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Influences of 1-methylcyclopropene-containing papers on the metabolisms of membrane lipids in Anxi persimmons during storage

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Abstract

Objectives: The aim of this work was to analyse the effects of 1-methylcyclopropene (1-MCP) treatment on the metabolisms of membrane lipids in postharvest Anxi persimmons during storage.
Materials and methods: Anxi persimmon (*Diospyros kaki* L. f. cv. Anxi) fruits were treated by paper containing 1-MCP with a concentration of 1.35 μl/l. The cellular membrane permeability was analysed by the electric conductivity meter. The activities of lipoxygenase (LOX), phospholipase (PLD) and lipase were determined by spectrophotometry. The component and relative amounts of membrane fatty acids were determined using gas chromatograph (GC).
Results: The 1-MCP-treated Anxi persimmons manifested a lower electrolyte leakage rate, lower LOX, PLD and lipase activities, higher levels of unsaturated fatty acids (USFAs), higher ratio of USFAs to saturated fatty acids (SFAs) (U/S), higher index of USFAs (IUFA), but lower levels of SFAs.
Conclusions: The degradation and the metabolisms of membrane lipids could be suppressed by 1-MCP treatment, which might be accountable for the delaying softening of postharvest Anxi persimmons during storage.

Key words: Anxi persimmon; membrane lipids; membrane-degrading enzymes; membrane peroxidation; 1-methylcyclopropene (1-MCP).

Introduction

Persimmon (*Diospyros kaki* L.) is an important fruit in Asian countries including China, Japan and Korea (Zhang *et al.*, 2010). Anxi persimmon (*Diospyros kaki* L. f. cv. Anxi) is a major cultivar in southern China and is popular among consumers because of its appealing flavour and high nutritive values (Lin *et al.*, 2008). However, the Anxi persimmons are prone to soften quickly after harvest because of the ethylene-accelerated ripening, which restricts its long-time storage and long-distance transportation (Lin *et al.*, 2008; Gao *et al.*, 2020; Ma *et al.*, 2020; Wang *et al.*, 2020).

Fruit ripening and softening are considered as the protracted forms of senescence which is characterized by cellular membrane disintegration (Singh *et al.*, 2012). It was reported that the peroxidation and degradation of membrane lipid might be caused by the activities of lipoxygenase (LOX), phospholipase (PLD) and lipase during ripening of fruit such as Japanese plum (Singh *et al.*, 2012), pear (Sheng *et al.*, 2016), mango (Jincy *et al.*, 2017), tomato (Pak Dek *et al.*, 2018) and kiwifruit (Huang *et al.*, 2019). PLD can catalyse the hydrolysis of phospholipids. Then the products can be degraded to free fatty acids (FFAs) by lipase. LOX can catalyse the peroxidation of unsaturated fatty acids (USFAs), leading to the injury of the cellular membrane and the ion leakage (Shi *et al.*, 2018; Lin *et al.*, 2019). In addition, the reduced ratio of USFAs to saturated fatty acids (SFAs) (U/S) of membrane lipids was thought

to be related to fruit ripening and senescence (Wang and Jiao, 2001; Wang *et al.*, 2015).

Ethylene is a crucial factor that induces climacteric fruit ripening. During the ripening of climacteric fruit, ethylene production is usually induced, accompanied by many changes on primary and secondary metabolisms including carbohydrate, proteins and lipids metabolisms (Nakano *et al.*, 2003). Previous studies showed that ethylene had strong effects on fatty acid (FA) pathway via regulating related gene expression and enzymatic activity including LOX, which was in relation to fruit ripening and aroma volatile production (Griffiths *et al.*, 1999; Zhang *et al.*, 2010). The 1-methylcyclopropene (1-MCP) can block the downstream ethylene signal pathway by binding to ethylene receptors (Chen *et al.*, 2017). Previous studies indicated that 1-MCP treatment could influence the membrane lipid metabolism during ripening of kiwifruit (Zhang *et al.*, 2009) and tomato fruit (Tiwari and Paliyath, 2011), chilling injury of 'Nanguo' pears (Cheng *et al.*, 2015) and leaf yellowing of pak choi (Song *et al.*, 2020). Persimmon is a climacteric fruit (Nakano *et al.*, 2002, 2003; Besada *et al.*, 2010). Anxi persimmon shows a respiratory climacteric during ripening (Wang *et al*, 2018b). The ripening of persimmon can be inhibited by 1-MCP treatment (Harima *et al.*, 2003; Luo *et al.*, 2007; Yin *et al.*, 2012; Wang *et al.*, 2018b). However, there is a lack of knowledge about the effects of 1-MCP treatment on membrane lipids metabolisms in Anxi persimmons during storage.

