Evidence supporting the involvement of MHO in the formation of superficial scald in 'Dangshansuli' Pears

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Abstract : The development of superficial scald in 'Dangshansuli' pears (*Pyrus bretschneideri* Rehd) was investigated in relation to 6-methyl-5-hepten-2-one (MHO). Over the course of a 180-day cold storage at 2 ± 0.5 °C and a 9-day post storage at 20 °C, the effect of endogenous MHO was investigated in chemically untreated pears, and pears treated with diphenylamine (DPA) and 1-methylcyclopropene (1-MCP) after harvest. In the untreated pears, MHO began to accumulate in peel tissue after 60 d of cold storage and continued to do so until the end of cold storage, by which time superficial scald had developed. In DPA- and 1-MCP-treated pears, on the other hand, there was very little MHO accumulation during cold storage and, at the end of this period, no scald had developed. Results from the cold storage period, therefore, indicate a positive relation between scald incidence and endogenous MHO concentration. Results from 9-day post-storage reveal also a strong positive correlation between scald and endogenous MHO concentration, with a correlation coefficient of 0.97936 ($P < 0.05$).

The effect of exogenous MHO was investigated before and after cold storage in DPA- and 1-MCP-treated pears, as well as untreated pears. The application of 0.5, 1.0, and 2.0 mL L⁻¹ MHO both before and after cold storage caused scald develop in a

concentration-dependent manner: The higher the MHO concentration, the higher the scald incidence. The application of DPA and 1-MCP prior to application of MHO did not affect scald development before cold storage. However, after cold storage, DPA-treated pears were found to have a lower scald incidence and MHO concentration than untreated pears. Moreover, the results indicate that MHO directly induces superficial scald in ‘Dangshansuli’ pears.

Key words : 6-methyl-5-heptene-2-one; superficial scald; ‘Dangshansuli’ pear; *Pyrus bretschneideri*

1. Introduction

Superficial scald is a physiological disorder, which causes the skins of pears and apples to develop irregular brown or black patches (Lurie and Watkins, 2012). It generally occurs after several months of storage at low temperature and rapidly intensifies when the fruits are transferred from cold storage to room temperature. Despite the fruit internal quality is not significantly affected, its commercial value is lost, because it becomes unsuitable for the market as a fresh commodity (Rui et al., 2012). ‘Dangshansuli’ pear is one of the most widely grown varieties and one of the main export fruits in China, however, superficial scald is pointed out by producers and as one of the major pome fruit cold-storage disorders that may lead to great economic losses in the postharvest storage periods or the shelf periods (Hui et al., 2011).

Superficial scald is attributed to autoxidation products of α -farnesene (Mir et al., 1999; Whitaker, 2004), including conjugated triene hydroperoxides (Huelin and Coggiola, 1970a; Whitaker et al., 2009; Jordi et al., 2013), intermediary free radicals (Anet, 1972; Rowan et

al., 2001), and 6-methyl-5-hepten-2-one (MHO) (Mir et al., 1999). Whitaker indicated (2007) that oxidation products of α -farnesene associated with superficial scald development in d'Anjou pear fruits are conjugated trienols. Li and Wang (2009) found that the contents of α -farnesene and conjugated trienols in 1-MCP-treated or cold-treated fruits were significantly lower than in untreated 'Akemizu' pear, and α -farnesene and conjugated trienols had crucial roles in development of superficial scald in oriental pears. Guan et al. (2014) demonstrated that the reduction of superficial scald by 1-MCP was closely correlated with the inhibition on production of the conjugated trienol of peel in 'Wujiuxiang' pear. The results of Zhao et al. (2016) revealed a direct role of CTols during development of scald, however, antioxidant systems may play an important role in α -farnesene oxidation to CTols and scald susceptibility in 'd'Anjou' pears. To inhibit the oxidation of α -farnesene and the consequent production of conjugated trienes, diphenylamine (DPA) has been used to successfully control scald in apples (Zanella, 2003; Jung and Watkins, 2008). Besides, research (Hui et al., 2011) has indicated that the appropriate concentrations of 1-MCP and DPA could restrain and defer the occurrence of superficial scald of 'Dangshansuli' pear remarkably in cold storage period and shelf life respectively, although the mechanism of which were different. If DPA is banned, the most effective chemical replacement now available is 1-MCP, which exerts its effect indirectly by inhibiting ethylene action (Lurie and Watkins, 2012). Navid et al. (2011) showed that accumulation of α -farnesene and conjugated trienols were slower in skin of 1-MCP treated fruit compared with controls, and Asian pears with 1-MCP followed by cold storage maintained textural characteristics with less scald incidence. Findings of Gao et al. (2015) indicated that 1-MCP regulates the activities of

