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1-Methylcyclopropene Alleviates Postharvest Chilling Injury of Snap Beans by Enhancing Antioxidant Defense System

Running title: 1-MCP Alleviates Chilling Injury of Snap Bean

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SUMMARY

Research background. Chilling injury is an important disorder affecting the quality of tropical and subtropical vegetables during low temperature storage. Snap bean (*Phaseolus vulgaris* L.) is sensitive to chilling injury. The main purpose of the present study was to investigate the alleviating effects of 1-methylcyclopropene (1-MCP) on chilling injury of snap bean. In addition, the related mechanisms were also detected from the perspective of the changes of antioxidant defense system.

Experimental approach. Snap beans were exposed to different concentrations of 1-MCP. After 24 h of treatment, snap beans were stored at 4 °C for up to 14 days. Chilling injury index, electrolyte leakage, titratable acidity, total soluble solids were detected. Contents of chlorophyll, ascorbic acid and malondialdehyde were assessed. The total antioxidant capacity, ferrous ion chelating capacity, scavenging capacities on free radicals and activities of antioxidant enzymes were detected. Total phenol content and activities of related metabolic enzymes were determined.

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Results and conclusions. 1-MCP treatment reduced chilling injury index, electrolyte leakage rate and malondialdehyde content of snap beans. The levels of total soluble solids, titratable acid, ascorbic acid and total chlorophyll in 1-MCP treated snap beans were significantly higher than that of control. 1-MCP treated snap beans showed stronger total antioxidant capacity and metal chelating activity. 1-MCP treatment enhanced scavenging effects of snap beans on superoxide radical, hydroxyl radical and 1,1-diphenyl-2-trinitrophenylhydrazine radical. The activities of peroxidase, ascorbate peroxidase, superoxide dismutase and catalase in 1-MCP treated group were higher than control. 1-MCP treatment enhanced the accumulation of phenolic compounds in snap beans by regulating activities of phenolic metabolizing enzymes such as shikimate dehydrogenase, phenylalanine ammonia lyase enzyme, cinnamic acid-4-hydroxylase and polyphenol oxidase. In conclusion, 1-MCP has the effect of avoiding chilling injury of snap bean. The mechanism involved activation of enzymatic and non-enzymatic antioxidant systems.

Novelty and scientific contribution. This study gives insights into whether 1-MCP can regulate postharvest cold resistance in vegetables by enhancing the enzymatic antioxidant system as well as inducing non-enzymatic antioxidants accumulation. Considering the results, 1-MCP treatment could be an effective method to alleviate postharvest chilling injury of snap beans during low temperature storage.

Keywords: snap bean (*Phaseolus vulgaris* L.); chilling injury; 1-methylcyclopropene; antioxidant systems; phenolic compounds

INTRODUCTION

Temperature is an important environmental factor affecting metabolic process, quality and storage period of fruits and vegetables. In general, most of fruits and vegetables should be stored at low temperatures after harvest, as low temperatures reduce the respiration of fruits and vegetables. However, many tropical or subtropical vegetables are sensitive to low temperatures. They are vulnerable to chilling injury when they are stored in the low temperature environment above 0 °C. As a kind of chilling-sensitive vegetable, snap bean (*Phaseolus vulgaris* L.) is prone to chilling injury under low temperature stress for a long time. Chilling injury in snap bean is characterized by rusty spots on the surface, dark watery patches and discoloration (*1*). Quality control of snap bean during low temperature storage is closely related to the interests of consumers and business operators. In the last few years, many studies have been devoted to looking for ways to control chilling injury.

The relationship between reactive oxygen species (ROS) burst and chilling injury has been widely revealed. Environmental stress such as chilling stress can promote the generation of ROS and

destroy the balance of ROS in plant cells. The accumulation of ROS can damage the integrity of cell membrane. Then the membrane fatty acids undergo lipid peroxidation to form malondialdehyde (MDA) (*2*). Therefore, maintaining intracellular ROS homeostasis is very important for alleviating chilling injury of vegetables during low temperature storage. The intracellular antioxidant system with ROS clearance is divided into non-enzymatic and enzymatic antioxidant system. The enzymatic antioxidant systems consist of a series of antioxidant enzymes such as catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and ascorbate peroxidase (APX), which immediately quench the ROS to protect the membrane from oxidative damage. The non-enzymatic antioxidants include ascorbic acid, polyphenols, tocopherol, and so on (*3*).

1-methylcyclopropene (1-MCP) is a small cyclic hydrocarbon molecule. It is a type of ethylene receptor inhibitor. With the receptors inactivated, the tissue no longer responds to ethylene even if it is present. Recently, the application of 1-MCP as postharvest treatments has been considered for enhancing the quality of horticultural crops (*4,5*). However, there are few studies on the prevention and control of 1-MCP on chilling injury of snap beans during low temperature storage. The main purpose of the present study is to investigate the alleviating effects of 1-MCP on chilling injury of snap beans. In addition, the related mechanisms were also detected from the perspective of the changes of antioxidant defense system.

MATERIALS AND METHODS

Chemicals

The chemicals used in this study were of analytical grade. 1-MCP, pyrogallol, NADP, polyvinylpyrrolidone, polyethylene glycol, trans-cinnamic acid, trichloroacetic acid, ascorbic acid, 2,6-dichloroindophenol, guaiacol, EDTA and salicylic acid were obtained from Yuanye, Jiangsu, China. 1,1-diphenyl-2-trinitrophenylhydrazine radical, pyrogallol, ferrozine, TPTZ, riboflavin and phosphate buffer were obtained from Aladdin, Shanghai, China. Thiobarbituric acid, gallic acid, nitroblue tetrazolium and glacial acetic acid were purchased from Suolaibao, Beijing, China.

Snap beans material and treatment

Snap beans (*Phaseolus vulgaris* L. cv. 'Jiuyueqing') were harvested during a typical commercial ripening period from a farm in Changchun, China. Then they were immediately delivered to the laboratory within 1 h. All samples are uniform in size and color without mechanical damage. Each treatment had three replicates with 400 snap beans in each replicate. Snap beans of each treatment were placed in a 40 L sealed container and exposed to 1-MCP at different concentrations (0.5, 1, 1.5, 2, 2.5 μL/L). Control beans exposed to air. Mini fan was used to keep air circulation. After 24 h of

treatment, all snap beans were stored at 4 °C and 75 % RH for up to 14 days. Snap beans were randomly taken at 2-day interval. Chilling injury index was assessed immediately after removal from 4 °C. 20 snap beans were used to determine electrolyte leakage, titratable acidity and the content of malondialdehyde in pericarp. The remaining beans were immediately frozen in liquid nitrogen and stored at −80 °C for further analysis. All experiments were performed in triplicate.

Chilling injury index

Chilling injury (CI) of snap beans is characterized by rusty spot on the surface, dark watery patches and discoloration (*1*). The CI level was arbitrated as follows: 0, no abnormality; 1, small watery patches or rusty spots, no discoloration; 2, moderate watery patches or rusty spots, no discoloration; 3, severe watery patches or rusty spots, discoloration; 4, extremely severe watery patches, large rusty spots, discoloration of the entire pod. CI index was determined by using the following formula:

$$\text{CI index}=\sum[(\text{CI}_{\text{grade}})\cdot(N(\text{fruit})_{\text{CIgrade}})]/(4\cdot N(\text{fruit})_{\text{treated total}}) \quad /1/$$

Electrolyte leakage, malondialdehyde, total soluble solids, titratable acidity, chlorophyll and ascorbic acid content

Electrolyte leakage of snap beans was expressed by the total conductivity using the method described by Wang *et al*. (*6*). Bean pod plate was made with a 7 mm diameter punch. A test tube was filled with 2 g of bean pods and 20 mL of deionized water. After shaking, a conductimeter (DDS-11A; Suoshen Co., Shanghai, China) was used to detect the conductivity. Then, tubes were boiled for 15 min. After cooling down, the total conductivity of the solution was tested again. Thiobarbituric acid reactive substances (TBARS) method was used to measure malondialdehyde (MDA) content (*2*). Results were represented as micromoles of MDA per kilogram of snap bean pods. Total soluble solids (TSS) content was measured using refractometer (WYT; Chengdu Taihua Optical Co., LTD, China). Results were represented as a mass fraction percentage. Titratable acidity (TA) content was measured by titration (*7*). A mass of 20 g of bean pods was homogenized in 250 mL of distilled water. After centrifugation at 10 000×*g* for 30 min, the supernatant was collected and used for measuring the content of titratable acidity*.* A volume of 20 mL of supernatant was titrated with 0.01 M NaOH until the color of the solution changed to pink (phenolphthalein indicator) for 30 s. Results were represented as g of malic acid per 100 g of bean pods. A method described by Hmmam *et al*. (*8*) was used to detect the content of chlorophyll. Under dim light, 5 g of bean pods was grinded with 50 mL acetone, 0.1 g $CaCO_3$ in a prechilled mortar. After centrifugation at 12 000×*g* for 10 min, the supernatant was adjusted to 50 mL with acetone. Optical density was determined at 663 nm (chlorophyll a) and 645

nm (chlorophyll b), using acetone 95 % as a blank. 2,6-dichlorophenol-indophenol method (*9*) was adopted to analyze ascorbic acid content.

