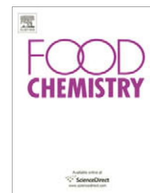
Food Chemistry 115 (2009) 1458–1463

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem



Methyl jasmonate reduces chilling injury and enhances antioxidant enzyme activity in postharvest loquat fruit

Shifeng Cao, Yonghua Zheng *, Kaituo Wang, Peng Jin, Huaijing Rui

College of Food Science and Technology, Nanjing Agricultural University, Weigang 1, Nanjing 210095, PR China

ARTICLE INFO

Article history:
Received 16 October 2008
Received in revised form 8 December 2008
Accepted 27 January 2009

Keywords:
Loquat fruit
Methyl jasmonate
Chilling injury
Antioxidant enzyme
Lipoxygenase
Fatty acids

ABSTRACT

Loquat fruit were pre-treated with 10 μmol/l methyl jasmonate (MeJA) for 24 h at 20 °C, and then stored at 1 °C for 35 days to investigate the effect of MeJA treatment on chilling injury and changes in the antioxidant system. Loquat fruit developed chilling injury, manifested as increased fruit firmness, decreased extractable juice rate and internal browning during storage. These chilling injury symptoms were significantly reduced by MeJA treatment. MeJA also markedly delayed the increases in $O_2^{-\cdot}$ production rate and H_2O_2 content. Meanwhile, the MeJA-treated fruit exhibited significantly higher activities of superoxide dismutase, catalase and ascorbate peroxidase, and lower activity in lipoxygenase than control fruit during the storage. The ratio of unsaturated/saturated fatty acid in MeJA-treated fruit was also significantly higher than that in control fruit. These results suggest that the reduction in chilling injury by MeJA may be due to enhanced antioxidant enzyme activity and higher unsaturated/saturated fatty acid ratio.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

Loquat (*Eriobotrya japonica* Lindl.) fruit, native to subtropical China, is famous for its soft, juicy taste and nutritional value and highly favoured by consumers worldwide. Loquat fruit mature in the hot and rainy season and have a short postharvest life because they are susceptible to microbial decay, mechanical damage, moisture and nutritional losses. Low temperature storage is commonly used for loquat to inhibit fruit decay and extend postharvest life. However, the fruit of red-fleshed loquat cultivars develop some physiological disorders including stuck peel, firm and juiceless flesh (leatheriness), and internal browning after 2–3 weeks of storage at 1–5 °C, which are regarded as chilling injury symptoms (Zheng, Li, & Xi, 2000). The development of these chilling disorders reduces consumer acceptance for this fruit, thus limiting the storage life. Although various methods such as modified atmosphere packaging, low temperature conditioning, and applications of polyamine, salicylic acid or 1-methylcyclopropene have been demonstrated to reduce these disorders of the fruit, there is still a need for development of more effective techniques for loquat fruit storage (Cao & Zheng, 2008).

Chilling injury is an economically important postharvest problem that reduces the overall quality and marketability of many tropical and subtropical fruits and vegetables. The development of chilling injury symptoms can be the consequence of oxidative stress from excess reactive oxygen species (ROS) that induce peroxidation and breakdown of unsaturated fatty acids in membrane lipids (Lyons, 1973). ROS scavengers, on the other hand, increase the degree of unsaturation of 18-carbon fatty acids in the polar lipids and act as antioxidants to reduce the severity of chilling injury (Wang & Baker, 1979). The metabolism of ROS is controlled by an array of interrelated antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX). Superoxide anion radical ($O_2^{-\cdot}$) is efficiently converted to H_2O_2 by the action of SOD, while H_2O_2 is destroyed predominantly by APX and CAT (Mittler, 2002). Previous studies have shown that a positive relationship exists between the antioxidant enzyme activity and the chilling tolerance in harvested fruits (Sala, 1998; Wang, 1995). Lipoxygenase (LOX) catalyses peroxidation of polyunsaturated fatty acids and is believed to be a major contributor to chilling-induced membrane damage in plant tissue (Pinhero, Paliyath, Yada, & Murr, 1998). An increase in LOX activity was observed in chilling injured cucumber fruit (Mao, Pang, Wang, & Zhu, 2007). These results suggest that enhanced antioxidant enzyme system and reduced peroxidation of membrane lipids may be involved in chilling tolerance in harvested fruit.

Jasmonic acid and its volatile methyl ester, methyl jasmonate (MeJA), are a class of cyclopentanone compounds, regarded as endogenous regulators that play an important roles for regulating the stress response, plant growth and development (Creelman & Mullet, 1997). In recent research, MeJA has been applied to reduce the development of chilling injury symptoms in a number of horticultural crops, including mango, sweet pepper, and tomato fruit

* Corresponding author. Tel.: +86 25 84399080; fax: +86 25 84395618.
E-mail address: zhengyh@njau.edu.cn (Y. Zheng).