Currently, 1-MCP has been used commercially to restrain ripening of climacteric fruit including medlar (Selcuk and Erkan, 2015), mango (Razzaq *et al.*, 2015), plum (Lin *et al.*, 2018) and tomato (Pak Dek *et al.*, 2018). However, it is difficult to control the 1-MCP concentration precisely with the powder form of reagent. A 1-MCP-containing paper is an innovative approach for applying the 1-MCP treatment, in which the 1-MCP concentration can be accurately calculated by the paper size (Chen *et al.*, 2015, 2017). It was found that the paper containing 1-MCP could delay the softening of harvested Huanghua pears (Chen *et al.*, 2015, 2017) and plums (Li *et al.*, 2012; Lin *et al.*, 2018). Our previous works showed that the paper containing 1-MCP treatment lowered fruit respiration rate, delayed fruit respiratory peak, reduced fruit weight loss, delayed the changes in chromaticity values of a^*, b^*, C^* L^* and hue angle ($h°$) on fruit surface colour, kept higher fruit firmness by suppressing cell wall degradation, maintained higher contents of total soluble solid (TSS), titratable acid (TA), total sugars, sucrose, reducing sugar, and vitamin C, and thus retarded fruit senescence, and extended the shelf life of Anxi persimmons (Wang *et al.*, 2018b, 2018c, 2020).

This work investigated the effects of paper containing 1-MCP on LOX, PLD and lipase activities, the relative contents of USFAs and SFAs, the index of USFAs (IUFA), and the U/S of Anxi persimmons during postharvest storage. The results of this study would provide insight into the influence of 1-MCP on membrane lipid metabolism of postharvest Anxi persimmons, which is beneficial for the maintenance of its postharvest quality.

Materials and Methods

Materials and treatments

The size of one piece of the 1-MCP containing paper (AnsiP-S, Taiwan Lytone Enterprise, Inc. Taibei, China) was 25 cm × 20 cm. The concentration of 1-MCP gas was calculated according to Chen *et al.* (2017) and Wang *et al.* (2020). In this study, 1.35 μl/l of 1-MCP gas could be released from 1 + 1/2 pieces of 1-MCP containing paper by spraying the distilled water in an 80 l of airtight foam box.

Anxi persimmon fruits at 90% maturity (180 d after full bloom, the fruit with firmness about 180.57 N, TSS content about 18%. The hue angle $h°$ and the chromaticity values of a^*, b^*, C^* and L^* on fruit surface were 79.47, 10.60, 58.90, 60.06 and 65.96, respectively) were harvested from an orchard in Anxi, Fujian, China. The fruits were selected with uniform colour, size and maturity for the following treatment.

Based on our prior works, the paper containing 1-MCP treatment at 1.35 μl/l for 12 h was chosen in this experiment, because it displayed the most effective in maintaining quality, retarding senescence, and extending the shelf life of Anxi persimmon fruit during storage at 25 ± 1 °C (Wang *et al.*, 2018b, 2020).

Anxi persimmons (N = 225) were divided into two groups: 1-MCP-treated group (105 persimmons) or control group (105 persimmons). The remaining 15 Anxi persimmons were used to measure the physiological parameters at storage day 0. One group of Anxi persimmons were put into an air-tight foam box containing 1-MCP (1.35 μl/l) and were sealed up for 12 h. The Anxi persimmons in the control group were put into and sealed up in the same box with no 1-MCP for 12 h. Then every five Anxi persimmons were packaged into a polyethylene film bag with a thickness of 0.015 mm. The storage temperature was 25 ± 1 °C. The relative humidity of the storage environment was 85%. Three bags of Anxi persimmons (N = 15) were taken separately from each group for determining the following physiological indices every 5 storage days.

Determination of cell membrane permeability

The flesh discs with diameters of 2 mm (2.0 g) from the equator area of 5 Anxi persimmon fruit were dipped in 25 ml of distilled water (25 °C, 2 h). The electrolyte leakage (C_1) of Anxi persimmon flesh discs extraction was measured with a conductivity meter. After measurement of C_1, the total electrolyte leakage (C_2) was tested when the above Anxi persimmon extraction with flesh discs was boiled for 30 min, then diluted to 25 ml with distilled water, and cooled quickly to 25 °C. The relative leakage rate was presented as $(C_1/C_2) \times 100\%$.

Analyses of activities of PLD, lipase and LOX

Activities of PLD, lipase and LOX were analysed based on protocols of Liu *et al.* (2011) and Zhang *et al.* (2018).

PLDs were extracted from flesh tissue of five Anxi persimmon fruits. The persimmon flesh (5.0 g) was ground in an ice bath with 5 ml of sodium acetate buffer (0.1 M, pH 5.6), then centrifuged at 4 °C (15 000×*g*, 20 min). The supernatant was collected and diluted to 10 ml with the sodium acetate buffer (0.1 M, pH 5.6) as the enzyme extract solution. Then, 3.0 ml of enzyme extract solution was mixed with 3.0 ml of 0.4 M lecithin solution, reacted for 1 h at 28 °C, and then washed by petroleum ether for three times. Thereafter, the water phase was added with 3.0 ml of ammonium reineckate, then centrifuged at 4 °C (15 000×*g*, 15 min). The sediment was collected and fully dissolved with 5 ml of propanone. Then the absorbance was determined at 520 nm. One unit (U) of PLD activity was defined as a change of 1 in the absorbance per hour.

