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# Effect of 1-methylcyclopropene treatment on green asparagus quality during cold storage**

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A b s t r a c t. Green asparagus was treated with 1-methylcyclopropene at three concentration levels at room temperature for 24 h after harvest to evaluate the postharvest quality during cold storage at 4°C. Comparing with the controls, the loss of vitamin C, decomposition of chlorophyll, and accumulation of the malonydiadehyde under treatments of 1-methylcyclopropene were reduced during storage. The enzyme activities in asparagus including peroxidase and phenylalanine ammonia lyase were inhibited by 1-methylcyclopropene treatments, while the activity of superoxide dismutase was enhanced. Based on non-significant difference of the treated samples with 6 μl $l^{-1}$, 1-methylcyclopropene treatments at 4 μl $l^{-1}$ could be selected to maintain postharvest quality of green asparagus and provide long storage life.

K e y w o r d s: green asparagus, quality, cold storage 1-methylcyclopropene, respiration

## INTRODUCTION

Asparagus (*Asparagus officinalis* L.) is an important vegetable with a high market value. Asparagus has been planted widely in China since 1970 years, and now the area of planting has exceeded 40 000 ha (Pan *et al.,* 2001). The demand of international market has been increasing, especially for fresh asparagus in the recent years, because the price of fresh-keeping asparagus is 47.1 and 45.9% higher than that of the potted and fast frozen asparagus, respectively (Wang *et al*., 2006). This situation results in 20% export increases of fresh-keeping asparagus every year in China. The asparagus spear, which is crisp and tender with a distinctive taste, is an abundant source of vitamins, amino acids and trace elements. However, asparagus is highly perishable and non-climacteric resulting in a very short shelf-life, mainly due to its high respiratory activity during storage (Albanese *et al*., 2007; Li and Zhang, 2006; Villanueva *et al*., 2005). The quality of fruit and vegetable is determined by several external factors (shape, mass, colour) and internal factors (firmness, absence or presence of internal defects) (Gómez *et al*., 2005; Wang *et al*.,2006). The loss of quality is mainly perceived by consumers owing to the wrinkling of stems, toughness, loss of the green and brightness. Toughness of asparagus is a major factor in determining spear quality, and largely related to the degree of lignifications of the spears in both fiberring and vascular bundles. The lignifications are controlled by enzymes, including phenylalanine ammonia lyase (PAL), peroxidase (POD) and isoperoxidases (An *et al*., 2006; 2007).

Ethylene is associated with the regulation of several plant metabolic processes (Mullins *et al*., 2000). The role of ethylene in inducing fruit and vegetable senescence has been well documented in many studies. Ethylene promotes processing characteristics of senescence, such as softening, decline in chlorophyll and photosynthesis, reduction in the levels of proteins and starch, and increase in the activities of many hydrolytic enzymes (Liguori *et al*., 2004; Zhang and De Baerdemaeker, 1996).

The 1-methylcyclopropene (1-MCP) is an inhibitor of ethylene perception that apparently binds to the cellular ethylene receptors, blocking or delaying the processes of maturation and senescence normally triggered with ethylene (Blankenship and Dole, 2003; Pre-Aymard *et al*., 2005). Since it is non-toxic and odourless, 1-MCP is potentially applied to extend the shelf life and quality of plant products. 1-MCP is registered as SmartFresh$^{TM}$ for use on ornamentals

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and fruit in several countries including the USA, New Zealand, Columbia, Holland, Austria, England, Switzerland and Turkey. The main research has focused on effect of 1-MCP on postharvest quality of many different vegetables and fruits at temperatures ranging from 2 to 30°C, active concentrations from 0.7 nl $l^{-1}$ to 24 µl $l^{-1}$ and treatment durations from 6 to 24h (Blankenship and Dole, 2003). The reported results suggest that the treatment temperature with 1-MCP seems to be important for a given fruit due to special receptor sensitivity. In practice, 1-MCP has been used successfully for perishable and non-climacteric fruit. Up to date, there is no paper about effects of 1-MCP on postharvest quality of green asparagus during cold storage.

