African Journal of Biotechnology Vol. 10(34), pp. 6581-6589, 11 July, 2011
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Effect of 1-methylcyclopropene and modified atmosphere packaging on chilling injury and antioxidative defensive mechanism of sweet pepper

Xihong Li[1], Juan Yun[1]*, Xuetong Fan[2], Yage Xing[1] and Yao Tang[1]

[1]Key Laboratory of Food Nutrition and Safety, Ministry of Education, Tianjin University of Science and Technology, Tianjin, 300457, People's Republic of China.
[2]U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 E. Mermaid Lane, Wyndmoor, PA 19038, USA.

Accepted 15 April, 2011

Some sweet peppers (*Capsicum annuum* L.) are chilling sensitive and can develop injury when stored at temperatures less than 7°C. This study was conducted to investigate the effect of 1-methylcyclopropene (1-MCP) (650 ppb) and modified atmosphere packaging (MAP) on chilling injuries (CI) of sweet pepper during 30 days storage at 4°C. The results showed that, 1-MCP and MAP reduced chilling injury symptoms which were correlated with decreased electrolyte leakage and malondialdehyde content. The combination of 1-MCP and MAP further reduced chilling injury. Atomic force microscope (AFM) images showed that, the surface of the sweet peppers with 1-MCP and MAP treatments were smoother than of the control samples. The activities of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) of sweet peppers were also influenced by 1-MCP and MAP. SOD, CAT and POD activities of sweet peppers were 87.3, 28.97 and 0.248 U·g^{-1}, respectively at the beginning of the storage. The activities decreased during the first 15 days of storage followed by an increase during the later period of storage. Treatment with 1-MCP, MAP alone and in combination frequently reduced the activities of those enzymes during storage. These results suggested that, combination of 1-MCP treatment and MAP is a promising treatment for reducing chilling injuries of peppers stored at 4°C.

Key words: 1-MCP, chilling injuries, modified atmosphere packaging, sweet pepper.

INTRODUCTION

There is an increasing demand for high quality fruits and vegetables. However, many fruits and vegetables, such as sweet peppers, are sensitive to low temperatures and may be easily injured after a period of exposure to chilling temperatures (Paull, 1990; Wang, 1990). When chilling stress is prolonged, a variety of chilling injury symptoms, such as surface lesions, internal discoloration and water-soaking of the tissue would take place (Saltveit and Morris, 1990). Sweet peppers are one of the most nutritive vegetables containing a high amount of ascorbic acid, an essential human nutrient (Davey et al., 2002). However, one of the most significant problems for postharvest storage of sweet peppers is chilling injury when stored below 7°C (Meir et al., 1995). Sweet pepper varieties with a high susceptibility to chilling injury showed sheet pitting, alternaria rot on pods and calyxes and darkening of seeds (Chien, 1997). It is a challenge to reduce the chilling injury and maintain freshness of sweet peppers (Nilprapruck et al., 2008).

1-Methylcyclopropene (1-MCP) is a blocker of ethylene receptors and is being used as an agent to investigate the role of ethylene in ripening and senescence of many fruits and vegetables (Fan et al., 1999a; Blankenship and Dole, 2003, Marin et al., 2009; Zhang et al., 2010; Mao et al., 2004). Studies have shown that, chilling injury can be either increased or decreased by 1-MCP depending on commodities. For example, 1-MCP reduced the development of apple superficial scald (Fan et al., 1999b), a physiological disorder that occurs in the skin

*Corresponding author. E-mail: yunjuan_1@yahoo.com.cn. Fax: +86 2260601341.

during prolonged low-temperature storage.

1-MCP can reduce ethylene production and maintain firmness and acidity in climacteric peppers (Menniti et al., 2004; Candan et al., 2006). However, the effect of 1-MCP on chilling injury of sweet peppers has not been investigated. 1-MCP effectively reduces chilling injury symptoms in plums. Chilling injury in plums is related to the climacteric behaviour of the cultivar and to the quick induction on a short-term basis of higher capability to produce ethylene in the cold (Candan et al., 2006). Additionally, the effects of 1-MCP treatment are due to not only its action on ethylene but also a direct influence on the antioxidant potential (Larrigaudière et al., 2004; Vilaplana et al., 2006).

On the other hand, modified atmosphere package (MAP), in terms of reduced O_2 and elevated CO_2, helps to maintain freshness and visual appearance of fruits and vegetables by reducing their respiration rate as well as inhibiting metabolic activity, ethylene sensitivity and production, and physiological and pathological deterioration during storage (Gorris and Tauscher, 1999). For the MAP technology, initial gas flushing is sometimes used to accelerate gas composition modification to avoid product exposure to high concentrations of unsuitable gases like O_2, CO_2, C_2H_4, moisture, flavours and odours (Vermeiren et al., 1999). The storage life of fresh fruits and vegetables is considerably increased by modifying the atmosphere around the food (Jayas and Jeyamkondan, 2002). Therefore, they are normally packaged in modified atmospheres at refrigerated temperatures during storage, manufacture, distribution and retailing.

Nonetheless, there is little information about the effect of 1-MCP and MAP on chilling injury and antioxidative defense system of sweet pepper (Fallik et al., 1999; Serrano et al., 2010; Banks and Nicholson, 2000). Therefore, in this study, we investigated the effect of the combined application of 1-MCP and MAP on chilling injury of sweet pepper in relationship to antioxidant enzymes.

MATERIALS AND METHODS

Samples preparation and 1-MCP treatment

Sweet peppers (*Capsicum annuum L.*) were harvested from a commercial farm in Tianjin, China. Pepper fruits were transported immediately after harvest to the Key Laboratory of Food Nutrition and Safety, Ministry of Education, Tianjin University of Science and Technology. Fruits of regular shape and uniform size were selected. Four different treatments were used: (1) control, (2) 1-MCP, (3) MAP and (4) 1-MCP +MAP. The experimental design was a randomized block with three replicates of 90 fruits for each treatment; 5 fruits (350 to 400 g) were used to analyze various physicochemical indices. Then, 650 ppb 1-MCP (EthyBloc®, BioTechnologies for Horticulture, IL, USA) was applied to the fruits placed in sealed air-tight polyethylene (PE) bags (30 µm thickness, 4 m^3) and the rest were exposed to air at 10°C for 5 h under the same conditions. The 1-MCP concentration was calculated according to the percentage of active ingredient in the SmartFresh™ powder and the free head-space of the sealed bags. The samples were stored at 4°C for 30 days and the fruits left in air at the same temperature were used as the control. Before the establishment of storage conditions, the PE bag was opened and the chamber was thoroughly aerated. Triplicate samples were prepared for every treatment and two determinations were carried out per triplicate sample (n=6). The changes of CO_2 and O_2 concentration in the packages were measured using a checkpoint O_2/CO_2 analyzer (PBI–Dansensor A/S, Denmark).