Total antioxidant capacity, metal chelating activity and free radical scavenging activity

FRAP method was used to detect the total antioxidant activity of snap beans (*10*). 10 g of bean pods was homogenized with 50 mL of distilled water and centrifugated at 10 000×*g* for 20 min. 3 mL of ferric-TPTZ reagent was mixed with 250 µL supernatant. The reaction solution was bathed in water at 37 °C for 10 min. The absorbance of the solution was measured at 593 nm using spectrophotometer. The standard curve was constructed with ferrous sulfate solution (25–800 µM). FRAP value was expressed as millimoles of ferrous iron equivalents per kilogram of snap beans. The method of Deng *et al*. (*11*) was used to estimate the chelation of ferrous ions. The reaction mixture consisted of 1 mL of sample extract, 3.7 mL of ethanol and 0.1 mL of 2 mM solution of $FeCl_2$. The reaction was started with adding 0.2 mL of 5 mM ferrozine. Then the mixture was shaken vigorously and kept at room temperature for 10 min. The increase in absorbance at 562 nm was recorded. Results were expressed as clearance percentage. The clearance percentage was given by the following formula:

$$\text{Clearance percentage}=((A_0-A_1)/A_0)\cdot 100 \qquad /2/$$

where A_0 is the absorbance of the control, and A_1 is the absorbance in the presence of the samples. The control did not contain compound or standard.

The experimental procedure recorded by Zuo *et al*. (*12*) was used to measure the superoxide radical ($O_2^{\cdot-}$) scavenging rate. 10 g of bean pods was homogenized with 300 mL distilled water and centrifugated at 10 000×*g* for 30 min. The reaction system included 0.5 mL of supernatant and 4.43 mL of 50 mM Tris-HCl buffer solution (pH 8.2). Then the mixture was placed at 25 °C for 20min. Afterward, 70 µL of 15 mM pyrogallol solution was added. The absorbance was measured at 325 nm. Fenton reaction was used to detect hydroxyl radical (·OH) scavenging rate. 5 g of bean pods was homogenized with 10 mL of distilled water. After centrifugation at 10 000×*g* for 30 min at 4 °C, the supernatant was collected. The reaction system consisting of 2 mL of sample extract, 2 mL of 9 mM salicylic acid–ethanol solution, and 1 mL of 9 mM ferrous sulfate solution was kept at 37 °C for 1 h. The reaction was initiated by the addition of 2 mL of 8.8 mM hydrogen peroxide. The absorbent value was determined at 510 nm. The method described by Sridhar and Charles (*13*) was used to measure 1,1-diphenyl-2-trinitrophenylhydrazine radical (DPPH·) scavenging rate. 5 g of bean pods was extracted with 10 mL of distilled water and the homogenate was centrifuged at 10 000×*g* for 20 min. The reaction mixture contained 2 mL of supernatant and 2 mL of 20 µM DPPH and placed under dark for 30 min. The increase in absorbance at 517 nm was recorded. Results were expressed as clearance percentage. The clearance percentage was determined by the Eq 2.

Antioxidant enzyme activity

A mass of 2.5 g of bean pods from each treatment was homogenized with 10 mL of 0.2 M cold potassium phosphate buffer. After centrifugation at 10 000×*g* for 30 min at 4 °C, the obtained supernatant was the crude extract of enzyme. The method described by Sanches *et al*. (*14*) was used to assay APX activity. The activity was determined in a reaction mixture consisting of 3 mL of reaction mixture (contained 50 mM pH7.0 potassium-phosphate, 0.1mM EDTA disodium and 0.3 mM ascorbate), 0.5 mL of enzyme extract and 0.5 mL of 0.1mM H_2O_2). The increase in absorbance at 290 nm was recorded. CAT activity was measured by the method of Zuo *et al*. (*12*). The reaction mixture contained 1mL of distilled water, 1 mL of 0.2 M potassium-phosphate buffer, 0.5 mL of enzyme extract and 0.5 mL of 0.1 M H_2O_2. The absorbance was measured at 240 nm. The POD activity was detected by a method described by Guo *et al*. (*15*). The reaction mixture contained 3 mL of 25 mM guaiacol solution, 1 mL of 0.5 M H_2O_2 and 0.5 mL of enzyme extract. The POD activity was calculated by measuring the absorbance at 470 nm. Nitroblue tetrazolium (NBT) reduction method was used to determine the activity of SOD, as described by Zuo *et al*. (*12*). An enzyme activity unit is expressed as the amount of enzyme required for a 0.01 change in absorbance per minute. Results were expressed as U/g.

Total phenolic content and enzyme activity associated with phenolic metabolism

For determination of the content of total phenolic, 2 g of bean pods was homogenized in 5 mL of methanol. After centrifugation at 10 000×*g* for 30 min, the supernatant was collected. The reaction system included 1 mL of Folin-Ciocalteu reagent, 0.5 mL of supernatant and 3 mL of 1 M sodium carbonate. Then the total volume of mixture was adjusted to 10 mL with distilled water. After the mixture had been kept at 25 °C for 1 h, the absorbance value was measured at 760 nm (*16*). The result was expressed as the mass of gallic acid equivalents on a fresh mass basis in mg/g.

For determination of shikimate dehydrogenase (SKDH), 2 g of bean pods was homogenized in 6 mL of 50 mM potassium-phospate buffer, pH 6.8. And then sample was centrifuged at 10 000×*g* for 30 min at 4 °C. The reaction system included 0.2 mL of supernatant, 1.9 mL of 100 mM Tris-HCl, pH 9.0, 1.45 mL of 2 mM shikimic acid and 1.45 mL of 0.5 mM NADP (*17*). The absorbent value was determined by the reduction of NADP at 340 nm. For determination of phenylalanine ammonolyase (PAL), 5 g of bean pods was homogenized in 5 mL extraction buffer, containing 4 % polyvinylpyrrolidone, 0.002 M EDTA and 0.005 M β-mercaptoethanol. The mixture was then centrifuged at 10 000×*g* for 30 min at 4 °C. The reaction mixture consisting of 0.2 mL of supernatant, 1 mL of 0.6 mM L-phenylalanine and 2 mL of 0.2 M borate buffer (pH 8.8) was kept at 37 °C for 1 h. The increase in absorbance at 290 nm was recorded. For determination of cinnamate-4-hydroxylase

(C4H), 1 g of bean pods was homogenized in 3 mL of 50 mM pH 8.9 Tris-HCl buffer solution which contained 4 mM magnesium sulfate, 5 mM ascorbic acid, 10 % glycerinum and 0.15 % polyvinylpyrrolidone. Then 0.5 mL of supernatant was mixed with 2.5 mL reaction solution which contained 50 mM pH 8.9 Tris-HCl buffer, 2 μM NADP and 2 m M trans-cinnamic acid. The increase in absorbance at 340 nm was recorded. For determination of polyphenol oxidase (PPO) 5 g of bean pods was homogenized in 5 mL of precooled extraction buffer containing 1 % TritonX-100, 1 mM polyethylene glycol and 4 % polyvinylpyrrolidone. PPO activity was measured by the method of Wang *et al*. (*18*). The increase in absorbance at 420 nm was recorded. Activities were expressed on a fresh mass basis as U/g, where U=(0.01 Δ*A*)/min.

Statistical analysis

All experiments were repeated three times. Data were presented as mean±SD. Data were statistically analyzed by analysis of variance (ANOVA) with SPSS statistical software 26.0.0 (*19*). Significant differences were performed by Duncan's multiple range tests. A probability of $p<0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Sensory quality of snap beans and CI index

The most typical chilling symptoms of snap beans include discoloration, dark watery patches and rusty spot on the surface (*1*). Snap bean is sensitive to chilling temperature and very vulnerable to chilling injury. In fact, the sensitivity of snap beans to CI has significant difference in cultivars (*20*). There was no sign of CI when 'Opus' snap beans stored at 1 °C. 'Romano' snap beans were only slightly affected when stored at 5 °C for 2 weeks. Due to chilling injury, postharvest storage time of 'Tendergreen' and 'Top Crop' snap beans was reduced by 40 %. Chilling injury symptoms of 'Leon' snap beans became apparent 2 days after exposure to 1 or 5 °C and 3 days after exposure to 10 °C (*1*). In our study, CI symptoms in control group appeared 2 days after exposure to 4 °C. After 6 days of storage at 4 °C, bean pods showed obvious symptoms of chilling injury. At that time, the sensory quality of snap beans reached the limit of acceptable. Supporting Fig. S1 showed the differences in appearance of snap beans on the 14th day. Bean pods were severely affected and showed many rusty spots and dark watery patches on the surface, especially for control and 2.5 μL/L 1-MCP treated groups. Fig. 1 showed 1-MCP treatment significantly reduced the CI index of snap beans. 1-MCP at 1 μL/L was the most effective concentration. However, CI index of 2.5 μL/L 1-MCP treated group was higher than that of control. It indicates high concentration of 1-MCP aggravated chilling damage of snap bean, and led to a more rusty spots appearance at the end of storage. 1-MCP can irreversibly

bind to ethylene receptors, thereby avoiding subsequent ethylene response. It has been demonstrated that 1-MCP prevents deterioration of quality by delaying senescence, as well as by inducing chilling tolerance in many vegetables. Although postharvest treatment with 1-MCP is effective and non-toxic, its effectiveness is highly variable. This study showed that its effectiveness is directly related to the concentration used in the treatment.

