Journal of Food Technology Research
2014 Vol. 1, No. 2, pp. 146-155
ISSN(e): 2312-3796
ISSN(p): 2312-6426
DOI: 10.18488/journal.58/2014.1.2/58.2.146.155

EFFECT OF CARBON MONOXIDE ON ACTIVE OXYGEN METABOLISM OF POSTHARVEST JUJUBE

Shaoying Zhang[1] --- Qin Li[2] --- Yulan Mao[3]
[1,2,3]*College of Food Science, Shanxi Normal University, Linfen City, Shanxi, China*

ABSTRACT
To prolong the shelf life postharvest jujube, the effect of carbon monoxide (CO) on senescence of postharvest jujube in relation to active oxygen metabolism was investigated. Jujubes were fumigated with CO gas at 5, 10, 20 or 40μmol/L for 1 h, and then stored for 30 days at room temperature. Changes in membrane permeability, malonaldehyde (MDA), H_2O_2, $O_2^{\cdot -}$ content, and activities of active oxygen metabolism associated enzymes including superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) in fruit were measured. The results indicated that a little of CO could exhibit positive physiological effect on controlling senescence of winter jujube during storage. The study further showed that fumigating jujube fruit with 10 μmol/L CO could reduce increase of MDA content and the relative membrane permeability, effectively inhibit oxidative damage to lipids, decrease H_2O_2 and $O_2^{\cdot -}$ content and maintain high activities of SOD, CAT and POD. The present data suggest that exogenous CO treatment might delay the jujube fruit senescence by regulating active oxygen metabolism.

Keywords: Carbon monoxide, Jujube, Active oxygen, Metabolism, Senescence, Preservation.

Contribution/ Originality

This study documents for the first time the effect of carbon monoxide (CO) on H_2O_2, $O_2^{\cdot -}$ content and activity of antioxidant enzymes (SOD, CAT and POD) in relation to active oxygen metabolism during senescence of postharvest jujube.

1. INTRODUCTION

Jujube is a fruit with particular flavours and abundant nutrients, such as amino acids, vitamin C, flavonoids, and so on (Li *et al.*, 2007), but the shelf life of post-harvest jujube usually was shortened owing to subjecting to water loss and decay. Thus, restraining the fruit senescence and postponing the shelf life of post-harvest jujube are quite necessary in terms of market demand and economic benefit (Wang *et al.*, 2011). Similar to other fruit and vegetable, jujube mature and senescence are influenced by internal factors, such as plant hormones and other endogenous regulatory factors. For example, NO had been verified to regulate the mature and senescence of fruit and vegetable as an endogenous gaseous signal molecule (Zhu and Zhou, 2007).

Carbon monoxide (CO) is a diatomic gaseous small molecule, and was traditionally deemed poisonous to human and environment. It was apt to combine with hemoglobin, restraining oxygen bonds chemically to the hemoglobin and generating poisoning reaction (Guzman, 2012). Nevertheless, in recent years, people found that CO existed in the tissues or organs of many plants (Vreman *et al.*, 2011). Similar to (nitric oxide) NO, CO is also a signal molecule of plant and animal cell, generated through the catalysis of heme oxygenas. And it can regulate multiple metabolisms in cell. As for plant at present, CO may promote the plant enhances the abiotic stress resistance, including heavy metal, ultraviolet irradiation, nutrients deficiency, and so on (Kong *et al.*, 2010; Santa-Cruz *et al.*, 2010; Shen *et al.*, 2011).

Reactive oxygen species (ROS), such as singlet and triplet oxygen, $O2^{\bullet -}$, $OH^{\bullet}$ and H_2O_2, are generated as a part of normal metabolism, increasing tempestuously under stress circumstances (Mittler, 2002). During the storage of fruit and vegetable, ROS produce in large amounts with the time extension. The equilibrium of ROS metabolism was destroyed and the fruit and vegetable gradually become senescent (Buchanan-Wollaston *et al.*, 2003). Therefore, the ROS metabolism level is one of important indexes of postharvest physiology of fruit and vegetable.