Analytical methods

Chilling injury index

The severity of chilling injuries was evaluated every 5 days. The degree of chilling injury was measured by the extent of surface browning or blackening of the pulp, using the following scale: (1) no damage (2) black less than 5% of total area, (3) black less than 30% of total area, (4) black 30 to 50% of fruit area and (5) more than 50% of fruit area black (Wongsheree et al., 2009). Three packages of fruits were randomly sampled each time.

Determination of MDA content and membrane permeability

Malondialdehyde (MDA) content was assayed with the thiobarbituric acid (TBA) reaction, according to the reported method (Li, 2000). About 0.2 g of pulp was dissolved in 10 ml of 10% (w/v) trichloroacetic acid; the mixture was centrifuged at 4000 *g* for 10 min at 4°C. After incubating of a 2 ml of the supernatant with 2 ml 6.7 g L^{-1} TBA for 30 min at 95°C, the mixture was quickly cooled in an ice bath and further centrifuged at 4000 *g* for 10 min. The absorbance of the supernatant was read at 532, 450 and 600 nm, respectively, with the 756-PC UV/VIS spectrophotometer (Shanghai Spectrum Instruments Co., Ltd., China) and the results were expressed as µmol g^{-1}.

MDA content (µmol g^{-1}) = $6.45 \times (OD_{532} - OD_{600}) - 0.56 \times OD_{450}$.

Membrane permeability, expressed by relative leakage rate, was determined according to the method of Zhang et al. (2005). Pepper fruits were sliced into small discs (0.2 cm thick, 1.0 cm diameter) and washed three times with deionised water to remove surface-adhered electrolytes. After blotting out the surface moisture with filter paper, 10 discs were placed in closed vials containing 30 ml deionized water and shaken at 25°C on a rotary shaker for 30 min; subsequently, electrical conductivity of the solution was determined, using a conductivity meter (model DDSJ-308A, Shanghai Precision and Scientific Instrument Co., Ltd., China). The vials with solution were then boiled for 10 min, quickly cooled and the total electrical conductivity was obtained. Relative leakage rate was expressed as percent of total electrolytes.

Atomic force microscopy (AFM) imaging and analysis

Samples were cut into thin pieces of the epicarp which fit into AFM imaging and were stuck onto scanned platform by double-sided tape (Yang et al., 2004). Tapping mode was carried out using a multimode (JSPM-5200 AFM JEOL, Japan) equipped with a Si_3N_4 cantilevered scanner with a 80 mm × 80 mm scan size and a 3.51 µm vertical range. Resolution was about 0.1 nm for vertical range and 1 nm for lateral. To make the results comparable, the images were obtained from the center area of each surface. At least 10 images were analyzed for each sample to obtain reliable results.

Three images of different zones were examined and the images

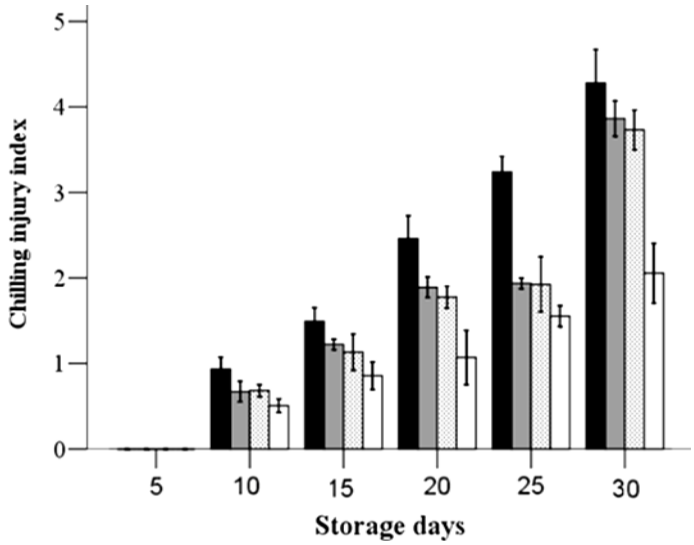

Figure 1. Chilling injury index in the pulp of sweet pepper treated with air (control), MAP, 1-MCP, and 1-MCP + MAP, after 30 days storage at 4°C. Each point represents the mean value ± SD (n=6). ■, Control; ▒, 1-MCP; ▒, MAP; □,1-MCP + MAP.

were analyzed with the WinSPM processing software in order to average the roughness value. The height variation was represented by a color scale in which bright color denoted higher areas and dark color denoted lower areas for all images. Different scales were used in the vertical and horizontal directions. Two amplitude parameters were used. The arithmetic average roughness, R_a and the root mean square (RMS) roughness, R_q were given by:

$$R_a = \frac{1}{n_x n_y} \sum_{i=1}^{n_x} \sum_{j=1}^{n_y} \left| Z(i,j) - Z_{ave} \right|, \quad (1)$$

$$R_q = \sqrt{\frac{\sum_{i=1}^{n_x} \sum_{j=1}^{n_y} \left[Z(i,j) - Z_{ave} \right]^2}{n_x n_y}}, \quad (2)$$

Where Z (*i,j*) denote the topography data for the surface after specimen tilt-correction; Z_{ave} is the average surface height; *i* and *j* corresponds to pixels in the *x* and the *y* direction. The maximum number of pixels in the two directions was given by n_x and n_y (Lindseth et al., 1999).

Determination of the activities of SOD, CAT and POD

SOD, CAT and POD activities were analyzed according to the methods of Wang et al. (2005). All the chemicals were from Sigma Company (St. Louis, MO, USA). Pulp weighing 5 g was homogenized in 10 ml 25 mmol L^{-1} potassium phosphate buffer (PBS), at pH 7.8, containing 0.8 g L^{-1} polyvinyl polypyrrolidone (PVPP) and 1 mmol L^{-1} ethylenediaminetetraacetic acid (EDTA) and then, was centrifuged at 10,000 *g* for 20 min at 4°C. The resulting supernatants were used directly for POD, SOD and CAT assays. All operations were carried out at 4°C and all the enzymatic activities were measured in fresh extracts at a defined temperature.

