



Effect of 1-MCP intermittent treatments on quality and antioxidative enzymes activities of plums (*Prunus salicina* L. cv. 'Oishi Wase')

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Abstract

1-MCP intermittent treatment was developed to evaluate the effects on antioxidative enzyme activities and ripening of plums during storage at 25°C. Plum fruit treated with 1-MCP 1µL/L for 12 h at 25°C every 3 days for one, two or three times, respectively, were investigated in this study. Control fruit were stored at 25°C (85-90% RH) without exposure to 1-MCP after harvest. The results showed that contents of titratable acidity, soluble sugar and ascorbic acid in plum fruit were maintained higher in all the fruit treated with 1-MCP than in control fruit during storage. Firmness of the control fruit declined rapidly to 0.2 kg cm⁻² after 6 days of storage at 25°C, which was 97.4% lower than that in the fruit of twice treatment. The MDA content in the fruit increased gradually during storage, but reduced by the 1-MCP intermittent treated until day 9 of storage. The activities of SOD and POD in the 1-MCP intermittent treated-fruit were significantly lower than that in control before day 9 of storage. SOD activity in fruit of twice treatment and triple treatment were 11.9% and 12.0% lower than that in control fruit on day 6 of storage, respectively. POD activity in the fruit of twice treatment and triple treatment were 32.7% and 45.5% lower than that in control fruit on day 9 of storage, respectively. The 1-MCP treatment enhanced the activity of CAT significantly in plum fruit before day 9 of storage. The CAT activity in twice treatment and triple treatment were 55.9% and 47.4% higher than that in control fruit on day 6, respectively. In conclusion, 1-MCP intermittent treatment significantly delayed plum fruit ripening and maintained the quality properties of fruit, and it may hold promise as an alternative approach to extend plum fruit shelf life at the room temperature.

Key words: 1-MCP intermittent treatment, antioxidative enzymes, ripening, quality.

Introduction

Plum is one of the most favored specialty fruits and is very popular world-wide. The postharvest life of plums is limited due to a rapid rate of ripening at the room temperature¹. Several attempts, including cold temperature², polyamine³, heat and calcium⁴, modified atmosphere packaging⁵ and coating⁶ treatments, have been made to maintain plum quality during storage.

1-Methylcyclopropene (1-MCP) is an inhibitor of ethylene receptors and can delay ripening of horticultural products. The use of 1-MCP is a potentially useful tool for commercial application to reduce the ripening process, maintain quality and extend shelf life of fruit, vegetables and ornamental species⁷. The degree of response to 1-MCP varies greatly by cultivar and harvest maturity of plums⁸, and 1-MCP delayed the climacteric increase in ethylene production of plums, even when fruit were harvested close to the climacteric peak⁹. Moreover, recent research has demonstrated that the beneficial effects of 1-MCP treatment are not exclusively due to its action on ethylene but also through a direct effect on the antioxidative potential¹⁰.

Previous studies have shown that accumulation of reactive oxygen species (ROS) during senescence initiated the degradation of intracellular membrane structures and decompartmentation¹¹.

Antioxidative system, composed of both antioxidative enzymes and antioxidants, is equipped in fruits to prevent oxidative damage¹². The intracellular levels of ROS are tightly controlled, not only by antioxidative enzymes including superoxide dismutase, catalase, peroxidase, but also by non-enzymatic components such as ascorbic acid¹¹. It was suggested that the decrease in antioxidative enzyme activities and ascorbate contents during senescence is often associated with a reduction in the capacity to prevent oxidative damage¹³.

The 'Oishi Wase' plums (Japanese types) show a ripening pattern typical of climacteric fruit and effect of 1-MCP on 'Oishi Wase' plums, treated only one time, was limited because new ethylene receptors may become rapidly available in the room temperature. We found that 1-MCP intermittent treatment could extend plum fruit shelf life remarkably more than that of plums treated only one time, but we knew little about changes of plums quality and antioxidative enzymes after 1-MCP intermittent treatment. The objective of our study was to show the effects of 1-MCP intermittent treatment on ripening and antioxidative enzymes of 'Oishi Wase' plums and to learn how the mechanisms might be involved in 1-MCP intermittent treatment.