H₂O₂-scavenging enzymes to inhibit the accumulation of H₂O₂ and thereby reduces cell membrane damage and inhibits the accumulation of conjugated trienols, thus, 1-MCP could decrease the incidence of superficial scald in ‘Wujiuxiang’ pear. What’s more, DPA and/or 1-MCP also can inhibit the development of superficial scald in Whangkeumbae (Li et al., 2007), ‘Laiyang’ pear (Li et al., 2013) and ‘Abate’ pear (Zhu et al., 2015). However, Huelin and Coggiola (1970b) found that applying synthetic conjugated trienes to apples did not cause peel browning, and Du et al. (1993) showed that apples with large amounts of naturally-occurring conjugated trienes had no incidence of superficial scald when stored at 20 °C. Furthermore, Watkins et al. (1995) reported that, among apples with similar conjugated triene contents in their peels, the disorder only occurred at temperatures lower than 20 °C, namely 0, 4 or 10 °C. While, on the one hand, these facts clearly underscore the importance of temperature, they also appear to contradict the idea of α -farnesene being the principal cause of scald.

To further illuminate the role of α -farnesene, other scientists have investigated the oxidation products of the conjugated triene and their relationship to scald. Whitaker and Saftner (2000) found that the major oxidation product of conjugated trienes was MHO. Meanwhile, Mir et al. (1999) showed that peel browning in apples increased concomitantly with MHO content, and Mir and Beaudry (1999) detected MHO production only in scalded apples. In addition to this, the exogenous application of MHO vapor induced scald-like skin browning in nine apple cultivars. MHO sensitivity was also shown to be higher in scald-susceptible cultivars than in scald-resistant cultivars (Song and Beaudry, 1996). These findings appear to suggest the importance of MHO in scald development. However, Wang

and Dilley (2000a) discovered that, while all hypobarically-stored apples produced MHO when transferred to air at 20 °C, they did not develop scald. This was true both for scald-resistant and scald-susceptible apples, which produced similar levels of MHO. Similarly, Ju and Curry (2002) reported that MHO treatment increased MHO concentration in apple peels, but it did not affect scald development. In 2009, study showed that even transgenic apples suppressed for ethylene biosynthesis genes can produce α -farnesene, which in turn can oxidize to free radicals and MHO, leading to scald development (Pesis et al., 2009). And Pesis et al. (2010) found that low O₂ pretreatments induced volatile alcohols and reduced ethylene and MHO production, thereby reducing superficial scald development of ‘Granny Smith’ apples after 8 months at 0 °C plus 3 days at 20 °C. Pesis et al. (2014) assumed that low O₂ and 1-MCP pretreated fruit remained free of physiological disorders, due to the reduced production of ethylene and the oxidation product MHO during cold storage. In order to perform a more broad investigation, a PTR-ToF–MS (proton transfer reaction—time of flight—mass spectrometry) was employed to monitor the volatile organic compounds (VOCs) production along with the progression of scald in fruit of ‘Granny Smith’ apples, and the rapid and non-destructive analysis of the VOC array carried out by PTR-ToF–MS identified three specific groups of metabolites in the skin, among which MHO markedly associated with superficial scald (Farneti et al., 2015). Then Asanda et al. (2016) investigated the relationship between scald severity and metabolic changes in ‘Granny Smith’ apples, and demonstrated that scald severity is not directly related to some scald-associated metabolites in ‘Granny Smith’ apples. While increases in ethylene production, α -farnesene and MHO corresponded with the onset and progression of scald severity, this relationship

did not hold in high to severely affected fruit.