0308-8146/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2009.01.082

(Ding, Wang, Gross, & Smith, 2001; Fung, Wang, Smith, Gross, & Tian, 2004; González-Aguilar, Fortiz, & Wang, 2000). Thus, MeJA has a potential application in postharvest treatment by alleviating chilling injury and maintaining quality. Recently, we found that MeJA treatment at 10 μmol l^{-1} was effective in alleviating chilling injury of cold-stored loquat fruit (Cao et al., 2007). However, the mode of action of MeJA in reducing chilling injury and quality deterioration has not been clearly elucidated. The objective of this study was to determine whether the MeJA-induced changes in the antioxidant system and fatty acid composition are linked to the enhanced tolerance to chilling injury in cold-stored loquat fruit.

2. Materials and methods

2.1. Plant material and treatments

Loquat fruit (*E. japonica* L. cv. Fuyang) were hand-harvested at ripe stage from an orchard in Jiangsu, PR China, and transported within 2 h to our laboratory. Fruit were selected for uniform size and colour and the absence of visual defects, then randomly divided into two lots. The first lot of fruit was treated with 10 μmol/l MeJA (Aldrich, Chemical Company, Wilwaukee, WI, USA) in sealed chambers at 20 °C for 24 h, whereas the second lot of fruit was subjected to the same condition without exposure to MeJA (control). Following treatment, the chambers were opened, and both lots of fruit were stored at 1 °C and approx. 95% relative humidity for up to 35 days. There were three replicates of five kilograms of fruit each per treatment, and the experiment was conducted twice. Fruit samples were taken after MeJA treatment (time 0) and at 7 day intervals during storage for measurements of fruit firmness, extractable juice, internal browning, superoxide anion production, H_2O_2 content, fatty acid composition, and activities of SOD, CAT, APX and LOX.

2.2. Determinations of fruit firmness, extractable juice rate and internal browning index

Fruit firmness was measured on two paired sides of 10 fruit from each replicate (skin removed) with a TA-XT2i texture analyser (Stable Micro System Ltd., UK) with a 5 mm diameter probe at a speed of 1 mm s^{-1}.

Extractable juice rate was estimated from the weight loss from placental tissue plugs in response to low-speed centrifugation. Four plugs (7 mm wide and 10 mm thick) were placed over sterile cotton in a 50 ml centrifuge tube and centrifuged for 10 min at 1700*g* at room temperature. The results are expressed as fresh weight loss of the tissue plugs after centrifugation.

Internal browning (IB) index manifested as browning discoloration near the core was evaluated visually using 10 fruit from each replicate after cutting the fruit longitudinally in half. For each fruit, IB was scored according to a 5-grade scale, where 0 = none; 1 = slight; 2 = moderate; 3 = moderately severe; 4 = severe. Results were expressed as an IB index calculated using the following formula: IB index (between 0 and 4) = [(IB level) × (number of fruit at the IB level)]/(total number of fruit in the treatment).

2.3. Measurements of $O_2^{-\cdot}$ and H_2O_2

$O_2^{-\cdot}$ production was measured using the method of Elstner (1976) with some modifications. Five grams of flesh tissue was ground in 5 ml of 50 mM phosphate buffer (pH 7.8). The homogenate was centrifuged at 10,000*g* for 20 min at 4 °C. The supernatant was used for the determination of $O_2^{-\cdot}$ production. The reaction mixture of 1 ml of 1 M hydroxyammoniumchloride and 1 ml crude extract was incubated at 25 °C for 1 h. Then 2 ml ether was added to the incubation mixture in order to prevent the interference of chlorophyll and the mixture was centrifuged at 10000*g* for 5 min. after that 1 ml mixture from the water layer, 1 ml of 17 mM p-aminophenylsulfonic acid (in glacial acetic acid:-H_2O = 3:1) and 7 mM α-naphthylamine (in glacial acetic acid:-H_2O = 3:1) were added and the mixture incubated at 25 °C for a further 20 min, followed by immediate measurement of absorbance at 530 nm. $O_2^{-\cdot}$ production was calculated against the standard curve using sodium nitrite as a standard and expressed as nmol g^{-1} FW min^{-1}.

For H_2O_2 determination, 2 g of fresh tissue was homogenised with 5 ml of chilled 100% acetone and then centrifuged at 10,000*g* for 20 min at 4 °C. The supernatant was collected immediately for H_2O_2 analysis according to the method of Patterson, Mackae, and Ferguson (1984). H_2O_2 content was expressed as nmol g^{-1} FW.