Materials and Methods

Mature green 'Oishi Wase' plums were harvested from 8-year old trees grown in commercial orchard located in Hebei Province of China and transported to laboratory immediately. The fruit were selected for uniform size and freedom from defects. 1-Methylcyclopropene (1-MCP) was released from a commercial powdered formulation (EthylBloc, Rohm and Haas China, Inc).

Treatment: Following harvest, plum fruit were randomly divided into four groups for experiments. Each group contained three replicates. All the treatments were performed at 25°C as follows:

Control: 200 plums were treated without exposure to 1-MCP and stored at 25°C (85-90% RH).

Once treatment: 200 fruits were exposed to 1 µL/L 1-MCP in a plastic-film tent for 12 h at 25°C, and stored at 25°C (85-90% RH).

Twice treatment: 200 fruits were treated with 1 µL/L 1-MCP for 12 h after harvest as described above, and were treated again with the same conditions after stored for 3 d at 25°C (85-90% RH).

Triple treatment: 200 fruits were treated with 1 µL/L 1-MCP for 12 h after harvest as described in once treatment, and then the fruit were treated for the second and the third treatments with the same conditions after stored for 3 and 6 d at 25°C (85-90% RH), respectively.

Enzyme assays: To analyze activities of antioxidative enzymes, 3 g of frozen plum tissue was homogenized in 5 mL of 0.05 M potassium phosphate buffer, pH 7.8. The homogenate was centrifuged at 10,000×g at 4°C for 30 min and the supernatant was collected for the enzyme assays.

Superoxide dismutase (SOD; EC 1.15.1.1) activity was determined by the method of Zhao *et al.*¹⁴. One unit of SOD was defined as the amount of enzyme that caused a 50% decrease of the SOD-inhibitable NBT reduction. The SOD activity was expressed as units per gram fresh weight (FW).

Catalase (CAT; EC 1.11.1.6) activity was determined by the method of Sala¹⁵. The reaction mixture consisted of 2 mL of 50 M sodium phosphate buffer (pH 7.0), 0.5 mL of 40 M H₂O₂ and 0.5 mL of enzyme. The decomposition of H₂O₂ was measured by the decline in absorbance at 240 nm.

Peroxidase (POD, EC 1.11.1.7) activity was assayed according to Zauberman *et al.*¹⁶. The results were expressed as units per gram fresh weight (FW). One unit of enzyme activity was defined as the amount of enzyme which caused a change of 0.001 in absorbance per min.

Quality properties assays: Fruit firmness was measured using a firmness tester (4 mm diameter probe, Model GY-1) at the equator of the fruits from opposite sides of each fruit. Titratable acidity (TA) was determined by titration with 0.1 M NaOH and expressed as

percentage of citric acid. The soluble sugar concentration was determined according to the method of Dubois *et al.*¹⁷. Content of ascorbic acid (AsA) was assayed according to the method of Liu *et al.*¹⁸. AsA content was titrated with 2,6-dichlorophenolindophenol and expressed as mg g⁻¹ FW. Malondialdehyde (MDA) was measured according to the method of Hodges *et al.*¹⁹. Data on quality properties represent the means of measurements for three times.

Data analysis: All statistical analyses were performed with SPSS 10.0. Data were analysed by one-way analysis of variance (ANOVA). Mean separations were performed using the least significant difference method (LSD test). Each experiment had three replicates and all experiments were run three times with similar results.