Similar to NO, research has found that exogenous CO probably resists biotic and abiotic stresses by strengthening the antioxidant ability of plant tissue. The antioxidant ability contains increasing the level of antioxidant substances such as ASA, GSH and polyphenol, or enhancing the activities of antioxidant enzymes including SOD, POD, CAT and APX. Thus, exogenous CO may participate in the physiological regulation of plant (Huang *et al.*, 2011). Other studies have found that treatment with exogenous CO donors could restrain the cut-flower senescence by regulating the antioxidant enzymes of plant tissue (Ling *et al.*, 2006). But so far, the influences of CO on the metabolism of active oxygen of fruits and vegetables during storage have not been reported. The objective aims to enrich the research of CO to plant physiology.

2. MATERIALS AND EXPERIMENTAL METHODS

2.1. Materials

Jujubes were purchased from an orchard in the vicinity of the Shanxi Normal University, and the cultivars were *Ziziphus jujuba* Mill. cv. Dongzao. They were picked at a preclimacteric but physiologically mature stage in the noon. About 50 kg of Jujube was selected to acquire uniform shape, size and colour, and then they were transported to the laboratory quickly using open cartons.

CO (99.99%) was purchased from Beijing Huaneng Special Gases Co., Ltd. (Beijing, China). Sinopharm Chemical Reagent Co., Ltd. (Shanghai China) supplied thiobarbituric acid, nitroblue tetrazolium and methionine (biochemical reagent). Other reagents (analytical grade) were purchased frome Alfa Aesar Company (Tianjin, China).

2.2. Fruit Treatment

According to screening experiment, the jujubes were fumigated at 0.5, 1, 1.5 and 2 h with 10 μmol L^{-1} CO, and the jujubes treated with 1 hour showed the best effect. In formal experiment, the jujubes were fumigated with CO gas at different concentrations (5, 10, 20, or 40 μmol L^{-1}) for 1 h under ambient temperature (about 20 ± 2℃). Approximately 3 kg of jujubes was placed in a glass container 40 cm in height and diameter, and then sealed with a lid. CO gas was injected into the glass container through a port in the lid. Jujubes not fumigated with CO gas were also sealed in a glass container for 1 h to serve as control samples. After treatment, all samples were placed in plastic bags. The bags were no holes and the humidity was controlled through adjusting the size of opening. And the relative humidity was measured using hygrometer (G337, Mingle Metal Company, Shenzhen, China). All the samples were stored under ambient temperature with 85% relative humidity. The reactive oxygen species including $O_2^{\bullet-}$ and H_2O_2 and their related metabolism enzymes containing SOD, POD and CAT of the jujubes were measured periodically.

2.3. Determination of Malonaldehyde (MDA) Content and Membrane Permeability

MDA (Malonaldehyde) was measured essentially as described previously (Xing *et al.*, 2008). Membrane permeability was assayed according to the method of Duan *et al.* (2011). In the experiment, pericarp discs were taken from the equatorial region with a cork borer (10 mm in diameter).

2.4. Determination of Hydrogen Peroxide H_2O_2 and $O_2^{\bullet-}$ Content

The endogenous H_2O_2 level was assayed according to the modified method described previously by Goraj *et al.* (2012). And the superoxide anion ($O_2^{\bullet-}$) was determined as described by Kan *et al.* (2011).

2.5. Determination of Related Enzyme Activities

Superoxide dismutase (SOD) activity was assayed with a modified method (Zhao *et al.*, 2009). Peroxidase (POD) activity was analyzed using a modified method (Yang *et al.*, 2009). And catalase (CAT) activity was determined using the method described by Garcı´A *et al.* (2007).