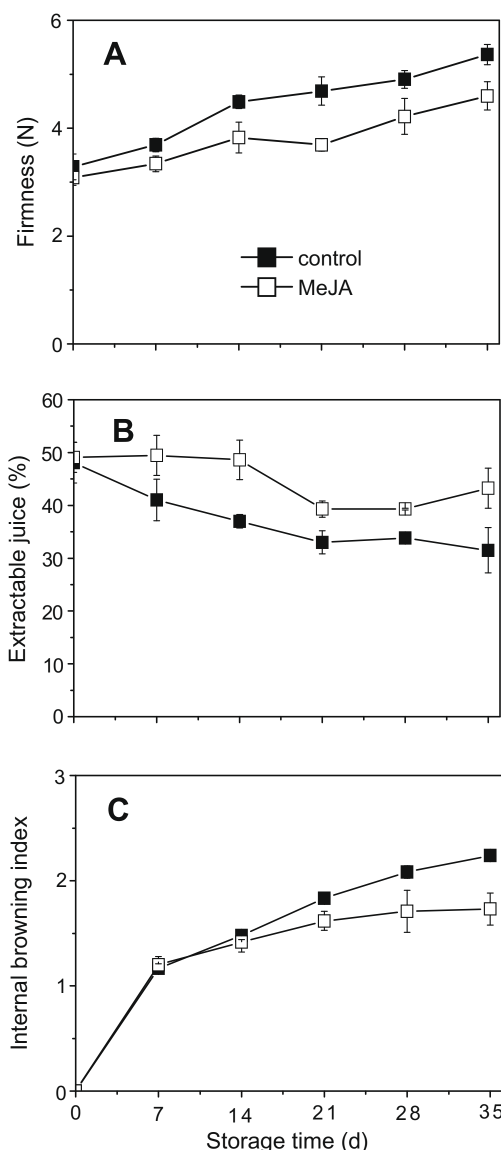


Fig. 1. Effects of MeJA on fruit firmness (A), extractable juice (B), and internal browning index (C) of loquat fruit during storage at 1 °C. Values for firmness and internal browning are the means ± SE of triplicate samples of ten fruit each. Values for extractable juice are the means ± SE of triplicate assays. Vertical bars represent the standard errors of the means.

2.4. Enzyme assays

All enzyme extract procedures were conducted at 4 °C. For SOD, 1 g of flesh tissue was ground with 5 ml of 50 mM sodium phosphate buffer (pH 7.8). Flesh tissue (1 g) was ground with 5 ml of 50 mM sodium phosphate buffer (pH 7.0) for CAT, or 50 mM sodium phosphate buffer (pH 7.0), containing 0.1 mM EDTA, 1 mM ascorbic acid and 1% polyvinyl-pyrrolidone (PVP) for APX. For LOX, 5 g of flesh tissue was ground with 5 ml of 50 mM Tris–HCl (pH 8.0) containing 10 mM KCl, 500 mM sucrose and 0.5 mM phenylmethylsulfonylfloride at 4 °C. The extracts were then homogenised and centrifuged at 10,000*g* for 20 min at 4 °C. The supernatants were used for the enzyme assays.

SOD activity was determined photochemically by the method of Rao, Paliyath, and Ormrod (1996). The reaction mixture contained 50 mM sodium phosphate (pH 7.8), 14 mM methionine, 3 μM EDTA, 1 μM nitro-blue-tetrazolium (NBT), 60 μM riboflavin and 0.1 ml crude enzyme extract in a total volume of 3 ml. The formation of blue formazan was monitored by recording the absorbance at 560 nm. One unit of SOD activity was defined as the amount of enzyme that causes a 50% inhibition of NBT reduction under assay conditions.

CAT activity was assayed according to the method of Chance and Maehly (1955). One unit of CAT was defined as the amount of enzyme that decomposes 1 μmol of H_2O_2 min^{-1}.

APX activity was carried out as described the method of Nakano and Asada (1989). One unit of APX was defined as the amount of enzyme that oxidises 1 μmol ascorbate min^{-1}.

LOX activity was assayed using the method of Todd, Paliyath, and Thompson (1990). One unit of LOX is defined as the amount of enzyme which causes an increase in absorption at 234 nm of 0.01 min^{-1} at 25 °C when linoleic acid is used as the substrate.

Protein content in the enzyme extracts was estimated using the Bradford (1976) method, using bovine serum albumin as a standard. Specific activity of the enzymes was expressed as units per milligram protein.

2.5. Fatty acid quantification

Total lipids were extracted according to Valero, López-Frías, Llópis, and López-Jurado (1990). Briefly, 20 g of flesh tissue were homogenised in 10 ml chloroform:methanol:0.1 N HCl (200:100:1) and then 10 ml of 0.1 N HCl were added before centrifugation at 4000*g* for 10 min. The organic phase was collected and taken to dryness. Methylation of fatty acids was carried out by adding 1 ml boron trifluoride/methanol at boiling temperature for 10 min. Methylated fatty acids were extracted with hexane, taken to dryness and redissolved in 200 μl chloroform before injection. Fatty acids were separated and quantified according to Mirdehghan et al. (2007) by gas chromatography (GC, Hewlett–Packard model 6890) equipped with a flame ionisation detector (FID). Identification and quantification of fatty acids were performed by comparing retention times and peak areas with authentic standards (Sigma Chemical Co., St. Louis, MO, USA). The unsaturated/saturated fatty acid ratio was calculated by the formula: (18:1 + 18:2 + 18:3)/(16:0 + 18:0) where 16:0 = palmitic acid; 18:0 = stearic acid; 18:1 = oleic acid; 18:2 = linoleic acid; 18:3 = linolenic acid.