For analysis of the lipase activity, 5.0 g of flesh tissue from 5 Anxi persimmons was ground in an ice bath with 5 ml of phosphate buffer (0.2 M, pH 7.8) containing 0.05 M mercaptoethanol, then centrifuged at 4 °C (15 000×*g*, 20 min). The supernatant was collected as the enzyme extract for the lipase activity analysis. The enzymatic reaction solution contained 1.0 ml enzyme extract, 2.3 ml phosphate buffer (0.2 M, pH 7.8), 0.5 ml of 0.5 % (*w*/*v*) α-naphthyl acetate. The reaction was performed for 30 min at 25 °C, followed by a chromogenic reaction with 0.15% (*w*/*v*) Fast Blue B Salt. Then

the absorbance was determined at 520 nm. One unit of lipase activity was defined as a change of 0.01 in absorbance per minute.

As for LOX activity, the persimmon flesh (5.0 g) from 5 Anxi persimmon fruit was ground in an ice bath with 5 ml of phosphate buffer (0.1 M, pH 6.8), then centrifuged at 4 °C (15 000×*g*, 15 min). The supernatant was used as the crude enzyme extract for LOX activity. The reaction mixture contained 2.75 ml of 0.1 M phosphate buffer (pH 6.8), 0.05 ml of 0.01 M sodium linoleic acid and 0.2 ml enzyme extract solution. After reaction for 5 min at 30 °C, the absorbance at 234 nm was recorded per minute. One unit of LOX activity was defined as a change of 0.1 in absorbance per minute.

The protein content in the enzyme extract solution was measured by the method of Bradford (1976). The activities of PLD, lipase and LOX were represented as U/kg protein.

Analyses of composition and relative contents of membrane fatty acids

The composition and relative contents of membrane fatty acids (FAs) in Anxi persimmons were analysed referring to methods of Cao *et al.* (2011) and Zhang *et al.* (2018).

Ten grams of flesh tissue from five Anxi persimmons were dried at 100 °C for 30 min to inactivate the lipase, then were dried to a constant weight at 50 °C. Then 0.5 g of dried flesh tissue (0.5 g) of Anxi persimmon was added with 1.5 ml of 0.4 M potassium hydroxide solution in methanol. The hydrolysis of lipids and the methylation of FA were performed by shaking of this mixture solution for 3 h. Thereafter, the solution was mixed with benzene/petroleum ether (1:1, *v*/*v*), then added with double-distilled water for separation. The supernatant was used for the composition analysis of FAs.

The composition and relative contents of membrane FAs were analysed using the gas chromatograph (7890A, Agilent Technologies Inc., USA) with a 0.32 mm × 60 m quartz glass column filled with 20% diethylene glycol succinate (DB-23, Agilent Technologies Inc.) and a flame ionization detector. The temperatures of the injector and detector were 250 °C and 280 °C, respectively. The flow rate of H_2 and air carrier gas was 30 and 400 ml/min, respectively. The mixed FAs was used as external standard. The relative contents of individual FAs were measured by comparing their retention time and the peak area with standards:

$$\text{U/S}: \quad \text{total relative contents of USFAs/total relative contents of SFAs.}$$

$$\text{IUFA}: \quad [\Sigma(\text{relative content of USFAs} \times \text{corresponding double-bond number})] \times 100.$$

Statistical analyses

All the above experiments were performed triply. The data were expressed as the mean ± standard error (n = 3). Data of significant differences between the control Anxi persimmons and the 1-MCP-treated Anxi persimmons during storage were analysed using the SPSS software program, version 21.0. The asterisks were used to indicate differences (*P < 0.05 or **P < 0.01).

Results and discussion

Influences of 1-MCP treatment on the cell membrane permeability of postharvest Anxi persimmons

The relative leakage rate is commonly used to reflected the cell membrane permeability (Yi *et al.*, 2008; Zhang *et al.*, 2018). The postharvest Anxi persimmons showed an increasing trend of relative leakage rate during storage (Figure 1). The relative leakage rate of the control Anxi persimmons increased sharply within 0–5 days, increased slowly during 5–20 days, and increased faster during 20–35 days. Correlation analysis showed that the relative leakage rate (Figure 1) showed a negative correlation (r = –0.933, P < 0.01) with the firmness (Supplementary Figure S1) of the control Anxi persimmons during 0–35 days. These data indicated that the cell membrane permeability increased during softening of postharvest Anxi persimmons during storage. These results were consistent with the previous researches on other fruit species. It was found that cell membrane disintegration occurred during the ripening and senescence processes of fruit such as kumquat (Li *et al.*, 2008), sweet cherry (Pasquariello *et al.*, 2015), longan (Lin *et al.*, 2017) and pear (Shi *et al.*, 2018). The disruption of cell membrane structure may lead to the loss of cellular compartmentation, resulting in the accelerated ripening and senescence (Huang *et al.*, 2019).