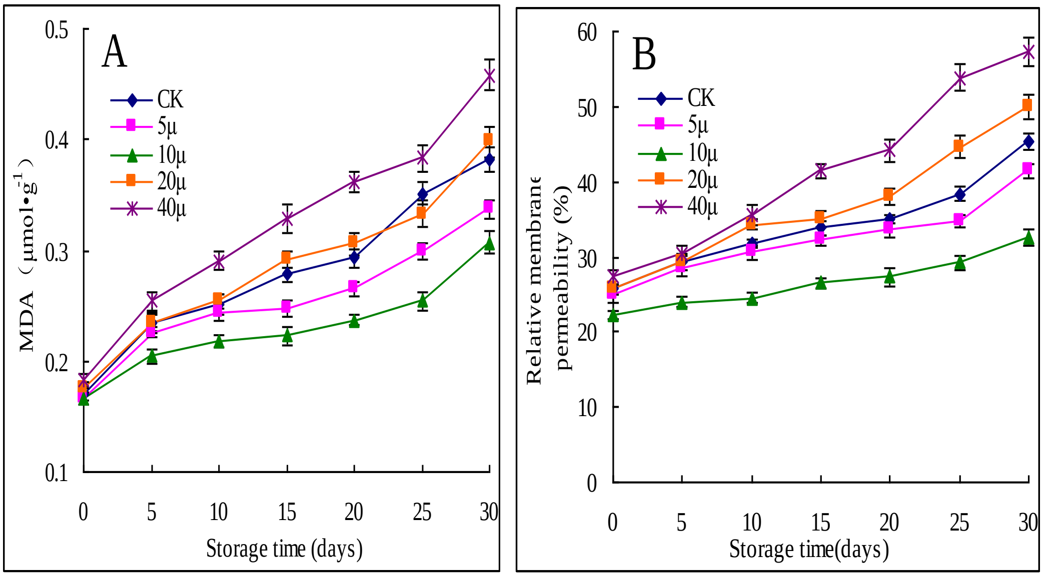
2.6. Statistical Analysis

The data were analyzed using the DPS7.05 statistical software with ANOVA. The number values were the means ± standard error of three replicates. Mean separations were performed with the Tukey's test; the probability indicating statistical significance was $P < 0.05$.

3. RESULTS

3.1. MDA and Membrane Permeability

Fig-1. Effect of CO fumigation on MDA content (A) and relative membrane permeability (B) of postharvest jujube. Each point represents the mean value ± SD.