Results and Discussion

As shown in Fig. 1A, firmness of the control fruit declined rapidly to 0.2 kg cm⁻² after 6 days of storage at 25°C, which was 97.4% lower than that in fruit of twice treatment. The decrease of firmness in plum was significantly ($p < 0.01$) slowed down by 1-MCP intermittent treatment during storage. Firmness in fruit of control and once treatment was undetectable on day 9 of

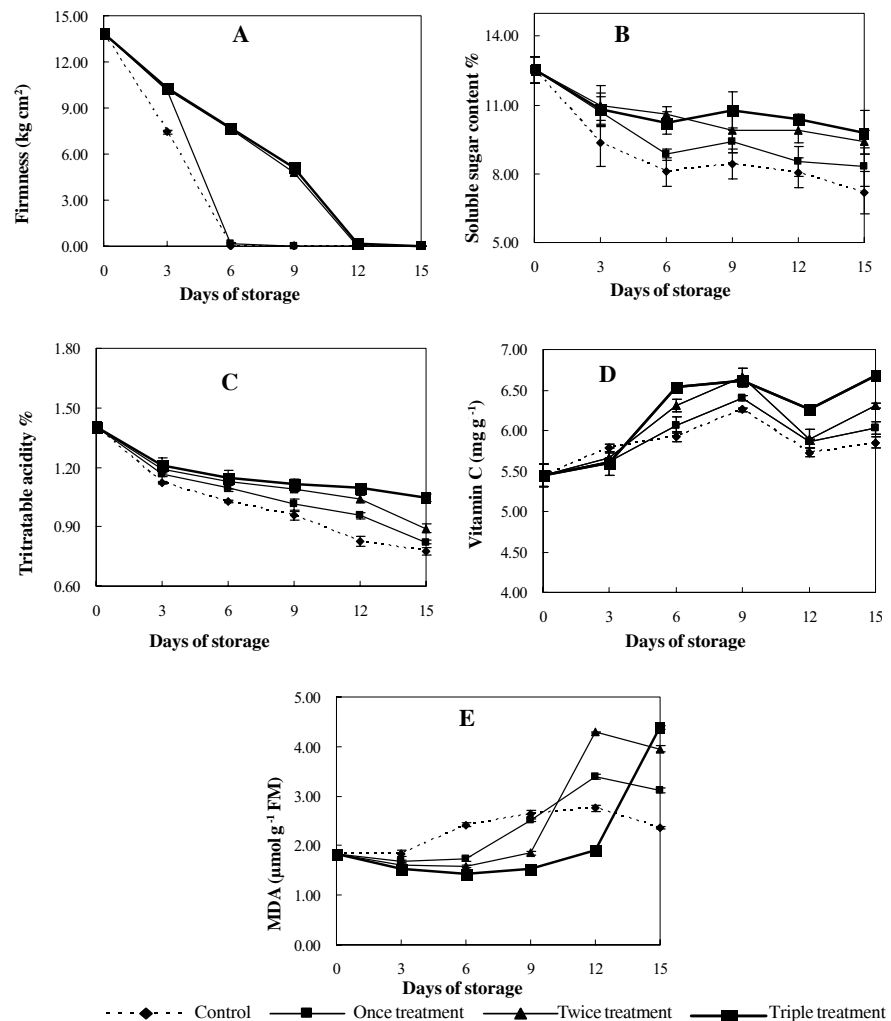


Figure 1. Effect of 1-MCP intermittent treatment on levels of firmness (A), soluble sugar content (B), titratable acidity (C), vitamin C (D), MDA content (E) in plum fruits stored for 15 days at 25°C and 8590 % RH (n = 3). Vertical bars indicate the standard errors of three replicates. Mean separations were performed using the least significant difference method (LSD test).

storage. In contrast, the firmness in fruit of twice treatment and triple treatment was significantly ($p < 0.01$) higher than that in fruit of control and once treatment on day 9 of storage.