The aim of this study was to determine the effect of 1-methylcyclopropene treatment on postharvest quality of green asparagus during cold storage.

## MATERIALS AND METHODS

The raw asparagus spears were delivered to laboratory in 4 h after harvest. The green asparagus spears from Hua-Lin Agriculture Company, Nantong, China, was 200-240 mm in length, 9-11 mm in diameter and average mass of roughly 500 g each bundle.

1-MCP (active ingredient value of 0.14 g 100 $g^{-1}$) was released from a commercial powder formulation (Smart Fresh$^{TM}$ Rohm and Haas China Inc., Beijing, China). The samples of asparagus spears were divided into four groups: untreated control, treated with 2, 4, and 6 µl $l^{-1}$ 1-MCP for 24 h at 20°C in the fumigation tank. The treated samples were stored at 4°C and 80% relative humidity (RH). The observations were carried at storage of 0 (control), 6, 12, 20, and 26 day until the raw materials lost commercial value in three replications.

The static-measuring method under room temperature (18±3°C) was adopted. 500 g randomly taken asparagus were put into in a Petri dish filled with 20 ml of 0.4 N NaOH and kept in an airtight desiccator (dia in 260 mm) for 30 min. The residual NaOH in the Petri dish was then titrated with 0.2 N oxalic acid ($C_2H_2O_4$). The respiratory intensity was calculated from the volume of oxalic acid consumed.

The vitamin C content in the supernatant was determined by the 2,6-dichlorphenolindophenol sodium salt method (Hang, 1989). 10 g edible part of asparagus tissue were sufficiently homogenized with 100 ml of 2 ml/100 ml oxalic acid and centrifuged at 4°C at 6 000 g for 10 min. Total chlorophyll was extracted with 80% acetone and quantified by spectrophotometric analysis. Absorbance readings were taken at 645, 652 and 663 nm using a spectrophotometer (752, Shanghai Exact Science Instrument Ltd., Shanghai, China) and final results were expressed as milligram of chlorophyll per gram fresh tissue. The assay of malonyldialdehyde (MDA) was extracted with 10 ml/100 ml trichloroacetic acid (TCA). The homogenate was centrifuged at 4 000g for 10 min. 2 ml of 0.6 ml/100 ml thiobarbituric acid (TBA) in 10 ml/100 ml TCA was added to 2 ml resulting supernatant. The solution was heated in a boiling water bath for 15 min, immediately cooled and then centrifuged at 6 000 g for 10 min. Absorbance (D values) was measured at 450, 532 and 600 nm using the spectrophotometer. MDA content was calculated as:

$$\text{MDA } (\mu\text{mol } l^{-1}) = 2\,[6.45\,(D_{532}\,D_{600})\,0.56\,D_{450}].$$

POD activity was determined by measuring the absorbance at 430 nm. One unit of POD activity was defined as changing 1.0 in absorbance per minute. Plant material was extracted in 50 mmol phosphate buffer (pH 7.0) containing 1 mol NaCl. The homogenate was centrifuged at 3 000 g for 15 min. The assay medium contained 0.1 ml of enzyme extract, 0.1 ml 1% o-phenylene diamine/ethanol solution, 0.2 ml 0.3 ml/ 100 ml $H_2O_2$, and 2.6 ml 100 mmol phosphate buffer (pH 7.0). The superoxide dismutase (SOD) activity was determined by reagent box of SOD (A001-1, Nanjing Jiancheng Bioengineering Institute, Nanjing, China).Plant tissue was extracted in 50 mmol phosphate buffer (pH 7.8) containing 0.1mM EDTA. The homogenate was centrifuged at 10 000 g for 20 min. Phenylalanine ammonia lyase (PAL) activity was determined by measuring the absorbance at 290 nm. One unit of PAL activity was defined as the change in absorbance of enzyme extract. Plant tissue was extracted in frozen Tris-$H_2SO_4$ buffer (pH 8.3) containing 7 mmol mercaptoethanol and 1 mmol EDTA-Na. The homogenate was centrifuged at 10 000 g for 30 min. The assay medium contained 0.5 ml of enzyme extract, 1.0 ml 20 mmol L-phenylalanine, and 2 ml 50 mM Tris-$H_2SO_4$ buffer (pH 8.8). The mixture was incubated at 30°C for 30 min.