Electrolyte leakage, MDA, TSS, TA, chlorophyll and ascorbic acid

Membrane permeability is usually indicated by the change in electrolyte leakage. Electrolyte leakage is a good qualitative index of chilling sensitivity. As shown in Fig. 2a, conductivity rates of all groups increased with the storage time. The conductivity rate in control group increased from 34.26 to 65.14%. However, in contrast with the control group, 1 μL/L 1-MCP treatment delayed electrolyte leakage. Chilling injury can enhance membrane lipid peroxidation and produce MDA (*8*). Oxidative stress in fruits and vegetables can be detected directly as accumulation of MDA. Fig. 1 and Fig. 2b showed the content of MDA increased with the increase of CI index under low temperature stress, indicating that the chilling stress had exacerbated the degradation of membrane lipids and may lead to the deletion of cells integrity. Compared with the control group, the content of MDA in 1 μL/L 1-MCP treatment group was the lowest (Fig. 2b). These results clearly demonstrated that 1-MCP could inhibit the accumulation of MDA and reduce electrolyte leakage, suggesting that the membrane integrity was maintained when exposed to 1 μL/L 1-MCP. Similar results were reported in 1-MCP induced chilling tolerance in nectarine (*3*) and persimmon (*21*).

TSS and TA are are important quality indexes of vegetables (*22*). Chilling injury induced rapid plant senescence and had negative effects on these quality attributes (*23*). As can be seen from Fig. 2c, TSS contents in all groups decreased continuously during refrigeration storage. TSS content in control group decreased from 5.10 to 3.07 %. 0.5 and 1 μL/L 1-MCP treatment significantly prevented the decrease of TSS. However, the other groups had no statistical significance compared with control ($p>0.05$). Fig. 2d showed TA continuously decreased throughout 14-day storage in all groups. However, the content of TA was noticeably higher in 0.5−1.5 μL/L 1-MCP treatment compared with the control. The content of TA in 1 μL/L 1-MCP treatment group was the highest, which may be related to the lowest CI index in this group, thus inhibiting the rapid senescence of snap beans.

Discoloration is one of the most common symptoms of CI observed in snap beans. Chlorophyll is an important factor to determine the acceptability of snap beans by consumer. The chlorophyll degradation usually reflects the quality deterioration of snap beans (*24*). Chlorophyll content significantly decreased as the color of the snap bean pods turned from a bright green to a more yellowish green color. Fig. 2e showed the total content of chlorophyll decreased from 77.01 to 32.20

mg/kg fresh mass in the control group. However, 1 μL/L 1-MCP treatment delayed the degradation of chlorophyll. Ascorbic acid is often considered as an important non-enzymatic antioxidant bioactive compound which scavenges ROS (*25*). The decline of ascorbic acid levels is usually associated with the aging process in plants. Fig. 2f showed the content of ascorbic acid in snap beans decreased continuously during storage. Treatment with 1 μL/L 1-MCP significantly prevented the decline of ascorbic acid.

The above results suggested that 1 μL/L was the optimal concentration of 1-MCP used for cold storage of snap beans and consequently this concentration was used for the next experiments.

Total antioxidant capacity, metal chelating activity and free radical scavenging rates

Results from Fig. 3a showed the FRAP value increased initially and then decreased. At the end of storage, a maximal increase of 1.66-fold of control was seen with 1 μL/L 1-MCP treatment group. Similarly, as shown in Fig. 3b, compared to untreated control, 1 μL/L 1-MCP treated snap beans showed 1.93-fold higher metal chelating activity on the 14th day. This result indicates that 1-MCP can inhibit the formation of Fe^{2+}-ferrozine complex in snap beans.

ROS such as $O_2^{\cdot-}$ and·OH are the most prevalent radicals in plant cell. DPPH is widely used in the evaluation of reducing substances as experimental free radicals. Figs. 3c–3e showed free radicals scavenging rate increased during the initial ten days of storage and decreased thereafter. Scavenging rates of $O_2^{\cdot-}$,·OH and DPPH·in control beans were 46.52, 55.30 and 41.86 % lower than those in 1 μL/L 1-MCP treatment beans at 14th day of storage, respectively. The enhanced tolerance to chilling injury in 1-MCP treated snap beans was associated with increased levels of free radicals scavenging rate, which could be related to changes in antioxidant enzyme activities.

Antioxidant enzyme activities

It has been reported that the chilling tolerance of vegetables is positively correlated with the activity of antioxidant defense system (*26*). Low temperature stress is also a kind of stress which not only negatively affects the membrane structure of chilling sensitive vegetables, but also reduces the activity of antioxidant enzymes (*27*). POD, CAT, APX and SOD are important components of antioxidant system in vegetables and have the ability to remove ROS (*28*). To investigate the effect of 1-MCP on enzymatic antioxidant system of snap beans, activities of POD, APX, SOD and CAT were detected. As shown in Fig. 4, snap beans exposed to 1 μL/L 1-MCP showed significantly higher activities of POD, APX, SOD and CAT in comparison with control beans. These results clearly indicated the enhanced antioxidant activity of 1-MCP treated snap beans was achieved by inducing the activities of POD, APX, SOD and CAT antioxidant enzymes.

Total phenol content and activities of SKDH, PAL, C4H and PPO

Non-enzymatic antioxidants such as phenolic compounds also play an important role in the removal of ROS during stress tolerance (*29*). As shown in Fig. 5a, 1-MCP treatment delayed the decrease of total phenolic content during the whole storage period. During storage from 8 to 14 days, the total phenolic content in control group decreased from 2.49–2.28 mg/g fresh mass, and in 1 μL/L 1-MCP treated group decreased from 2.63–2.34 mg/g fresh mass. Considering the results, the improvement of free radical scavenging abilities could be associated with the increase of total phenolic content, which prevents membrane lipid peroxidation (*30*). Snap beans treated with 1-MCP showed significantly ($p<0.05$) higher activities of SKDH, PAL and C4H as compared with control during cold storage (Figs. 5b–5d). Fig. 5e showed the PPO activity of snap beans exposed to 1-MCP was significantly lower than that of control($p<0.05$). The metabolism of phenolic compounds is closely related to the activities of SKDH, PAL, C4H and PPO. SKDH is the key enzyme that catalyzes shikimic acid to produce L-phenylalanine. PAL and C4H are key and rate-limiting enzymes for the synthesis of phenolic compounds. PPO plays an important role in the browning of cold-damaged horticultural crops (*31*). In this study, the PPO activity of snap beans treated with 1-MCP was lower, which may be related to the lower intensity of browning and chilling injury. Taken together, our results suggested that 1-MCP treatment promoted total phenol content by enhancing the activities of SKDH, PAL and C4H, inhibited the activity of PPO, which increased the total phenol content in snap beans.

CONCLUSIONS

Results from this study showed that 1-MCP had the effect of avoiding chilling injury of snap beans. Postharvest treatment of snap beans with 1-MCP inhibited the accumulation of MDA and reduced electrolyte leakage. 1-MCP treatment decreased the consumption of organic acids as respiratory substrates. Treatment with 1-MCP significantly prevented the decline of TSS and total chlorophyll. 1-MCP treated snap beans showed stronger total antioxidant capacity and metal chelating activity. 1-MCP treatment enhanced scavenging effects of snap beans on superoxide radical, hydroxyl radical and 1,1-diphenyl-2-trinitrophenylhydrazine radical. Its effectiveness is directly related to the concentration used in the treatment. The optimal concentration of 1-MCP to avoid chilling injury in snap beans is 1.0 μL/L. The mechanism involved activation enzymatic and non-enzymatic antioxidant systems. Treatment with 1-MCP stimulated the activities of ascorbate peroxidase (APX), peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT) in snap beans which are important enzymes in the enzymatic antioxidant system. Besides, 1-MCP treatment enhanced the accumulation of non-enzymatic antioxidants such as ascorbic acid and phenolic compounds in snap beans. The increase of total phenol content in 1-MCP treated snap beans was related to the regulation

of shikimate dehydrogenase, phenylalanine ammonia lyase enzyme, cinnamic acid-4-hydroxylase and polyphenol oxidase. Accordingly, 1.0 μL/L 1-MCP treatment is probably a good way to maintain the storage quality of snap beans during low-temperature storage.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

N. Lv carried out the experimental, data analysis, wrote and revised the manuscript. C. Wang and H. Zhou participated in the antioxidant activity experiment. C. Guo and H. Zhang were involved in the statistical analysis and design of figures. D. Ren participated in designing and supervising the entire work. All authors have participated in the preparation and revision of the manuscript and agree with the final version of the manuscript.

SUPPLEMENTARY MATERIAL

Supplementary material is available at www.ftb.com.hr.