It is clear from the foregoing that scald development in apples is still not clearly understood. Although existing research has shown the mechanisms of scald development in both pears and apples to be similar, this mechanism in pears is even less understood. More specifically, no research has been reported regarding the relationship between MHO and superficial scald in pears. This study, therefore, has sought to examine the effects of MHO on scald development in pears. Because they are one of the most popular pear varieties cultivated in China and because are highly susceptible to superficial scald, this study was conducted using ‘Dangshansuli’ pears (*Pyrus bretschneideri* Rehd. cv, Dangshansuli).

2. Materials and methods

2.1 Handling and Treatment

2.1.1. Pear Collection

‘Dangshansuli’ pears (*Pyrus bretschneideri* Rehd.) were harvested on the morning of September 25th at a commercial orchard in Shaanxi Province, China. The harvested pears were sorted to create a group, whose members were uniform in size and color, and were without sunburn or blemishes. This group of pears was then transferred to the laboratory the same day.

2.1.2. Anti-scald treatment

The next day, the pears were randomly divided into several batches. One of the batches was not given any anti-scald treatment (control). A second batch was treated with DPA (DPA batch). The pears in this batch were soaked in a 2000 mg L⁻¹ DPA solution (Productos Citrosol, S.A., Valencia, Spain; 31 %, w/v) for 1 minute at 20 °C. The third batch was treated

with 1-MCP (1-MCP batch) as follows. The pears were placed in a 4 M³ air-tight room at ambient temperature. 1.0 $\mu\text{L L}^{-1}$ 1-MCP (SmartFreshTM, AgroFresh Inc., Springhouse, PA) was then released into the room's atmosphere by mixing a powdered reagent with an aqueous solution according to the manufacturer's instructions. The pears were left in the room undisturbed for 24 h at 20 °C, and then removed to the cold storage.

2.1.3. Phases of the Experiment

The experiment was conducted in three phases elaborated below: 1) Post-harvest MHO treatment to evaluate the effect of exogenous MHO prior to cold storage (post-harvest MHO phase). 2) Cold storage followed by post-storage without MHO treatment to evaluate the effect of endogenous MHO (cold storage and non-MHO post-storage). 3) Cold storage followed by MHO treatment to evaluate the effect of exogenous MHO after cold storage (MHO post-storage). Each phase used pears from all three treatment batches. Therefore, each batch was divided into three replicates, one for each phase.

2.1.4. Post-harvest MHO treatment (post-harvest MHO phase)

The post-harvest MHO DPA and 1-MCP replicates (50 pears each) were both treated with 1.0 mL L^{-1} MHO, namely pears were sealed in a 30 L chamber with 30 mL MHO for 24 h under dark condition. The post-harvest MHO control replicate (250 pears) was divided into five sub-replicates of 50 pears each. The first three of these sub-replicates were placed in 3 chambers (30 L/chamber) with 15, 30 and 60 mL MHO for 24 h under dark condition, that is, these 3 groups were treated with 0.5, 1.0, and 2.0 mL L^{-1} MHO, respectively. To account for the effect of ketone on peel browning, the fourth sub-replicate was treated with 1.0 mL L^{-1} acetone by the similar way. The fifth sub-replicate was left untreated to form an MHO control.

All MHO treatments were performed according to the methods of Ju and Curry (2002). Following treatment, all seven replicates and sub-replicates were stored at 20 °C for 14 d. At the end of this period, scald grade, scald index, scald incidence, and the MHO emissions rate were determined for each replicate and sub-replicate.