The average and standard deviation were obtained over three replicates. Data were further analyzed using the SPSS System (SPSS Inc., USA) by the analysis of variance (ANOVA). The significant difference of average values among the treatments was determined at the level of $p \leq 0.05$.

## RESULTS AND DISCUSSION

The changing trend of respiratory intensity of 4 groups was generally similar, having the same peak time (Fig. 1a). The asparagus respiratory intensity declined at the beginning of storage (0-6 days), then rapidly increased to peak value after 12 days storage, then declined dramatically to the lowest value, and increased gradually at the final stage of storage. The same type of phenomena was observed by Pan *et al.* (2001). Before treatments, the respiratory intensity of the green asparagus spears increased sharply after harvest, reached a peak in 3 h roughly, and descended gradually in following 24 h. It could be primarily due to a large quantity of wounds caused during harvest. Owing to its self-protection mechanism, the asparagus might switch to higher respiratory intensity to speed up the formation of wound-recovering tissue (Li and Zhang, 2006; Li *et al*., 2008). Therefore, asparagus would reach a respiratory peak in a very short time

mainly caused by coercion. The initial decline/rise trends during storage might be because coercive respiratory intensity peak had already passed when the asparagus was sent to the laboratory. In this experiment, the changing trends could be due to respiratory intensity which coincides with asparagus senescence and deterioration. The significant differences were observed between control and the three 1-MCP treatment concentrations ($p<0.05$) after 6 days storage, and increasing the 1-MCP concentration had progressively greater effects. The treatment with 2 μl $l^{-1}$ 1-MCP did not significantly affect the respiratory intensity until the 6th day. No significant difference was observed between 4 and 6 μl $l^{-1}$ 1-MCP treatment during the whole storage. Furthermore, the peak value of respiratory intensity using 6 μl $l^{-1}$ 1-MCP was only 68.5% of that in controls. However, in the latter period of storage (>26 days), increasing the 1-MCP concentration had no significant effects on the respiratory intensity. The results indicated that 1-MCP could effectively control the respiratory intensity at a relatively lower level.

A decrease of vitamin C (VC) content of all the 4 treated asparagus spears was observed over the storage period but with a sharp decrease at the first 12 days (Fig. 1b). The decrease of VC in samples treated with 1-MCP treatments was significantly higher than that in controls after the first 6 days of storage ($p<0.05$). Increasing the 1-MCP concentration obviously enhanced the VC retention rate because the VC retention rate at 6 μl $l^{-1}$ 1-MCP treatment was 32.7%, which was remarkably higher than that (22.0%) for controls after 37 days of storage. Thus, 1-MCP could effectively restrain the decline of VC content. 1-MCP slows vitamin C loss in Chinese jujube (Jiang *et al*., 2004), peaches (Liu *et al*., 2005), pineapples (Selvarajah *et al*., 2001), and minimally processed lettuce (Tay and Perera, 2004) and pineapple (Budu and Joyce, 2003).

No significant difference in chlorophyll content of the green asparagus was found between 2 μl $l^{-1}$ 1-MCP treatments and controls during the first 12 days of storage ($p>0.05$), but with increasing the storage time, the differences became significant (Fig. 1c). A significant difference in chlorophyll content was observed between the higher 1-MCP concentration treatments and controls over the whole storage period. However, increasing the 1-MCP concentration from 4 to 6 μl $l^{-1}$ had no significant effects on chlorophyll content during the most of storage time. It was found that chlorophyllase activity was reduced in 1-MCP-treated broccoli florets and avocado fruit (Gong and Mattheis, 2003; Hershkovitz *et al*., 2005).