For SOD determination, the reaction mixture (3 ml) contained 50 mmol L^{-1} sodium phosphate buffer (pH 7.8), 13 mmol L^{-1} methionine, 750 µmol L^{-1} nitroblue tetrazolium (NBT), 10 µmol L^{-1} EDTA, 2 µmol L^{-1} riboflavin and 0.1 ml of the enzyme extract. The mixtures were illuminated by light (4000lx) for 10 min and the absorbance at 560 nm was then determined at 25°C. Identical solutions held in the dark served as blanks. The volume of enzyme corresponding to 50% inhibition of NBT reduction at 560 nm was considered as one enzyme unit (U). The SOD activity was expressed as U g^{-1} FW.

For CAT determination, the reaction mixture consisted of 2 ml sodium phosphate buffer (50 mmol L^{-1}, pH 7.0), 0.5 ml H_2O_2 (40 mmol L^{-1}) and 0.5 ml enzyme extract. The decomposition of H_2O_2 was measured by the decline in absorbance at 240 nm at 25°C. The CAT specific activity was expressed as U g^{-1} FW, per U = 0.1Δ $absorbance_{240\ nm}$ min^{-1}.

For POD determination, 0.5 ml of enzyme extract was incubated in 2 ml buffered substrate (100 mmol L^{-1} sodium phosphate, pH 6.4 and 8 mmol L^{-1} guaiacol) for 5 min at 30°C and the increasing absorbance was measured at 460 nm every 30 s for 150 s after adding 1 ml of H_2O_2 (24 mmol L^{-1}). POD activity was expressed as U g^{-1} FW, per U = 0.01Δ $absorbance_{470\ nm}$ min^{-1}.

Statistical analysis

Experimental data was analyzed using SPSS 13.0 software (SPSS Inc. Chicago, IL, USA). The mean values were calculated and reported as the mean ± S.D. (n=6). The data were processed with variance analysis (ANOVA) according to least significant difference (LSD) test at P = 0.05 to compare means between treated fruit and controls.

RESULTS AND DISCUSSION

Chilling injury index

Chilling injury (CI) index of the sweet peppers are presented in Figure 1. The CI index of the pulp in sweet pepper increased during storage. Browning symptoms associated with CI were observed in the surface of the fruit. After 15 days of storage at 4°C, the treated and control samples showed different browning symptoms. 1-MCP and/or MAP treatment appeared to have a lower CI index (35.6% of surface area) than that of the control sample. The CI index of the treatment with 1-MCP + MAP, which was 2.2, was lower than those of the treatments of 1-MCP (3.8), MAP (3.6) and the control (4.3) after 30 days of storage.

CI often occurs when some vegetables and fruits have been exposed to low temperature for a certain period of time (Nilprapruck et al., 2008). Exposure of sweet peppers to temperatures below 7°C for more than 10 days caused CI as indicated by dot-pitting followed by sheet-pitting. These morphological lesions may lead to alternaria-induced rot on pods and calyxes, seed darkening and fruit shrinkage due to moisture loss (Hardenburg et al., 1986). The abnormality may be caused by physiological changes in the cell membrane

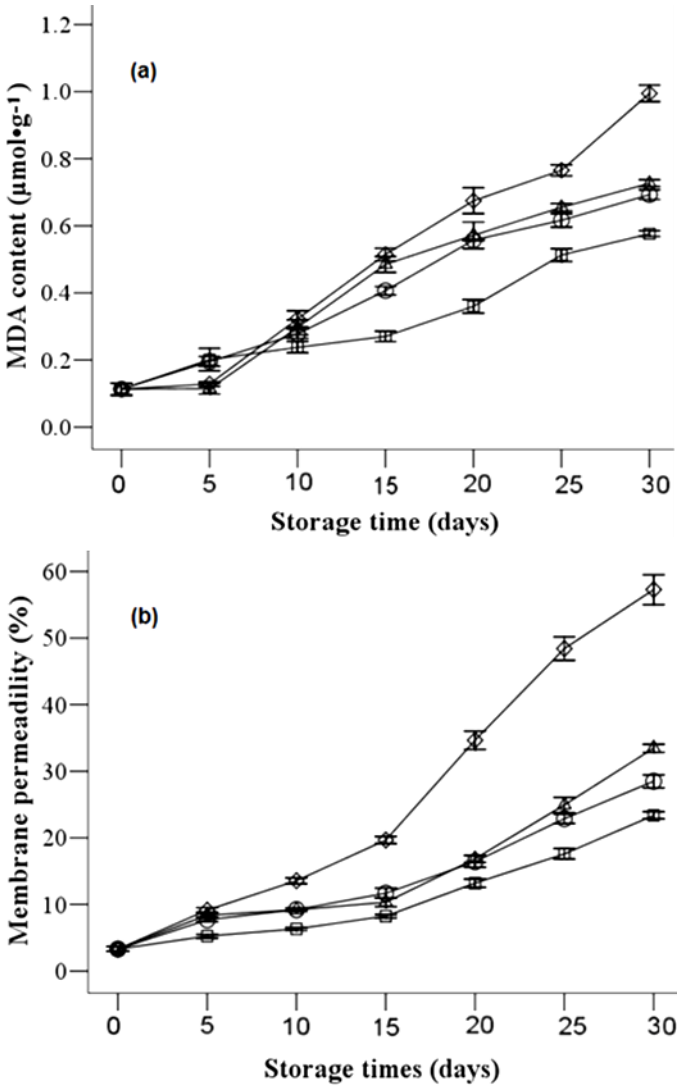

Figure 2. MDA (a) and membrane permeability (b) of the samples after 30 days storage at 4°C. Each point represents the mean value ± SD (n=6). ◇, control; △1-MCP; ○ MAP; □ 1-MCP + MAP.

where the liquid-crystalline state had been changed to a solid gel state. However, the sweet pepper treated with 1-MCP and MAP had less severe (P = 0.05) browning symptom than the group without treatments. 1-MCP and MAP treatments resulted in a reduction of CI symptom. In 'Larry Ann' plums, the 1-MCP treatment completely inhibited the cold-induced stress response observed in the cold-stored fruit (Larrigaudière et al., 2009). Forney et al. (1990) reported that, packing pepper fruit inside a plastic bag created a modified atmosphere within the package and thus reduced its susceptibility to CI.