2.1.5. Cold storage followed by post-storage without MHO treatment (cold storage and non-MHO post-storage)

All three cold storage replicates (one from each batch) were placed in cold storage (2 ± 0.5 °C, 80-85 % RH) for 180 d. Beginning on the 30th day, the MHO emissions rate was measured every 30 d, with the final measurement being taken on the 180th day. Upon removal from cold storage, scald grade, scald index, and scald incidence were determined for all three replicates. The pears were then stored at 20 °C for 9 d without being treated with MHO (non-MHO post-storage). On days 1, 3, 5, 7, and 9, measurements were taken to determine α -farnesene and conjugated trienes concentrations, MHO emissions, and scald incidences for the DPA and control replicates. (These data were not taken for the 1-MCP replicate.)

2.1.6. Cold storage followed by MHO treatment (MHO post-storage)

The three MHO post-storage replicates were placed in cold storage (2 ± 0.5 °C, 80-85 % RH) for 180 d. After being removed from cold storage, eight sub-replicates were created: 2 DPA sub-replicates, 2 1-MCP sub-replicates, and 4 control sub-replicates. The first DPA sub-replicate consisted of 50 DPA-treated pears randomly selected and treated with 1.0 mL L^{-1} MHO, while the second DPA sub-replicate consisted of the remaining 50 DPA pears untreated with MHO. The first 1-MCP sub-replicate was composed of 50 randomly selected 1-MCP pears treated with 1.0 mL L^{-1} MHO, and the second 1-MCP sub-replicate contained the

remaining 50 1-MCP pears untreated with MHO.

The first three control sub-replicates each contained 50 pears which were randomly selected from the control batch and then treated with 0.5, 1.0, and 2.0 mL L⁻¹ MHO, respectively. The fourth control sub-replicate was comprised of pears from the control not treated with MHO (MHO control). All MHO treatments were conducted following the methods of Ju and Curry (2002). After these treatments, all eight sub-replicates were stored at 20 °C for 7 d. At the end of this period, scald grade, scald index, scald incidence, and the MHO emissions rate were determined for each sub-replicate.

2.2 Measurements

2.2.1. MHO emissions rate measurement

Using a brass cork borer (2.0 cm diameter), 20 peel discs (about 3 mm thick) were removed from 5 pears within each replicate or sub-replicate. The discs were weighed and then placed in a 15 mL inclosed glass headspace vial for 3 h at 25 °C. After a 3-hour enclosure, the headspace was sampled using solid phase micro-extraction (SPME). The fiber was equilibrated (PDMS) for 16 min to absorb volatiles emitted from the peels. The amount of MHO partitioned in the fiber's epicuticular wax was analyzed using an Agilent 6890N gas chromatograph, as indicated by Song et al. (1997), and improved: It was injected in the splitless injection port and lasted 2 min, the initial temperature was 40 °C and kept 2 min, then increased to 250 °C by 50 °C/min and running 2 min. A flame ionization detector was used and the detection temperature was 250 °C. A standard of MHO purchased from Sigma was used to obtain a reference retention time and mass spectrum. MHO emissions rate was then calculated and reported in units of $\mu\text{L kg}^{-1} \text{ h}^{-1}$, using standard MHO curves with a

concentration range of 0-500 μL .

