004; Ding et al., 2007). The 1-MCP has been shown to preserve higher antioxidant enzyme activities for scavenging ROS, and maintain cell-membrane integrity (Larigaudère et al., 2004; Yuan et al., 2010).

The aim of this work was to investigate the effects of

1-MCP on softening, yellowing and H₂O₂ production in ‘Jingbaili’ pear fruits during and after cold storage, to explore the role of ethylene and H₂O₂ in fruit softening and senescence.

Materials and Methods

Materials and Treatments

‘Jingbaili’ pear (*Pyrus ussuriensis* Maxim.) fruits were harvested from Daxing District, Beijing, China, at the commercially mature stage (September 10, 2010) and transported to the laboratory within 4 h. Approximately 280 kg fruits were selected for uniformity of weight (mean weight 133.5 ± 13.2 g per fruit) and shape without any visual defects.

Pear fruits were randomly divided into two groups. One group was put into plastic boxes (60 cm × 40 cm × 30 cm, L × W × H) and sealed tightly with a plastic bag (65 cm × 85 cm × 90 cm, L × W × H), then exposed to 1.0 μL·L⁻¹ 1-MCP (Dow Chemical, Beijing, China) as reported by Wei et al. (2009) at 25 ± 2°C for 24 h. The second group (the control) was exposed to air after being sealed under the same conditions. After treatment, most fruits from each group were immediately transferred to cold storage at 0°C, 75% relative humidity, and meanwhile, some fruits were sampled to determine respiration rate and ethylene production rate at 25°C, and also firmness, soluble solids content (SSC), chlorophyll (*a*, *b*, and *a+b*) and H₂O₂ content, which were indicated as initial value before storage. After 60 and 120 days of cold storage, the fruits from each group were placed at 20 ± 2°C and 75% relative humidity for shelf-life testing. For determination of respiration rate and ethylene production rate during storage, pear fruits from each group were sampled at 10, 20, 30, 60, and 120 days of cold storage and measured at 0°C, and did at 2-day intervals for 10 days at shelf storage and measured at 20°C. Three replicates were performed, 12 fruits each replicate. For measurements of firmness, SSC, chlorophyll and H₂O₂ content, pear fruits from each group were removed at 10, 20, 30, 60, and 120 days of cold storage and 5-day intervals for 10 days of shelf-life. Three replicates were performed, 10 fruits each replicate. H₂O₂ content measurements were performed with fresh peel and flesh tissue.

Firmness and SSC

Firmness was determined by using a digital fruit hardness meter (GY-4, Top Instrument, Hangzhou, Zhejiang, China), which was fixed to the fruit hardness testing shelf and equipped with a pressure head 11.1 mm in diameter. The firmness of each fruit was measured at two equidistant points on the equatorial region with the skin removed; it was manually assessed and the insertion depth of the pressure

head was held at 10 mm. Peak values were automatically calculated and expressed in Newton (N). Flesh from three equidistant points was pressed and the juice was measured for soluble solids content (SSC) using a pocket digital refractometer (PAL-1, Atago, Tokyo, Japan).

Respiration Rate and Ethylene Production Rate

For measurement of respiration rate, pear fruits were sealed in gas-tight containers (4 L) for 2 h. According to Zhang et al. (2005), a 1.0 mL gas sample was withdrawn using a gas-tight syringe to analyze respiration rate with an infrared CO₂ analyzer (HWF-1A, Kexi Instrument, Jiangsu, China). Data were expressed as mg·kg⁻¹·h⁻¹.

For measurement of ethylene production rate, pear fruits were sealed in gas-tight containers (4 L) for 4 h. A 1.0 mL gas sample was withdrawn using a gas-tight syringe and injected into a gas chromatograph (GC-9800, Kechuang Instrument, Shanghai, China) equipped with a GDX-102 column and a flame ionization detector. The column temperature was 78°C and the injection temperature was 120°C. The carrier gas was N₂ with a rate of 40 mL·min⁻¹. Ethylene production rate was calculated and data were expressed as μL·kg⁻¹·h⁻¹.