The changing trend of MDA content was similar for all the four treatments (Fig. 1d). The MDA content of samples treated with 1-MCP was significantly lower than that in controls before 26 days of storage ($p<0.05$), and increasing the 1-MCP concentration had progressively greater effects. However, no significant difference was observed among the three 1-MCP treatments at the first 6 (or 12) days of storage, and between the controls and the three 1-MCP treatments only at 37th day of storage. The decreasing trend of MDA contents during the latter period of storage might be caused by the increased antioxidant enzymes activity and their enhanced abilities to scavenge superoxide anion ($O_2^-$). Thus, the accumulation of MDA contents declined might be due to the production of membrane lipid peroxidation.

The POD activities of all 4 treated samples presented a similar trend at the first 20 days with a peak at 12th day, which is mainly stimulated by harvest injury (Fig. 1e). The average POD values in treated samples with 1-MCP were lower than those in controls during this period. After 20th day, the POD in controls and 2 μl $l^{-1}$ 1-MCP treatments declined continuously, but that in 4 and 6 μl $l^{-1}$ 1-MCP treated samples presented a small fluctuation and reached another peak in the 26th day. The similar phenomena were reported for broccoli, which might be resulted from the increased substrate during the latter period of storage (Wang and Win , 2002).

The SOD activity increased with the extension of storage time in all the four treated samples (Fig. 1f). No significant differences in SOD activity were found between the samples treated with 1-MCP and controls before 6 days of storage ($p>0.05$). During following period of storage, the increase of SOD activity of the samples treated with 1-MCP was significantly higher than that in controls. But the SOD difference was not significant between the two higher concentration treatments. The similar SOD activity enhanced by 1-MCP was also found for broccoli and cabbage (Li, 2004; Wang and Win, 2002).

Results from Fig. 1g showed a similar PAL activity trend for all the 4 treated samples, which was in accordance with reports about fresh-cut green asparagus in MAP storage (An *et al*., 2007). PAL activity appeared a marked growth in first 6 days of storage time, then decreased sharply until 12 day. The reason is that wounding generally induced PAL activity (Rico *et al*., 2007). PAL activities in the three 1-MCP treatments were significantly lower than those in controls over the whole storage period ($p<0.05$), and increasing the 1-MCP concentration gradually reduced the PAL values. The peak value of PAL with 6 μl $l^{-1}$ 1-MCP treatments was only 60.5% of controls on the 6th day. However, no significant difference was observed between the two higher 1-MCP concentration treatments.

The mechanisms to prevent oxidation are associated with the defense system, including antioxidant enzymes and endogenous antioxidations (Wang and Win, 2002). By means of regulating the antioxidant enzyme system, 1-MCP might help to delay the senescence of green asparagus and could influence the biosynthesis, signaling and action of ethylene. SOD and catalase (CAT) could also inhibit the formation of ethylene. The further fundamental research is needed to determine the mechanism of 1-MCP treatment in delaying the senescence of green asparagus and its relationship with the antioxidant enzymes.

