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ORIGINAL ARTICLE

Retention of iceberg lettuce quality by low temperature storage and postharvest application of 1-methylcyclopropene or gibberellic acid

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Abstract This study was conducted to evaluate the changes in quality of iceberg lettuce during storage at different temperatures and the effects of postharvest treatments of 1-methylcyclopropene or gibberellic acid at high temperature. The results showed that quality of the lettuce was remarkably retained during storage at 0 °C, but significantly declined at 20 °C. However, quality of the vegetable at shelf-temperature (20 °C, 85~95% RH) was effectively delayed by the treatment with 1-methylcyclopropene (1-MCP) or gibberellic acid (GA). Browning of the lettuce leaves was significantly inhibited by the storage at low temperature and by treatment with1-MCP and GA. The biochemical analysis further indicated that the reduction of soluble protein and sugar, decrease in activity of polyphenol oxidase (PPO) and peroxidase (POD) and accumulation of free amino acids in the lettuce leaves during storage could be remarkably prevented by low temperature, treatment with1-MCP or GA. Our result suggested that 1-MCP or GA treatment would provide a potential way for controlling quality of the lettuce under suboptimal postharvest temperature conditions.

Keywords Quality · *Lactuca sativa* L. · Postharvest · Low temperature

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Introduction

Iceberg lettuce is one of the most highly favorable fresh vegetables, being a traditional ingredient in salads, sandwiches, and other items found in self-service restaurants (Da Cruz et al. 2008). Nevertheless, it is known quality of iceberg lettuce declines rapidly at ambient temperature, which deeply limits storage and consumption of the vegetable. Edible quality both sensorial and nutritional of iceberg lettuce declines rapidly after harvest and considerable losses may occur during storage.

Deterioration of postharvest quality of vegetables could be inhibited by application of some treatments. Studies from Chandra et al. (2010) indicated that a longer shelf-life along with a higher quality of iceberg lettuce could be ensured by storing the vegetable at low temperature. However, in practice iceberg lettuces are usually handled at ambient temperature during transportation and on shelf in the market in China and many other countries, and may loose quality severely in hot weather.

1-Methylcyclopropene (1-MCP) is a competitive inhibitor of ethylene action which binds to the ethylene receptor to regulate tissue responses to ethylene (Sisler and Serek 1997). Previous studies showed that 1-MCP could extend storage life of iceberg lettuce at low temperature (Tay and Perera 2004; Wills et al. 2002). It has been shown that reducing the ethylene level can extend storage life of lettuce packaged in polyethylene bags by delaying leaf browning at both temperatures of 20 °C and 0 °C (Kim and Wills 1995). However, little attention has been devoted to the effect of 1-MCP on the quality of iceberg lettuce on shelf-life at ambient temperature.

Gibberellic acid (GA) is a growth regulator which shows important effect on senescence. Retarding effects of GA on postharvest quality decline were demonstrated in a few leafy vegetable species (Jiang et al. 2002; Lers et al. 1998). The effects of growth regulators on various senescence-related processes can vary, depending on factors such as plant species, leaf age, experimental system and conditions (Van Staden et al. 1988). Until recently, there is lack of knowledge about the influence of GA on quality of iceberg lettuce during storage at room temperature.

The objects of this research reported here were to evaluate changes of quality of iceberg lettuce during storage at different temperatures (0 °C, 10 °C or 20 °C, 85~95% RH) and to investigate the effects of postharvest treatment with 1-MCP or GA on quality of the lettuce at room temperature (20 °C, 85~95% RH).

Material and methods

Plant materials and treatments

Iceberg lettuces (*Lactuca sativa* L. var. asparagina) were grown under standard commercial conditions, in a vegetable farm in Beijing. Iceberg lettuces were harvested and packed in the early morning and immediately transported to the laboratory. Iceberg lettuces were selected for uniform size with whole head, and wrapper leaves were discarded. Each replication contained 20 lettuce heads and a total of 60 heads were used for 3 replicates in the experiments.

In experiment I, each 20 lettuce heads were placed in a plastic basket and packed with an unsealed polyethylene (PE) bag, then stored at 0 °C, 10 °C or 20 °C, 85~95% RH.