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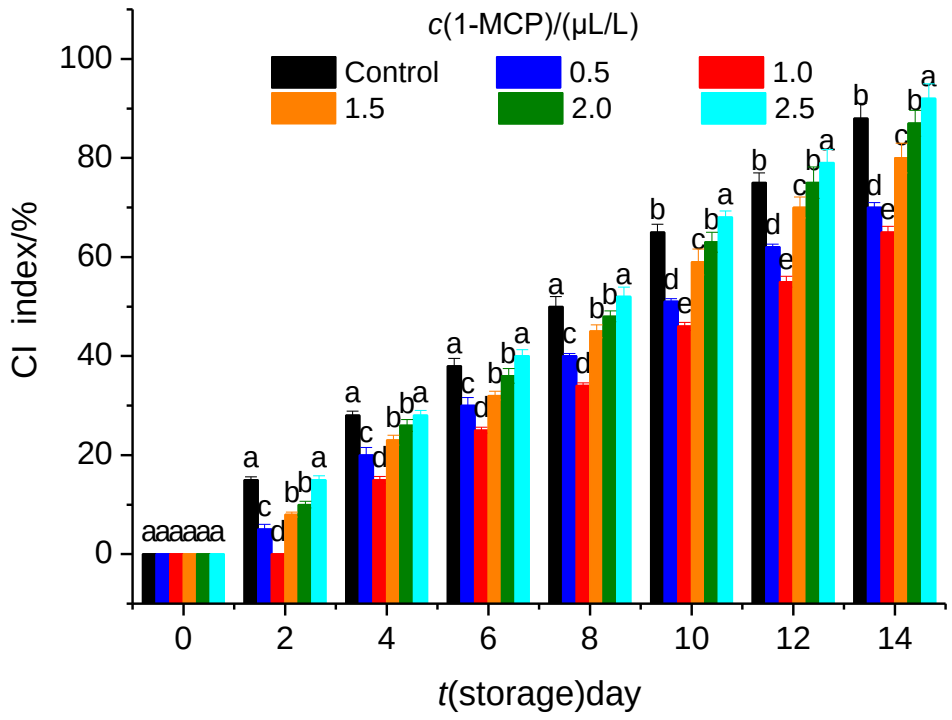
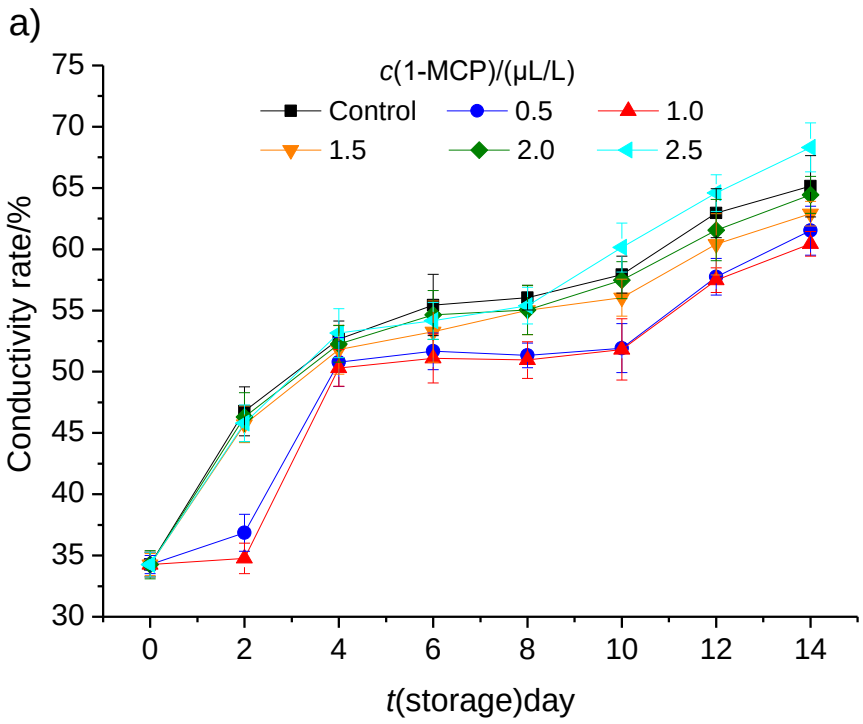
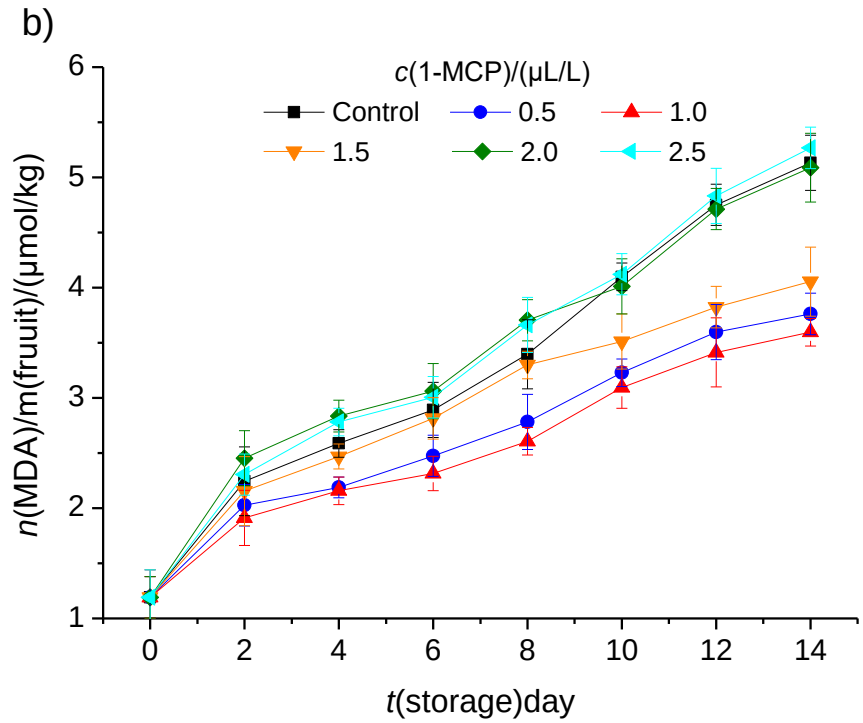


Fig. 1. CI index of snap beans treated with 1-MCP and control. Snap beans were stored at 4 °C for up to 14 days. Data are presented as mean value±S.D. of three replications. Different letters

indicate significant differences among treatments according to Duncan's test at p=0.05. No differences were detected whose letters are absent





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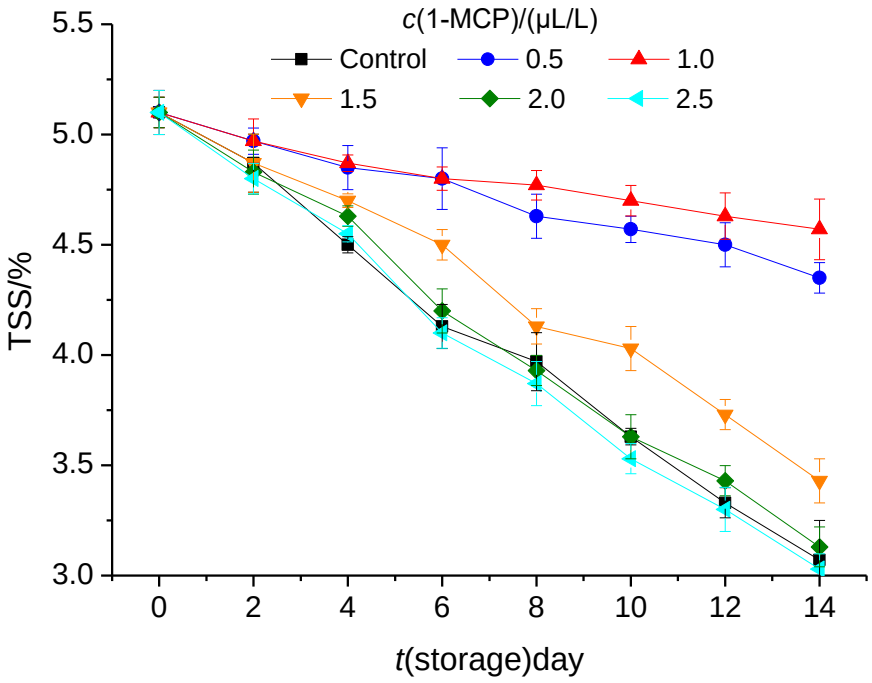

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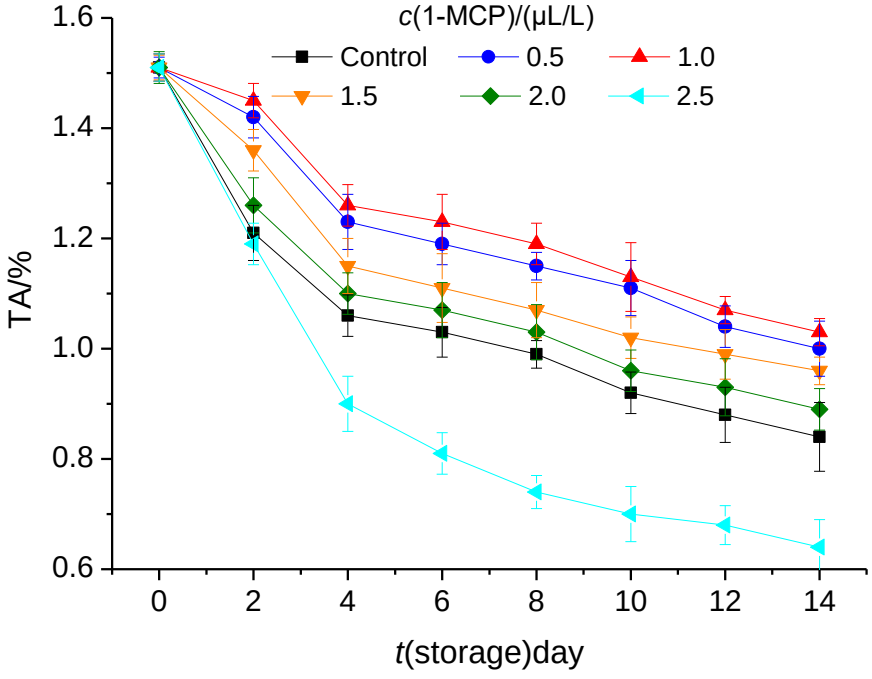