MDA content and electrolyte leakage

Malondialdehyde (MDA) content and electrolyte leakage have been used as direct indicators of membrane injury. MDA is often used as one index of cell oxidative damage, which is the final product of lipid peroxidation (Xu et al., 2009). Maintenance of membrane integrity at low temperature has been considered important in the resistance to low temperature (Saruyama et al., 2004). Shackel et al. (1991) hypothesized that, fruit softening in tomato resulted from turgor loss of cells accompanied with the damage of cellular membranes. Thus, MDA has also been used as an indicator of the degree of plant oxidative stress (Hodges et al., 1999). In this study, there was a continuous increase in MDA content in all the treated fruits stored at 4°C (Figure 2a), yet the application of 1-MCP and MCP to the harvested sweet peppers significantly delayed the increase of MDA. As a result, the MDA content in the 1-MCP+MAP treated peppers increased at a slower pace than the control and the MAP-treated peppers. Accumulation of MDA is often taken as an indicator of CI (Wongsheree et al., 2009; Wang et al., 2008). The CI damage is often accompanied by a low activity of antioxidant enzymes such as CAT (MacRae and Ferguson, 1985; Posmyk et al., 2005).

Membrane permeability changes during storage were assessed by determining the intensity of electrolyte leakage (Xu et al., 2009; Li et al., 2007). This parameter was included in order to have more information on membrane stability and thereby on the relative ion content in the apoplastic space. In this study, the change in membrane permeability shared similar trends with MDA content (Figure 2b). No significant change was observed at the beginning of the storage, while the untreated fruit exhibited higher electrolyte leakage activity than the treated ones after the cold storage. The initial electrolyte leakage was 3.3% in the sweet peppers. The electrolyte leakage contents of 1-MCP+MAP treated peppers (22%) had significantly lower relative leakage rates than the control (57%), 1-MCP (33%) and MAP (37%) treated fruits, at the end of storage. These results indicated that, higher membrane integrity was maintained with 1-MCP and MAP treatment. The rigidity of the cell membrane accelerated the leakage of cell content and caused electrolyte leakage from the cell (Lyons, 1973; Paull, 1990), causing weight loss and browning in sweet peppers.

AFM images

Figure 3 shows the typical plane and three-dimensional profiles obtained for sweet peppers stored at 4°C in the areas of 5.0 μm × 5.0 μm at the 30th day. As shown in Figure 3, the cell wall and small bump were observed on the epicarp of the sweet pepper. However, the cells that formed epicarp of the sweet pepper in the control were not smooth, as shown in Figure 3a, indicating a rougher surface. The R_a of the sample stored at 4°C treated with 1-MCP and MAP was 31.9 nm, while the control sample had Ra of 78.4 nm (Table 1). It can be seen clearly from