Chlorophyll Content

Twenty peel discs (1 cm²) from each group were extracted in 15 mL 80% (v/v) acetone. Absorbance was measured at 645 nm and 663 nm in a spectrophotometer (UV-2100, Unico Instrument, Dayton, NJ, USA). Chlorophyll (*a*, *b*, and *a+b*) content was calculated according to the equations of Arnon (1949). Data were expressed as mg·g⁻¹.

Hydrogen Peroxide Content

The H₂O₂ content was determined as described by Belincampi et al. (2000) with slight modifications. Fresh peel and flesh tissue (4.0 g) were homogenized in 8.0 mL 0.1 M phosphate buffer (pH 7.2) containing 10% (w/v) polyvinyl-pyrrolidone (PVPP). The homogenate were centrifuged at 10,000 g at 4°C for 15 min. A 500 μL aliquot of the supernatant was added to 1.0 mL assay reagent (500 μM ammonium ferrous sulfate, 50 mM H₂SO₄, 200 μM xylenol orange and 200 mM sorbitol) and 500 μL distilled water. The absorbance was measured at 560 nm. The mixture was incubated in the dark at 25°C for 30 min and H₂O₂ content was calculated from a standard curve using known concentrations of H₂O₂. Data were expressed as nmol·g⁻¹ fresh weight.

Statistical Analysis

All experiments were performed using a completely randomized design. All values are expressed as mean ± standard error (SE). Comparisons of all data were analyzed using a

simple analysis of variance (ANOVA) ($p < 0.05$). Statistical analysis was carried out using the SPSS statistical package (Version 13.0, SPSS Inc., Chicago, IL, USA).

Results

Changes in Firmness and SSC

Fruit firmness decreased 31.0 and 24.7% in the control and 1-MCP-treated fruits, respectively, after 120 days of cold storage (Fig. 1A). SSC in the control and 1-MCP-treated fruits gradually increased during cold storage (Fig. 1B). However, during subsequent storage at $20 \pm 2^\circ\text{C}$, firmness was more obviously decreased in the control fruits subjected to 120 days of cold storage than in the fruits subjected to 60 days of cold storage, and it was significantly higher in 1-MCP-treated fruits than that in the control fruits ($p < 0.05$) (Figs. 1C and 1E). There was less difference between control and 1-MCP-treated fruits in SSC during subsequent storage at $20 \pm 2^\circ\text{C}$ (Figs. 1D and 1F).