In all the treatment groups, TA and soluble sugar content in the plum fruit decreased gradually during storage, and this reduction was inhibited by 1-MCP intermittent treatment. As shown in Fig. 1B, Soluble sugar content in fruits of twice treatment and triple treatment was 23.2% and 29.0% higher ($p < 0.05$) than that in control fruit on day 12 of storage. TA in fruits of twice treatment and triple treatment was 26.5% and 32.5% higher ($p < 0.05$) than that in control fruit on day 12 of storage, respectively

The ascorbic acid (AsA) content was increased gradually at the beginning of storage and then decreased until day 9 of storage, as shown in Fig. 1D. The AsA content in the control was lower than that all 1-MCP treatments. The AsA content in fruit of twice treatment and triple treatment was 6.4% and 10.5% ($p < 0.05$) higher than that in control fruit, respectively

Malondialdehyde (MDA) is a final product in lipid peroxidation of cell membrane, and its concentration can reflect the degree of lipid peroxidation. The MDA content in the fruit increased gradually during storage, and was reduced by the 1-MCP intermittent treatment until day 9 of storage. MDA content in the fruit of twice treatment and triple treatment was 30.3% and 41.7% higher ($p < 0.01$) than that in control fruit on day 9 during storage, respectively (Fig. 1E).

As shown in Fig. 2A, SOD activity in control fruit was higher than that in 1-MCP treatment at the beginning 9 days of storage, and then gradually declined. SOD activity in control fruit increased rapidly at the beginning of storage and reached the peak at day 6, 1-MCP treatment retarded the peak. SOD activity in fruit of twice treatment and triple treatment was 11.9% and 12.0% lower ($p < 0.05$) than that in control fruit on day 6 of storage, respectively.

POD activity in control fruit increased rapidly before 6 days storage and hold high level until day 12. However, it was reduced by 1-MCP intermittent treatment (Fig. 2B). POD activity in the fruit of twice treatment and triple treatment was 32.7% and 45.5% lower ($p < 0.01$) than that in control fruit on day 9 of storage, respectively.

The 1-MCP treatment markedly enhanced the CAT activity in plum fruit in the first 9 days during storage. CAT activity in control fruit increased rapidly on day 9 and reached the peak on day 12, then declined rapidly (Fig. 2C). The CAT activity in twice treatment and triple treatment was 55.9% and 47.4% higher ($p < 0.01$) than that in control fruit on day 6, respectively.

1-Methylcyclopropene (1-MCP) has resulted in an explosion of research on its effects on fruits and vegetables, both as a tool to further investigate the role of ethylene in ripening and senescence, and as a commercial technology to improve maintenance of product quality²⁰. 1-MCP prevented or delayed the climacteric increase in ethylene production and softening of the fruit²¹. However, we found that 1-MCP treated only one time showed limited effect in plums of the so-called climacteric cv. 'Oishi Wase' during storage at 25°C. In order to effectively use 1-MCP to maximize profits for 'Oishi Wase' plums and provide a quality consumer product, 1-MCP intermittent treatment was developed to extend fresh fruit shelf life. In our study, the firmness in the fruit of 1-MCP intermittent treatment was significantly higher than that in control fruit before day 12 of storage. 1-MCP intermittent treatment significantly inhibited the increase of MDA and the

decrease of titratable acidity and soluble sugar in 'Oishi Wase' plums. These results support the observations of Dong *et al.*²² and Menniti *et al.*²³ with other cultivars treated by 1-MCP.

Antioxidants are important components in plum. For stress regulation, the antioxidative enzymes involved in their metabolism are of prime importance²⁴. The effective destruction of active oxygen species requires the action of several antioxidative enzymes acting concomitantly with non-enzymatic antioxidant. The superoxide radical (O_2^-) is efficiently converted to H_2O_2 by the action of SOD. H_2O_2 may be toxic for the cells and should not be allowed to accumulate. The main enzymes responsible for converting H_2O_2 to water are CAT and POD¹⁰. This study showed that 1-MCP intermittent treatment markedly affected the activity of antioxidative enzymes in 'Oishi Wase' plums. The CAT activity in plum was enhanced, but the SOD and POD activity was inhibited