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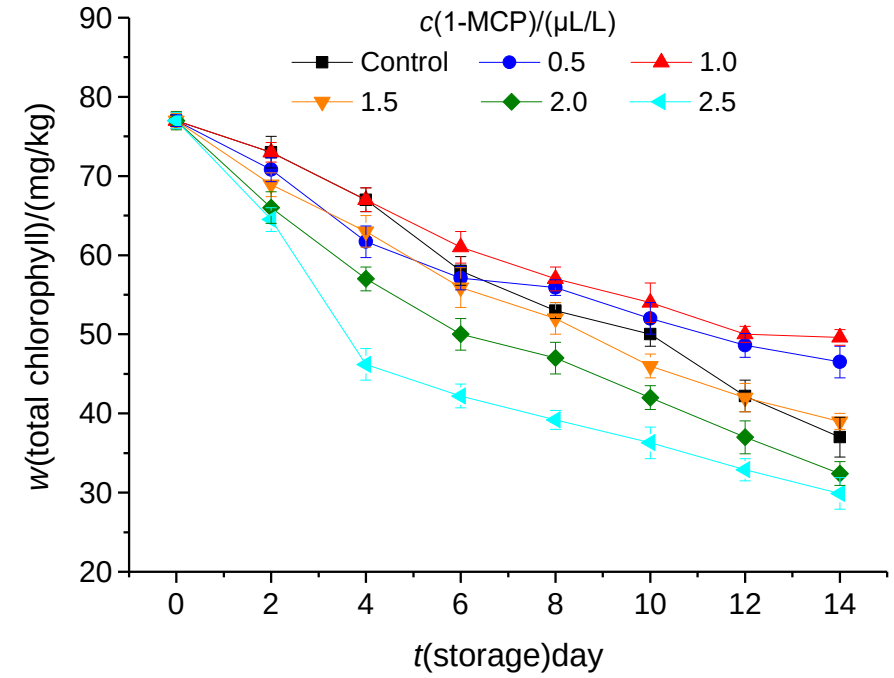

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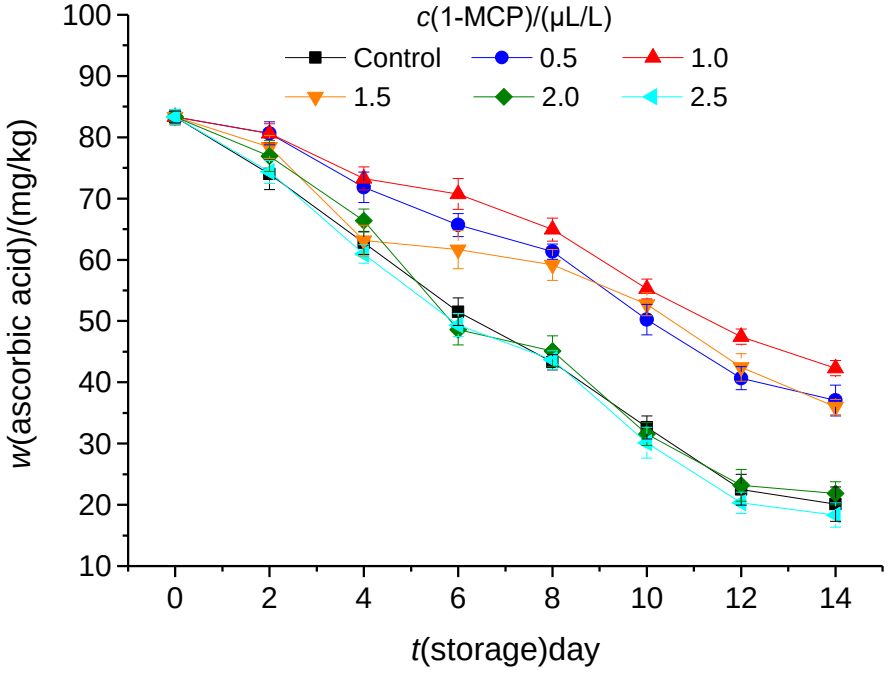
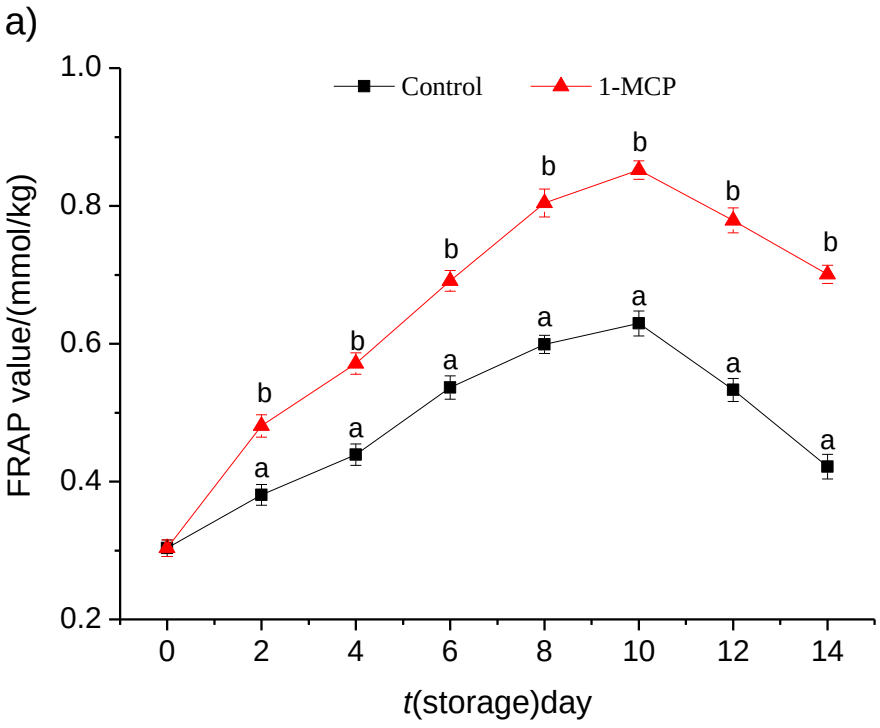
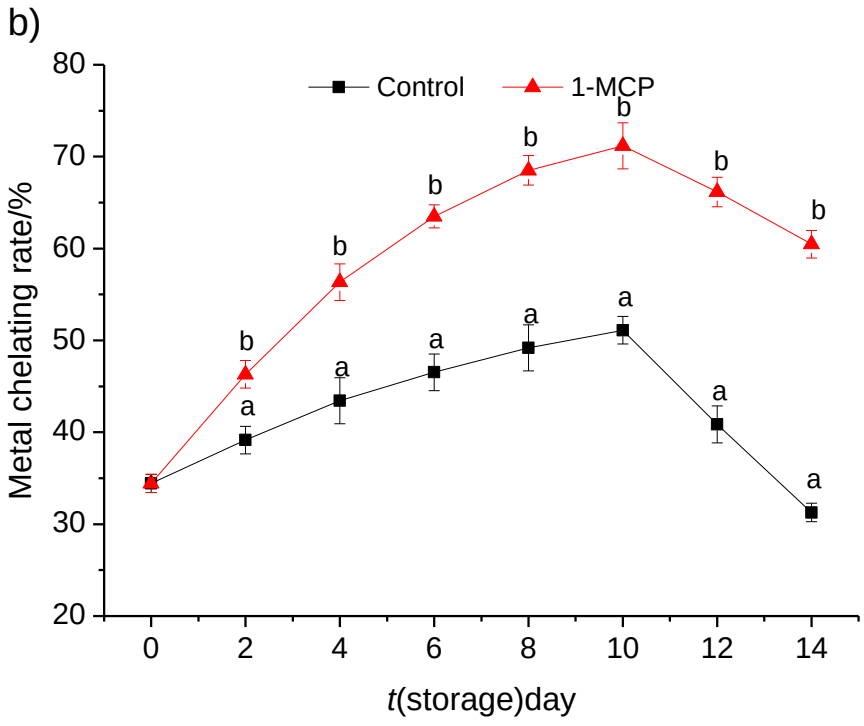
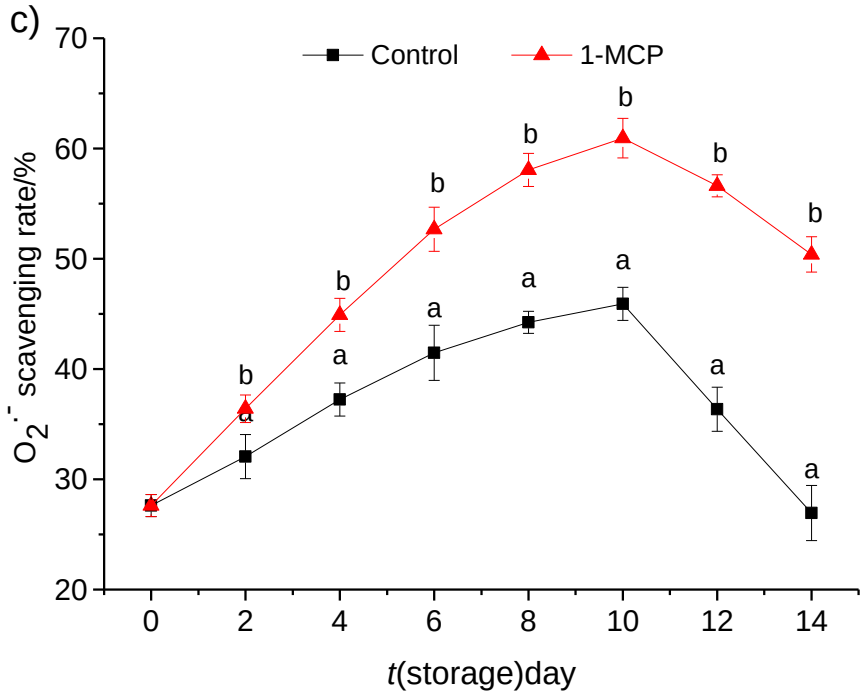
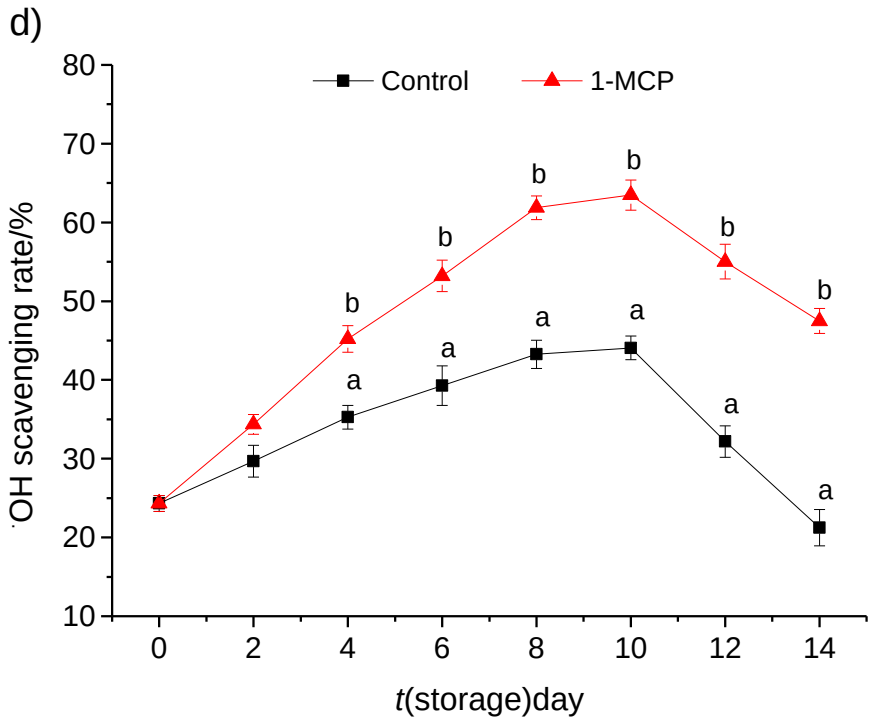