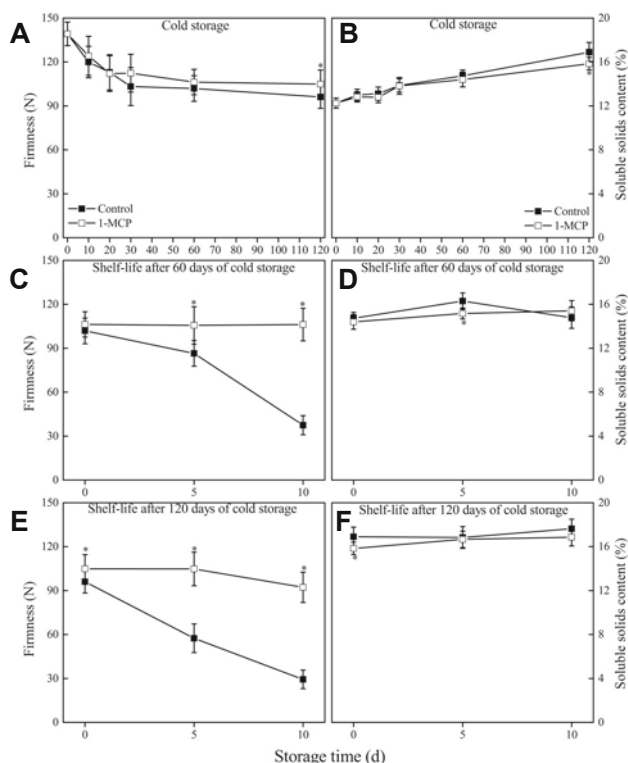


Fig. 1. Firmness and soluble solids content of 'Jingbaili' pear fruits treated with $1.0 \mu\text{L}\cdot\text{L}^{-1}$ 1-MCP (□) and the control (■) during cold storage (A and B), and during storage at $20 \pm 2^\circ\text{C}$ after 60 days (C and D) and 120 days (E and F) of cold storage. Each value is the mean \pm standard error (SE) of three replicates in one experiment. Vertical bars represent the standard errors of the means. Values with asterisks (*) indicate where significant differences are found between the control and 1-MCP treatment.

Changes in Respiration and Ethylene Production Rate

Rates of respiration and ethylene production were measured immediately after finish of 1-MCP treatment, the initial respiration rates were 22.74 and $15.63 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ and ethylene production rates were 5.90 and $1.31 \mu\text{L}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ in control and 1-MCP-treated fruits, respectively. Respiration rate showed more relatively constant while ethylene production rate had an increased trend in control and 1-MCP-treated fruits at cold storage (Figs. 2A and 2B). The rates of respiration and ethylene production had marked peaks at shelf storage subsequent to 60 days of cold storage, but no to 120 days. 1-MCP-treated fruits had significant lower rates of respiration and ethylene production than those in the control fruits at cold and shelf storage, and this was more obvious at shelf storage (Figs. 2C to 2F).

Changes in Chlorophyll Content

Chlorophyll content of the peel in the 1-MCP-treated fruits was higher than that in the control fruits after 30 days of

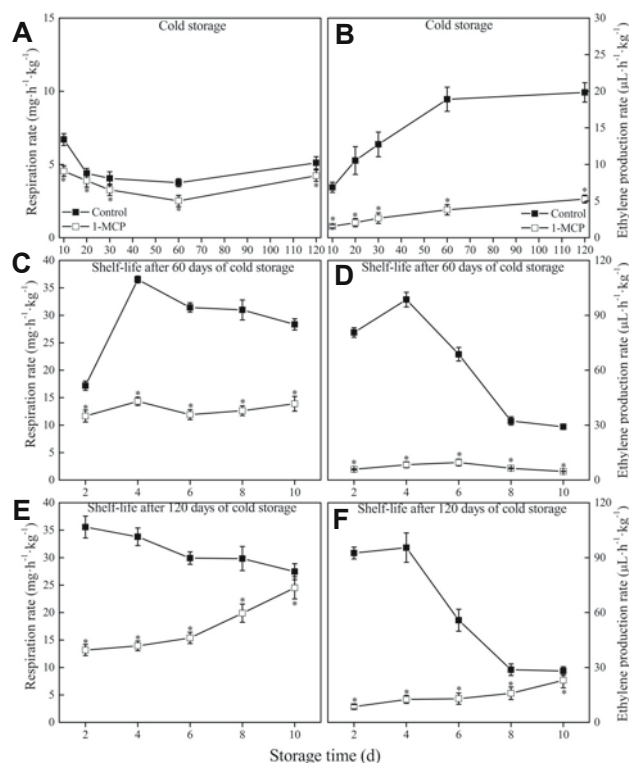


Fig. 2. Respiration rate and ethylene production rate of 'Jingbaili' pear fruits treated with $1.0 \mu\text{L}\cdot\text{L}^{-1}$ 1-MCP (□) and the control (■) during cold storage (A and B), and during storage at $20 \pm 2^\circ\text{C}$ after 60 days (C and D) and 120 days (E and F) of cold storage. Each value is the mean \pm standard error (SE) of three replicates in one experiment. Vertical bars represent the standard errors of the means. Values with asterisks (*) indicate where significant differences are found between the control and 1-MCP treatment.