2.6. Statistical design

Experiments were performed using a completely randomized design. All statistical analyses were performed with SPPS (SPSS Inc., Chicago, IL, USA). Data were analysed by one-way analysis of variance (ANOVA). Mean separations were performed by Duncan's multiple range tests. Differences at $p < 0.05$ were considered as significant.

3. Results

3.1. Occurrence of chilling injury symptoms

Fruit firmness, internal browning and extractable juice rate were used to evaluate the development of chilling injury in loquat fruit (Fig. 1). Ripe loquat fruit flesh is soft and juicy. However, instead of softening that tends to occur in most fruits, fruit firmness increased while extractable juice rate decreased in loquat fruit during cold storage, which resulted in a dry and firm fruit texture (flesh leatheriness) and reduced the edible quality. The control fruit exhibited severe flesh leatheriness and higher internal browning index after 21 days of storage at 1 °C. Treatment with 10 μmol/l MeJA significantly ($P < 0.05$) inhibited the increases in fruit firmness, internal browning and maintained higher extractable juice rate, thereby delaying the development of chilling injury symptoms and maintaining fruit quality.

3.2. Effect of MeJA treatment on $O_2^{-\cdot}$ production and H_2O_2 content

Levels of $O_2^{-\cdot}$ and H_2O_2 remained relatively stable and no significant differences were observed between the control and MeJA-

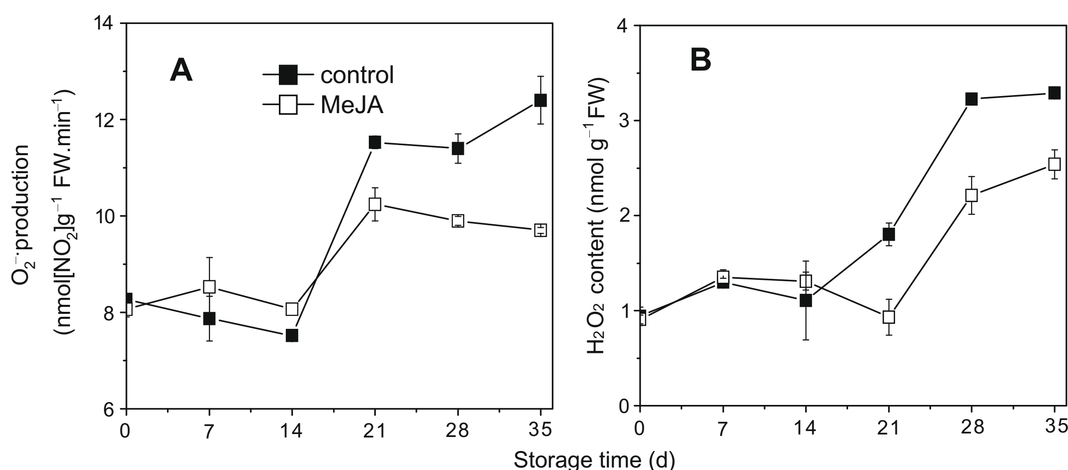


Fig. 2. Effects of MeJA on levels of $O_2^{-\cdot}$ (A), and H_2O_2 (B) of loquat fruit during storage at 1 °C. Values are the means ± SE of triplicate assays. Vertical bars represent the standard errors of the means.

treated fruit during the initial 14 days of cold storage. Thereafter, both $O_2^{-\cdot}$ and H_2O_2 contents increased rapidly, treatment with 10 μmol/l MeJA significantly ($P < 0.05$) inhibited the increases in $O_2^{-\cdot}$ and H_2O_2 levels (Fig. 2). Contents of $O_2^{-\cdot}$ and H_2O_2 in control fruit were 21.8% and 22.8% higher than those in MeJA-treated fruit on the 35th day of storage, respectively.

3.3. Effect of MeJA treatment on SOD, CAT, APX and LOX activities

SOD activity in both control and MeJA-treated fruit increased with storage time, fruit pre-treated with MeJA maintained remarkably higher SOD activity throughout the storage (Fig. 3A). The changes of CAT and APX activities in loquat fruit exhibited a similar pattern during the cold storage. The activities of both enzymes in control fruit increased slightly during the first 7 days of storage, and then decreased gradually during the remainder of storage. MeJA treatment significantly ($P < 0.05$) promoted the increases and delayed the decreases in activities of CAT and APX, the activities of both enzymes were significantly higher ($P < 0.05$) in MeJA-treated fruit than those in control fruit during the whole storage period (Fig. 3B and C). LOX activity increased gradually during storage. The increase of LOX activity was significantly ($P < 0.05$) inhibited by MeJA treatment (Fig. 3D).