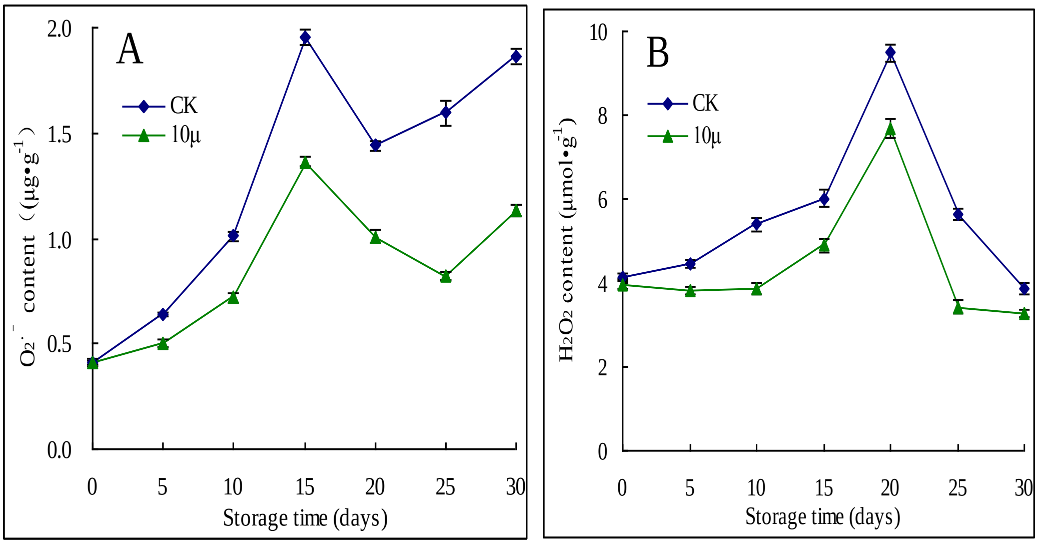


Membrane permeability, expressed as relative leakage rate, is an important index of membrane integrity.As shown in Figure 1 A, the relative membrane permeability of all the samples tended to increase with storage time extension. Compared with the control, treatment with CO at low concentrations (5 μmol L^{-1} and 10 μmol L^{-1}) exhibited inhibitory effect on increase of the relative membrane permeability in a dose-dependent manner, while CO at 20 μmol L^{-1} and 40 μmol L^{-1} obviously promoted the increase of relative membrane permeability. The relative membrane permeability of sample treated with 10 μmol L^{-1} CO was significantly lower than that of other treatments and the control. At day 30, the relative membrane permeability of sample with 10 μmol L^{-1} CO-treatment was 32.55%, which was 28.18% lower than the control (45.32%).

Similarly to the relative membrane permeability, an increase in MDA content was showed in Figure 1 B. Fumigating jujube with 5 μmol L^{-1} and 10 μmol L^{-1} CO could inhibit the increase of MDA content during storage, especially the treatment with 10 μmol L^{-1} CO. At day 30, MDA content of jujube pulp treated with 10 μmol L^{-1} CO was the lowest in all samples and it was 33.48% lower than that of control sample. There were no significant difference in 20 μmol L^{-1} CO treated and the control. MDA content of sample treated with 40 μmol L^{-1} CO was higher than that of control between 5 and 30 days, which indicated that fumigation with CO for high concentration was unfavorable condition to jujube fruit. The reason was probably that fumigation with CO for high concentration was toxic to jujube fruit, which was similar to nitric oxide (Pun *et* *al.*, 2010). Since treatment with 10 μmol L^{-1} CO was shown to be optimal in anti-senescence and cell oxidative damage of jujube during storage, it was used in all subsequent experiments.

3.2. $O_2^{\bullet-}$ and H_2O_2

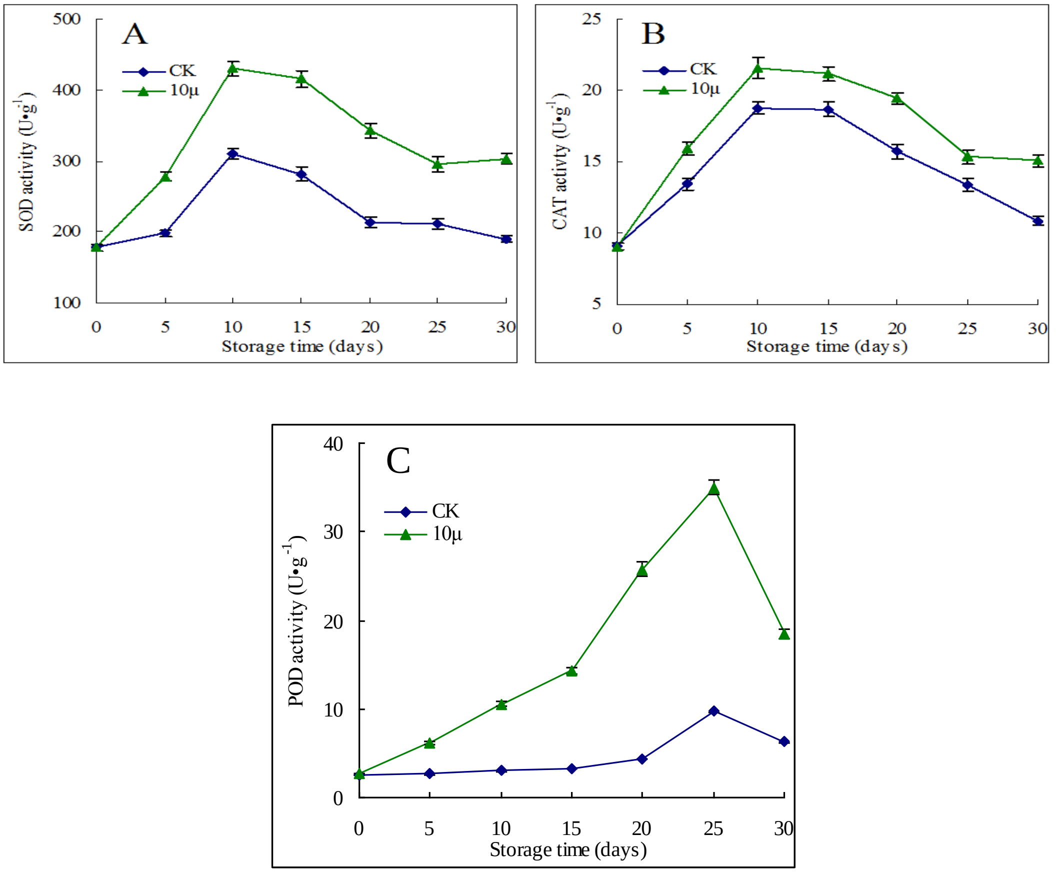
Fig-2. Effect of CO fumigation on $O_2^{\cdot-}$ content (A) and H_2O_2 content (B) of postharvest jujube. Each point represents the mean value ± SD.