Fig. 2. Conductivity (a), MDA (b), TSS (c), TA (d), chlorophyll content (e) and ascorbic acid content (f) of snap beans. Snap beans were stored at 4 °C for up to 14 days. Data are presented as mean value±S.D. of three replications









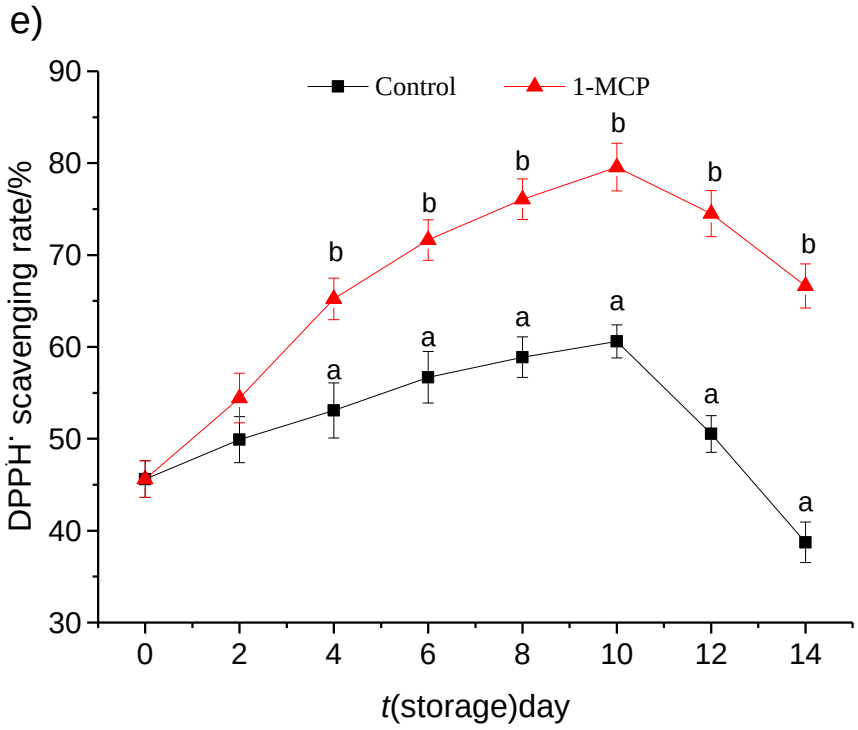
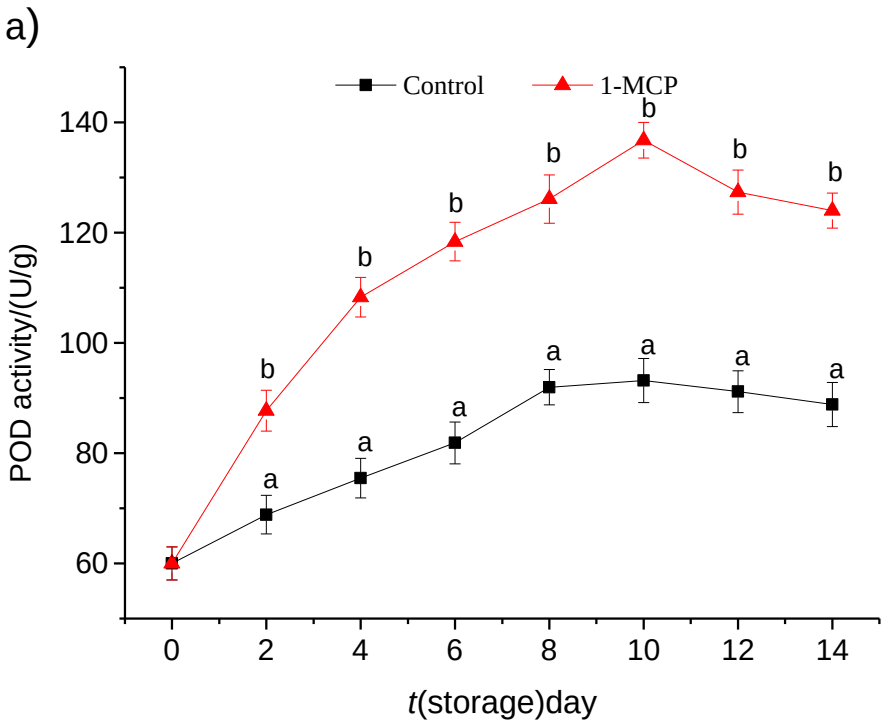
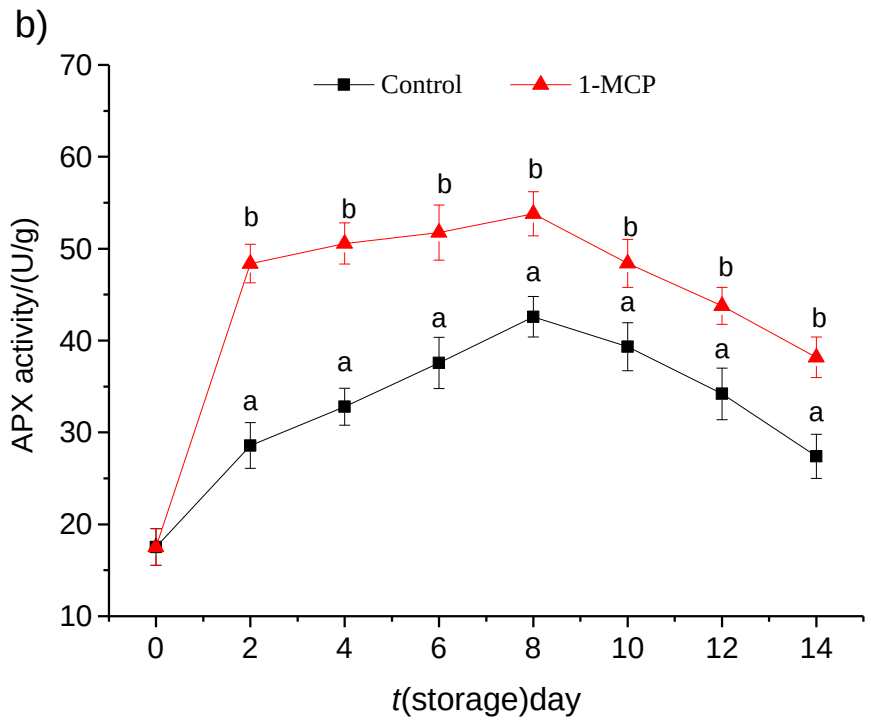