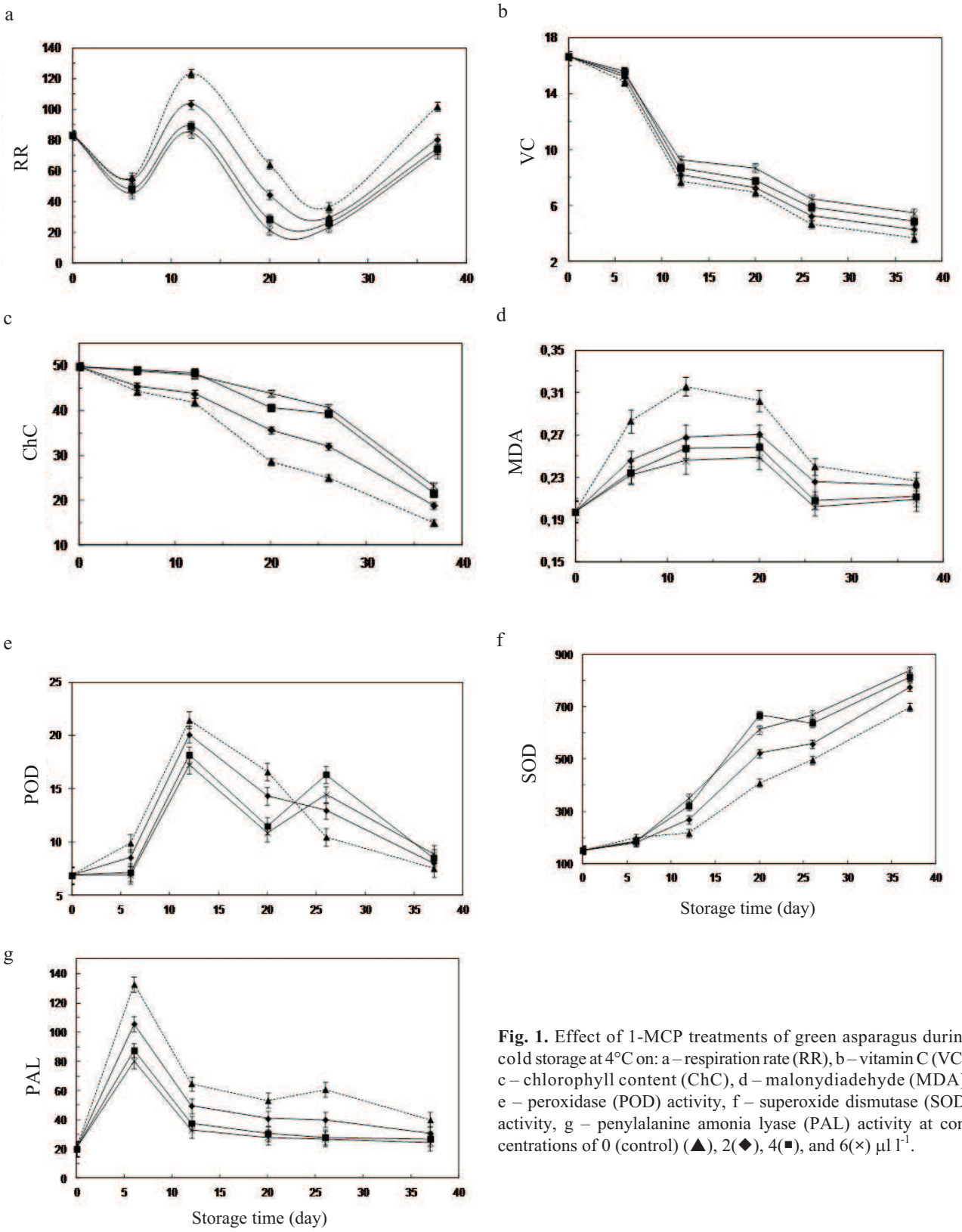


**Fig. 1.** Effect of 1-MCP treatments of green asparagus during cold storage at 4°C on: a – respiration rate (RR), b – vitamin C (VC), c – chlorophyll content (ChC), d – malonydiadehyde (MDA), e – peroxidase (POD) activity, f – superoxide dismutase (SOD) activity, g – penylalanine amonia lyase (PAL) activity at concentrations of 0 (control) (▲), 2(◆), 4(■), and 6(×) µl $l^{-1}$.

## CONCLUSIONS

1. The treatment of 1-methylcyclopropene inhibited the respiration, reduced the loss of vitamin C and chlorophyll, and weaken phenylalanine ammonia lyase activity and the accumulation of malonyldialdehyde during storage as compared with the controls.

2. The superoxide dismutase activities were higher in 1-methylcyclopropene treated samples than in the control.

3. The increase of 1-methylcyclopropene concentration enhanced the treatment effect, but the difference of effects was not significant between the two higher concentration treatments.

4. The treatment of 1-methylcyclopropene clearly delayed the process of lignification and senescence, and extended the postharvest quality of green asparagus.

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