Journal of Applied Botany and Food Quality 94, 132 - 139 (2021), DOI:10.5073/JABFQ.2021.094.016

[1]Institute of Genetics and Physiology, Hebei Academy of Agriculture and Forestry Sciences, Shijiazhuang, China
[2]Hebei Plant Genetic Engineering Center, Shijiazhuang, China

The influence of 1-MCP on the fruit quality and flesh browning of 'Red Fuji' apple after long-term cold storage

Jingang He[1,2], Yunxiao Feng[1,2], Yudou Cheng[1,2], Shen Wang[1], Yaxiong Fu[1], Junfeng Guan[1,2*]

(Submitted: January 25, 2021; Accepted: June 28, 2021)

Summary

This study assessed the influence of 1-MCP treatment on the fruit quality and flesh browning of 'Red Fuji' apple at shelf life after long-term cold storage. The 'Red Fuji' fruit were stored at 0±0.5 °C for 270 days after treating with 1.0 µL L^{-1} 1-methylcyclopropylene (1-MCP). Fruit quality, browning rate of stem-end flesh, chlorogenic acid content, polyphenol oxidase (PPO) activity were analyzed at shelf-life under 20±0.5 °C, the expression profile of ethylene receptors (*MdERS*1), phenylalnine ammonia lyase genes (*MdPAL1*, *MdPAL2*), quinate hydroxycinnamoyl/hydrxycinnamoyl CoA shikimate gene (*MdHCT3*), polyphenol oxidase genes (*MdPPO1*, *MdPPO5*) and lipoxygenase gene (*MdLOX*) were measured by real-time quantitative PCR.

1-MCP treatment improved the fruit storage quality, decreased stem-end flesh tissue browning, and fruit decay. In addition, the fruit respiration rate and ethylene production rate increased at shelf-life, but this increase could be inhibited by 1-MCP. The same rule was observed in the changes of chlorogenic acid content and PPO activity, the expression of *MdERS1*, *MdPAL1*, *MdPPO1* and *MdLOX* were inhibited by 1-MCP as well in the stem-end flesh. Thus, 1-MCP treatment improves the fruit quality of 'Red Fuji' apple at shelf-life after long-term cold storage, and inhibits the browning of stem-end flesh by decreasing the chlorogenic acid content and PPO activity. *MdPAL1*, *MdHCT3*, *MdPPO1* and *MdLOX* participate in the flesh browning progress.

Keywords: *Malus* × *domestica*; 1-methylcyclopropylene; storage quality; chlorogenic acid; polyphenol oxidase

Introduction

1-methylcyclopropene (1-MCP), an inhibitor of ethylene perception, delays fruit ripening and extends the storage and shelf life by impeding binding of ethylene to its receptors (Sisler et al., 2003). 1-MCP based technology is widely used by apple industries around the world to help maintain fruit quality, especially texture (Watkins, 2008; Mattheis et al., 2008).

Apples are characterized by valuable nutritional and taste qualities, and among the most popular fruit species in China. 'Red Fuji' apple (*Malus* × *domestica* cv. Red Fuji) accounts for about 72% of the total apple yield in China. Low-temperature storage is generally used as commercial application to prolong the storage period. However, a number of physiological disorders limit its quality in cold storage and shelf life, including flesh browning and superficial scald (He et al., 2016).

Flesh browning is a result of membrane damage and it is usually associated with enzymatic oxidation of phenolic compounds by polyphenol oxidase (PPO) to *o*-quinones, which polymerize non-enzymatically to produce heterogeneous black, brown or red pigments commonly called melanins (Tomás-Barberán et al., 2010). The occurrence of browning is mainly due to disruption of cellular compartmentalization, allowing the enzymatic oxidation of phenolic substrates in the vacuole by PPO located in the cytoplasm. This oxidation generates *o*-quinones that react with other compounds and result in polymerization to produce the browning appearance (Franck et al., 2007; Sun et al., 2011; Lin et al., 2014).

Chlorogenic acid is an important substrate for PPO enzymatic browning reaction (Tomás-Barberán et al., 2010). The biosynthetic pathway of chlorogenic acid is initiated from the deamination of phenylalanine to cinnamic acid by phenylalanine ammonia lyase (PAL), followed by hydroxylation and methylation of cinnamic acid by cinnamate 4-hydroxylase (C4H) and 4-hydroxycinnamoyl-CoA ligase (4CL), respectively. Thus *ϱ*-coumaric acid is produced, then hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase (HCT/HQT) pathway begins, and chlorogenic acid is synthesized finally (Niggeweg et al., 2004; Mahesh et al., 2007; Zhao et al., 2013). Furthermore, PAL, 4CL and HCT/HQT are considered as key regulators related to chlorogenic acid biosynthesis (Lallemand et al., 2012; Escamilla-Treviño et al., 2014; Yuan et al., 2014).

Damage of membrane integrity is considered the primary event that ultimately leads to the development of browning (Kou et al., 2015). Previous studies have demonstrated that the membrane lipid metabolism is correlated to lipoxygenase (LOX) (Mao et al., 2007). LOX is potentially involved in senescence, membrane alter-actions and lipid degradation in plants under stress (Gao et al., 2015). LOX catalyzes peroxidation of plasma membrane lipids, increases lipid unsaturation, and thus changes membrane fluidity (Wang, 2001), resulting in the loss of membrane integrity and increased membrane permeability (Aghdam et al., 2013). It has been proven that normal tissue does not develop browning. In fruit suffering from environment stress, membrane lipid composition, the structure and function of cell membranes are all altered, thereby cellular compartmentalization is broken down, which results in the release of enzymes to contact with their substrates, and then promoting browning (Pongprasert et al., 2011; Lin et al., 2016).

The effects of 1-MCP on the incidence of physiological disorders of apples have been variable (Watkins, 2007). Our previous research found that 1-MCP can effectively inhibit the flesh softening and reduce the flesh browning in 'Red Fuji' apple (He et al., 2016). The objective of the current study was to investigate the effects of 1-MCP treatment on fruit quality, flesh browning and its regulation mechanism of flesh browning in 'Red Fuji' apple during cold storage and shelf life. With regard to 'Red Fuji' apples, it is important to discuss the potential relationship of browning, the metabolism of phenols and membrane lipid during cold storage and shelf life.