3.4. Effect of MeJA treatment on fatty acid composition

In this study, five fatty acids were identified and quantified in membrane lipids of loquat fruit flesh tissue, two saturated (palmitic acid and stearic acid), one mono-unsaturated (oleic acid) and two polyunsaturated (linoleic acid and linolenic acid). Among all the fatty acids, linoleic acid (C18:2) was predominant (approx. 31%). Levels of palmitic, stearic, and oleic acids increased (Fig. 4A–C), while contents of linoleic, linolenic acid and the ratio of unsaturated/saturated fatty acid decreased (Fig. 4D–F) during the whole storage period. MeJA treatment significantly ($P < 0.05$) inhibited the increases in palmitic, stearic, and oleic acids levels and delayed the decreases in linoleic and linolenic acid contents, thus it maintained significantly ($P < 0.05$) higher unsaturated/saturated fatty acid ratio compared to the control fruit.

4. Discussion

MeJA has been implicated in the signalling pathway mediating induced defence responses in chilling-stressed plants and the onset of the tolerance has often been correlated with the accumulation of defence-related enzymes and compounds (Creelman & Mullet, 1997). When exogenously applied, MeJA has been shown to result in an improved chilling tolerance and reduced incidence of chilling injury in several fruit (Ding et al., 2001; Fung et al., 2004). In this study, we found that MeJA treatment could effectively reduce flesh leatheriness and internal browning, the typical chilling injury symptoms in loquat fruit (Fig. 1). This indicates that the chilling tolerance of loquat fruit was also enhanced by postharvest treatment with MeJA. Since MeJA treatment is easy to set up and inexpensive, it could be a useful technique to maintain quality during loquat fruit storage.

An increasing amount of evidence suggests that oxidative stress from excess production of ROS, such as $O_2^{-\cdot}$, singlet oxygen, H_2O_2 and hydroxyl radical, may contribute to the development of chilling injury and that antioxidant enzymes, SOD, CAT and APX may play important roles in detoxifying ROS and alleviating chilling injury (Hariyadi & Parkin, 1991; Sala, 1998; Zheng, Raymond, Wang, & Wang, 2008). Therefore, protection from oxidative injury is crucial to cell survival under chilling stress and is thought to be a

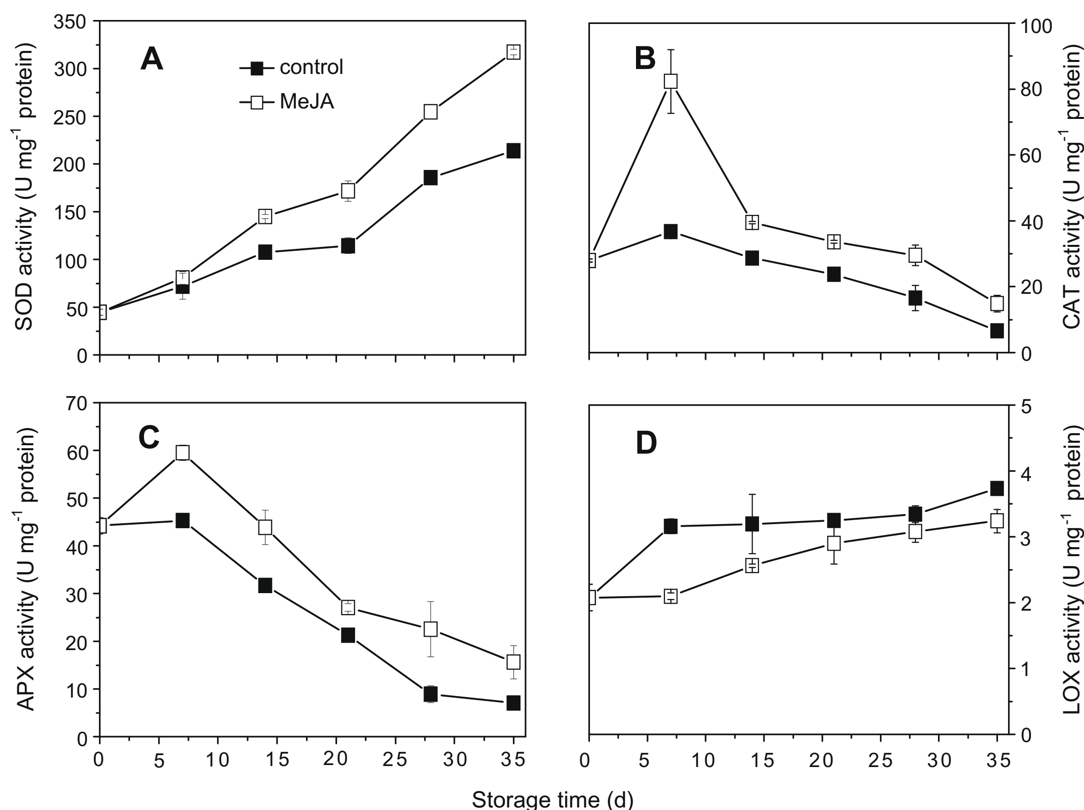


Fig. 3. Effects of MeJA on activities of SOD (A), CAT (B), APX (C), and LOX (D) of loquat fruit during storage at 1 °C. Values are the means ± SE of triplicate assays. Vertical bars represent the standard errors of the means.