In addition, the relative leakage rate of 1-MCP-treated Anxi persimmons increased gradually during 0–35 days (Figure 1). Moreover, compared to the control fruit, during 5–35 days of storage, the 1-MCP-treated Anxi persimmons showed a noticeably lower relative leakage rate (P < 0.05) (Figure 1), but a clearly higher firmness (P < 0.05) (Supplementary Figure S1). These results indicated that 1.35 μl/l 1-MCP treatment could suppress the increase of relative leakage rate in postharvest Anxi persimmons, which helped retain the structural integrity of the cellular membrane and delayed fruit softening and senescence. Similarly, it was reported that 1-MCP treatment could inhibit the electrolyte leakage and delayed ripening of watermelon (Mao *et al.*, 2004), pear (Li *et al.*, 2013) and kiwifruit (Huang *et al.*, 2019).

Influences of 1-MCP treatment on activities of PLD and lipase in postharvest Anxi persimmons

The PLD and lipase are two key enzymes catalysing the degradation of membrane phospholipids (Zhang *et al.*, 2018; Lin *et al.*, 2019). The structural phospholipids can be hydrolysed by PLD to phosphatidic acid and diacylglycerol, which can be further degraded to FFAs by lipase (Wang *et al.*, 2018a; Lin *et al.*, 2019). In this study, the PLD activity (Figure 2A) in the control Anxi persimmons decreased within 0–5 days, thereafter increased from 10 to 25 days, and declined from 25 to 35 days. The lipase activity of the control Anxi persimmons increased within 0–5 days, but decreased from 5 to 10 days, then increased gradually from 10 to 30 days, and dropped quickly from 30 to 35 days (Figure 2B). Moreover, the PLD activity

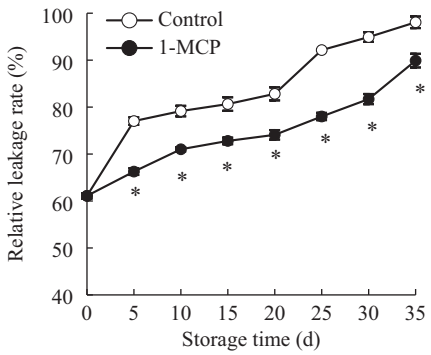

Figure 1. Influences of paper-containing 1-MCP treatment on cell membrane permeability of postharvest Anxi persimmons. * and ** represent differences (*p < 0.05 or **p < 0.01) between 1-MCP treated and control Anxi persimmons on the same storage days based on the independent samples t-test. 1-MCP, 1-methylcyclopropene.

(Figure 2A) showed a positive correlation (r = 0.897, P < 0.05) with the relative leakage rate (Figure 1), and a negative correlation (r = –0.915, P < 0.05) with the firmness (Supplementary Figure S1) within 5–25 days. These results indicated that the enhanced membrane permeability and fruit softening of Anxi persimmons were related to the increased PLD and lipase activities during storage. The increased PLD and lipase activities could induce cell membrane degradation and enhance membrane permeability (Purwanto *et al.*, 2016). Pak Dek *et al.* (2018) found that the PLD activity of tomato fruit increased during the ripening process. The PLD and lipase activities of pears displayed increasing tendencies during storage (Shi *et al.*, 2018).

In addition, 1-MCP-treated persimmons showed similar trends but lower (P < 0.05) levels of PLD and lipase activities compared with the control persimmons during storage (Figure 2A and B). Similar results of 1-MCP treatment on PLD and lipase activities were observed in watermelon (Mao *et al.*, 2004), tomato (Pak Dek *et al.*, 2018) and longan (Lin *et al.*, 2017). These data indicated that the 1-MCP treatment could reduce lipase and PLD activities, which might suppress the degradation of membrane phospholipids and maintained the cell membrane integrity, and thus delayed softening and senescence of Anxi persimmons during storage. However, the responses of the contents of membrane phospholipids including structural phospholipids, phosphatidic acid and diacylglycerol to 1-MCP treatment, and their relation to activities of PLD and lipase in Anxi persimmon fruit should be further investigated in the future.