In experiment II, 1-Methylcyclopropene (1-MCP) was released from a commercial powdered formulation (Ethlyl-Bloc, Rohm and Haas China, Inc). Concentration of 1-MCP was determined by a gas chromatograph equipped with a hydrocarbon separated column and calibrated against butane. The iceberg lettuces placed in a plastic basketry together with a beaker containing 1 mol/L KOH to absorb CO_2 from respiration of the lettuce, were sealed in a PE bag. An aliquot of 1-MCP was injected into the bag to the level of 0.2 μL/L 1-MCP. After being incubated with 1-MCP at 20 °C for 24 h in darkness, the bags were unsealed and stored at 20 °C, 85~95% RH. Gibberellic acid (GA) was purchased from Sigma-Aldrich Co., Ltd (Shanghai, China). The lettuces were treated by spraying the GA solution (0.1 mg/L GA, 0.1% Triton X-100, 2 mL/per head), then placed in plastic basket and packed with an unsealed PE bag, and stored at 0 °C, 10 °C or 20 °C, 85~95% RH. The control lettuces were placed in plastic basket and packed with an unsealed PE bag, and stored at 0 °C, 10 °C or 20 °C, 85~95% RH.

The visual quality and browning spot level were measured on the same day of sampling as indicated in the results. For the other biochemical parameters, including soluble sugar or protein content, free amino acids, PPO and POD activities, the sample materials were stored at −18 °C before carrying the extractions.

Visual quality evaluation

The visual quality of lettuce was evaluated by 10 panelists randomly selected from the research students in the College of Food Science and Nutritional Engineering, China Agricultural University. They have been trained up before conducting their real experiment. Ten lettuce heads were evaluated for each treatment by a panelist. Lettuce visual quality for marketability was evaluated using the scoring system of Kader et al. (1973). Overall visual quality (OVQ) (Sharma et al. 2011; Kishore et al. 2011) was rated on a scale from 9 to 1, where 9=excellent (essentially free from defects) and 1=extremely poor (not useable), 3, 5, and 7 representing poor (excessive defects, limit of salability), fair (slightly to moderately objectionable defects; lower of sales appeal), and good (minor defects; not objectionable) quality respectively. An OVQ rating of 6 was considered the limit of salability (Nunes et al. 2009; Liu 2011). The samples were inspected for visual quality attributes every 2 days over a period of 6 days.

Browning evaluation

Skin appearance was assessed as the extent of the total browned area on 30 lettuces, comprising 10 lettuces collected randomly from each of three bags. Subjective evaluation of the browning on the lettuce leaf surface was determined visually using: 0, no browning (excellent quality); 1, browning spots on all the leaves in single lettuce head ≤4, spot diameter ≤2 mm); 2, browning spots on all the leaves in single lettuce head ≤8, spot diameter 2~3 mm; 3, browning spots on all the leaves in single lettuce head ≤10, spot diameter 3~4 mm and 4, browning spots on all the leaves in single lettuce head >10, spot diameter ≥4 mm (poor quality) The browning index was calculated as $\sum(\text{browning level} \times \text{number of lettuces with that browning level})/(\text{total number of lettuces})$ (López-Gálvez et al. 1996; Rennie et al. 2001). The samples were inspected for visual quality attributes every 2 days over a period of 6 days.

Extractions and measurements

Greenish white leaves from the middle part of lettuce head were used for biochemical assay. Four gram samples were homogenized in 5 mL 50 mmol/L phosphate buffered saline (pH 7.5). Following centrifugation at 4 °C for 30 min at 12,000×*g*, the supernatant was collected. Soluble sugar content in the supernatant was determined by a colorimetric method according to the method of Dubois et al. (1956)

using glucose (Sigma-Aldrich trading Co., Ltd, Shanghai, China) as sugar standard. Two mL of the extract were mixed with 0.05 mL 80% phenol, then added in with 5 mL concentrated H_2SO_4 (98%), mixed thoroughly; after being incubated at room temperature for 25 min, absorbance of the mixture were measured at 485 nm.

Greenish white leaves from the middle part of lettuce head samples (2 g) were ground in ice-cold mortar and pestle with 2 mL of 300 mmol/L Tris–HCl buffer (pH 7.8). The homogenate was centrifuged at 12,000×*g* at 4 °C for 30 min and the supernatant was collected and used for determination of soluble protein and amino acids. Soluble protein contents of lettuce samples were measured according to the method of Bradford (1976) using bovine serum albumin (Sigma-Aldrich Co., Ltd, Shanghai, China) as protein standard. Amino acids contents of lettuce samples were measured according to the method of Yemm and Cocking (1954) using methionine (Sigma-Aldrich Co., Ltd, Shanghai, China) as the standard.