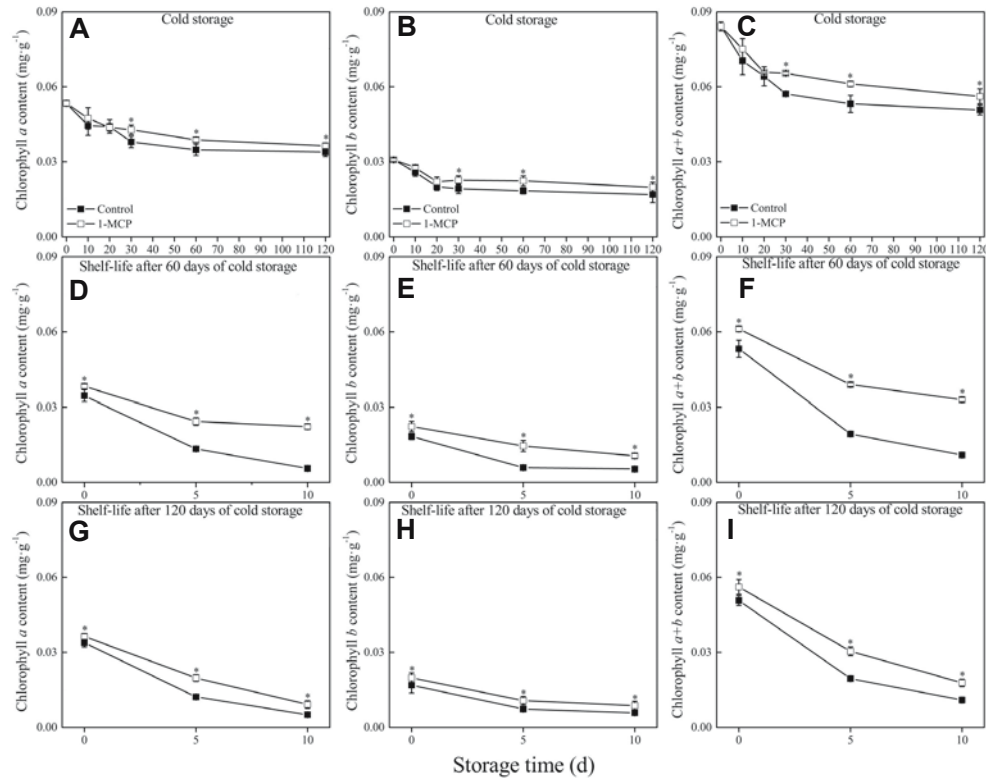


Fig. 3. Chlorophyll a, chlorophyll b, and chlorophyll a+b contents of 'Jingbaili' pear fruits treated with $1.0 \mu\text{L}\cdot\text{L}^{-1}$ 1-MCP (\square) and the control (\blacksquare) during cold storage (A, B, and C), and during storage at $20 \pm 2^\circ\text{C}$ after 60 days (D, E and F) and 120 days (G, H and I) of cold storage. Each value is the mean \pm standard error (SE) of three replicates in one experiment. Vertical bars represent the standard errors of the means. Values with asterisks (*) indicate where significant differences are found between the control and 1-MCP treatment.

cold storage (Figs. 3A to 3C). After fruits were transferred to storage at $20 \pm 2^\circ\text{C}$, the chlorophyll content in the control fruits decreased rapidly, and was inhibited by 1-MCP. The suppressive effect of 1-MCP treatment on the decrease of chlorophyll content was more marked in fruits subjected to 60 days of cold storage than those subjected to 120 days of cold storage (Figs. 3D to 3I).

Changes in H_2O_2 Content

The H_2O_2 content increased in both the peel and flesh tissue during and after cold storage, and this trend was more obvious in shelf-life after cold storage and was inhibited by 1-MCP treatment. The accumulation of H_2O_2 in peel tissue was higher than that in flesh tissue, and it was higher in peel and flesh tissue at shelf storage than that at cold storage (Fig. 4).