Fig. 3. Total antioxidant capacity (a), metal chelating activity (b) and free radicals $O_2^{\cdot-}$ (c), ·OH (d) and DPPH· (e) scavenging rates of snap beans. Snap beans were stored at 4 °C for up to 14 days. Data are presented as mean value±S.D. of three replications. Different letters indicate significant differences among treatments according to Duncan's test at p=0.05. No differences were detected whose letters are absent





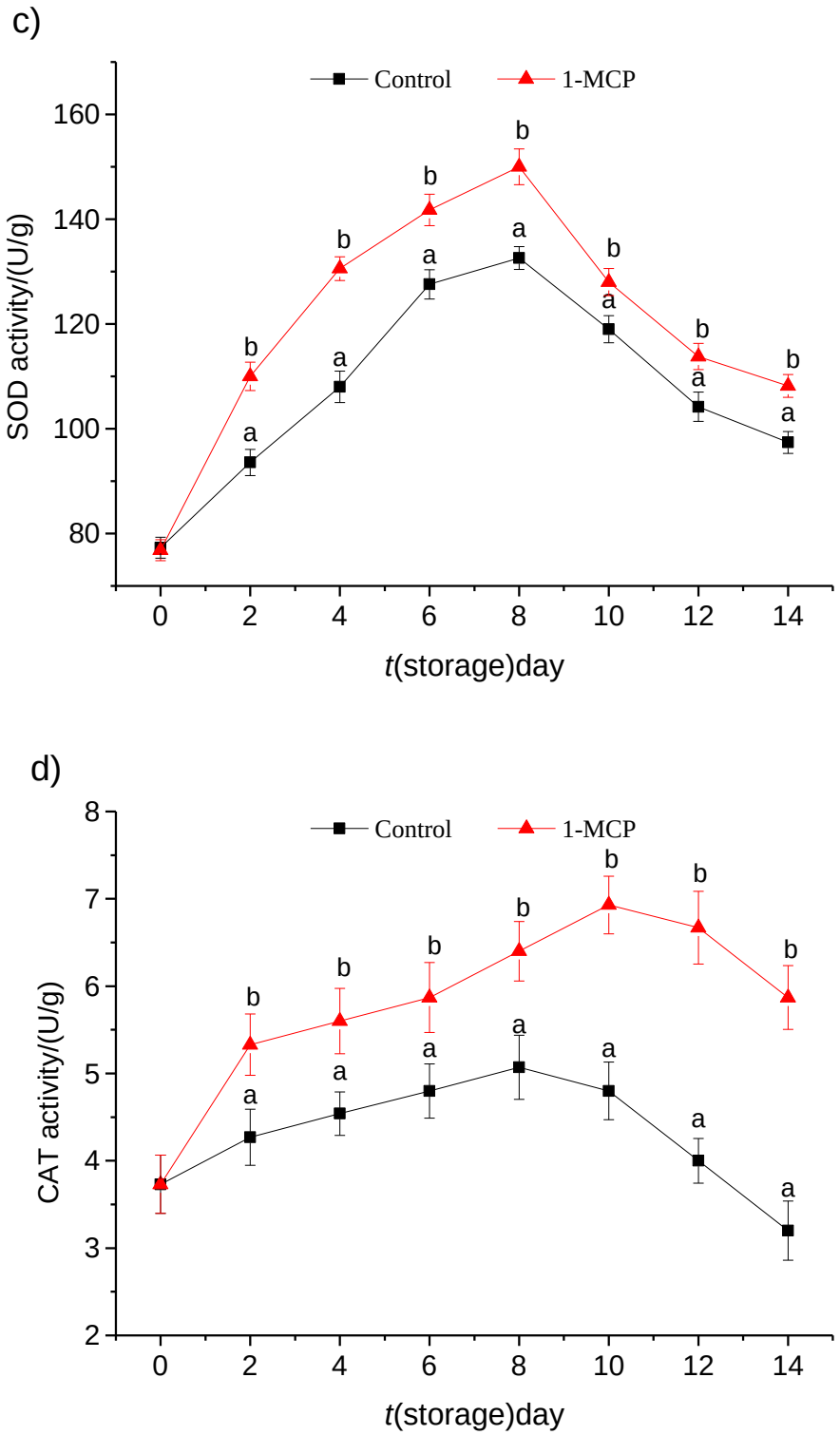
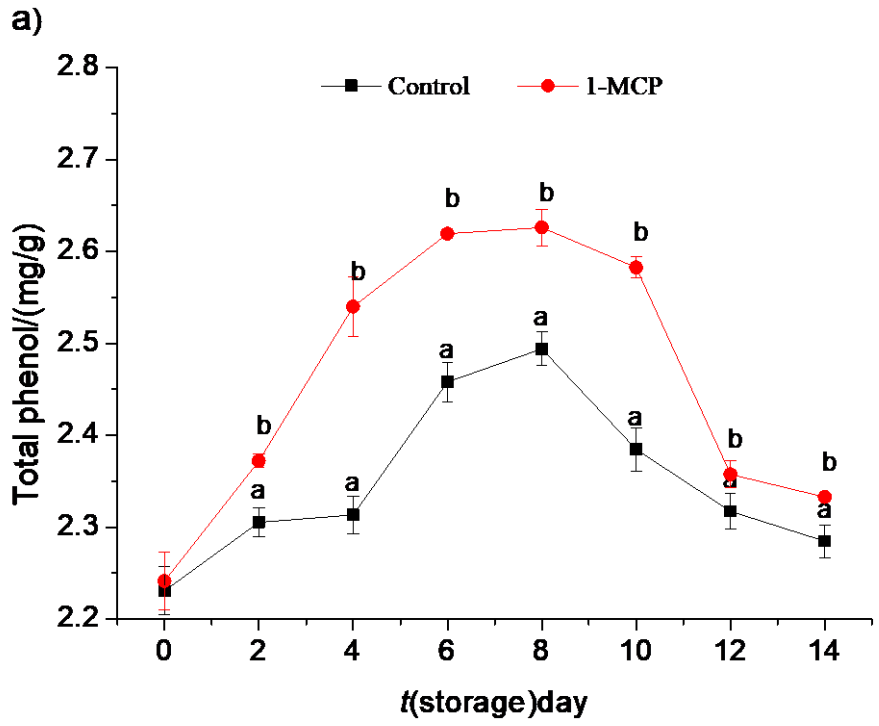
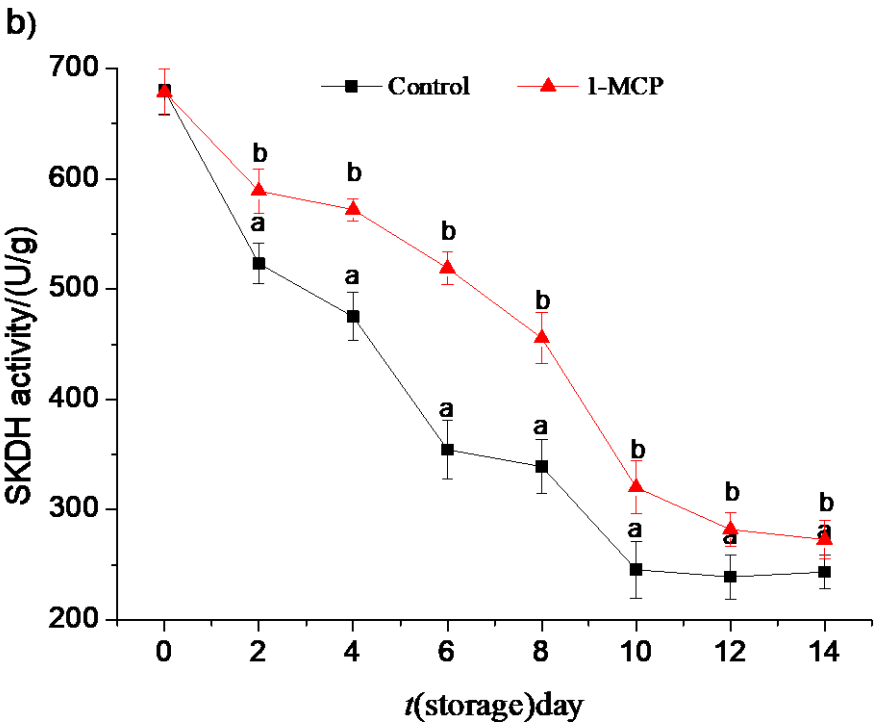


Fig. 4. Activities of POD (a), APX (b), SOD (c) and CAT (d) of snap beans. Snap beans were stored at 4 °C for up to 14 days. Data are presented as mean value±S.D. of three replications. Different

letters indicate significant differences among treatments according to Duncan's test at p=0.05. No differences were detected whose letters are absent