Our specific aim was to elucidate the role of ethylene, chlorogenic acid and membrane lipid metabolism in stem-end browning. To achieve the objective, we assessed the activity of key enzyme PPO and its gene expression, and investigated the gene expression pattens in chlorogenic acid biosynthesis pathway and membrane lipid peroxidation gene.

* Corresponding author

Materials and methods

Materials and 1-MCP treatment

'Red Fuji' apples were harvested from a commercial orchard during the optimum commercial harvest period in Hebei province of China (N38°23′41.92 E114°27′21.66). The apple fruit (average single fruit weight of 237.73 ± 20.42 g) were randomly divided into 2 groups of 48 kg fruit each. Fruit of each group were divided into 3 equal parts and carefully put into 3 sealed plastic boxes (60 L), then were exposed to 0, and 1.0 μL L^{-1} 1-MCP (Dow Chemical Co., Midland, MI, USA) at 25 ± 2 °C for 24 h. The group without 1-MCP (0 μL L^{-1}) was set as control (He et al., 2016). After treatment, the fruit were packed with microporous plastic film (15 μm in thickness) and packed into paper box. Each box that contained 30 fruits was stored at 0 ± 0.5 °C with 85%-95% relative humidity. The fruit were stored at 20 ± 0.5 °C as shelf life after 270 days of cold storage. The respiration rate and ethylene production rate were measured at 1, 3, 5 and 7 days of shelf life, and the fruit quality was measured on the harvest day (0 d), the end of cold storage (270 d), 1 d (270+1 d) and 7 d (270+7 d) at shelf life.

Fruit quality

Fruit firmness was measured at two equidistant points on the equatorial region with the skin removed, using a digital fruit hardness meter (Model: GY-4, Top Instruments Co., Ltd., Hangzhou, China) with a pressure head 8 mm in diameter. The firmness was automatically calculated and expressed in kg cm^{-2}. The flesh juice was squeezed from two equidistant points and measured for soluble solids content (SSC) by a PAL-1 pocket digital refractometer (Atago Co., Ltd.,Tokyo, Japan). Titratable content (TA) was determined by acid base titration with 0.01 mol L^{-1} NaOH up to pH 8.1, and the result was calculated as malic acid (%).

Browning rate of stem-end flesh was calculated as the percentage of browning fruit number in the total fruit number. Fruit decay rate was calculated as the percentage of rotten fruit in the total fruit number. Three replicates of 10 fruit each were measured for each treatment at every sampling time.

Sampling

For each treatment, three replicates were set and there were 10 fruits in each replicate at every sampling time (0 d, 270+1 d, 270+7 d). After firmness and SSC measurement, we cut flesh of 0.5 cm wide and 1.0 cm deep, about 1 cm from the stem of each fruit being tested, and the stem-end flesh was frozen and ground into powder with liquid nitrogen immediately, then stored at -80 °C for chlorogenic acid content, PPO activity analysis and RNA isolation.

Respiration rate and ethylene production rate

Respiration rate was measured by HFY-1a CO_2 infrared analyzer (Kexi Instrument Co., Ltd., Jiangsu, China) after the fruits were sealed in the container for 1 h, 10 mL of gas was withdrawn from the container and injected to the analyzer, respiration rate was expressed as CO_2 release rate (μL kg^{-1} h^{-1}). For ethylene measurements, 1 mL of gas was withdrawn from the container after the fruits were sealed for 3 h, and then injected into a gas chromatograph (Model: GC9790II, Fuli Instruments Technology Co., Ltd., Wenling, China) equipped with a GDX-502 column and a flame ionization detector (FID). The temperatures of column, vaporization oven and FID were 78 °C, 120 °C and 200 °C, respectively. N_2 was used as the carrier gas with a rate of 40 mL min^{-1}. Ethylene production rate was expressed as μL kg^{-1} h^{-1}. 10 fruits were sealed in each container, and three replicates were measured for each treatment.

Chlorogenic acid content and PPO activity

Chlorogenic acid was extracted from 2 g frozen powder of stem-end flesh and its content was measured via high-performance liquid chromatography (HPLC) according to the method of He et al. (2017). With a HITACHI L2000 HPLC System (Hitachi, Tokyo, Japan), which consisted of a Hitachi pump L-2130, a Hitachi automatic sample injector L-2200, and a Hitachi UV detector L-2400 and equipped with a Lachrom C18 column (250 mm × 4.6 mm, 5 μm), HPLC was performed in a mobile phase of 5% acetic acid (solvent A), H_2O (solvent B) and 100% acetonitrile (solvent C) at a flow rate of 1 mL min^{-1} and a temperature of 30 °C, and monitored at a wave length of 280 nm with injection of 5 μL samples. Samples were tested using external standard method, which used retention time to determine the quality and peak area for quantification. The analysis was performed in triplicates for each sample to obtain the mean value and standard deviation.

For PPO (EC 1.14.18.1) extraction and activity measurement, 1 g of frozen stem-end flesh tissue was powdered in liquid nitrogen with a mortar and blended in 5 mL of 100 mmol L^{-1} phosphate buffer (pH 7.0) containing 0.3 g PVP. Next, the solution was centrifuged at 12 000 × *g* for 15 min at 4 °C, after which the supernatant was collected as the crude enzyme extract. The reaction mixture contained 100 mmol L^{-1} catechol in 50 mmol L^{-1} phosphate buffer (pH 6.0) (Cheng et al., 2015). Changes in the absorbance at 420 nm were measured by using a spectrophotometer (Model: UV-2100, UNICO Instrument, Dayton, USA). The PPO active unit (U) was determined as the change value of absorbance increased by 0.01 per minute per gram of flesh sample and the result was expressed as U g^{-1} FW.

RNA isolation and real-time quantitative PCR analysis

Total RNAs were isolated from fruit stem-end flesh, using improved hexadecyltrimethylammonium bromide (CTAB) method (Gasic et al., 2004). Genomic DNAs were eliminated with PrimeScript RT Reagent Kit with gDNA Eraser (Perfect Real Time) (TaKaRa Bio Inc., Japan) and first strand of cDNA was generated by reverse transcription.