Relationship between Firmness, Chlorophyll a+b Content and H_2O_2 Content

There were significant linear relationships between firmness, chlorophyll a+b content and H_2O_2 content during and after cold storage in fruits. The significant negative correlation were observed between H_2O_2 content and chlorophyll a+b

content of peel ($r = -0.897^{**}$) (Fig. 5A), and between flesh H_2O_2 content and firmness ($r = -0.95^{**}$) (Fig. 5B).

Discussion

Fruit softening usually accompanied with more ethylene production. 1-MCP was involved in competition with ethylene receptor and has reduced the ethylene production (Sisler and Serek, 1997). In our experiment, 1-MCP showed a marked inhibition on respiration and ethylene production at shelf-life after cold storage (Fig. 2). However, the effect of 1-MCP became much weaker and was limited during shelf-life storage after a long-term of cold storage. These results were in agreement with effect of 1-MCP treatment on kiwifruit during shelf-life (Koukounaras and Sfakiotakis, 2007).

The changes in peel chlorophyll content reflect the external fruit quality during storage (Kuckenberg et al., 2008; Medlicott et al., 1986). In general, it was demonstrated that the main source of H_2O_2 in higher plants is from the photosynthetic electron transport chain and the location of H_2O_2 production is the thylakoid membrane in chloroplasts (Ivanov and Khorobrykh, 2003; Mubarakshina et al., 2006). If the system of H_2O_2 scavenging in the chloroplast broken down, they

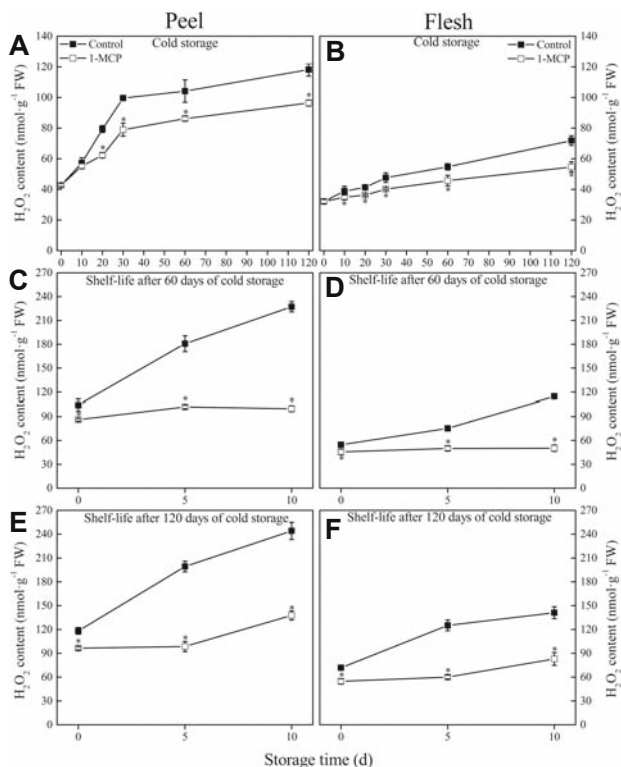


Fig. 4. H_2O_2 content of 'Jingbaili' pear fruits treated with $1.0 \mu\text{L}\cdot\text{L}^{-1}$ 1-MCP (\square) and the control (\blacksquare) during cold storage (A and B), and during storage at $20 \pm 2^\circ\text{C}$ after 60 days (C and D) and 120 days (E and F) of cold storage. Each value is the mean \pm standard error (SE) of three replicates in one experiment. Vertical bars represent the standard errors of the means. Values with asterisks (*) indicate where significant differences are found between the control and 1-MCP treatment.

would be transformed into reactive oxygen species, which induced lipid peroxidation and accelerated the fruit senescence (Bowler et al., 1992). And more, chlorophyll breakdown usually initial occur in chlorophyll *a* degradation (Ito et al., 1993; Scheumann et al., 1998). Our results supported this notion that the H_2O_2 content in peel tissue was higher than that in flesh tissue, and more chlorophyll *a* is the main ingredient in chlorophyll of peel and was showed a faster degradation than chlorophyll *b* (Fig. 3), and also there were significantly negative correlation between chlorophyll and H_2O_2 content of the peel (Fig. 5A).