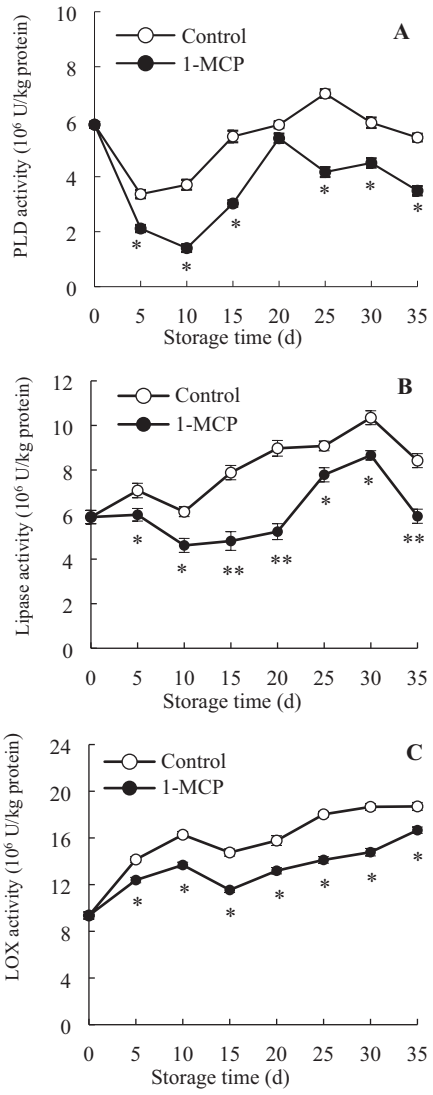

Figure 2. Influences of paper-containing 1-MCP treatment on activities of PLD (A), lipase (B) and LOX (C) in harvested Anxi persimmons. * and ** represent differences (*p < 0.05 or **p < 0.01) between 1-MCP treated and control Anxi persimmons on the same storage days based on the independent samples t-test. 1-MCP, 1-methylcyclopropene; PLD, phospholipase; LOX, lipoxygenase.

Influences of 1-MCP treatment on the LOX activity of postharvest Anxi persimmons

LOX is a key enzyme in the peroxidation of membrane USFAs, which catalyses the formation of SFAs from USFAs in the cell membrane, and the production of malondialdehyde (MDA) that is a biomarker of membrane USFA peroxidation, leading to the reduced membrane fluidity, the damaged structure of cell membrane, and the increased electrolyte leakage of the cell membrane (Yi *et al.*, 2009; Chomkitichai *et al.*, 2014; Lin *et al.*, 2019; Liang *et al.*, 2020). Lv *et al.* (2014) reported that ABA treatment could increase the LOX activity and accumulate the MDA content, resulting in the accelerated softening of 'Fuping Jianshi' persimmon fruit. Ren *et al.* (2017) found that sodium nitroprusside (SNP) treatment could suppress the softening of 'Tainong' mango fruit, which was associated with the reduced LOX activity and the reduced accumulation of MDA. Pasquariello *et al.* (2015) found that chitosan coating reduced cell membrane damage and maintained membrane integrity of sweet cherry by suppressing LOX activity and delaying MDA accumulation. As shown in Figure 2C, the LOX activity in control persimmons increased quickly within 0–10 days, then dropped slightly during 10–15 days, thereafter increased slowly from 15 to 35 days. In addition, the MDA content (Supplementary Figure S2) in the control Anxi persimmons increased quickly during 0–35 days. Correlation analyses found that, during 15–35 days, the LOX activity (Figure 2C) in the control Anxi persimmons had a clearly positive correlation (r = 0.941, P < 0.05) with the relative leakage rate (Figure 1). In addition, the MDA content (Supplementary Figure S2) showed positive correlations with the LOX activity (r = 0.880, P < 0.01) and the relative leakage rate (Figure 1) (r = 0.927, P < 0.01) during 0–35 days. These findings indicated that the increased LOX activity might cause the accumulation of MDA and the damage of cell membrane integrity, resulting in the accelerated softening of postharvest Anxi persimmons. Coincidently, the increased LOX activity was shown during the ripening of kiwifruit (Zhang *et al.*, 2009), Golden papaya (Resende *et al.*, 2012), Japanese plums (Singh *et al.*, 2012) and grapevine berries (Pilati *et al.*, 2014).

Previous studies had found that the transcriptional expression and activity of LOX was affected by ethylene pathway during fruit ripening (Ferrie *et al.*, 1994; Griffiths *et al.*, 1999; Schaffer, 2007; Zhang *et al.*, 2010). In this study, compared to control persimmons, a lower (P < 0.05) LOX activity (Figure 2C), a lower (P < 0.05) MDA content (Supplementary Figure S2), a lower (P < 0.05) relative leakage rate (Figure 1), but a higher firmness (P < 0.05) (Supplementary Figure S1) were displayed in 1-MCP treatment Anxi persimmon fruit within 10–35 days. These data indicated the 1-MCP treatment could suppress LOX activity and reduce the accumulation of MDA, which were beneficial for the suppressed peroxidation of membrane USFAs and the maintenance of cell membrane integrity, and thus delayed softening and senescence of Anxi persimmons during storage. These results agreed with the work of Cai *et al.* (2018) who reported that the delayed ripening of peaches

by 1-MCP treatment was related to the lower LOX activity. Besides, Zhang *et al.* (2009) indicated that 1-MCP treatment could retard the expression of *AdLox1* and *AdLox5* during kiwifruit ripening. Therefore, the 1-MCP treatment plays an important role in membrane lipid metabolism via influencing transcriptional expression and activity of LOX.