As described in Figure 2 A, CO treatment significantly impaired $O_2^{\bullet-}$ generation after the fruit were treated for 5 days ($P<0.05$). On day 30, $O_2^{\bullet-}$ content in the 10 μmol L^{-1} CO treated fruit only was 1.13 μg g^{-1}, lower 39.27% than the control (1.86 μg g^{-1}). Similarly, the increase of H_2O_2 content in the fruit was inhibited after CO treatment (Figure 2 B), but the maximum value of H_2O_2 content emerge after 20 days at room temperature both in control and in CO-treated fruit. CO significantly decreased the H_2O_2 generation of jujube fruit after treated for 5 days and lasted for 30 days.

3.3. SOD\ CAT\ POD

FIG-3. Effect of CO fumigation on SOD (A), CAT (B), and POD (C) activities of postharvest jujube. Each point represents the mean value ± SD.



Both SOD and CAT activities showed a peak on the 10 th day, and then declined (Figure 3 A, B). It was clear that SOD and CAT activities were significantly higher ($P<0.05$) in CO-treated fruit than that of the control after 5 days of treatment, until the 30 th day the SOD and CAT activities was 1.59-fold and 1.39-fold higher than the control, respectively. Different from the changes of SOD and CAT activities, the peak of POD activity emerged at day 25 (Figure 3 C), CO treatment significantly promoted the increase of POD activity after the fruit were treated for 5 days ($P<0.05$), and the POD activity in CO-treated fruit was 2.88 times to 3.60 times higher than that of control after 5 days of treatment.

4. DISCUSSION

As is known to all, the research and application of CO in storage technology mainly center on preventing discoloration of meat. Studies showed treatment with exogenous CO may postpone the senescence of plant leaf and cut-flower, prolong the shelf life of cut rose (Ling *et al.*, 2006). Zhang *et al.* (2013) also found that CO may restrain the browning of fresh cut lotus during the storage time, and extend its shelf life. In our previous study, we found that fumigating jujubes with 10 μmol L^{-1} CO for 1 h could restrain the browning and decay of postharvest jujubes during the storage time, maintain the jujube quality, and postpone the shelf life (Zhang and Li, 2014), however, the mechanism is not clear. The results in the paper showed that CO fumigation might restrain the MDA increase of fruit tissue and postpone the enhancement of membrane permeability. After fumigation, the protective enzyme activities including SOD, CAT and POD were also maintained higher level. Thus, the accumulation of active oxygen such as $O_2^{\bullet -}$ and H_2O_2 was restrained during the storage time, and accordingly the senescence of postharvest jujubes was delayed.