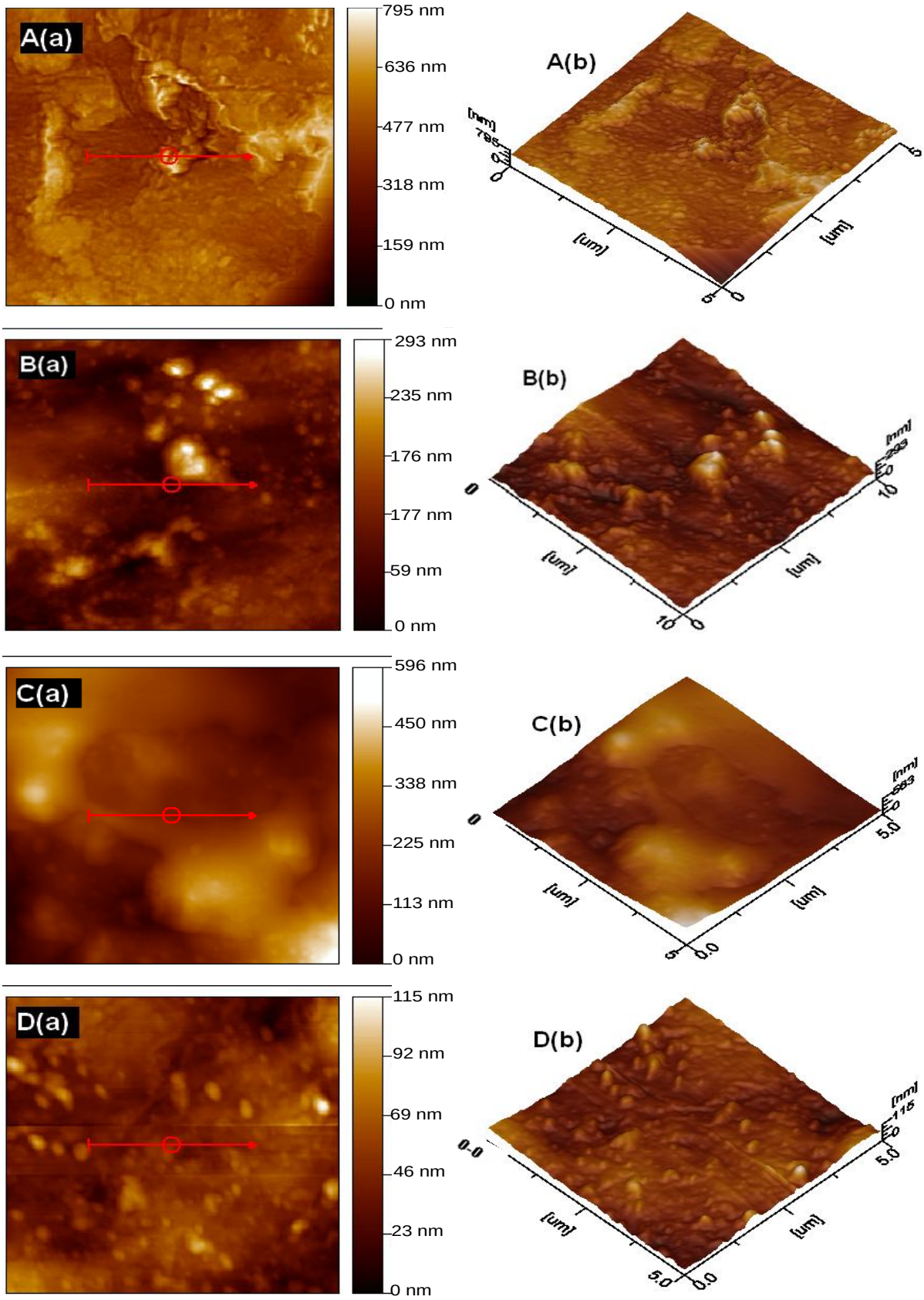

Figure 3. Plane and three-dimensional profiles of sweet pepper skin after 30 days storage at 4°C by AFM. (a), plane profiles; (b) three-dimensional profiles. Scan area = 5.0 μm × 5.0 μm. A, Control; B, 1-MCP; C, MAP; D, 1-MCP + MAP.

Table 1. Effects of storage temperature and treatment on roughness average (R_a) and quadratic root average of the roughness (R_q) (nm) of sweet pepper skin (mean ± SD, n= 6).

Group	Ra*	Rq*
Control	61.0^{c} ± 11.12	93.5^{d} ± 8.62
1-MCP	31.6^{b} ± 10.74	40.9^{b} ± 13.41
MAP	78.4^{d} ± 17.07	81.2^{c} ± 21.57
1-MCP+MAP	11.5^{a} ± 5.36	14.2^{a} ± 9.07

*Each data represents the mean value ± SD. Different letters within columns indicate significant differences at P = 0.05.

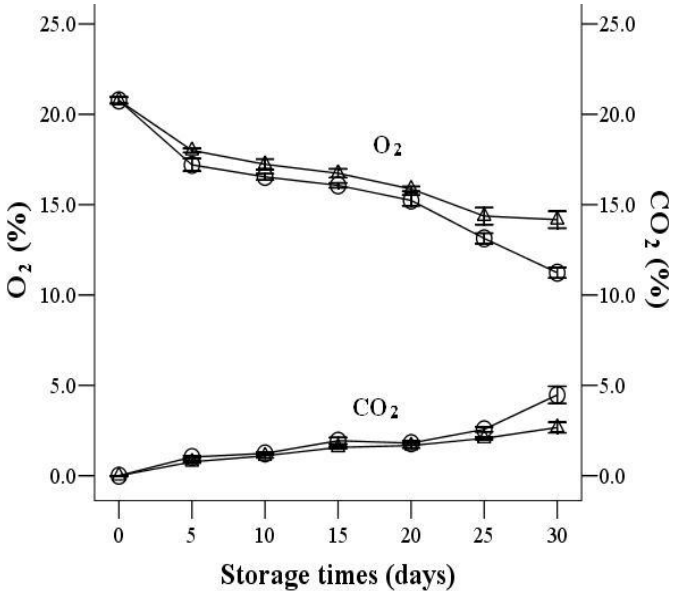

Figure 4. O_2 and CO_2 concentration in the sample packaging after 30 days storage at 4°C. Each point represents the mean value ± SD (n=6). ○, MAP; △, 1-MCP + MAP.

Figure 3 that, sweet peppers of the different treatments had different roughness. The surface of sweet pepper which was treated with1-MCP and1-MCP+MAP stored at 4°C was less rough compared with that of the control and the Ra and Rq of sweet peppers were smaller than those of the control group. The higher the number of R_a and R_q was, the more severe was the injury and withering of the vegetable.

Chilling injury (CI), as characterized by skin withering, was induced at 4°C. Change in the MDA content and electrolyte leakage was closely related to the occurrence of chilling-injury; that is, dehydration caused the contraction of protoplast.

The roughness analysis gained from AFM was effective in determining the shrinkage degree of the sweet pepper during storage. With the increment of time, the surface of the control group became rougher than the 1-MCP+MAP treatment group. As reported by Ensikat et al. (2000) and Verran et al. (2000), the wax platelet assayed of 7 to 10 nm and unpolished stainless steel was 75 ± 29 nm of Ra. Compared with these values, the results of sweet peppers in this paper were credible. The topology of plant materials was studied by AFM (Yang et al., 2004). Hershko and Nussinovitch (1998) compared the roughness between the onion skin surface and the chloroform-cleaned onion surface. AFM images might be helpful when evaluating topographical data, since these are well established methods for characterizing surfaces (Lindseth and Bardal, 1999). However, scanning electron microscope (SEM) in combination with the AFM to appreciate the quality of homogeneity of the samples on a large scale need further research (Darrort et al., 1995).

Atmosphere composition

Within PE pouches, a classical modification of the internal atmosphere was observed; O_2 partial pressure decreased whereas CO_2 partial pressure increased, reaching a steady state different from air, a so-called equilibrium modified atmosphere (MA) (Charles et al., 2008). The changes in headspace gas composition in the package of sweet pepper are shown in Figure 4. It was evident that O_2 concentration of the sweet peppers with MAP treatment was lower than that of the samples with 1-MCP + MAP treatment, reaching the minimum concentration of 11.2% (MAP treatment) and 14.1% (1-MCP+MAP treatment) at the end of the storage (Figure 4). During the later storage days, O_2 concentration in the MAP group and in the 1-MCP+MAP group declined, while CO_2 concentration increased continuously. However, the O_2 concentration of MAP treatment was lower (P = 0.05) than that of the group of 1-MCP+MAP treatment at the 20th day in contrast to the CO_2 concentration.

The results on headspace O_2 and CO_2 levels suggested that, the respiration rate of sweet peppers treated by 1-MCP+MAP was reduced compared with the MAP treated peppers. According to the result reported by Gorris et al. (1999), MAP inhibited respiration rate by the function of reduced O_2 and elevated CO_2 and then reduced metabolic activity and ethylene sensitivity in sweet peppers during storage time. On the other hand, 1-MCP can also delay or even impair ripening in a wide range of vegetables because 1-MCP inhibits ethylene perception by binding to the ethylene receptors to block the effects of endogenous and exogenous ethylene (Sisler et al., 1996; Larrigaudière et al., 2009). The lower O_2 and high CO_2 levels in the 1-MCP+MAP packages suggested that, 1-MCP treatment reduced the reparation rate in pepper fruit. Similar results were reported by Mao et al. (2007), on fresh-cut apples.