Influences of 1-MCP treatment on membrane fatty acids of postharvest Anxi persimmons

The composition of membrane FAs was found to have an important impact on the integrity and fluidity of cell membranes (Yi *et al.*, 2009). High levels of USFAs were beneficial for the membrane fluidity of kiwifruit (Zhang and Tian, 2009; Zhang *et al.*, 2009; Huang *et al.*, 2019). Wang *et al.* (2001) found that the enhanced peroxidation of membrane USFAs and the decreased U/S contributed to the reduced membrane fluidity and membrane deterioration, which might be associated with the ripening and senescence of blackberry fruit.

This study analysed the main compositions and their relative contents of cellular membrane FAs of Anxi persimmon fruit on the storage day 0, as well as their dynamic changes during storage (Table 1, Figure 3). As shown in Table 1, the main USFAs including linolenic acid ($C_{18:3}$) (25.77%), oleic acid ($C_{18:1}$) (21.93%), linoleic acid ($C_{18:2}$) (2.85%) and palmitoleic acid ($C_{16:1}$) (12.22%) accounted

Table 1. Composition and relative contents of membrane FAs in harvested Anxi persimmons on storage day 0.

Category	Fatty-acid composition of membrane lipid	Relative content (%)
$C_{16:0}$	Palmitic acid	25.91 ± 0.28
$C_{18:3}$	Linolenic acid	25.77 ± 0.14
$C_{18:1}$	Oleic acid	21.93 ± 0.52
$C_{18:0}$	Stearic acid	12.32 ± 0.27
$C_{16:1}$	Palmitoleic acid	12.22 ± 0.18
$C_{18:2}$	Linoleic acid	2.85 ± 0.09

Note: *m* and *n* in '$C_{m:n}$', respectively, represent carbon atom number and unsaturated bond number in fatty acid.

FAs, fatty acids.