The H_2O_2 mediate a series of responses to fruit ripening and senescence (Brennan and Frenkel, 1977). In this study, dramatic changes in H_2O_2 levels were observed in pear fruits treated with 1-MCP during storage, which correlated with fruit softening and chlorophyll degradation. Some studies have shown that H_2O_2 has no effect on membrane damage at steady-state levels, whereas higher concentrations H_2O_2 can result in degradation of fruit color, lipid peroxidation, and softening (Brennan and Frenkel, 1977; Jimenez et al., 2002; Scandalios, 1993). In addition, 1-MCP treatment led to de-

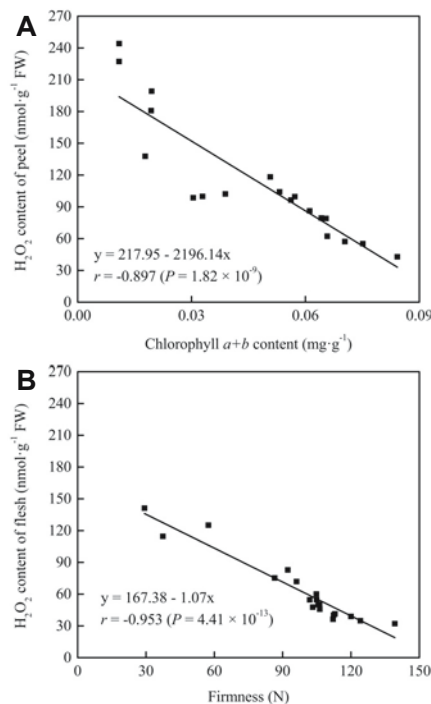


Fig. 5. The correlation between H_2O_2 content and chlorophyll (*a+b*) content of peel (A), and flesh H_2O_2 content and firmness (B) of 'Jingbaili' pear fruits treated with $1.0 \mu\text{L}\cdot\text{L}^{-1}$ 1-MCP and the control during and after cold storage.

creased levels of H_2O_2 and lipid peroxidation (Dong et al., 2011; Meng et al., 2011; Singh and Dwivedi, 2008), concomitant with increased activities of antioxidant enzymes including CAT and SOD, which alleviated the toxic effects of ROS (Fu et al., 2007; Yuan et al., 2010). Our studies showed that 1-MCP-treated fruits had lower H_2O_2 contents in peel and flesh tissue compared with the control fruits during and after cold storage (Fig. 4). Linked with our data showed that the H_2O_2 content was negatively related to firmness and chlorophyll *a+b* content (Fig. 5), thus, the effect of 1-MCP on H_2O_2 content might be contributed to the delaying of softening and yellowing.

In conclusion, 1-MCP application ($1.0 \mu\text{L}\cdot\text{L}^{-1}$) in post-harvest 'Jingbaili' pear fruits showed softening and yellowing, and reduced the accumulation of H_2O_2 content during and after cold storage. After long-term cold storage, 1-MCP appears to effectively maintain higher fruit quality and improve shelf-life.

Acknowledgement: This research was supported by the Earmarked Fund for China Agriculture Research System (with Project No. CARS-29-7B).