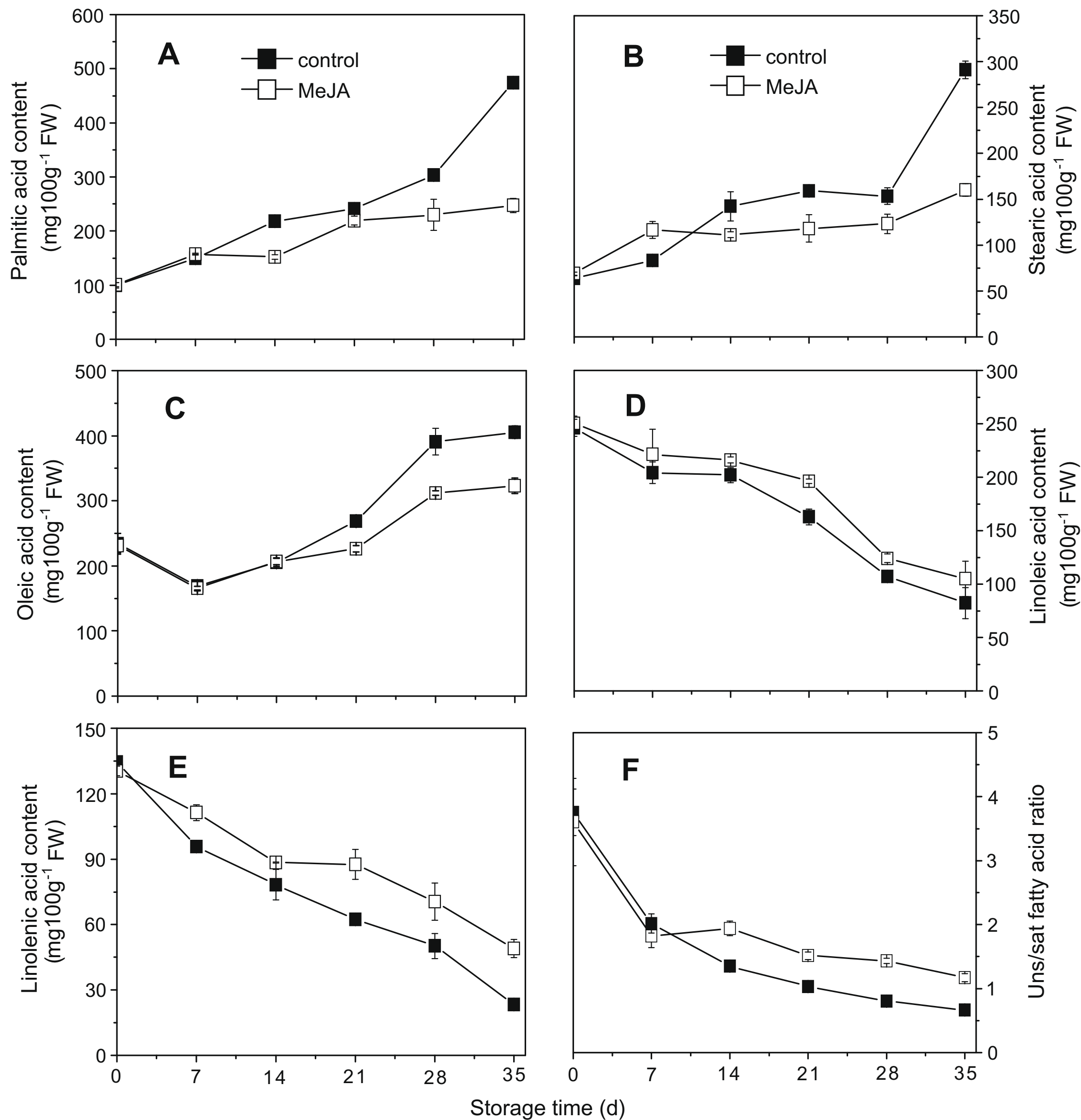


Fig. 4. Effects of MeJA on contents of palmitic (A), stearic (B), oleic (C), linoleic (D), linolenic (E) acid and unsaturated/saturated fatty acid ratio (F) of loquat fruit during storage at 1 °C. Values are the means ± SE of triplicate assays. Vertical bars represent the standard errors of the means.

major mechanism of resistance to chilling stress. It has been reported that that the improvement of chilling tolerance in harvested horticultural crops is related to enhancement in activities of antioxidant enzymes. Sala (1998) found that the chilling-tolerant mandarins have a higher antioxidant enzyme activity than the chilling-sensitive cultivars. A number of postharvest treatments that induce chilling tolerance and alleviate chilling injury (e.g. heat shock, low temperature conditioning and superatmospheric oxygen treatment) also enhance antioxidant enzyme activity (Sala & Lafuente, 2000; Wang, 1995; Zheng et al., 2008). In the present work, CAT and APX activities decreased (Fig. 3B and C) while $O_2^{-\cdot}$ and H_2O_2 contents increased (Fig. 2A and B) significantly during the development of irreversible chilling injury symptoms (flesh leatheriness and internal browning, Fig. 1) in control fruit. This indicates that the oxidative stress may be also involved in the development of chilling injury in loquat fruit. Treatment with MeJA significantly reduced these chilling injury symptoms and increased the activities of SOD, CAT, and APX under chilling stress. The increased SOD activity could enhance the ability of the tissue to dismutate $O_2^{-\cdot}$, while the enhanced CAT and APX activities would contribute to the stronger elimination of H_2O_2, which may account for the lower levels of $O_2^{-\cdot}$ and H_2O_2 observed in MeJA-treated fruit (Fig. 2A and B). These results suggest that effect of MeJA in reducing the occurrence of chilling injury was correlated to enhanced antioxidant enzyme activity. The linked action of CAT, APX and SOD may be one of the major factors that trigger the chilling resistance in MeJA-treated loquat fruit.