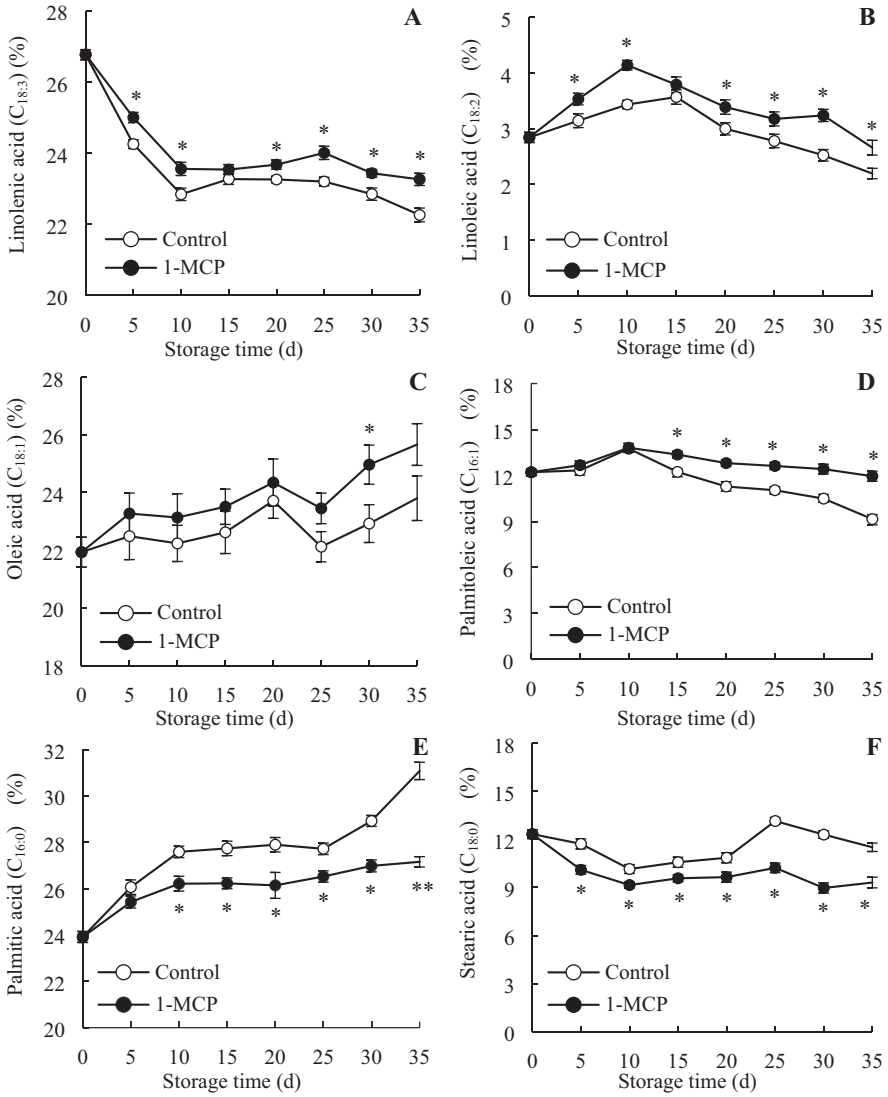

Figure 3. Influences of paper-containing 1-MCP treatment on relative contents of membrane FAs in harvested Anxi persimmons. * and ** represent differences (*$p < 0.05$ or **$p < 0.01$) between 1-MCP treated and control Anxi persimmons on the same storage days based on the independent samples *t*-test. 1-MCP, 1-methylcyclopropene; FAs, fatty acids.

for 62.77% of the total membrane FAs, whereas the main SFAs including palmitic acid ($C_{16:0}$) (25.91%) and stearic acid ($C_{18:0}$) (12.32%) accounted for 38.23%.

Figure 3A shows that the level of linolenic acid ($C_{18:3}$) of control Anxi persimmon dropped quickly during 0–10 days, then changed little during 10–25 days, thereafter decreased gradually from 25 to 35 days. The level of linoleic acid ($C_{18:2}$) of control Anxi persimmon increased slowly from 0 to 15 days, then decreased quickly from 15 to 35 days (Figure 3B). The level of oleic acid ($C_{18:1}$) of control Anxi persimmon increased slightly during storage (Figure 3C). The relative content of palmitoleic acid ($C_{16:1}$) of control Anxi persimmon showed little change during 0–5 days, then an increase from 5 to 10 days, thereafter a gradual decrease from 10 to 35 days (Figure 3D). The level of palmitic acid ($C_{16:0}$) of control Anxi persimmons increased rapidly within 0–10 days, changed little during 10–25 days, thereafter increased quickly from 25 to 35 days (Figure 3E). The level of stearic acid ($C_{18:0}$) of control Anxi persimmons decrease quickly during 0–10 days, changed little within 10–20 days, then increased quickly from 20 to 25 days, thereafter dropped rapidly from 25 to 35 days (Figure 3F). Moreover, the U/S dropped rapidly from 0 to 5 days, decreased slowly from 5 to 20 days, then decreased quickly from 20 to 35 days (Figure 4A). The IUFA of control Anxi persimmons dropped quickly within 0–10 days, changed little within 10–15 days, thereafter decreased quickly during 15–35 days (Figure 4B). Further analyses indicated the relative leakage rate (Figure 1) showed negative correlations with the U/S (r = –0.987, P < 0.01) and the IUFA (r = –0.971, P < 0.01) during 0–35 days.

These results illustrated that the unsaturation of membrane FAs showed a declining tendency, while SFA content showed an increasing tendency. Thus the ratio of USFAs to SFAs (U/S) in cell membrane decreased with a storage time during the ripening of Anxi persimmons. High ratio of U/S was reported to be beneficial for maintaining membrane fluidity of postharvest fruit (Yi *et al.*, 2009; Zhang *et al.*, 2009; Huang *et al.*, 2019). Therefore, the decreasing U/S could reduce membrane fluidity of postharvest Anxi persimmons, which had an adverse effect on cell membrane integrity.

LOX plays key roles in the formation of SFAs from USFAs in cell membranes, and the U/S and IUFA are closely related to the contents of USFAs and SFAs. The USFAs can be oxidized by LOX with the production of SFAs and hydroperoxide, resulting in the decreases of the U/S and IUFA, and consequently leading to the membrane damage and the disruption of cellular compartmentalization (Wang *et al.*, 2018a; Lin *et al.*, 2019). In this work, the level of linolenic acid ($C_{18:3}$) (Figure 3A) displayed negative correlations with the LOX activity (Figure 2C) during 0–10 days (r =–0.998, P < 0.05) and 20–35 days (r = –0.978, P < 0.05), respectively. The level of linoleic acid ($C_{18:2}$) (Figure 3B) showed a negative correlation with the LOX activity (Figure 2C) (r = –0.942, P < 0.05) during 15–35 days. The content of palmitoleic acid ($C_{16:1}$) (Figure 3D) showed a negative correlation with the LOX activity (r = –0.991, P < 0.01) (Figure 2C) during 15–35 days. Moreover, during 15–35 days of storage, the dropped U/S (Figure 4A) and IUFA (Figure 4B) in the control Anxi persimmons displayed a clearly negative correlation with the raised LOX activity (Figure 2C), the correlation coefficients r were –0.957 (P < 0.05) and –0.981 (P < 0.01), respectively. Therefore, the reduction of USFAs of Anxi persimmon during storage could be attributed to the increased LOX activity, and thus resulting in the increased electrolyte leakage. These findings were coincided with previous works about longan that the reduction of USFAs was related to higher LOX activities (Lin *et al.*, 2016; Wang *et al.*, 2018a).