Primers for real-time quantitative PCR (qRT-PCR) were designed according to apple (*Malus × domestica*) nucleotide sequences registered in GenBank of NCBI by DNAMAN 6.0 software. The sequence of primers was shown in Tab. 1. qRT-PCR analysis was performed on ABI 7500 Real-Time System using TB Green™ Premix ex Taq II quantitative PCR kit (TaKaRa Bio Inc., Japan). The relative gene expression amount was calculated with the formula $2^{-\Delta\Delta CT}$ by Excel 2007 (Livak and Schmittgen, 2001). The *MdActin* gene (GenBank ID: XM_029089583.1) was used as internal reference and the amount of gene expression in the stem-end flesh at 0 d was defined as 1.0.

Statistical analysis

All statistical data analyses were done by SPSS (18.0). The data were analyzed by one-way analysis of variance (ANOVA). Means were separated using Duncan method at $p<0.05$. Data are shown as means ± standard errors. Fig. 1-8 were generated by GraphPad Prism 8.0, and different letters in Fig. 1-8 represented significant difference at $p<0.05$.

Results

Fruit quality, browning rate and decay rate

The fruit firmness, SSC and TA content decreased after 270 days of cold storage, and 1-MCP treatment could maintain higher firmness, SSC and TA, but there was no significant difference in SSC at 270+7 d (Fig. 1). This indicated that 1-MCP effectively delayed fruit

Tab. 1: Sequence of primers for qRT-PCR

Gene	Genbank ID	Primer sequences (5'-3')	
MdActin	XM_029089583.1	5'-TGACCGAATGAGCAAGGAAATTACT-3'	5'-TACTCAGCTTTGGCAATCCACATC-3'
MdERS1	NM_001328757.1	5'-GGAGATCTCGTTGGACGCAA-3'	5'-GGATGACTCTGACCACTGGC-3'
MdETR2	XM_008358087.3	5'-ATGTTATGTGGCAGCGCAGG-3'	5'-GGCACACAATTCTGCCAACA-3'
MdPAL1	XM_008357397.2	5'-GTGTTTAATTAGGCGGGGGA-3'	5'-AAATTGATTGCCTCATATCACAGAT-3'
MdPAL2	XM_029105821.1	5'-AGCACCACCTCTTTCCATTCC-3'	5'-GCCGGGAAAAACTAAGTGGG-3'
Md4CL2	XM_008364603.2	5'-CTCGATCGATGCGTGTTGAC-3'	5'-TGCCTTTGATCCCCGACTTC-3'
MdHCT3	XM_008344601.3	5'-TCACCATTTCCTTGCGCCTC-3'	5'-ACGTTGACTCCCTCACGGTA-3'
MdPPO1	NM_001319261.1	5'-GAGCTCATGAAGGCCCTACC-3'	5'-TTTGGAGCTCGAGTTCTGGG-3'
MdPPO5	JQ388482.1	5'-TCAAATCGACGCCAACCTCA-3'	5'-GTTTCGATTGAACCGGCCC-3'
MdLOX	NM_001294093.1	5'-AGCGACAAGAAAGAAGAACC-3'	5'-TACTGACCGTAGTTGATTGC-3'

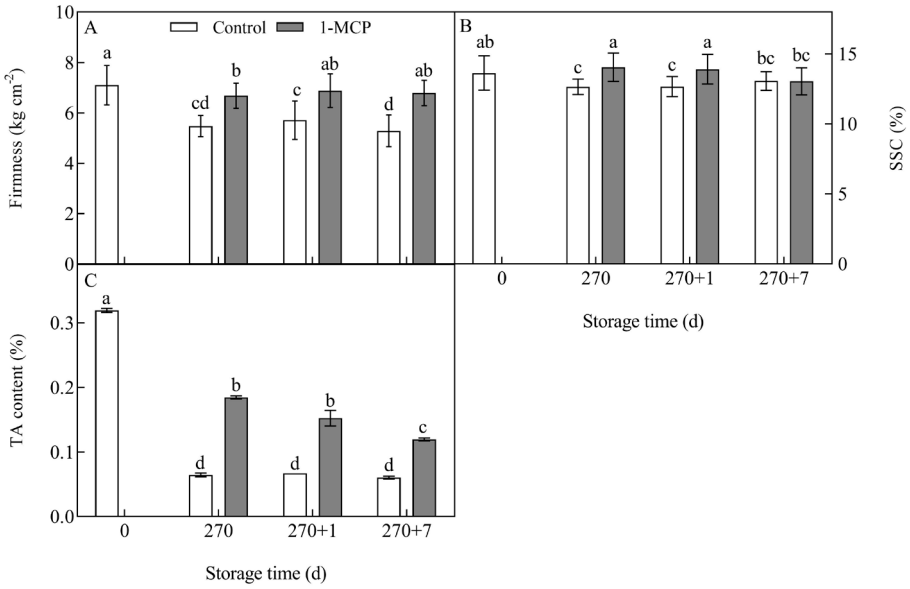

Fig. 1: Fruit firmness (A), SSC (B) and TA content (C) of control and 1-MCP treated 'Red Fuji' apple at shelf life after cold storage. Data are shown as means ± standard errors, different letters represent significant difference at $p<0.05$.

softening and maintained fruit quality at shelf life in 'Red Fuji' apple. In addition, the browning rate of stem-end flesh was as high as 46.7% in the control fruit at 270 d, while the value is 0 in the 1-MCP treatment fruit. The browning became more serious during the shelf life, but 1-MCP significantly delayed and reduced the browning rate (Fig. 2A). Meanwhile, 1-MCP effectively reduced fruit decay (Fig. 2B).

Respiration rate, ethylene production rate and the expression of ethylene receptor gene (*MdERS1*) in the stem-end flesh

During the shelf life, the respiration rate of fruit increased at first, reached the peak value at 270+5 d and then decreased. The respiration rate was significantly lowered by 1-MCP (Fig. 3A). The ethylene production rate of the control fruit displayed an obvious increase and reached the peak value at 27+3 d, but kept at a very low level in 1-MCP treatment fruit during shelf life (Fig. 3B). The ethylene production and the expression of *MdERS1* was effectively inhibited by 1-MCP treatment (Fig. 4).

Chlorogenic acid content and biosynthesis related genes expression

Compared with the initial stage of storage, the chlorogenic acid content of stem-end flesh increased gradually with the extension of storage time, which was markedly inhibited by 1-MCP (Fig. 5).