LOX is thought to play a primary role in generating peroxidative damage in membrane lipids in plant tissues (Lee et al., 2005; Siedow, 1991). An increase in LOX activity was observed in maize seedlings in response to chilling stress, which suggests that LOX may be involved in the occurrence of chilling injury (Pinhero et al., 1998). Therefore, in addition to investigating the relationship between antioxidant enzyme activity and chilling tolerance, we examined the possible role that LOX may play in response to chilling stress of loquat fruit in this study. We observed that the development of irreversible chilling injury symptoms, e.g., flesh leatheriness and internal browning, was accompanied by significant increase of LOX activity in the control fruit, while this increase of LOX activity and the development of chilling injury symptoms

were significantly inhibited by MeJA treatment (Fig. 3D). Similar results were also reported by Mao et al. (2007), who found that the development of chilling injury in cucumber fruit was accompanied with an increase of LOX activity when exposed to chilling stress, and that the enhanced tolerance to chilling by heat treatment was associated with the reduction in LOX activity. These results suggest that LOX may be involved in the induction of chilling injury in loquat fruit. The effect of MeJA in reducing the occurrence of chilling injury may result from inhibition of the increase in LOX activity.

Membranes are thought to be the primary sites for development of chilling injury. Increased peroxidation of membrane lipids has been noted in chilling-sensitive crops at chilling temperatures, which could reduce the degree of lipid unsaturation and induce phase transition of membrane lipids from a liquid-crystalline to a solid-gel state (Marangoni, Palma, & Stanley, 1996). There appears to be a correlation between fatty acid composition in membrane lipids and chilling tolerance. For example, Boonsiri, Ketsa, and van Doorn (2007) found that chilling-induced seed browning of hot peppers was related to lower levels of unsaturated fatty acids. A pre-storage heat treatment induced acclimation of pomegranate fruit to low temperature and thus reduced chilling injury by maintaining higher unsaturated/saturated fatty acid ratio (Mirdehghan et al., 2007). During the development of chilling injury in this study, levels of the two saturated fatty acids increased, while contents of the two major polyunsaturated fatty acids decreased with a concomitant reduction in the ratio of unsaturated/saturated fatty acid (Fig. 4F). Polyunsaturated fatty acids, e.g., linoleic and linolenic acid, are the main substrates of LOX action (Siedow, 1991). The decrease of their contents could be due to the increased LOX activity. Treatment with MeJA significantly reduced the severity of chilling injury and maintained significant higher unsaturated/saturated fatty acid ratio than in control fruit. These results suggest that the decrease of lipid unsaturation may be involved in the induction of chilling injury in loquat fruit. The higher unsaturated/saturated fatty acid ratio might contribute to the reduced chilling injury in MeJA-treated fruit.

In conclusion, our results suggest that the oxidative stress may be involved in the development of chilling injury in loquat fruit. MeJA treatment can effectively enhance chilling tolerance and reduce chilling injury of loquat fruit. The reduction in chilling injury by MeJA may be due to enhanced antioxidant enzyme activity and higher unsaturated/saturated fatty acid ratio.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (30671462) and National Scientific and Technical Supporting Program (2006BAD30B03, 2006BAD22B05).

References

Boonsiri, K., Ketsa, S., & van Doorn, W. G. (2007). Seed browning of hot peppers during low temperature storage. *Postharvest Biology and Technology, 45*, 358–365.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle-dye binding. *Analytical Biochemistry, 72*, 248–254.

Cao, S. F., & Zheng, Y. H. (2008). Postharvest biology and handling of loquat fruit. *Stewart Postharvest Review, 4*, 1–5.

Cao, S. F., Zheng, Y. H., Yang, Z. F., Ma, S. J., Li, N., Tang, S. S., et al. (2007). Effects of methyl jasmonate treatment on quality and decay in cold-stored loquat fruit. *Acta Horticulture, 750*, 425–430.

Chance, B., & Maehly, A. C. (1955). Assay of catalases and peroxidase. *Methods in Enzymology, 2*, 764–775.

Creelman, R. A., & Mullet, J. E. (1997). Biosynthesis and action of jasmonate in plants. *Annual Review of Plant Physiology and Plant Molecular Biology, 48*, 355–381.