As for higher plants, the breakdown of cell wall components and membrane disruption indicate the senescence degree, leading to cellular decompartmentation and tissue structure loss. It is demonstrated that active oxygen species (AOS) are largely involved in the process of ripening, particularly in membrane deterioration (Rogiers *et al*., 1998), since increased AOS levels in fruit during the ripening process not only are to alter membrane integrity, but also react with unsaturated fatty acids causing lipid peroxidation (Lacan and Baccou, 1998). ROS accumulation could cause oxidative damage to lipids and form toxic products such as MDA, which is a secondary end product of polyunsaturated fatty acid oxidation. Therefore, MDA content is usually an indicator of the degree of plant oxidative stress. Our results indicated that treating jujube with low concentration CO at 5 or 10 μmol L^{-1} might restrain the enhancement of membrane permeability and increase of MDA content, but a contrary effect appeared when treating jujube with high concentration CO at 20 and 40 μmol L^{-1}.

The cytotoxic and cytoprotective effects of CO in animals have been demonstrated, at least partially, that were dependent on the concentration applied as well as on the availability of iron or copper (Piantadosi, 2002). Similarly in plants, it has been agreed that CO in high concentrations normally inhibits plant growth and development (Xu *et al*., 2006). We further deduced that applications of CO gas dose-dependently alleviated the loss of membrane integrity and membrane lipid peroxidation during fruit senescence. Ling et al. found that low concentration of Hematin, CO releaser/donor (0.01 and 0.001 μmol L^{-1}) prolonged vase life of cut rose, while high concentration (0.1 μmol L^{-1}) resulted in the opposite effect suggesting the dual role of CO in plants. Therefore, we presumed that exogenous CO treatment with optimum concentration may restrain the enhancement of membrane permeability during the senescence of plant tissue, maintain cell membrane integrity, and prevent the MDA accumulation induced by lipid peroxidation. The result was similar to Ling *et al*. (2006).

Another possibility is that generation of ROS, such as H_2O_2, and $O_2^{\bullet -}$ contributes to accelerated senescence as it is well known that there is an increase of reactive oxygen species during senescence. Overproduction of ROS, in turn, up-regulates the synthesis of enzymes that degrade ROS and prevent damage induced directly or indirectly by ROS (Zhang *et al*., 2011). CO treatment of jujube results in lower H_2O_2 and $O2^{\bullet -}$ content and enhances the activities of antioxidative enzymes such as CAT, SOD, and POD. These enhanced activities maybe helpful in scavenging accumulated ROS and play a partial role in protecting cellular functions required to prevent senescence.

Carbon monoxide, a small diatomic gas, is low water solubility. Not similar with NO, CO is a stable non radical molecule and does not alternate between different redox species (García-Mata and Lamattina, 2013). Consequently, CO could not directly react with ROS of plant tissue to reduce the harm produced by the excessive amounts of ROS such as H_2O_2 and $O_2^{\bullet-}$. Interestingly, recent investigations have also found that CO plays an important role in relation to plant development and responds to many abiotic and biotic stresses. CO has been found to be efficacious to prolong vase life of fresh cut rose through regulating antioxidative metabolism (Ling *et al.*, 2006). Recently, CO has been highly appreciated for its dominant propensity to bind to the reduced centers of transitional metals in heme-containing proteins (such as guanylate cyclase and cytochrome c oxidase) (Pun *et al.*, 2010). Since CAT and POD is a heme-containing protein, we could speculate that CO increaseed CAT and POD activities in this way.

It is reported that treatment with exogenous CO might increase the activities of CAT and SOD through enhancing the gene expression of *CAT* and *Cu/Zn-SOD*, thus restraining the excessive production of ROS (Huang *et al.*, 2011). The result of Meng *et al.* (2011) suggested that exogenous CO could suppress the oxidative damage induced by Hg toxicity via heightening the activities of SOD, CAT and POD. In addition, several studies indicated that heme oxygenase（HO）widely existing in plants had the oxidation resistance function as well (Santa-Cruz *et al.*, 2010). Exogenous CO might induce the *HO* gene expression of plant tissue, and then increase HO activity. Accordingly, the various biotic and abiotic stresses were decreased (Han *et al.*, 2008).