Literature Cited

Apel, K. and H. Hirt. 2004. Reactive oxygen species: Metabolism,

- oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.* 55:373-399.
- Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.* 24:1-15.
- Bellincampi, D., N. Dipiero, G. Salvi, F. Cervone, and G. De Lorenzo. 2000. Extracellular H₂O₂ induced by oligogalacturonides is not involved in the inhibition of the auxin-regulated *rolB* gene expression in tobacco leaf explants. *Plant Physiol.* 122:1379-1385.
- Blankenship, S.M. and J.M. Dole. 2003. 1-Methylcyclopropene: A review. *Postharvest Biol. Technol.* 28:1-25.
- Bowler, C., M. Van Montagu, and D. Inzé. 1992. Superoxide dismutase and stress tolerance. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 43:83-116.
- Brennan, T., A. Rychter, and C. Frenkel. 1979. Activity of enzymes involved in the turnover of hydrogen peroxide during fruit senescence. *Bot. Gaz.* 140:384-388.
- Brennan, T. and C. Frenkel. 1977. Involvement of hydrogen peroxide in the regulation of senescence in pear. *Plant Physiol.* 59:411-416.
- Cheng, G., X. Duan, J. Shi, W. Lu, Y. Luo, W. Jiang, and Y. Jiang. 2008. Effects of reactive oxygen species on cellular wall disassembly of banana fruit during ripening. *Food Chem.* 109:319-324.
- Cheng, Y., Y. Dong, H. Yan, W. Ge, C. Shen, J. Guan, L. Liu, and Y. Zhang. 2012. Effect of 1-MCP on chlorophyll degradation pathway-associated genes expression and chloroplast ultrastructure during the peel yellowing of Chinese pear fruits in storage. *Food Chem.* 135:415-422.
- Ding, Z.S., S.P. Tian, X.L. Zheng, Z.W. Zhou, and Y. Xu. 2007. Responses of reactive metabolism and quality in mango fruit to exogenous oxalic acid or salicylic acid under chilling temperature stress. *Physiol. Plant.* 130:112-121.
- Dong, Y., L.Q. Liu, and J.F. Guan. 2011. Effects of 1-methylcyclopropene on NO content, NOS activity, and H₂O₂ content in postharvest Suli pears. *Agr. Sci. China* 10:797-804.
- Fu, L., J. Cao, Q. Li, L. Lin, and W. Jiang. 2007. Effect of 1-methylcyclopropene on fruit quality and physiological disorders in Yali pear (*Pyrus bretschneideri* Rehd.) during storage. *Food Sci. Technol. Int.* 13:49-54.
- Ito, H., Y. Tanaka, H. Tsuji, and A. Tanaka. 1993. Conversion of chlorophyll *b* to chlorophyll *a* by isolated cucumber etioplasts. *Arch. Biochem. Biophys.* 306:148-151.
- Ivanov, B. and S. Khorobrykh. 2003. Participation of photosynthetic electron transport in production and scavenging of reactive oxygen species. *Antioxid. Redox Sign.* 5:43-53.
- Jeong, J., D.J. Huber, and S.A. Sargent. 2002. Influence of 1-methylcyclopropene (1-MCP) on ripening and cell-wall matrix polysaccharides of avocado (*Persea americana*) fruit. *Postharvest Biol. Technol.* 25:241-256.
- Jimenez, A., G. Creissen, B. Kular, J. Firmin, S. Robinson, M. Verhoeven, and P. Mullineaux. 2002. Changes in oxidative processes and components of the antioxidant system during tomato fruit ripening. *Planta* 214:751-758.
- Koukounaras, A. and E. Sfakiotakis. 2007. Effect of 1-MCP prestorage treatment on ethylene and CO₂ production and quality of 'Hayward' kiwifruit during shelf-life after short, medium and long term cold storage. *Postharvest Biol. Technol.* 46:174-180.
- Kuckenbergh, J., I. Tartachnyk, and G. Noga. 2008. Evaluation of fluorescence and remission techniques for monitoring changes in peel chlorophyll and internal fruit characteristics in sunlit and shade sides of apple fruit during shelf-life. *Postharvest Biol. Technol.* 48:231-241.