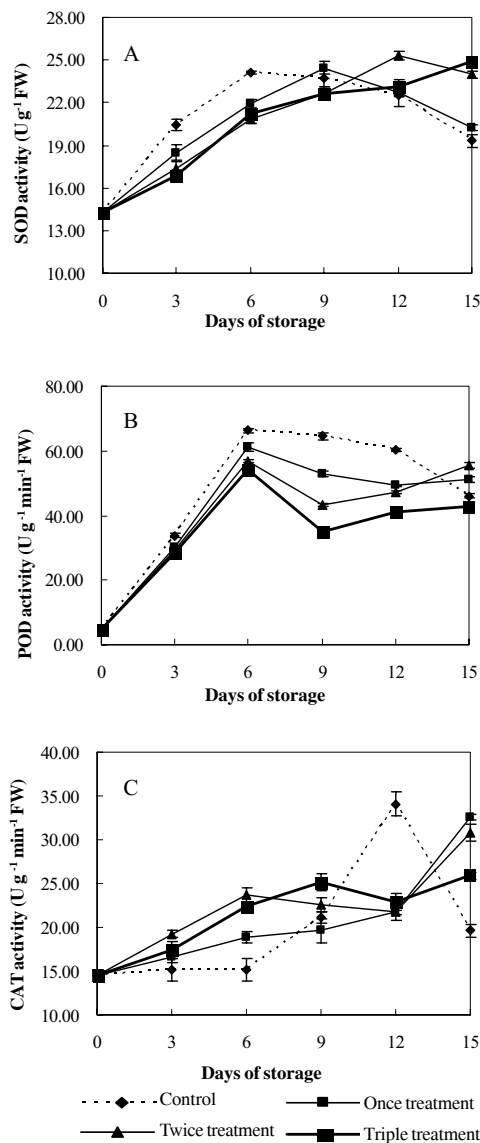


Figure 2. Effect of 1-MCP intermittent treatment on activities of SOD (A), POD (B), CAT (C) in plum fruits stored for 15 days at 25°C and 85-90% RH (n = 3). Vertical bars indicate the standard errors of three replicates. Mean separations were performed using the least significant difference method (LSD test).

by 1-MCP intermittent treatment before day 9 of storage. In addition, as the non-enzymatic antioxidant, AsA content in plum fruit was slightly enhanced by the 1-MCP intermittent treatment during storage. These could be contributed to delay the fruit senescence.

The present study showed that twice treatment with 1 µL/L 1-MCP was most effective at maintaining the quality of plum during storage at 25°C. In our study, the plum fruit of triple treatment was characterized as abnormal ripening and poor colour developed. Further research is needed to clarify this issue. It suggested that 1-MCP intermittent treatment could hold promise as an alternative approach to delay 'Oishi Wase' plums ripening and extend plum shelf life at the room temperature.