Peroxidase (POD; E.C.1.11.1.7) was assayed according to Zeng et al. (2006), 100 μL of the extract was incubated with 2.5 mL 25 mmol/L guaiacol (Sigma-Aldrich Co., Ltd., Shanghai, China) and 200 μL 250 mmol/L H_2O_2 at 24 °C for 3 min, and absorbance at 470 nm measured with an spectrophotometer (Purkinje General instrument Co., Ltd., Beijing, China). The POD activity was expressed as 0.01 ΔA_{470} min^{-1} mg^{-1} protein. Polyphenoloxidase (PPO; EC 1.10.3.1) was assayed according to Zhao et al. (2009), 100 μL of the extract was incubated with 2 mL 0.05 mol/L phosphate buffer (pH 7.0) and 0.5 mL 0.5 mol/L catechol at 24 °C for 2 min, and absorbance at 420 nm measured with an spectrophotometer. The PPO activity was expressed as 0.01 ΔA_{420} min^{-1} mg^{-1} protein.

Statistical analysis

Data were analyzed by analysis of variance (ANOVA) with SPSS 11.0 statistical software (SPSS Inc., Chicago, IL, USA). Significant differences were performed by Duncan's new multiple range tests. Differences at $P \leq 0.05$ were considered as significant. In all experiments, each treatment was carried out on three replicates, and all experiments were repeated three times with similar results.

Results and discussion

Effects of temperature, 1-MCP or GA on visual quality of iceberg lettuce

Visual analysis provides a critical additional means to evaluate the quality of fruits and vegetables (Mattheis and Fellman 1999). The results from the visual quality of iceberg lettuce are presented in Fig. 1. Visual quality of the lettuce was remarkably retained by low temperature (0 °C) but reduced by shelf temperature (20 °C) at storage period (Fig. 1a). The overall visual quality of the lettuce stored at 10 and 20 °C was not useable after 6 days of storage. However, higher temperatures are often encountered during handling, transportation and marketing in many developing countries. In order to resolve this problem, some treatments should be used for retention the quality. The use of 1-MCP can significantly reduce ripening and red index on green mature tomatoes stored at 25±1 °C (Paul et al. 2010). The stored pear fruits also retained post-storage shelf life by 1-MCP for 3 days at ambient conditions and 6 days at supermarket conditions (Mahajan et al. 2010). Therefore the effectiveness of 1-MCP or GA on retention quality of iceberg lettuce at 20 °C was evaluated. The result has shown as Fig. 1b, visual quality of the lettuce was remarkably retained by 0.2 μL/L 1-MCP or 0.1 mg/L GA when the lettuce leaves were stored in shelf-life at 20 °C. Even at the 6th day of shelf period, the quality of the lettuce treated by 1-MCP or GA was acceptable. No significant differences were found in lettuce appearance between the samples treated with 1-MCP and GA, however, was still much higher than the control. Many similar results have been reported in the other postharvest fruits and vegetables (Nanthachai et al. 2007). For example, 1-MCP was effective in extending plum fruit quality (Khan et al. 2009). The treatment of GA gave the best results in sweet cherry fruit for appearance and texture at the end of shelf life (Koyuncu et al. 2008).

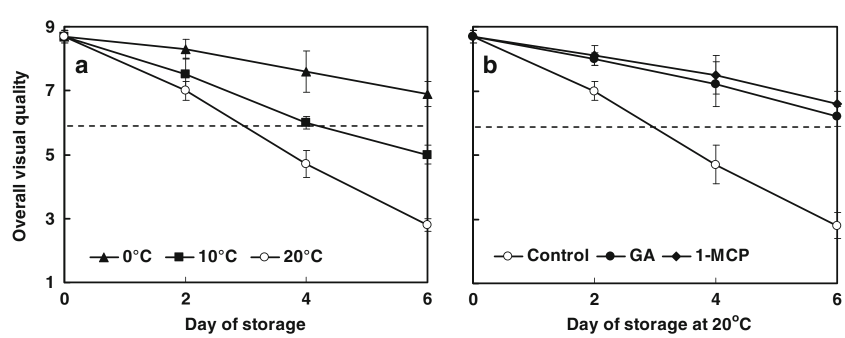

Fig. 1 Effects of temperature, 1-MCP or GA on visual quality of iceberg lettuce. **a** Effects of storage temperature on the visual quality of iceberg lettuce. **b** Effects of 1-MCP or GA on the visual quality of iceberg lettuce stored at 20 °C, 85~95% RH. Each point represents the mean± S.E (as *vertical bars*), *n*=10