Activities of SOD, CAT and POD

Senescence is considered to be associated with the antioxidative defensive system, including antioxidant

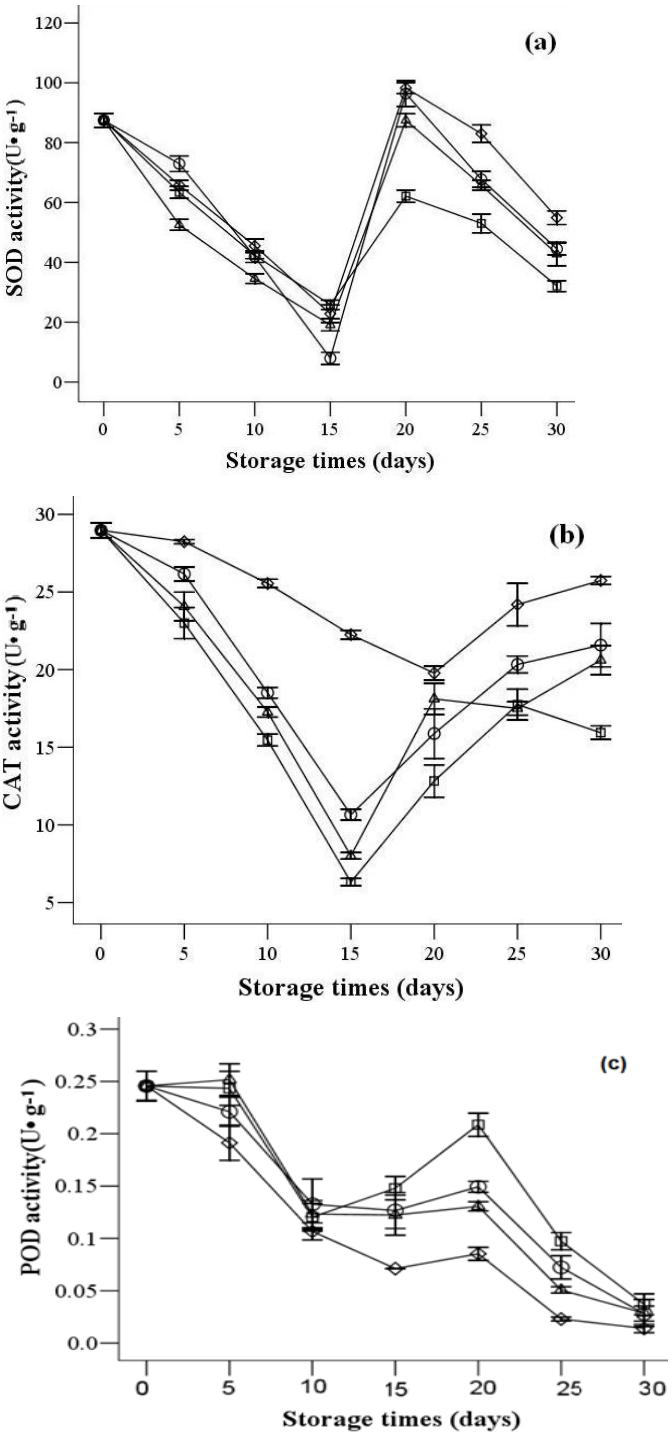

Figure 5. SOD (a), CAT (b) and POD (c) enzyme activities of the samples after 30 days storage at 4 °C, respectively. Each point represents the mean value ± SD (n=6). ◇, control; △1-MCP; ○ MAP; □ 1-MCP + MAP.

enzymes such as POD, CAT and SOD and antioxidants (Han et al., 2006; Sigaud-Kutner et al., 2005). The SOD activities decreased during the 15 days storage at 4°C and then increased afterward, reaching the highest level (32.07 to 54.91 U g^{-1}) on day 20 (Figure 5a). The SOD activity of the pepper fruits treated with 1-MCP + MAP was lower than that of other groups during the later period of storage (20 to 30 days). A lower SOD activity in MAP treated and 1-MCP treated samples, compared with the control was observed on days 25 and 30, which correlated with the development of chilling injury.

As revealed in Figure 5b, CAT activity declined in the control peppers during the first 20 days of storage and in other samples during the first 15 days of storage and then, increased rapidly. Samples treated with 1-MCP or MAP had lower CAT activity than the control during the entire storage period, while CAT activity in 1-MCP + MAP treated peppers was the lowest at most of the sampling days, which indicated the synergistic effect of 1-MCP and MAP on the CAT activity.

At 4°C, POD activity in all the treatments decreased gradually during the first 10 days of storage (Figure 5c). The POD activity of samples treated with 1-MCP and MAP increased significantly ($P < 0.05$) between days 10 to 20, while the control had little change in POD activity during the same period. After 20 days, POD activity of the control peppers decreased from 0.209 U g^{-1} on day 20 to 0.133 U g^{-1} at the end of the storage. POD activity levels in peppers treated with 1-MCP + MAP was the highest during the storage period.

As oxyradical detoxification enzymes, SOD, CAT and POD play an important role in the degradation oxidative species in plant tissue (Han et al., 2006; Mittler, 2002; Sigaud-Kutner et al., 2005). The superoxide radical ($O_2^{\bullet -}$) is efficiently converted to H_2O_2 by the action of SOD. H_2O_2 may be toxic to the plant cells and should not be allowed to accumulate. One important enzyme responsible for converting H_2O_2 to water is CAT. POD is a ubiquitous enzyme that has diverse biochemical functions in a variety of fruits and vegetables (Gaspar et al., 1981). Blackening or browning as a result of chilling injury might be produced by POD because POD catalyzes browning reactions through the generation of hydrogen peroxide, using free phenolics or quinones as substrates (Wang et al., 2005). Zauberman et al. (1988) reported that, the activity of POD has been found to increase during storage at chilling temperatures in mangoes. This increase was suggested as part of the chilling injury syndrome. The raise on POD activity was correlated with the chilling injury observed in those peppers stored at 4°C. The result indicated that, POD activity might be a factor that determines the higher tolerance to chilling injury in 1-MCP + MAP treated peppers. These facts together with the evidence in this work suggested that, the decreased activities of SOD, CAT and POD (Figure 2) in peppers induced by 1-MCP, might induce chilling injury resistance during storage as well.