Ding, C. K., Wang, C. Y., Gross, K. C., & Smith, D. L. (2001). Reduction of chilling injury and transcript accumulation of heat shock protein genes in tomatoes by MeJA and MeSA. *Plant Science, 161*, 1153–1159.

Elstner, E. F. (1976). Inhibition of nitrite formation hydroxylam-mouium-chloride: A simple assay for superoxide dismutase. *Analytical Biochemistry, 70*, 616–620.

Fung, R. W. M., Wang, C. Y., Smith, D. L., Gross, K. C., & Tian, M. S. (2004). MeSA and MeJA increase steady-state transcript levels of alternative oxidase and resistance against chilling injury in sweet peppers (*Capsicum annuum* L.). *Plant Science, 166*, 711–719.

González-Aguilar, G. A., Fortiz, J., & Wang, C. Y. (2000). Methyl jasmonate reduces chilling injury and maintains postharvest quality of mango fruit. *Journal of Agriculture and Food Chemistry, 48*, 515–519.

Hariyadi, P., & Parkin, K. L. (1991). Chilling induced oxidative stress in cucumber fruits. *Postharvest Biology and Technology, 1*, 33–35.

Lee, S. H., Ahn, S. J., Im, Y. J., Cho, K., Chung, G. C., Cho, B. H., et al. (2005). Differential impact of low temperature on fatty acid unsaturation and lipoxygenase activity in fig leaf gourd and cucumber roots. *Biochemical and Biophysical Research Communications, 330*, 1194–1198.

Lyons, J. M. (1973). Chilling injury in plants. *Annual Review of Plant Physiology, 24*, 445–466.

Mao, L. C., Pang, H. G., Wang, G. Z., & Zhu, C. G. (2007). Phospholipase D and lipoxygenase activity of cucumber fruit in response to chilling stress. *Postharvest Biology and Technology, 44*, 42–47.

Marangoni, A. G., Palma, T., & Stanley, D. W. (1996). Membrane effects in postharvest physiology. *Postharvest Biology and Technology, 7*, 193–221.

Mirdehghan, S. H., Rahemi, M., Martínez-Romero, D., Guillén, F., Valverde, J. M., Zapata, P. J., et al. (2007). Reduction of pomegranate chilling injury during storage after heat treatment: Role of polyamines. *Postharvest Biology and Technology, 44*, 19–25.

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

Nakano, Y., & Asada, K. (1989). Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. *Plant Cell Physiology, 22*, 867–880.

Patterson, B. D., Mackae, E. A., & Ferguson, I. B. (1984). Estimation of hydrogen peroxide in plant extracts using titanium. *Analytical Biochemistry, 139*, 487–492.

Pinhero, R. G., Paliyath, G., Yada, R. Y., & Murr, D. P. (1998). Modulation of phospholipase D and lipoxygenase activities during chilling. Relation to chilling tolerance of maize seedlings. *Plant Physiology and Biochemistry, 36*, 213–224.

Rao, M. V., Paliyath, G., & Ormrod, D. P. (1996). Ultraviolet-B- and ozone-induced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana*. *Plant Physiology, 110*, 125–136.

Sala, J. M. (1998). Involvement of oxidative stress in chilling injury in cold-stored mandarin fruits. *Postharvest Biology and Technology, 13*, 255–261.

Sala, J. M., & Lafuente, M. (2000). Catalase enzymes activity is related to tolerance of mandarin fruits to chilling. *Postharvest Biology and Technology, 20*, 81–89.

Siedow, J. N. (1991). Plant lipoxygenase: Structure and function. *Annual Review of Plant Physiology and Plant Molecular Biology, 42*, 145–188.

Todd, J. F., Paliyath, G., & Thompson, J. E. (1990). Characteristics of a membrane associated lipoxygenase in tomato fruit. *Plant Physiology, 94*, 1225–1232.

Valero, D., López-Frías, M., Llópis, J., & López-Jurado, M. (1990). Influence of dietary fat on the lipid composition of perirenal adipose tissue in rats. *Annals of Nutrition and Metabolism, 34*, 327–332.

Wang, C. Y. (1995). Effect of temperature preconditioning on catalase, peroxidase, and superoxide dismutase in chilled zucchini squash. *Postharvest Biology and Technology, 5*, 67–76.

Wang, C. Y., & Baker, J. E. (1979). Effects of two free radical scavengers and intermittent warming on chilling injury and polar lipid composition of cucumbers and sweet pepper fruits. *Plant Cell Physiology, 20*, 243–251.

Zheng, Y. H., Li, S. Y., & Xi, Y. F. (2000). Changes of cell wall substances in relation to flesh leatheriness in cold-stored loquat fruits. *Acta Phytophysiologica Sinica, 26*, 306–310.

Zheng, Y. H., Raymond, W. M. F., Wang, S. Y., & Wang, C. Y. (2008). Transcript levels of antioxidative genes and oxygen radical scavenging enzyme activities in chilled zucchini squash in response to superatmospheric oxygen. *Postharvest Biology and Technology, 47*, 151–158.