2.2.2. *α -farnesene and conjugated trienes measurements*

Two discs of skin tissue were removed from 5 pears (10 skins) in each replicate using a brass cork borer (2.0 cm diameter). The amount of hexane-extractable α -farnesene and conjugated trienes in these tissues were then analyzed according to the techniques of Anet (1972), although with some modifications. The 10 skins were immersed in 10 mL of purified n-hexane (100 mL of AR grade n-hexane was poured into 8 mL of concentrated sulfuric acid, stirred the solution with a magnetic stirrer for 2 h, and removed sulfuric acid by static separation. Washed it with saturated Na_2CO_3 solution and distilled water until neutral, then dried with anhydrous CaCl_2 . Filtered it to no absorption at 210~360 nm.) and agitated for 2 h. After being filtered, hexane-extractable α -farnesene and conjugated trienes contents were determined using a cellulose paper filter, whose absorbance was then measured with an ultraviolet spectrophotometer. The absorbance of α -farnesene was 232 nm and that of conjugated trienes was between 281 nm and 290 nm. These absorbances were used to calculate the concentration of α -farnesene ($\text{OD}_{232 \text{ nm}}$ and $E=27740$) and conjugated trienes ($\text{OD}_{281-290 \text{ nm}}$ and $E=25000$) in units of nmol m^{-2} .

2.2.3. *Scald grade, scald incidence and scald index*

(All) fifty pears from each replicate or sub-replicate were used to evaluate the scald incidence and index. Scald index was determined following the method of Zanella (2003). First, the total number of pears with scald was counted. Second, each pear with scald was assigned a scald grade based on what percentage of its surface area was affected by scald, namely, Grade 0: no scald; Grade 1: scald area $<25\%$ of total area; Grade 2: $25\% \leq \text{scald}$

area < 50 % of total area; Grade 3: scald area $\geq$ 50 % of total area. Finally, the scald incidence and the scald index for each grade were computed according to the following formulas:

Scald incidence = Number of scalded pears / Total number of pears $\times$ 100 %

Scald index = [Number of Pears $\times$ Grade] / [Total number of pears $\times$ 3 $\times$ (the highest grade)] $\times$ 100 %

2.3. Statistical analysis

The mean of three replicates was used to represent per measurement. Data were evaluated with Variance Analysis (L.S.D. test at $P < 0.05$), using SPSS 16.0 software. All figures were produced with Origin 6.1.

3. Results

3.1. Variation in MHO concentration during cold storage

The amount of MHO in the pear peels increased over the six-month cold storage period. In the first two months, MHO was not detected in any pear peel tissues. After 90 d, the MHO concentration of the control increased to $0.7 \mu\text{L kg}^{-1} \text{ h}^{-1}$, although MHO in DPA- and MCP-treated pears remained undetectable. MHO was first detected in the DPA-treated pears after 120 d. In the 1-MCP-treated pears, MHO was first detected after 150 d (Fig. 1).

Fig.1. Changes in MHO Concentration in the peel tissue of ‘Dangshansuli’ pears over a 180 d storage period at 2 ± 0.5 °C. Before storage, pears were treated with 2000 mg L^{-1} DPA, $1\mu\text{L L}^{-1}$ 1-MCP, or left untreated (control). Bars indicate S.D. of means. Every data was presented by the mean of three replicates.

3.2. Scald incidence at the end of cold storage

After 180 d of storage at 2 ± 0.5 °C, the scald incidence and scald index of control were 16.6 % and 12.5 %, respectively (Table 1). In the DPA and 1-MCP replicates, the scald incidence and scald index were both 0.0 % (Table 1).

Table 1 Scald incidence and scald index of ‘Dangshansuli’ pears after 180 d of storage at 2 ± 0.5 °C

Treatments	Control	DPA	1-MCP
Scald incidence (%)	16.6 a	0 b	0 b
Scald index (%)	12.5 a	0 b	0 b

Before storage, pears were treated with 2000 mg L^{-1} DPA, $1\mu\text{L L}^{-1}$ 1-MCP, or left untreated (control). Every data was presented by the mean of three replicates. The means followed with same letter are not significantly different according to L.S.D. test at $P<0.05$.

3.3. Post-Storage

3.3.1. Scald incidence during post-storage

Fig. 2A shows how scald incidence continually increased in both the DPA-treated pears

and the control during post-storage, and the values in the former were lower than that in the latter during the shelf period. On day 1, the scald incidence in both groups was similar. After this, however, scald incidence increased far more rapidly in the control, with an especially large increase between days 1 and 3. By day 9, scald incidence in the control was 83.4 %, while scald incidence in the DPA-treated pears was 18.6 %. It is, therefore, clear that DPA-treatment significantly inhibited scald incidence.