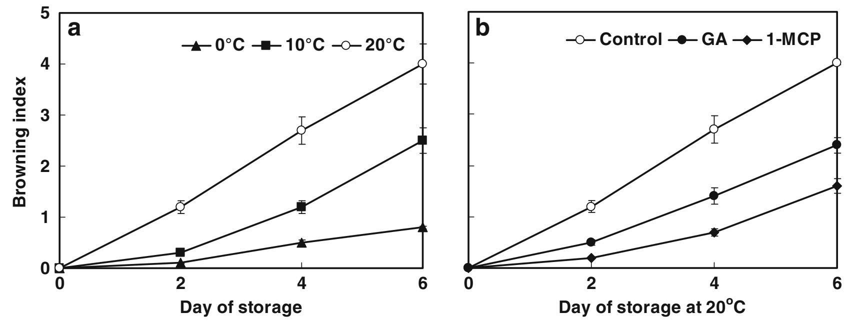

Fig. 2 Effects of temperature, 1-MCP or GA on development of browning spot of iceberg lettuce. **a** Effects of storage temperature on development of browning spot in iceberg lettuce. **b** Effects of 1-MCP or GA during shelf-life on development of browning spot in iceberg lettuce. Each point represents the mean±S.E (as *vertical bars*), n=10

Effects of temperature, 1-MCP or GA on browning spot of iceberg lettuce

Development of browning spot on leaves was observed during storage. Browning spot on leaves was remarkably prevented by low temperature (0 °C) and increased by shelf temperature (20 °C) at storage period (Fig. 2a). The effects of 1-MCP on inhibition of browning spot development on lettuce leaves during storage was also observed in several former studies (Fan and Mattheis 2000; Wills et al. 2002; Tay and Perera 2004), but how postharvest treatment especially 1-MCP or GA affect shelf-life was not clear. In order to investigate browning spot of iceberg lettuce on shelf-life, the effects of GA or 1-MCP on browning spot of iceberg lettuce at 20 °C was evaluated. Results from the browning index (Fig. 2b) indicated that 1-MCP or GA had effect on retarding the subsequent storage browning spot of the lettuce. Developing degree of the browning spot on the leaves was significantly (P<0.05) inhibited by 1-MCP or GA.

Effects of temperature, 1-MCP or GA on soluble sugar content of iceberg lettuce

Besides textural properties, lettuce quality depends on sensory characteristics and nutritional qualities affecting shelf-life (Chandra et al. 2010). Sweetness is an important factor affecting sensory of fresh vegetables. Treatment with low-temperature storage further retained sugar levels in the lettuce. Soluble sugar content (SSC) in the lettuce was significantly decline during storage at 20 °C (Fig. 3a). In order to detect SSC of iceberg lettuce on shelf-life, the effects of 1-MCP or GA on iceberg lettuce at 20 °C was evaluated. As shown in Fig. 3b, SSC in the lettuce leaves decline during shelf-life at 20 °C, whereas declining of SSC in the leaves was retarded by treatment with 1-MCP or GA. SSC in leaves treated with 1-MCP or GA was about 14% or 10% higher than that in control in the 6th d of shelf-life, respectively. Many other studies were also agreed with this result. The guava fruits treated with 1-MCP was also maintained acceptable quality in terms of total soluble solids and acidity (Mahajan and Gafandeep 2008).

Effects of temperature, 1-MCP or GA on soluble protein and free amino acids of iceberg lettuce

Low-temperature storage also retarded protein degradation in the lettuce leaves during storage (Fig. 4a). In order to investigate soluble protein of iceberg lettuce on shelf-life, the effects of 1-MCP or GA on iceberg lettuce at 20 °C was evaluated. As shown in Fig. 4b, 1-MCP or GA treatment retarded protein degradation in the lettuce leaves during shelf-life. Level of soluble protein in 1-MCP or GA treated leaves was 27% or 24% higher than that in control in the 6th d of shelf-life, respectively. Contrary to the trend in protein levels, level of free amino acids (FAA) in the lettuce leaves increased during storage. As shown in Fig. 4c, treating the lettuce with low-temperature retarded FAA accumulation in the leaves. Treating the lettuce with 1-MCP or GA also retarded FAA accumulation in the leaves (Fig. 4d). FAA level in 1-MCP or GA treated lettuce leaves