Conclusions

The combined treatment of MAP and 1-MCP reduced the

respiration rate of sweet peppers as indicated by the reduced O_2 and elevated CO_2 headspace levels in the packages. The activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) were influenced by the combined treatment of 1-MCP and MAP at 4°C. The values of the chilling injury index, electrolyte leakage and MDA content increased but were lower than that of the control peppers during storage. The AFM images showed that, the surface of stored sweet pepper without 1-MCP treatment was rougher than that with 1-MCP treatment. This study suggests that 1-MCP + MAP might have a synergistic effect on alleviating the chilling injury of sweet pepper and therefore, 1-MCP and MAP might be a promising candidate for extending the postharvest storage life of sweet peppers.

ACKNOWLEDGEMENTS

This study was partly supported by the 11th Five-Year Key Technologies R&D Program of China (2006BAD22B03). The authors also acknowledge Yate Color Printing and Packing Co. Ltd (Guangdong, China) for their kind gift of the packaging material.

REFERENCES

Banks NH, Nicholson SE (2000). Internal atmosphere composition and skin permeance to gases of pepper fruit. Postharv. Biol. Technol. 18(1): 33–41.

Blankenship SM, Dole JM (2003). 1-Methylcyclopropene: A review. Postharv. Biol. Technol. 28(1): 1–25.

Candan AP, Graell J, Crisosto C, Larrigaudière C (2006). Improvement of storability and shelf life of 'Blackamber plums' treated with 1-methylcyclopropene. Food Sci. Technol. Int. 12(5): 437–443.

Charles F, Guillaume C, Gontard N (2008). Effect of passive and active modified atmosphere packaging on quality changes of fresh endives. Postharv. Biol. Technol. 48(1): 22-29.

Chien YW (1997). Chilling and Freezing Injury. Produce Quality and Safety Laboratory, USDA, ARS. Henry A. Wallace Beltsville Agricultural Research Center, Beltsville, MD.

Darrort V, Troyon M, Ebothé J, Bissieux C, Nicollin C (1995). Quantitative study by atomic force microscopy and spectrophotometry of the roughness of electrodeposited nickel in the presence of additives. Thin Solid Films. 265(1-2): 52-57.

Davey MW, van Montagu M, Inze D, Sanmartin M, Kanellis A, Smirnoff N, Benzie IJJ, Strain JJ, Favell D, Fletcher J (2002). Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. J. Sci. Food Agr. 80(7): 825-860.

Ensikat HJ, Neinhuit C, Barthlott W (2000). Direct access to plant epicuticular wax crystals by a new mechanical isolation method. Int. J. Plant Sci. 161(1): 143-148.

Fallik E, Grinberg S, Alkalai S, Yekutieli O, Wiseblum A, Regev R, Regev H, Bar-Lev B (1999). A unique rapid hot water treatment to improve storage quality of sweet pepper. Postharv. Biol. Technol. 15(1): 25-32.

Fan XT, Blankenship SM, Mattheis JP (1999a). 1-methylcyclopropene inhibits apple ripening. J. Am. Soc. Hortic. Sci. 124: 690-695.

Fan XT, Mattheis JP, Blankenship SM (1999b). Development of apple superficial scald, soft scald, core flush, and greasiness is reduced by MCP. J. Agric. Food Chem. 47: 3063-3068.

Forney CF, Lipton WJ (1990). Influence of controlled atmosphere and packaging on chilling sensitivity. In: C.Y. Wang (Editor), Chilling Injury of Horticultural Crops. CRC Press, Boca Raton, Fla., pp. 257-268.

Gaspar T, Parel C, Thorpe TA (Eds.) (1981). Peroxidases. Geneva: University of Geneva Press.

Gorris L, Tauscher B (1999). Quality and safety aspects of novel minimal processing technology. In: Oliveira F. A. R. and Oliveira J. C. (ed) Processing of foods: Quality Optimization and Process Assessment. Boca Raton, FL: CRC Press, pp. 325-339.

Han JH, Tao WY, Hao HK, Zhang BL, Jiang WB, Niu TG (2006). Physiology and quality responses of fresh-cut broccoli florets pretreated with ethanol vapor. J. Food Sci. 71(5): 385–389.

Hardenburg RE, Watada AE, Wang CY (1986). The commercial storage of fruits, vegetables, and florist and nursery stocks. In: Agriculture Handbook, U.S. Dept. Agric. 66: 65.

Hershko V, Nussinovitch A (1998). Relationships between hydrocolloid coating and mushroom structure. J. Agric. Food. Chem. 46(8): 2988-2997.

Hodges DM, DeLong JM, Forney CF, Prange RK (1999). Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta, 207(4): 604–611.

Jayas DS, Jeyamkondan S (2002). PH-Postharvest Technology: Modified atmosphere storage of grains meats fruits and vegetables. Biosyst. Eng. 82(3): 235–251.

Larrigaudière C, Paula AC, Ubach D, Jordi G (2009). Physiological response of 'Larry Ann' plums to cold storage and 1-MCP treatment. Postharv. Biol. Technol. 51(1): 56–61.

Larrigaudière C, Vilaplana R, Soria Y, Recasens I (2004). Oxidative behaviour of Blanquilla pears treated with 1-methylcyclopropene during cold storage. J. Sci. Food Agric. 84(14): 1871–1877.

Li HS (2000). Principles and techniques of plant physiological biochemical experiment. Beijing: Higher Education Press, pp. 184-225.

Li T, Zhang M, Wang S (2007). Effect of modified atmosphere packaging with a silicon gum film as a window for gas exchange on Agrocybe chaxingu storage. Postharv. Biol. Technol. 43(3): 343–350.

Lindseth I, Bardal A (1999). Quantitative topography measurements of rolled aluminium surfaces by atomic force microscopy and optical methods. Surf. Coat. Tech. 111(2-3): 276–286.

Lyons JM (1973). Chilling injury in plant. Annual Review of Plant Physiology. Plant Physiol. 24: 445-456.

MacRae EA, Ferguson IB (1985). Changes in catalase activity and hydrogen peroxide concentration in plants in response to low temperature. Physiol. Plantarum. 65(1): 51–56.