3.3.2 α -farnesene concentration during post-storage

Fig.2B reveals that, for the both the control and the DPA-treated pears, α -farnesene concentration reached its maximum on day 3, were 129000, 116000 nmol m⁻² respectively, then steadily declined. By day 9, α -farnesene concentration in the control and the DPA-treated pears were 86000 and 82000 nmol m⁻², respectively. Throughout the 9 d shelf life, α -farnesene concentration in the DPA-treated pears was always slightly lower than that in control. The difference, however, was not significant ($P>0.05$).

3.3.3 Conjugated trienes concentration during post-storage

Fig.2C shows that, for both the DPA-treated and control pears, the level of conjugated trienes increased slightly between days 1 and 3, the values were 66000 and 22000 nmol m⁻² respectively. On day 3, the value were 72000 and 33000 nmol m⁻² respectively. After day 3, it tended to decline. In the control, this decline was more rapid than in the DPA-replicate. By the day 9, the conjugated trienes in control was 38000 nmol m⁻² while 18000 nmol m⁻² in DPA-treated pears. The conjugated trienes content of DPA-treated pears was always lower than that of the control during all shelf period, and this difference was significant ($P<0.05$).

3.3.4 MHO concentration during post-storage

Fig.2D indicates that, after the 6 months of RA storage, MHO emissions were low: $3.2 \mu\text{L kg}^{-1} \text{ h}^{-1}$ in control and $0.4 \mu\text{L kg}^{-1} \text{ h}^{-1}$ in DPA-treated pears. Upon removal from cold storage, these emissions then increased (more quickly the control pears and less quickly in the DPA-treated pears) until reaching their maximum of day 3, $4.3 \mu\text{L kg}^{-1} \text{ h}^{-1}$ in the control and $0.8 \mu\text{L kg}^{-1} \text{ h}^{-1}$ in DPA-treated pears, after which their values diminished. The control decreased very sharply between days 3 and 5, the value on 5th d was $1.7 \mu\text{L kg}^{-1} \text{ h}^{-1}$. Then level of MHO both in the DPA-treated and control pears slowly decreased. It had no significant difference ($P>0.05$) on day 9. However, the emissions of MHO in the control were higher than that in DPA-treated pears during 9 d shelf period.

Fig.2. Effects of 20 °C post-storage on: (A) superficial scald incidence, (B) α -farnesene concentration, (C) conjugated trienes concentration, and (D) 6-methyl-5-hepten-2-one emissions. Day 0 was when the pears were removed from the cold storage. Bars indicate S.D. of means. Every data was presented by the mean of three replicates.

3.4. Relationship between MHO concentration and grade of superficial scald

MHO concentration in the control progressively increased with the grade of scald (Fig.3A) after 180 d in cold storage and, accordingly, the greatest MHO concentration was found in pears most superficial scald, namely those with a scald grade of 3. Also, the differences in MHO concentration between the four grades of scald were significant ($P < 0.05$), and the data are about 1.7, 3.1, 4.4 and $7.4 \mu\text{L kg}^{-1} \text{h}^{-1}$, respectively. From these facts, it is clear that the severity of scald development was positively correlated with the MHO concentration in the ‘Dangshansuli’ pear peels. As showed in Fig.3B, the correlation coefficient between scald grade and MHO concentration was found to be 0.97936 ($P < 0.05$).

Fig.3. (A) Relationship between the grade of superficial scald and MHO concentration in the control peels after 180 d of cold storage. (B) The linear regression equation on the relationship between the grade of superficial scald and MHO concentration in the control peels after 180 d of cold storage. Every data was presented by the mean of three replicates. The means followed with same letter are not significantly different according to L.S.D. test at $P < 0.05$. Bars indicate S.D. of means.