5. CONCLUSIONS

In conclusion, a little of CO could exhibit positive physiological effect on controlling senescence of winter jujube during storage. The study further showed that fumigating jujube fruit with CO could reduce increase of MDA content and the relative membrane permeability, effectively inhibit oxidative damage to lipids, decrease H_2O_2 and $O_2^{\bullet-}$ content and maintain high activities of SOD, CAT and POD. Thus, it is suggested that exogenous CO treatment might delay the jujube fruit senescence by regulating active oxygen metabolism.

6. ACKNOWLEDGEMENTS

This work was supported by Program of the National Natural Science Foundation of China no. 31101359, by Program for the Innovative Talents of Higher Learning Institutions of Shanxi (2012), and by project for the 131 Leading Talent of Higher Learning Institutions of Shanxi under grant no. 447 (2013).

REFERENCES

Buchanan-Wollaston, V., S. Earl, E. Harrison, E. Mathas, S. Navabpour, T. Page and D. Pink, 2003. The molecular analysis of leaf senescence—a genomics approach. Plant Biotech, 1(1): 3–22.

Duan, X.W., T. Liu, D.D. Zhang, X.G. Su, H.T. Lin and Y.M. Jiang, 2011. Effect of pure oxygen atmosphere on antioxidant enzyme and antioxidant activity of harvested litchi fruit during storage. Food Research International, 44(7): 1905–1911.

Garcı´A, N.A.T., C. Iribarne, F. Palma and C. Lluch, 2007. Inhibition of the catalase activity from phaseolus vulgaris and medicago sativa by sodium chloride. Plant Physiology and Biochemistry, 45(8): 535-541.

García-Mata, C. and L. Lamattina, 2013. Gasotransmitters are emerging as new guard cell signaling molecules and regulators of leaf gas exchange. Plant Science, 201– 202: 66– 73.

Goraj, S., M. Libik-Konieczny, E. Surówka, P. Rozpadek, A. Kalisz, A. Libik, M. Nosek, P. Waligórski and Z. Miszalski, 2012. Differences in the activity and concentration of elements of the antioxidant system in different layers of Brassica pekinensis head. Journal of Plant Physiology, 169(12): 1158-1164.

Guzman, J.A., 2012. Carbon monoxide poisoning. Critical Care Clinics, 28(4): 537-548.

Han, Y., J. Zhang, X.Y. Chen, Z.Z. Gao, W. Xuan, S. Xu, X. Ding and W.B. Shen, 2008. Carbon monoxide alleviates cadmium-induced oxidative damage by modulating glutathione metabolism in the roots of medicago sativa. New Phytologist, 177(1): 155–166.

Huang, J.J., B. Han, S. Xu, M.X. Zhou and W.B. Shen, 2011. Heme oxygenase-1 is involved in the cytokinin-induced alleviation of senescence in detached wheat leaves during dark incubation. Journal of Plant Physiology, 168(8): 768–775.

Kan, J., H.M. Wang and C.H. Jin, 2011. Changes of reactive oxygen species and related enzymes in mitochondrial respiration during storage. Agricultural Sciences in China, 10(1): 149-158.

Kong, W.W., L.P. Zhang, K. Guo, Z.P. Liu and Z.M. Yang, 2010. Carbon monoxide improves adaptation of arabidopsis to iron deficiency. Plant Biotechnology Journal, 8(1): 88－99.

Lacan, D. and J.C. Baccou, 1998. High levels of antioxidant enzymes correlate with delayed senescence in nonnetted fruits. Planta, 204(3): 377–382.

Li, J.W., L.P. Fan, S.D. Ding and X.L. Ding, 2007. Nutritional composition of five cultivars of Chinese jujube. Food Chemistry, 103: 454–460.

Ling, T.F., B. Zhang, J.S. Lin, H. Liu, S.Y. Wei, Y.G. Sun and W.B. Shen, 2006. Effects of carbon monoxide on vase life and antioxidative metabolism in the cut rose flower. Acta Horticulturae Sinica, (In Chinese), 33(4): 779-782.