After 270 d cold storage, the *MdPAL1* expression level was nearly 200 times higher than the initial storage fruit, and then down-regulated at shelf life. 1-MCP treatment significantly decreased *MdPAL1* expression except that there was no significant difference with the control at 270+7 d (Fig. 6A). Different with *MdPAL1*, the *MdPAL2* expression was down-regulated during cold storage, and was gradually up-regulated at shelf life. However, both the down-regulation and up-regulation were alleviated by 1-MCP treatment (Fig. 6B).

The expression level of *Md4CL2* increased during cold storage, and then decreased during the shelf-life, but the expression of *Md4CL2* was inhibited by 1-MCP treatment especially at the end of shelf-life (Fig. 6C). The expression level of *MdHCT3* was significantly up-regulated during storage and was nearly 100 times higher at 270+7 d than that at the initial storage, and 1-MCP exhibited an obviously inhibition at the *HCT3* expression (Fig. 6D).

PPO activity and the expression level of *MdPPO*s

PPO activity increased during cold storage and shelf-life, which was significantly inhibited by 1-MCP (Fig. 7). The expression of *MdPPO1* reached the peak value at the end of cold storage, decreased at 270+1 d and increased again at 270+7 d in shelf life (Fig. 8A). There was no obvious change of *MdPPO5* expression during cold storage and earlier shelf-life, but a dramatical up-regulation was observed at 270+7 d in shelf-life (Fig. 8B). It is worth noting that the

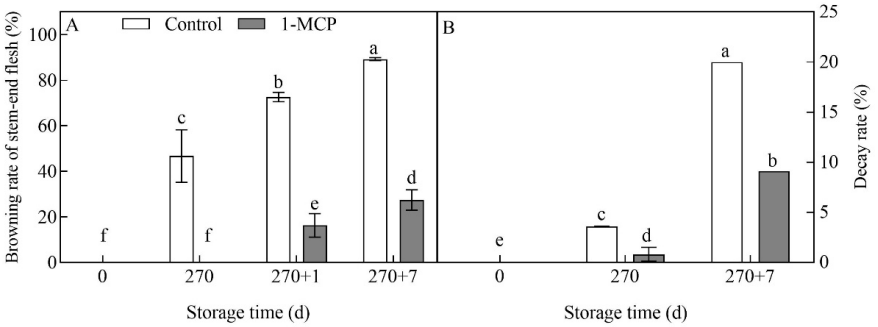

Fig. 2: Browning rate (A) of stem-end flesh and decay rate (B) of 'Red Fuji' apple at shelf life after cold storage. Data are shown as means ± standard errors, different letters represent significant difference at $p<0.05$.

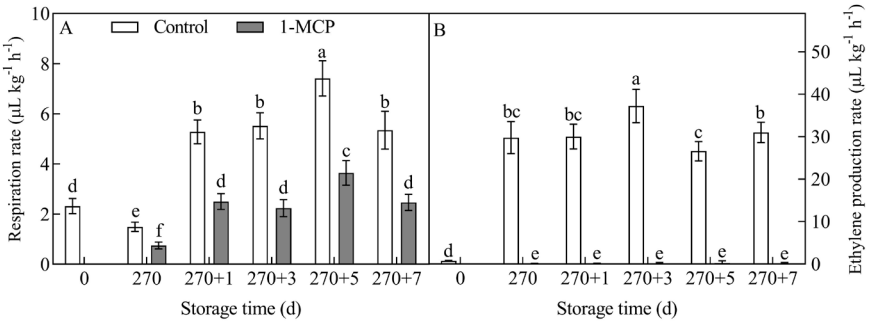

Fig. 3: Fruit respiration rate (A) and ethylene production rate (B) of 'Red Fuji' apple at shelf life after cold storage. Data are shown as means ± standard errors, different letters represent significant difference at $p<0.05$.

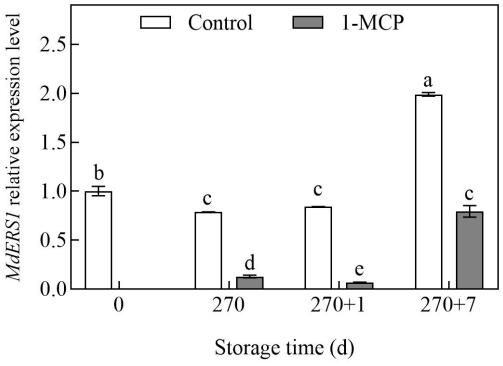

Fig. 4: *MdERS1* expression level of stem-end flesh tissue in 'Red Fuji' apple at shelf life after cold storage. Data are shown as means ± standard deviations, different letters represent significant difference at $p<0.05$.

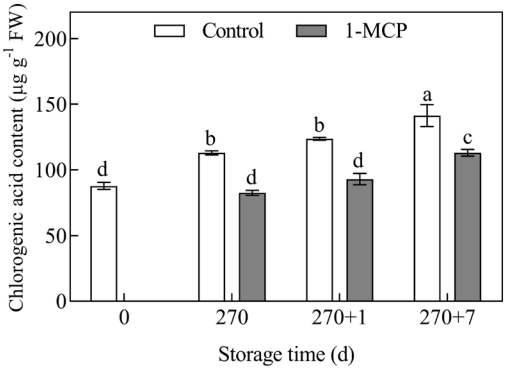

Fig. 5: Chlorogenic acid content of stem-end flesh tissue in 'Red Fuji' apple at shelf life after cold storage. Data are shown as means ± standard errors, different letters represent significant difference at $p<0.05$.

up-regulation of *MdPPO1* and *MdPPO5* was significantly inhibited by 1-MCP treatment.

The expression level of *MdLOX* gene significantly increased during cold storage, and subsequently decreased. In the control fruit, the expression of *MdLOX* showed an increasing trend. However, 1-MCP treatment effectively inhibited the expression and kept it at a very low level (Fig. 9).

Correlation analysis

According to the correlation analysis (Tab. 2), the browning rate of stem-end flesh is significantly correlated with chlorogenic acid content (r=0.962**), PPO activity (r=0.915**), the expression of *MdPAL1* (r=0.572**), *MdHCT3* (r=0.926**), *MdPPO1* (r=0.743**) and *MdLOX* (r=0.595**). The chlorogenic acid content was significantly correlated with PPO activity (r=0.865**), the expression amounts of *MdPAL1* (r=0.500*), *MdHCT3* (r=0.904**), PPO activity and *MdPPO1* expression (r=0.841**).