3.5. Effect of applied MHO

3.5.1. Effect of applied MHO on peel browning immediately after harvest

MHO was not detected in pear peel tissues at the time of harvest. When MHO was artificially applied to pears immediately after harvest, it caused varying degrees of skin browning, the severity of which depended upon the MHO concentration. The 2.0 mL L⁻¹ MHO treatment caused an average scald incidence of 80.0 % after 14 d, whereas the 1.0 mL L⁻¹ treatment brought about an average scald incidence of 63.3 %. This peel browning must have been due to the presence of MHO, and not simple chemical injury caused by the presence of ketone, because the acetone treatment did not induce peel browning. Unlike the other two MHO concentrations, the 0.5 mL L⁻¹ treatment did not induce peel browning, even though the average MHO emission rate from the peel, 1.3 $\mu\text{L kg}^{-1} \text{ h}^{-1}$, was much higher than that from untreated peels, which remained under the detection limit. And, the measured MHO emission of 1.0, 2.0 mL L⁻¹ MHO-treated peels was, 5.0, 6.6 $\mu\text{L kg}^{-1} \text{ h}^{-1}$, respectively, in fact, markedly higher than that of untreated pear peel. These facts suggest that MHO concentration in the epicuticular wax of ‘Dangshansuli’ pears is associated with scald development.

3.5.2. Effect of applied MHO on peel browning after 180 d of cold storage

At the end of the 180-day cold storage period, pears not treated with MHO had relatively low scald incidence (28.6 %) and MHO emission (3.4 $\mu\text{L kg}^{-1} \text{ h}^{-1}$). The application of MHO to pears after the 180 d of cold storage caused varying degrees of skin browning, whose severity increased with MHO concentration. At 20 °C for 7 d, the MHO emission rate of pears treated with 0.5, 1.0, 2.0 mL L⁻¹ MHO after 180 d storage was 16.3, 22.9 and 30.7 $\mu\text{L kg}^{-1} \text{ h}^{-1}$, respectively. And their scald incidences were 87.4 %, 91.8 % and 100 %, respectively.

respectively.

The pears treated after cold storage had a higher MHO concentration in the epicuticular pear wax than those treated after harvest. Furthermore, browning in the former occurred sooner than browning in the latter. This indicates that the endogenous MHO levels of pears treated after harvest was lower than after the 6 months of storage and that tissue sensitivity to exogenous MHO treatment increased dramatically after storage.

3.5.3 Effect of DPA and 1-MCP on peel browning

Lastly, both at harvest and after storage, treatment with 1.0 mL L⁻¹ MHO induced peel browning in both DPA- and 1-MCP-treated pears, there was no significant difference in peel browning (scald incidence and index) between these and the control. In other words, the application of DPA and 1-MCP could not prevent exogenous MHO-induced peel browning.

Table 2 Effects of treatments in a closed system at 20 °C on scald development and changes of MHO in fruit peel of ‘Dangshansuli’ pears

Storage	Days at 20 °C	Treatments	Concentration (mL L ⁻¹)	MHO (μL kg ⁻¹ h ⁻¹)	Scald incidence (%)	Scald index (%)
At harvest						
	14	Control		0 e	0 c	0 c
	14	MHO	0.5	1.3 d	0 c	0 c
	14	MHO	1.0	5.0 b	63.3 b	43.7 b
	14	MHO	2.0	6.6 a	80.0 a	82.3 a
	14	Acetone	1.0	0 e	0 c	0 c
DPA	14	MHO	1.0	3.7 c	62.5 b	37.0 b
1-MCP	14	MHO	1.0	1.5 d	68.7 b	45.8 b
After cold storage 180 d						
	0	Control		3.4 c	28.6 d	14.3 e
	7	Control		1.5 c	43.5 c	54.8 d
	7	MHO	0.5	16.3 b	87.4 b	62.5 c
	7	MHO	1.0	22.9 b	91.8 a	78.9 b
	7	MHO	2.0	30.7 a	100 a	93.4 a
RA+DPA	7	MHO	0	0.6 d	10.4 e	7.8 e
	7	MHO	1.0	21.8 b	92.4 a	75.7 b
RA+1-MCP	7	MHO	0	0.3 e	5.7 f	2.6 f

7	MHO	1.0	18.0 b	93.3 a	77.4 b
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Every data was presented by the mean of three replicates. The means followed with same letter are not significantly different according to L.S.D. test at $P < 0.05$.