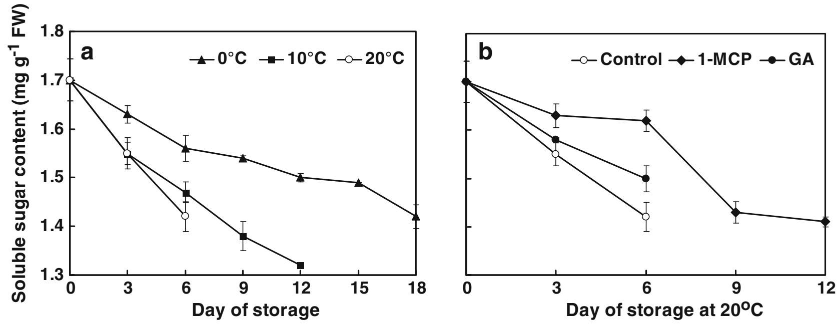

Fig 3 Effects of temperature, 1-MCP or GA on soluble sugar content of iceberg lettuce. **a** Effects of storage temperature on soluble sugar content in iceberg lettuce. **b** Effects of 1-MCP or GA during shelf-life on soluble sugar content in iceberg lettuce. Each point represents the mean of 3 replicates±S.E (as *vertical bars*)

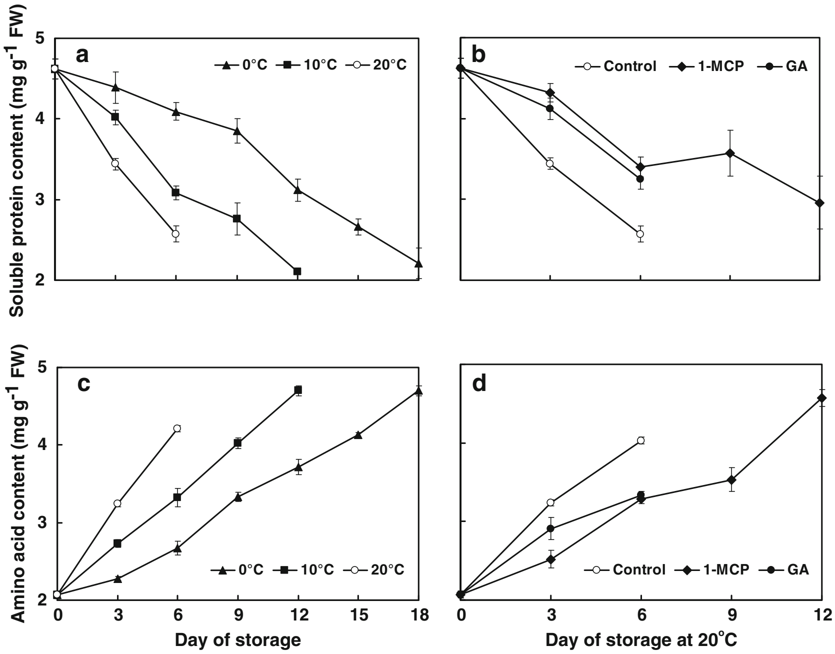

Fig. 4 Effects of temperature, 1-MCP or GA on soluble protein content and free amino acids level of iceberg lettuce. **a** Effects of storage temperature on soluble protein content in iceberg lettuce. **b** Effects of 1-MCP or GA during shelf-life on soluble protein content in iceberg lettuce. **c** Effects of storage temperature on free amino acids level in iceberg lettuce. **d** Effects of 1-MCP or GA during shelf-life on free amino acids level in iceberg lettuce. Each point represents the mean of 3 replicates±S.E (as *vertical bars*)

was about 18% lower than that in control in the 6th d of shelf-life. The trends in the visual quality scale of the leaves during storage are positively correlated to the trends in protein levels, and negatively correlated to the trend in free amino acids. It has been proposed that sensory quality of coriander leaf is related to the levels of protein and free amino acids in tissues, and the accumulation of free amino acids in leafy vegetables, such as methionine, can result in poor flavor quality (Jiang et al. 2002). This view is supported by the present study result that treatment with 1-MCP or GA could prevent accumulation of free amino acids in lettuce leaves and retain quality of iceberg lettuce.