Discussions

Postharvest treatment with 1-MCP, an inhibitor of ethylene action, has been shown to improve quality characteristics of apples, including reduced ethylene production and respiration, as well as improved firmness and acidity retention (Watkins, 2007; Deell et al., 2007). In our study, 1-MCP could effectively improve the quality of 'Red Fuji' apple fruit after long-term cold storage, and significantly reduce the respiration rate and ethylene production rate during cold storage and shelf life (Fig. 3), which was the physiological basis of 1-MCP function in delaying fruit senescence. Therefore, the fruit treated with 1-MCP kept higher fruit firmness and TA content, while maintaining a higher SSC (Fig. 1). 1-MCP treatment also significantly reduced

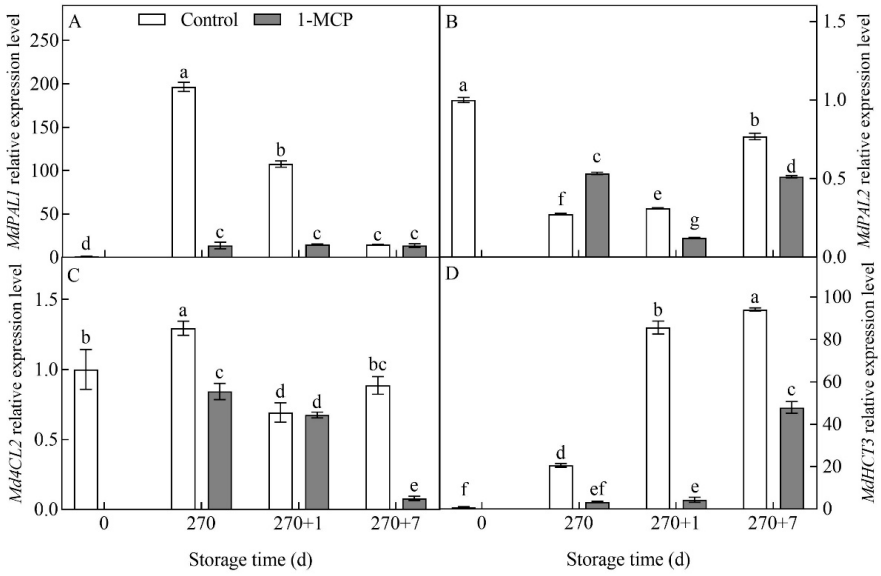

Fig. 6: The expression level of *MdPAL1* (A), *MdPAL2* (B), *Md4CL2* (C) and *MdHCT3* (D) of stem-end flesh tissue in 'Red Fuji' apple at shelf life after cold storage. Data are shown as means ± standard deviations, different letters represent significant difference at $p<0.05$.

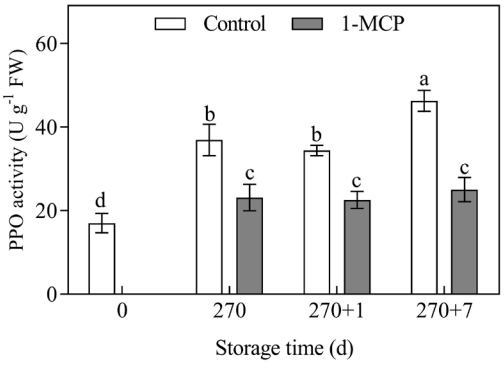

Fig. 7: PPO activity of stem-end flesh tissue in 'Red Fuji' apple at shelf life after cold storage. Data are shown as means ± standard errors, different letters represent significant difference at $p<0.05$.

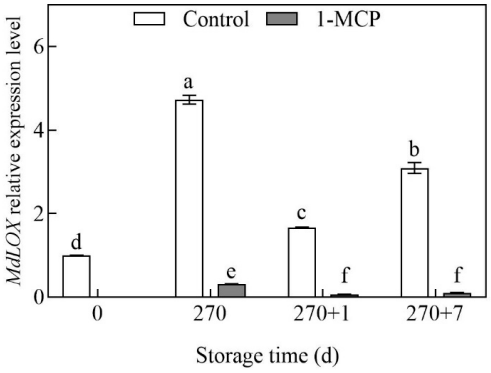

Fig. 9: *MdLOX* expression level of stem-end flesh tissue in 'Red Fuji' apple at shelf life after cold storage. Data are shown as means ± standard deviations, different letters represent significant difference at $p<0.05$.

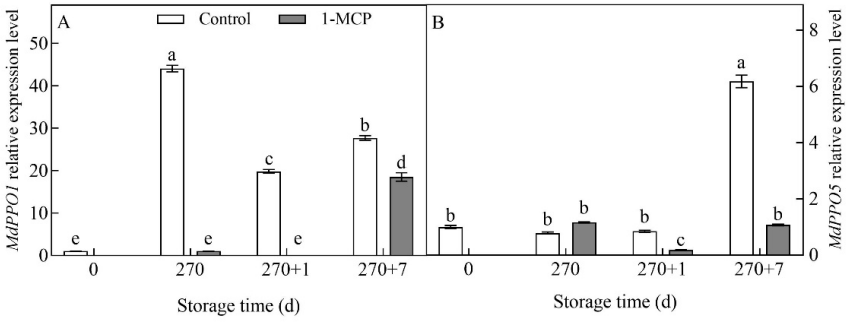

Fig. 8: *MdPPO1* (A) and *MdPPO5* (B) expression level of stem-end flesh tissue in 'Red Fuji' apple at shelf life after cold storage. Data are shown as means ± standard deviations, different letters represent significant difference at $p<0.05$.

fruit decay and stem-end flesh browning (Fig. 2). The physiological effect of ethylene begins with the recognition of ethylene signal by ethylene receptors (ETR) located in the endoplasmic reticulum (Alonso et al., 2003), and 1-MCP can delay and inhibit a series of fruit ripening and senescence processes caused by ethylene through the competition with ethylene binding sites on the receptors (Blankenship and Dole, 2003). Ireland et al. (2012) found that 100 μL L^{-1} ethylene in apples could up-regulate the expression of *ERS1* gene, while in our study, 1-MCP significantly inhibited the expression of *MdERS1* (Fig. 4), so it was speculated that *MdERS1* was involved in the flesh browning caused by ethylene signal.