Mao L C, Lu FJ, Wang GZ (2007). Application of 1-methylcyclopropene reduces wound responses and maintains quality in fresh-cut apple. Asia Pac. J. Clin. Nutr. 16(1): 111-115.

Mao L, Karakurt Y, Huber DJ (2004). Incidence of water-soaking and phospholipid catabolism in ripe watermelon (*Citrullus lanatus*) fruit: Induction by ethylene and prophylactic effects of 1-methylcyclopropene. Postharv. Biol. Technol. 33(1): 1-9.

Marin AB, Colonna AE, Kudo K, Kupferman EM, Mattheis JP (2009). Measuring consumer response to 'Gala' apples treated with 1-methylcyclopropene (1-MCP). Postharv. Biol. Technol. 51(1): 73-79.

Meir S, Rosenberger I, Aharon Z, Grinberg S, Fallik E (1995). Improvement of the postharvest keeping quality and colour development of bell pepper (cv. 'Maor') by packaging with polyethylene bags at a reduced temperature. Postharv. Biol. Technol. 5(4): 303–309.

Menniti AM, Gregori R, Donati I (2004). 1-Methylcyclopropene retards postharvest softening of plums. Postharv. Biol. Technol. 31(3): 269–275.

Mittler R (2002). Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7(9): 405–410.

Nilprapruck P, Pradisthakarn N, Authanithee F, Keebjan P (2008). Effect of Exogenous Methyl Jasmonate on Chilling Injury and Quality of Pineapple (*Ananas comosus* L.) cv. Pattavia. Silpakorn Univ. Sci. Technol. J. 2(2): 33-42.

Paull RE (1990). Chilling injury of crops of tropical and subtropical origin. In Chilling Injury of Horticultural Crops (Wang G. Y., ed). Florida: CRC Press, pp. 17-36.

Posmyk MM, Bailly C, Szafranska K, Janas KM, Corbineau F (2005). Antioxidant enzymes and isoflavonoids in chilled soybean (Glycinemax (L.)Merr.) seedlings. J. Plant Phys. 162(4): 403–412.

Saltveit ME, Morris LL (1990). Overview on chilling injury of horticultural crops. In: Chilling Injury of Horticultural Crops. Boca Raton, FL: CRC Press, pp. 3-15.

Saruyama H, Onodera H, Uemura M (2004). Transgenic rice plants expressing wheat catalase show improved tolerance for chilling-induced damage in membranes. In: Toriyama, K., Heong, K.L.,Hardy, B. (ed), Rice is Life: Scientific Perspectives in the 21st Century. Proceedings of the World Rice Research conference held in Tsukuba, Japan.

Serrano M, Zapata PJ, Castillo S, Guillén F, Martínez-Romero D, Valero D (2010). Antioxidant and nutritive constituents during sweet pepper development and ripening are enhanced by nitrophenolate treatments. Food Chem. 118(3): 497–503.

Shackel KA, Greve C, Labavitch JM, Ahmadi H (1991). Cell turgor changes associated with ripening in tomato pericarp tissue. Plant Physiol. 97(2): 814–816.

Sigaud-Kutner TCS, Neto AMP, Pinto E, Colepicolo P (2005). Diel activities of antioxidant enzymes, photosynthetic pigments and malondialdehyde content in stationary phase cells of *Tetraselmis gracilis* (Prasinophyceae). Aquat. Bot. 82(4): 239–249.

Sisler EC, Dupille E, Serek M (1996). Effects of 1-methylcyclopropene and methylcyclopropene on ethylene binding and ethylene action on cut carnation. Plant. Growth. Regul. 18(1-2): 169–174.

Vermeiren L, Devlieghere F, van Beest M, de Kruijf N, Debevere J (1999). Developments in the active packaging of foods. Trends Food Sci. Technol. 10(3): 77–86.

Verran J, Rowe DL, Cole D, Boyd RD (2000). The use of the atomic force microscope to visualize and measure wear of food contact surfaces. Int. Biodeterior. Biodegrad. 46(2): 99–105.

Vilaplana R, Valentines MC, Toivonen P, Larrigaudière C (2006). Antioxidant potential and peroxidative state of 'Golden Smoothee' apples treated with 1-methylcyclopropene. J. Am. Soc. Hortic. Sci. 131(1): 104–109.

Wang B, Wang J, Liang H, Yi J, Zhang J, Lin L, Wu Y, Feng X, Cao J, Jiang W (2008). Reduced chilling injury in mango fruit by 2,4-dichlorophenoxyacetic acid and the antioxidant response. Postharv. Biol. Technol. 48(2): 172-181.

Wang CY (1990). Chilling injury of horticultural crops. In: Relation of Chilling Stress to Membrane Permeability. Boca Raton, FL: CRC Press, pp. 201-210.

Wang YS, Tian SP, Xu Y (2005). Effects of high oxygen concentration on pro- and anti-oxidant enzymes in peach fruit during postharvest periods. Food Chem. 91(1): 99–104.

Wongsheree T, Ketsa S, van Doorn WG (2009). The relationship between chilling injury and membrane damage in lemon basil (Ocimum×Citriodourum) leaves. Postharv. Biol. Technol. 51(1): 91–96.

Xu W, Peng X, Luo Y, Wang J, Guo X, Huang K (2009). Physiological and biochemical responses of grapefruit seed extract dip on 'Redglobe' grape. J. Food Sci. Technol. 42(2): 471–476.

Yang HS, Feng GP, An H, Li Y (2004). Quantitative Roughness Analysis of Post-harvest Mushroom by Atomic Force Microscopy. Acta Bot. Sin. 46(10): 1249-1255.

Zauberman G, Fuchs Y, Rot I, Wexler A (1988). Chilling injury, peroxidase, and cellulase activities in the peel of mango fruit at low temperature. Hortic. Sci. 23(4): 732–733.

Zhang JH, Huang WD, Pan QH, Liu YP (2005). Improvement of chilling tolerance and accumulation of heat shock proteins in grape berries (*Vitis vinifera* cv.Jingxiu) by heat pretreatment. Postharv. Biol. Technol. 38(1): 80–90.

Zhang Z, Huber DJ, Rao J (2010). Short-term hypoxic hypobaria transiently decreases internal ethylene levels and increases sensitivity of tomato fruit to subsequent 1-methylcyclopropenetreatments. Postharv. Biol. Technol. 56(2): 131-137.