Effects of temperature, 1-MCP or GA on PPO and POD activity of iceberg lettuce

Although the effects of 1-MCP on inhibition of browning spot development on lettuce leaves during storage was observed in several former studies (Fan and Mattheis 2000; Wills et al. 2002; Tay and Perera 2004), mechanisms in the phenomenon was still unclear. It has been demonstrated that some enzymes, including PPO and POD, are involved in the oxidation of polyphenols into quinones that contributes the brown pigments in plant tissues (Mohammadi and Kazemi 2002; Saltveit 2004). PPO and POD have been shown to be

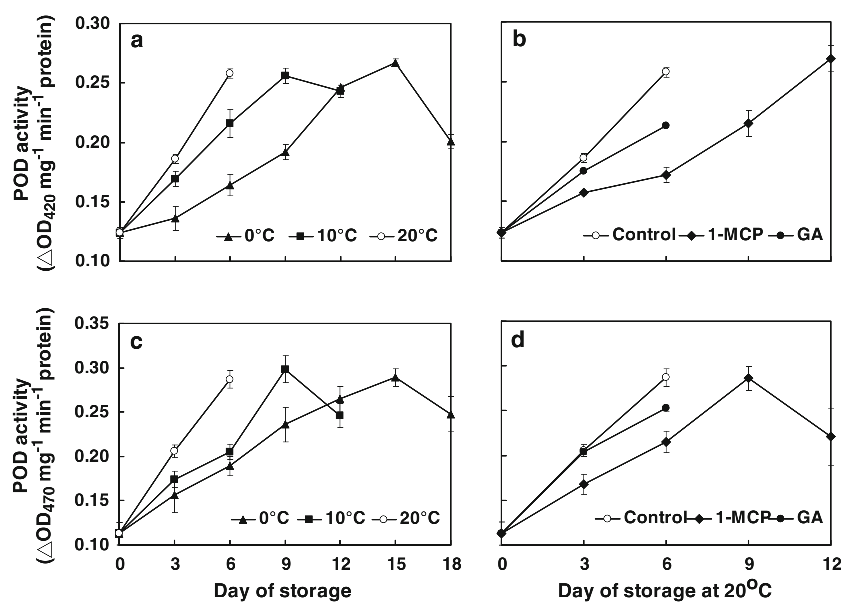

Fig. 5 Effects of temperature, 1-MCP or GA on PPO and POD activity of iceberg lettuce. **a** Effects of storage temperature on PPO activity in iceberg lettuce. **b** Effects of 1-MCP or GA during shelf-life on PPO activity in iceberg lettuce. **c** Effects of storage temperature on POD activity in iceberg lettuce. **d** Effects of 1-MCP or GA during shelf-life on POD activity in iceberg lettuce. Each point represents the mean of 3 replicates±S.E (as *vertical bars*)

responsible for browning reactions during postharvest handling, storage and processing of fruits and vegetables (Peiser et al. 1998). As shown in Fig. 5a, the increasing trends of PPO activity were significantly retarded by low temperature ($P<0.05$). The increasing trends of PPO activity were significantly retarded by 1-MCP or GA during shelf-life (Fig. 5b). PPO activity in the 1-MCP or GA treated lettuce leaves was 33% or 17% lower than that in control in the 6th d of shelf-life, respectively. POD activity in the lettuce tissue also increased during the storage. The increasing trends of POD activity were significantly retarded by low temperature (Fig. 5c). However, POD activity of the lettuce was accelerated stored at 20 °C. The increasing trends of POD activity were significantly retarded by 1-MCP or GA during shelf-life (Fig. 5d). POD activity of the 1-MCP or GA treatment was about 25% or 17% lower than that in control in the 6th d of shelf-life, respectively. This result was also similar to Peiser and co-worker's study (Peiser et al. 1998). They suggested PPO and POD may also play important roles in the developing russet spot on iceberg lettuce leaves during storage. Our study showed that both PPO and POD activity in the lettuce leaves were suppressed by 1-MCP or GA. These changes were negatively correlated ($P<0.05$) to the development of russet spot in the heads treated by 1-MCP or GA.

It has been found that reducing the ethylene level can delay leaf browning of lettuce stored at 20 °C or 0 °C (Kim and Wills 1995). This suggests that ethylene may play a important role in senescence of lettuce, which is further supported by our results of that 1-MCP could prevent leaf browning and reductions of soluble sugar and protein in the lettuce, and reduce activity of POD that involved in the oxidation of polyphenols into quinines (resulted in tissue browning).

In conclusion, the quality of lettuce was remarkably retained during storage at 0 °C. Furthermore, the decline in quality of the lettuce at high temperature (20 °C, 85~95% RH) could be effectively delayed by treatment with 1-MCP or GA. Therefore, commercial use of 1-MCP or GA may enhance iceberg lettuce quality under suboptimal postharvest conditions.

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