The development of fruit browning depends on the enzymatic action of the PPO. The apple's susceptibility to flesh browning is thought to be the result of complex interplay between the PPO enzyme and

Tab. 2: Correlation coefficient of flesh browning rate with chlorogenic acid content, PPO activity and the expression level of relative genes in 'Red Fuji' apple

	Browning Rate	Chlorogenic Acid Content	PPO Activity	*MdERS1*	*MdPAL1*	*MdPAL2*	*Md4CL2*	*MdHCT3*	*MdPPO1*	*MdPPO5*	*MdLOX*
Browning Rate	1.000										
Chlorogenic Acid Content	0.962**	1.000									
PPO Activity	0.915**	0.865**	1.000								
MdERS1	0.465*	0.516*	0.334	1.000							
MdPAL1	0.572**	0.500*	0.683**	-0.192	1.000						
MdPAL2	-0.184	-0.153	-0.167	0.666**	-0.601**	1.000					
Md4CL2	-0.020	-0.046	0.149	0.225	0.110	0.255	1.000				
MdHCT3	0.926**	0.904**	0.889**	0.397	0.483*	-0.100	-0.225	1.000			
MdPPO1	0.743**	0.720**	0.841**	0.396	0.619**	-0.017	0.347	0.683**	1.000		
MdPPO5	0.097	0.081	0.208	0.484*	-0.356	0.784**	0.077	0.273	0.247	1.000	
MdLOX	0.595**	0.551**	0.658**	0.581**	0.487*	0.179	0.679**	0.404	0.818**	0.161	1.000

** Significant correlation at 0.01 level (both sides);
* Significant correlation at 0.05 level (both sides)

polyphenol content (Di Guardo et al., 2014). However, the effects of 1-MCP on the incidence of physiological disorders of apples have been variable (Watkins, 2007). Ripening-related disorders such as senescent breakdown are greatly inhibited by 1-MCP (Moran et al., 2005; Deell et al., 2007), as are disorders such as superficial scald that is associated with ethylene production (Rupasinghe et al., 2000; Tsantili et al., 2007). The present results showed that the content of chlorogenic acid and PPO activity in the stem-end flesh increased after cold storage (Fig. 5, Fig. 7), which was positively correlated with the degree of flesh browning (Fig. 2, Tab. 2). 1-MCP significantly reduced the chlorogenic acid content and PPO activity, which was the biochemical basis of 1-MCP delaying the occurrence of flesh browning. However, it has been previously shown that 1-MCP treatment can make 'Empire' apples more sensitive to CO_2 concentration, and exacerbate flesh browning because of CO_2 injury (Jung and Watkins, 2011; Lee et al., 2012). Therefor, we deduced that the stem-end flesh browning in 'Red Fuji' apple is the consequence of senescence caused by ethylene, and 1-MCP could effectively inhibit the browning.

PAL, 4CL and HCT are involved in chlorogenic acid biosynthesis pathway in apple and pear fruit, and PAL is the enzyme that catalyzes the first step reaction of phenylpropane metabolic pathway (Cheng et al., 2015; Di Guardo et al., 2014; Both et al., 2018). This study demonstrated that 1-MCP treatment significantly decreased the expression of *MdPAL1* (Fig. 6A), *MdHCT3* (Fig. 6D) and *MdPPO1* (Fig. 8A), and displayed a lower chlorogenic acid content (Fig. 5) and PPO activity (Fig. 7) in the stem-end flesh. However, the expression trends of *MdPPO5* (Fig. 8B), *MdPAL2* (Fig. 6B) and *Md4CL2* (Fig. 6C) were not consistent with the changes of chlorogenic acid content and flesh browning.

LOX are the crucial enzymes of membrane lipid metabolism, and might induce degradation of phospholipids and unsaturated fatty acids (Wang, 2011; Aghdam and Bodbodak, 2014). High levels of unsaturated fatty acids could maintain normal cellular function and prevent the interaction of PPO with phenol substrates resulting in enzymatic browning (Liu et al., 2006). It has been proven that LOX activity was significantly higher and the gene expression increased when tissue browning occurred (Sheng et al., 2016). In our experiment, 1-MCP treatment reduced the *MdLOX* gene expression level in the stem-end flesh (Fig. 9) which was consistent with the obvious browning inhibition. This implied that 1-MCP plays an important role in the integrity maintaining of membrane lipid in the stem-end flesh of 'Red Fuji' apple.

Conclusion

Postharvest 1-MCP (1.0 μL L^{-1}) treatment can effectively improve the quality of 'Red Fuji' apple after long-term cold storage, and keep a low respiration rate and minimum ethylene production rate. Moreover, 1-MCP treatment markedly reduce the stem-end flesh browning by down-regulating the expression of *MdPAL1, MdHCT3, MdPPO1* and *MdLOX*, and then inhibiting the increase of chlorogenic acid content and PPO activity. In conclusion, 1-MCP based technology shows positive effects on quality control and reducing storage browning in 'Red Fuji' apple.

Acknowledgements

This research was funded by the HAAFS Agriculture Science and Technology Innovation Project (2019-2-1), Modern Agricultural Industry Technology Research System of Hebei Province (HBCT2018100207), and The Young Talents Fund of Hebei province.

Conflicts of interest

No potential conflict of interest was reported by the authors.