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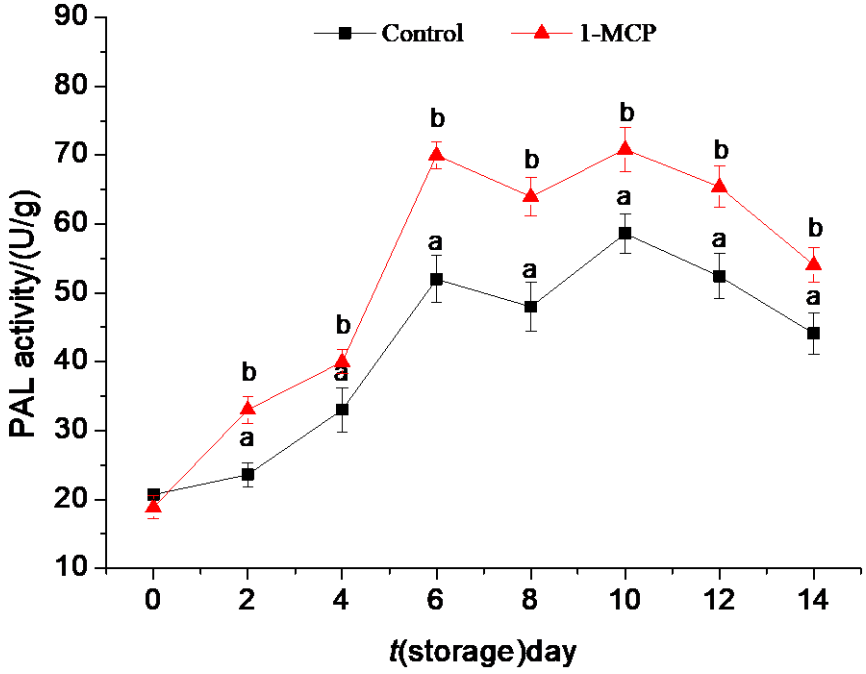

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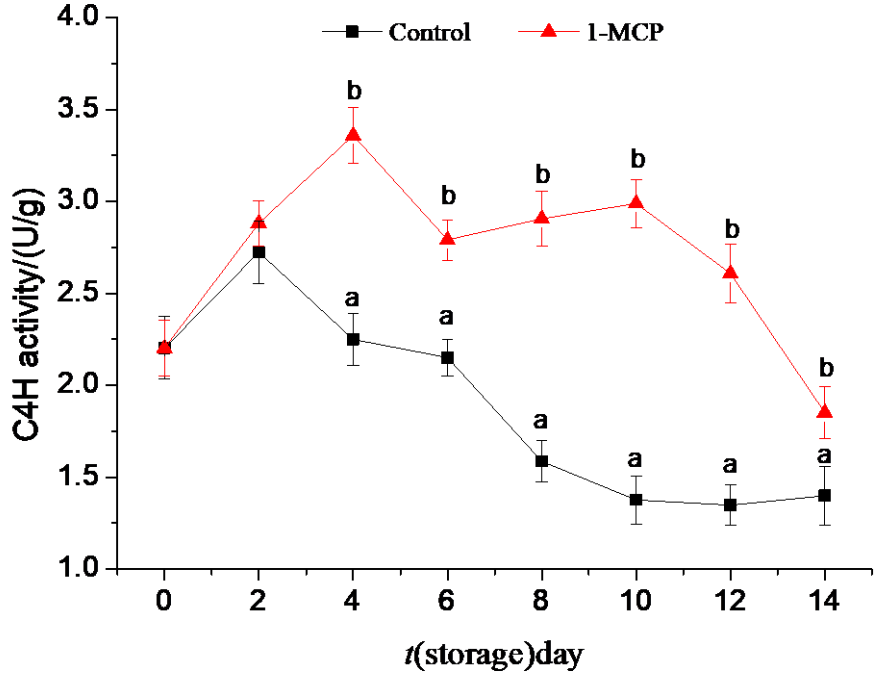

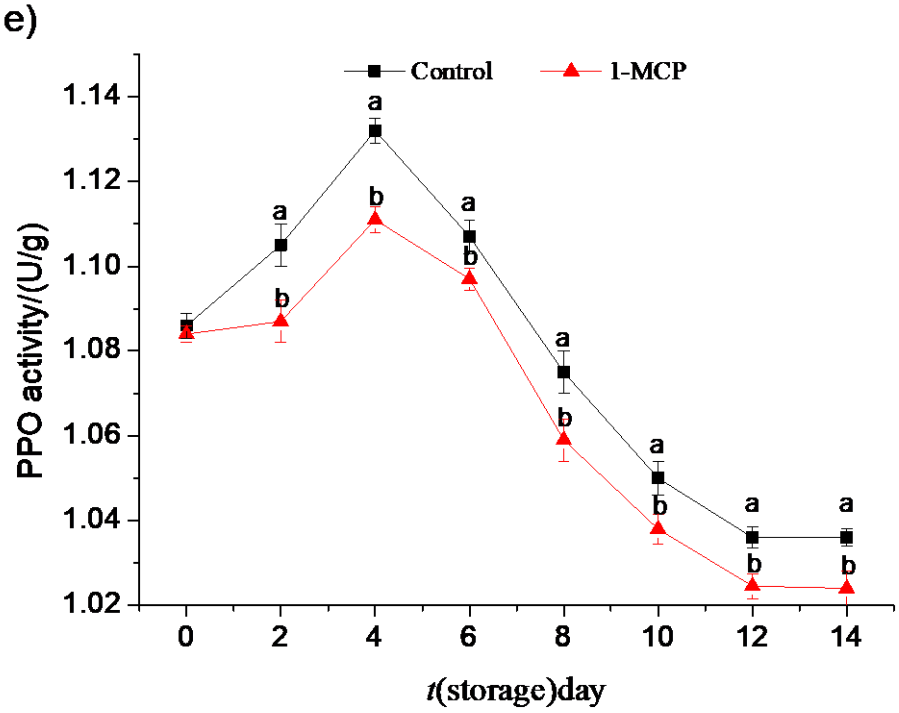

Fig. 5. Total phenolic content (a) and activities of SKDH (b), PAL (c), C4H (d) and PPO (e) of snap beans. Snap beans were stored at 4 °C for up to 14 days. Data are presented as mean value±S.D. of three replications. Different letters indicate significant differences among treatments according to Duncan's test at p=0.05. No differences were detected whose letters are absent

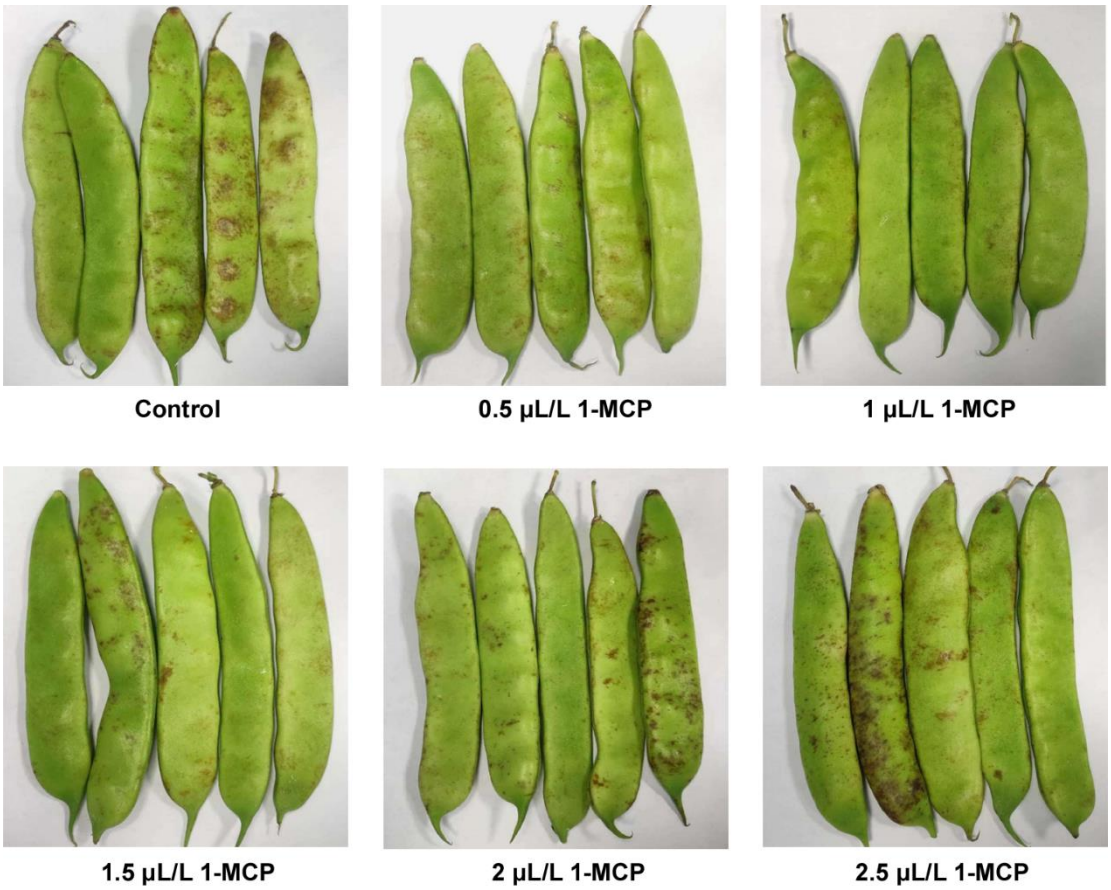

Fig. S1. The visual image of chilling injury in snap beans. Snap beans were stored at 4 °C for up to 14 days