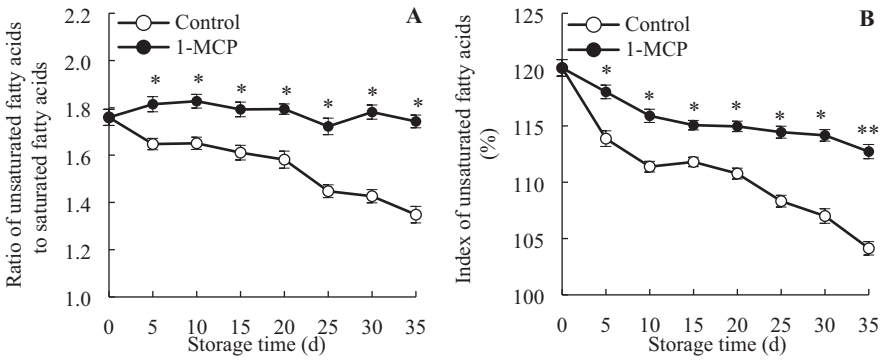

Figure 4. Influences of paper-containing 1-MCP treatment on the ratio of unsaturated fatty acids to saturated fatty acids (A) and the index of unsaturated fatty acids (B) in harvested Anxi persimmons. * and ** represent differences (*p < 0.05 or **p < 0.01) between 1-MCP treated and control Anxi persimmons on the same storage days based on the independent samples t-test. 1-MCP, 1-methylcyclopropene.

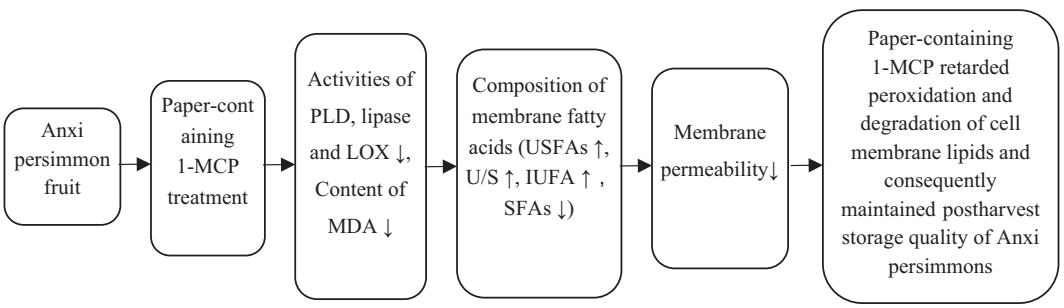

Figure 5. The probable mechanisms of paper-containing 1-MCP influencing metabolisms of membrane lipids of Anxi persimmons. 1-MCP, 1-methylcyclopropene; PLD, phospholipase; LOX, lipoxygenase; USFAs, unsaturated fatty acids; U/S, higher ratio of USFAs to saturated fatty acids (SFAs); IUFA, higher index of USFAs; SFAs, saturated fatty acids.

In addition, contrasted to control persimmons, the 1-MCP-treated fruit showed higher ($P < 0.05$) levels of linolenic acid ($C_{18:3}$) and linoleic acid ($C_{18:2}$) during 5–35 days, higher ($P < 0.05$) levels of palmitoleic acid ($C_{16:1}$) during 20–35 days and oleic acid ($C_{18:1}$) on the day 30, while lower ($P < 0.05$) levels of stearic acid ($C_{18:0}$) and palmitic acid ($C_{16:0}$) during 10–35 days (Figure 3A–F). Moreover, the decreases of IUFA and U/S in Anxi persimmons during storage were retarded by 1-MCP treatment (Figure 4A and B). Similar effects on preventing the reduction of USFAs by 1-MCP treatment were reported in kiwifruit (Huang *et al.*, 2019). Besides, Zhu *et al.* (2006) found that NO could restrain the lipid peroxidation, maintain a higher content of $C_{18:3}$ and consequently prevent the softening of peach fruit. Therefore, 1-MCP treatment could reduce the formation of SFAs and inhibit the peroxidation and degradation of USFAs via the restrained LOX activity, thus maintained the integrity and fluidity of the cellular membrane, prevented the ion leakage of cellular membrane, and consequently maintained postharvest storage quality of Anxi persimmons.

Conclusions

The treatment using papers containing 1.35 μl/l 1-MCP inhibited PLD, and lipase and LOX activities, reduced the degradation and peroxidation of membrane lipids, inhibited the accumulation of MDA, retarded the reduction of USFAs and consequently maintained the cell membrane integrity, resulting in the delayed softening and senescence of Anxi persimmons during storage. The possible mechanism about the influences of 1-MCP treatment on the metabolisms of membrane lipids of Anxi persimmons during storage is illustrated in Figure 5.

Supplementary material

Supplementary material is available at *Food Quality and Safety* online.

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Conflict of Interest

The authors declare no conflicts of interest.

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