References

Aghdam, M.S., Bodbodak, S., 2013: Physiological and biochemical mechanisms regulating chilling tolerance in fruits and vegetables under postharvest salicylates and jasmonates treatments. Sci. Hortic. 156, 73-85. DOI: 10.1016/j.scienta.2013.03.028

Alonso, J.M., Stepanova, A.N., Solano, R., Wisman, E., Ferrari, S., Ausubel, F.M., Ecker, J.R., 2003: Five components of the ethylene-response pathway identified in a screen for weak ethylene insensitive mutants in *Arabidopsis*. Proc. Natl. Acad. Sci. USA. 100(5), 2992-2997. DOI: 10.1073/pnas.0438070100

Blankenship, S.M., Dole, J.M., 2003: 1-Methylcyclopropene: a review. Postharvest Biol. Technol. 28, 1-25. DOI: 10.1016/S0925-5214(02)00246-6

Both, V., Brackmann, A., Thewes, F.R., Weber, A., Schultz, E.E., Ludwig, V., 2018: The influence of temperature and 1-MCP on quality attributes of 'Galaxy' apples stored in controlled atmosphere and dynamic controlled atmosphere. Food Packaging Shelf. 16, 168-177. DOI: 10.1016/j.fpsl.2018.03.010

Cheng, Y.D., Liu, L.Q., Zhao, G.Q., Shen, C.G., Guan, J.F., 2015: The effects of modified atmosphere packaging on core browning and the

expression patterns of *PPO* and *PAL* genes in 'Yali' pears during cold storage. LWT-Food Sci. Technol. 60(2), 1243-1248. DOI: 10.1016/j.lwt.2014.09.005

DEELL, J.R., AYRES, J.T., MURR, D.P., 2007: 1-methylcyclopropene influences 'Empire' and 'Delicious' apple quality during long-term commercial storage. HortTechnology. 17(1), 46-51. DOI: 10.21273/HORTTECH.17.1.46

ESCAMILLA-TREVIÑO, L.L., SHEN, H., HERNANDEZ, T., YIN, Y.B., XU, Y., DIXON, R.A., 2014: Early lignin pathway enzymes and routes to chlorogenic acid in switchgrass (*Panicum virgatum* L.). Plant Mol. Biol. 84(4), 565-576. DOI: 10.1007/s11103-013-0152-y

FRANCK, C., LAMMERTYN, J., QUANG, T.H., VERBOVEN, P., VERLINDEN, B., NICOLAI, B.M., 2007: Browning disorders in pear fruit. Postharvest Biol. Technol. 43, 1-13. DOI: 10.1016/j.postharvbio.2006.08.008

GAO, M.M., ZHOU, S., GUAN, J.F., ZHANG, Y.Y., 2015: Effects of 1-methylcyclopropene on superficial scald and related metabolism in 'Wujiuxiang' pear during cold storage. J. Appl. Bot. Food Qual. 88, 102-108. DOI: 10.5073/JABFQ.2015.088.014

GASIC, K., HERNANDEZ, A., KORBAN, S.S., 2004: RNA extraction from different apple tissues rich in polyphenols and polysaccharides for cDNA library construction. Plant Mol. Biol. Rep. 22(4), 437-438. DOI: 10.1007/BF02772687

GUARDO, D.M., TADIELLO, A., FARNETI, B., LORENZ, G., MASUERO, D., VRHOVSEK, U., COSTA, G., VELASCO, R., COSTA, F., 2014: A multidisciplinary approach providing new insight into fruit flesh browning physiology in apple (*Malus×domestica* Borkh.). PLoS ONE 8(10), e78004. DOI: 10.1371/journal.pone.0078004

HE, J.G., CHENG, Y.D., GUAN, J.F., ZHAO, Z., 2017: Changes of chlorogenic acid content and its synthesis-associated genes expression in Xuehua pear fruit during development. J. Integr. Agr. 16(2), 471-477. DOI: 10.1016/S2095-3119(16)61496-X

HE, J.G., FENG, Y.X., CHENG, Y.D., LI, L.M., GUAN, J.F., 2016: Effects of Postharvest 1-MCP and MAP treatments on physiological characteristics and quality of 'Fuji' apple during cold storage and subsequent shelf life. Food Sci. 37(22), 301-306. DOI: 10.7506/spkx1002-6630-201622046

IRELAND, H.S., GUILLÉN, F., BOWEN, J., TACKEN, E., 2012: Mining the apple genome reveals a family of nine ethylene receptor genes. Postharvest Biol. Technol. 72, 42-46. DOI: 10.1016/j.postharvbio.2012.05.003

JUNG, S.K., WATKINS, C.B., 2011: Involvement of ethylene in browning development of controlled atmosphere-stored 'Empire' apple fruit. Postharvest Biol. Technol. 59, 219-226. DOI: 10.1016/j.postharvbio.2010.08.019

KOU, X.H., WU, M.S., LI, L., WANG, S., XUE, Z.H., LIU, B., FEI, Y.Q., 2015: Effects of $CaCl_2$ dipping and pullulan coating on the development of brown spot on 'Huangguan' pears during cold storage. Postharvest Biol. Technol. 99, 63-72. DOI: 10.1016/j.postharvbio.2014.08.001

LALLEMAND, L.A., ZUBIETA, C., LEE, S.G., WANG, Y.C., ACAJJAOUI, S., TIMMINS, J., MCSWEENEY, S., JEZ, J.M., MCCARTHY, J.G., MCCARTHY, A.A., 2012: A structural basis for the biosynthesis of the major chlorogenic acids found in coffee. Plant Physiol. 160(1), 249-260. DOI: 10.1104/pp.112.202051

LEE, J., CHENG, L., RUDELL, D.R., WATKINS, C.B., 2012: Antioxidant metabolism of 1-methylcyclopropene (1-MCP) treated 'Empire' apples during controlled atmosphere storage. Postharvest Biol. Technol. 65, 79-91. DOI: 10.1016/j.postharvbio.2011.11.003

LIN, Y.F., LIN, H.T., ZHANG, S., CHEN, Y.H., CHEN, M.Y., LIN, Y.X., 2014: The role of active oxygen metabolism in hydrogen peroxide-induced pericarp browning of harvested longan fruit. Postharvest Biol. Technol. 96, 42-48. DOI: 10.1016/j.postharvbio.2014.05.001

LIN, Y.F., LIN, H.T., LIN, Y.X., ZHANG, S., CHEN, Y.H., JIANG, X.J., 2016: The roles of metabolism of membrane lipids and phenolics in hydrogen peroxide-induced pericarp browning of harvested longan fruit. Postharvest Biol. Technol. 111, 53-61. DOI: 10.1016/j.postharvbio.2015.07.030

LIVAK, K.J., SCHMITTGEN, T.D., 2001: Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25(4), 402-408. DOI: 10.1006/meth.2001.1262