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References

- ¹Singh, S. P., Singh, Z. and Swinny, E. E. 2009. Postharvest nitric oxide fumigation delays fruit ripening and alleviates chilling injury during cold storage of Japanese plums (*Prunus salicina* Lindell). *Postharvest Biol. Technol.* **53**:101-108.
- ²Guerra, M. and Casquero, P. A. 2008. Effect of harvest date on cold storage and postharvest quality of plum cv. Green Gage. *Postharvest Biol. Technol.* **47**:325-332.
- ³Valero, D., Martínez-Romero, D. and Serrano, M. 2002. The role of polyamines in the improvement of the shelf life of fruit. *Trends in Food Sci. and Technol.* **13**: 228-234.
- ⁴Serrano, M., Martínez-Romero, D., Castillo, S., Guillén, F. and Valero, D. 2004. Role of calcium and heat treatments in alleviating physiological changes induced by mechanical damage in plum. *Postharvest Biol. Technol.* **34**:155-157.
- ⁵Díaz-Mulaa, H. M., Martínez-Romero, D., Castillo, S., Serrano, M. and Valero, D. 2011. Modified atmosphere packaging of yellow and purple plum cultivars. 1. Effect on organoleptic quality. *Postharvest Biol. Technol.* **61**:103-109.
- ⁶Navarro-Tarazaga, M. L., Massa, A. and Pérez-Gago, B. M. 2011. Effect of beeswax content on hydroxypropyl methylcellulose-based edible film properties and postharvest quality of coated plums (cv. Angeleno). *LWT - Food Sci. Technol.* **44**:2328-2334.
- ⁷Khan, A. S. and Singh, Z. 2007. 1-MCP regulates ethylene biosynthesis and fruit softening during ripening of Tegan Blue plum. *Postharvest Biol. Technol.* **43**:298-306.
- ⁸Martinez-Romero, D., Dupille, E., Guillen, F., Valverde, J. M., Serrano, M. and Valero, D. 2003. 1-Methylcyclopropene increases storability and shelf life in climacteric and nonclimacteric plums. *J. Agric. Food Chem.* **51**:4680-4686.
- ⁹Salvador, A., Cuquerella, J. and Martinez-Javega, J. M. 2003. 1-MCP treatment prolongs postharvest life of Santa Rosa plums. *J. Food Sci.* **68**:1504-1510.
- ¹⁰Larrigaudière, C., Candan, A. P., Ubach, D. and Graell, J. 2009. Physiological response of Larry Ann plums to cold storage and 1-MCP treatment. *Postharvest Biol. Technol.* **51**:56-61.
- ¹¹Lacan, D. and Baccou, J. C. 1998. High levels of antioxidative enzymes correlate with delayed senescence in nonnetted fruits. *Planta* **204**:377-382.
- ¹²Noctor, G. and Foyer, C. H. 1998. Ascorbate and glutathione: Keeping active oxygen under control. *Rev. Plant Physiol. Plant Mol. Biol.* **49**:249-279.
- ¹³Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.* **7**:405-410.
- ¹⁴Zhao, Z. L., Jiang, W. B., Cao, J. K., Zhao, Y. M. and Gu, Y. H. 2006. Effect of cold-shock treatment on chilling injury in mango (*Mangifera indica* L. cv. Wacheng) fruit. *J. Sci. Food Agr.* **86**:2458-2462.
- ¹⁵Sala, J. M. 1998. Involvement of oxidative stress in chilling injury in cold-stored mandarin fruits. *Postharvest Biol. Technol.* **13**:255-261.
- ¹⁶Zauberman, G., Ronen, R. and Akerman, M. 1991. Postharvest retention of the red color of litchi fruit pericarp. *Sci. Hortic.* **47**:89-97.
- ¹⁷Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. 1956. Colorimetric method for determination of sugar and related substances. *Anal. Biochem.* **28**:350-356.
- ¹⁸Liu, H. X., Jiang, W. B., Bi, Y. and Luo, Y. B. 2005. Postharvest BTH treatment induces resistance of peach (*Prunus persica* L. cv. Jiubao) fruit to infection by *Penicillium expansum* and enhances activity of fruit defense mechanisms. *Postharvest Biol. Technol.* **35**:263-269.
- ¹⁹Hodges, D. M., DeLong, J. M., Forney, C. F. and Prange, R. P. 1999. Improving the thiobarbituric acid reactive-substance assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta* **207**:604-611.
- ²⁰Watkins, C. B. 2006. The use of 1-methylcyclopropene on fruits and vegetables. *Biotechnol. Adv.* **24**:389-409.
- ²¹Dong, L., Zhou, H. W., Sonogo, L., Lers, A. and Lurie, S. 2001. Ripening of Red Rosa plums: Effect of ethylene and 1-methylcyclopropene. *Aust. J. Plant Physiol.* **28**:1039-1045.
- ²²Dong, L., Lurie, S. and Zhou, H. W. 2002. Effect of 1-methylcyclopropene on ripening of Canino apricots and Royal Zee plums. *Postharvest Biol. Technol.* **24**:135-145.
- ²³Menniti, A. M., Gregori, R. and Donati, I. 2004. 1-Methylcyclopropene retards postharvest softening of plums. *Postharvest Biol. Technol.* **31**:269-275.
- ²⁴Foyer, C. H., Descourvières, P. and Kunert, K. J. 1994. Protection against oxygen radicals: An important defence mechanism studied in transgenic plants. *Plant Cell Environ.* **17**:507-523.