- Larigaudère, C., R. Vilaplana, Y. Soria, and I. Recasens. 2004. Oxidative behavior of Blanquilla pears treated with 1-methylcyclopropene during cold storage. *J. Sci. Food Agri.* 84:1871-1877.
- Medlicott, A.P., M. Bhogal, and S.B. Reynolds. 1986. Changes in peel pigmentation during ripening of mango fruit (*Mangifera indica* var. Tommy Atkins). *Annal. Applied Biol.* 109:651-656.
- Meng, K., Y. Dong, L. Liu, and J. Guan. 2011. Effect of 1-MCP on the content of H₂O₂ and activities of its relative enzymes in 'Lvbaoshi' pear during ripening and senescence. *J. Agri. Univ. Hebei* 34:27-31. (in Chinese)
- Mubarakshina, M., S. Khorobrykh, and B. Ivanov. 2006. Oxygen reduction in chloroplast thylakoids results in production of hydrogen peroxide inside the membrane. *Bioch. Biophys. Acta Bioenergetics* 1757:1496-1503.
- Porat, P., B. Weiss, L. Cohen, A. Daus, R. Goren, and S. Droby. 1999. Effects of ethylene and 1-methylcyclopropene on the post-harvest qualities of 'Shamuti' oranges. *Postharvest Biol. Technol.* 15:155-163.
- Purvis, A.C. and C.R. Barmore. 1981. Involvement of ethylene in chlorophyll degradation in peel of citrus fruits. *Plant Physiol.* 68:854-856.
- Scandalios, J.G. 1993. Oxygen stress and superoxide dismutases. *Plant Physiol.* 101:7-12.
- Scheumann, V., S. Schoch, and W. Rüdiger. 1998. Chlorophyll *a* formation in the chlorophyll *b* reductase reaction requires reduced ferredoxin. *J. Biol. Chem.* 273:35102-35108.
- Singh, R. and U.N. Dwivedi. 2008. Effect of ethrel and 1-methylcyclopropene (1-MCP) on antioxidants in mango (*Mangifera indica* var. Dashehari) during fruit ripening. *Food Chem.* 111:951-956.
- Sisler, E.C. and M. Serek. 1997. Inhibitors of ethylene responses in plants at the receptor level: Recent developments. *Physiol. Plant.* 100:577-582.
- Trebitsh, T., E.E. Goldschmidt, and J. Rivov. 1993. Ethylene induces *de novo* synthesis of chlorophyllase, a chlorophyll degrading enzyme, in *Citrus* fruit peel. *Proc. Natl. Acad. Sci. USA* 90:9441-9445.
- Trincherro, G.D., G.O. Sozzi, F. Covatta, and A.A. Frascina. 2004. Inhibition of ethylene action by 1-methylcyclopropene extends postharvest life of "Bartlett" pears. *Postharvest Biol. Technol.* 32:193-204.
- Wang, Y. and D. Sugar. 2013. Internal browning disorder and fruit quality in modified atmosphere packed 'Bartlett' pears during storage and transit. *Postharvest Biol. Technol.* 83:72-82.
- Watkins, C.B. 2006. The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. *Biotechnol. Adv.* 24:389-409.
- Watkins, C.B., J.F. Nock, and B.D. Whitaker. 2000. Response of early, mid and late season apple cultivars to postharvest application of 1-methylcyclopropene (1-MCP) under air and controlled atmosphere storage conditions. *Postharvest Biol. Technol.* 19:17-32.
- Wei, J., F. Ma, J. Guan, J. Yuan, and X. Zhu. 2009. Cell wall metabolism and its regulation in harvested *Pyrus ussuriensis* Maxin. cv. Jingbaili fruit during ripening. *Sci. Agricul. Sin.* 42:2987-2996. (in Chinese)
- Yuan, G., B. Sun, J. Yuan, and Q. Wang. 2010. Effect of 1-methylcyclopropene on shelf life, visual quality, antioxidant enzymes and health-promoting compounds in broccoli florets. *Food Chem.* 118:774-781.
- Zhang, S., X. Ren, and J. Rao. 2005. Effects of nitric oxide on active oxygen metabolism of postharvest tomato fruit. *Acta Hort. Sin.* 32:818-822. (in Chinese)