MAHESH, V., MILLION-ROUSSEAU, R., ULLMANN, P., CHABRILLANGE, N., BUSTAMANTE, J., MONDOLOT, L., MORANT, M., NOIROT, M., HAMON, S., KOCHKO, DE A., WERCK-REICHHART, D., CAMPA, C., 2007: Functional characterization of two *p*-coumaroyl ester 3'-hydroxylase genes from coffee tree: evidence of a candidate for chlorogenic acid biosynthesis. Plant Mol. Biol. 64(1-2), 145-159. DOI: 10.1007/s11103-007-9141-3

MAO, L.C., PANG, H.Q., WANG, G.Z., ZHU, C.G., 2007: Phospholipase D and lipoxygenase activity of cucumber fruit in response to chilling stress. Postharvest Biol. Technol. 44(1), 42-47. DOI: 10.1016/j.postharvbio.2006.11.009

MATTHEIS, J.P., RUDELL, D.R., 2008: Diphenylamine metabolism in 'Braeburn' apples stored under conditions conducive to the development of internal browning. J. Agr. Food Chem. 56(9), 3381-3385. DOI: 10.1021/jf703768w

MORAN, R.E., MCMANUS, P., 2005: Firmness retention, and prevention of coreline browning and senescence in 'Macoun' apples with 1-methylcyclopropene. HortScience. 40(1), 161-163. DOI: 10.21273/HORTSCI.40.1.161

NIGGEWEG, R., MICHAEL, A.J., MARTIN, C., 2004: Engineering plants with increased levels of the antioxidant chlorogenic acid. Nat. Biotechnol. 22, 746-754. DOI: 10.1038/nbt966

PONGPRASERT, N., SEKOZAWA, Y., SUGAYA, S., GEMMA, H., 2011: A novel postharvest UV-C treatment to reduce chilling injury (membrane damage, browning and chlorophyll degradation) in banana peel. Sci. Hortic. 130(1), 73-77. DOI: 10.1016/j.scienta.2011.06.006

RUPASINGHE, H.P.V., MURR, D.P., PALIYATH, G., SKOG, L., 2000: Inhibitory effect of 1-MCP on ripening and superficial scald development in 'McIntosh' and 'Delicious' apples. J. Hortic. Sci. Biotech. 75(3), 271-276. DOI: 10.1080/14620316.2000.11511236

SHENG, L., ZHOU, X., LIU, Z.Y., WANG, J.W., ZHOU, Q., WANG, L., JI, S.J., 2016: Changed activities of enzymes crucial to membrane lipid metabolism accompany pericarp browning in 'Nanguo' pears during refrigeration and subsequent shelf life at room temperature. Postharvest Biol. Technol. 117, 1-8. DOI: 10.1016/j.postharvbio.2016.01.015

SISLER, E.C., SEREK, M., 2003: Compounds interacting with the ethylene receptor in plants. Plant. Biol. 5(5), 473-480. DOI: 10.1055/s-2003-44782

SONG, L.L., JIANG, Y.M., GAO, H.Y., LI, C.T., LIU, H., YOU, Y.L., SUN, J., 2006: Effects of adenosine triphosphate on browning and quality of harvested litchi fruit. Am. J. Food Technol. 1(2), 173-178. DOI: 10.3923/ajft.2006.173.178

SUN, J., YOU, X.R., LI, L., PENG, H.X., SU, W.Q., LI, C.B., HE, Q.G., LIAO, B., 2011: Effects of a phospholipase D inhibitor on postharvest enzymatic browning and oxidative stress of litchi fruit. Postharvest Biol. Technol. 62(3), 288-294. DOI: 10.1016/j.postharvbio.2011.07.001

TOMÁS-BARBERÁN, F.A., ESPÍN, J.C., 2010: Phenolic compounds and related enzymes as determinants of quality in fruits and vegetables. J. Sci. Food Agric. 81(9), 853-876. DOI: 10.1002/jsfa.885

TSANTILI, E., GAPPER, N.E., ARQUIZA, J.M.R.A., WHITAKER, B.D., WATKINS, C.B., 2007: Ethylene and α-farnesene metabolism in green and red skin of three apple cultivars in response to 1-methylcyclopropene (1-MCP) treatment. J. Agr. Food Chem. 55(13), 5267-5276. DOI: 10.1021/jf063775

WANG, X.M., 2001: Plant phospholipases. Annu. Rev. Plant Physiol. Plant Mol. Biol. 2001, 52, 211-231. DOI: 10.1146/annurev.arplant.52.1.211

WATKINS, C.B., 2007: The effect of 1-MCP on the development of physiological storage disorders in horticultural crops. Stewart Postharvest Rev. 3(2), 11. DOI: 10.2212/spr.2007.2.11

WATKINS, C.B., NOCK, J.F., WHITAKER, B.D., 2000: Responses of early, mid and late season apple cultivars to postharvest application of 1-methylcyclopropene (1-MCP) under air and controlled atmosphere storage conditions. Postharvest Biol. Technol. 19(1), 17-32. DOI: 10.1016/S0925-5214(00)00070-3

WATKINS, C.B., 2008: Overview of 1-MCP trials and uses for edible horticultural crops. Hortscience 43(1), 86-94. DOI: 10.1007/s10658-007-9208-7

YUAN, Y., WANG, Z.Y., JIANG, C., WANG, X.M., HUANG, L.Q., 2014: Exploiting genes and functional diversity of chlorogenic acid and luteolin biosyntheses in *Lonicera japonica* and their substitutes. Gene 534(2), 408-416. DOI: 10.1016/j.gene.2012.09.051

ZHAO, S.C., TUAN, P.A., LI, X.H., KIM, Y.B., KIM, H., PARK, C.G., YANG, J.L., LI, C.H., PARK, S.U., 2013: Identification of phenylpropanoid biosynthetic genes and phenylpropanoid accumulation by transcriptome analysis of *Lycium chinense*. BMC Genomics. 14(1), 802. DOI: 10.1186/1471-2164-14-802

ORCID
Jingang He https://orcid.org/0000-0001-9986-8720
Yunxiao Feng https://orcid.org/0000-0003-0117-7062
Yudou Cheng https://orcid.org/0000-0002-5990-0041
Shen Wang https://orcid.org/0000-0002-1932-4641
Junfeng Guan https://orcid.org/0000-0001-8570-9219

Address of the corresponding author:
Junfeng Guan, Institute of Genetics and Physiology, Hebei Academy of Agriculture and Forestry Sciences, Shijiazhuang, China
E-Mail: junfeng-guan@263.net