Meng, D.K., J. Chen and Z.M. Yang, 2011. Enhancement of tolerance of Indian mustard (Brassica Juncea) to mercury by carbon monoxide. Journal of Hazardous Materials, 186(2-3): 1823–1829.

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

Piantadosi, C.A., 2002. Biological chemistry of carbon monoxide. Antioxid Redox Signal, 4(2): 259–270.

Pun, P.B.L., J. Lu, E.M. Kan and S. Moochhala, 2010. Gases in the mitochondria. Mitochondrion, 10(2): 83-93.

Rogiers, S.Y., M.G.N. Kumar and N.R. Knowles, 1998. Maturation and ripening of fruit of amelanchier alnifolia nutt. Are accompanied by increasing oxidative stress. Annals of Botany, 81(2): 203–211.

Santa-Cruz, D.M., N.A. Pacienza, A.H. Polizio, K.B. Balestrasse, M.L. Tomaro and G.G. Yannarelli, 2010. Nitric oxide synthase-like dependent no production enhances heme oxygenase up-regulation in ultraviolet-B-irradiated soybean plants. Phytochemistry, 71(14-15): 1700–1707.

Shen, Q., M. Jiang, H. Li, L.L. Che and Z.M. Yang, 2011. Expression of a brassia napus heme oxygenase confers plant tolerance to mercury toxicity. Plant Cell Environ., 34(5): 752—763.

Vreman, H.J., R.J. Wong and D.K. Stevenson, 2011. Quantitating carbon monoxide production from heme by vascular plant preparations in vitro. Plant Physiology and Biochemistry, 49(1): 61-68.

Wang, Y.F., F. Tang, J.D. Xia, T. Yu, J. Wang, R. Azhati and X.D. Zheng, 2011. A combination of marine yeast and food additive enhances preventive effects on postharvest decay of jujubes (Zizyphus Jujuba). Food Chemistry, 125(3): 835–840.

Xing, Z.T., Y.S. Wang, Z.Y. Feng and Q. Tan, 2008. Effect of different packaging films on postharvest quality and selected enzyme activities of hypsizygus marmoreus mushrooms. Journal of Agricultural Food Chemistry, 56(24): 11838-11844.

Xu, J., W. Xuan, B.K. Huang, Y.H. Zhou, T.F. Ling, C. Xu and W.B. Shen, 2006. Carbon monoxide induces adventitious roots development in mung bean [J]. Chinese Science Bulletin, 51(4): 409-414.

Yang, Z.F., Y.H. Zheng and S.F. Cao, 2009. Effect of high oxygen atmosphere storage on quality, antioxidant enzymes, and DPPH-radical scavenging activity of Chinese bayberry fruit. Journal of Agricultural Food Chemistry, 57(1): 176–181.

Zhang, H., S.L. Hu, Z.J. Zhang, L.Y. Hu, C.X. Jiang, Z.J. Wei, J. Liu, H.L. Wang and S.T. Jiang, 2011. Hydrogen sulfide acts as a regulator of flower senescence in plants. Postharvest Biology and Technology, 60(3): 251–257.

Zhang, S.Y. and N. Li, 2014. Effects of carbon monoxide on quality, nutrients and antioxidant activity of postharvest jujube. Journal of the Science of Food and Agriculture, 94(5): 1013-1019.

Zhang, S.Y., Y.W. Yu, C.L. Xiao, X.D. Wang and Y.Y. Tian, 2013. Effect of carbon monoxide on browning of fresh-cut lotus root slice in relation to phenolic metabolism. LWT - Food Science and Technology, 53(2): 555-559.

Zhao, Y., K. Tu, J. Su, S.C. Tu, Y.P. Hou, F.J. Liu and X.R. Zou, 2009. Heat treatment in combination with antagonistic yeast reduces diseases and elicits the active defense responses in harvested cherry tomato fruit. Journal of Agricultural Food Chemistry, 57(16): 7565–7570.

Zhu, S.H. and J. Zhou, 2007. Effect of nitric oxide on ethylene production in strawberry fruit during storage. Food Chemistry, 100(4): 1517–1522.