4. Discussion

4.1 Relationship between MHO and superficial scald in apples and pears

The biochemistry of scald etiology has focused almost exclusively on the involvement of α -farnesene and its oxidation (Lurie and Watkins, 2012). The oxidation of α -farnesene, conjugated trienes in apple peels produces MHO which has been implicated as a physiological predictor of superficial scald in apples (Mir and Beaudry, 1999; Wang and Dilly, 2000b; Whitaker, 2004; Pesis et al., 2010). The research of Farneti et al. (2015) found three specific groups of metabolites in ‘Granny Smith’ apple skin, among which MHO significantly associated with the development of the superficial scald. It has also been suggested that the scald mechanism in pears may be similar to that in apples (Zhou et al., 1984). However, until now, there has been no evidence demonstrating a relationship between MHO concentration and superficial scald in pears. The current study sought to shed light on this relationship. From different points of view, each of the three phases of the experiment indicated that MHO concentration was related to the development of superficial scald in ‘Dangshansuli’ pears. In so far as the mechanism of scald development in pears is similar to that in apples, one would expect to find a similar relationship between conjugated trienes and MHO in pears. The current study did not measure conjugated trienes during 2 ± 0.5 °C cold storage. However, it did find a positive correlation between these conjugated trienes and MHO concentration during non-MHO post-storage at 20 °C. It was also found that MHO production during cold storage occurred at a slower rate than in non-MHO post-storage. This evidence supports the hypothesis that the scald development mechanism in pears is similar to that in apples.

4.2 Effects of endogenous MHO on superficial scald in 'Dangshansuli' pears

Sabban-Amin et al. (2011) measured the accumulation of MHO in 'Granny Smith' apples after 8, 16, and 24 weeks of cold storage at 0 °C, and results showed that MHO in the control was higher than that of 1-MCP and low O₂ treatments during different periods. Likewise, MHO levels in the control were higher during cold storage in this experiment (Fig.1), and, by the end of cold storage, scald incidence in this replicate had increased from 0.0 % to 16.6 % (Table 1). The MHO concentrations of the cold-storage DPA and 1-MCP replicates, on the other hand, increased considerably less than that of the control, and the scald incidence of these replicates at the end of cold storage was 0.0 % (Table 1). Therefore, the DPA and 1-MCP treatments inhibited MHO production and completely prevented scald over 180 d of cold storage. This result was consistent with the research of Pesis et al. (2010), among which DPA and 1-MCP obviously inhibited MHO production and scald in cold storage, whereas the control's MHO significantly higher with serious scald. Besides, Fan et al. (1999) demonstrated that 1-MCP can delay the rise in production of α -farnesene and reduced accumulation of its oxidation products, CT and MHO in apples. What's more, Lurie and Watkins (2012) concluded that although MHO is produced during autoxidation of CTs and CTols, its production, and not its presence, is the important aspect involved in scald appearance. Moreover, in this study, scald did not occur at lower MHO concentrations, but did occur at higher ones, which suggests that there is an endogenous MHO concentration threshold above which scald occurs.

The grade of superficial scald and scald incidence were both positively correlated with endogenous MHO concentration. In addition, study has showed that apple occurred high

scald index with higher MHO production (Pesis et al., 2010). Scald incidence in particular was strongly correlated with endogenous MHO concentration, for the correlation coefficient relating these two factors was 0.97936 ($P < 0.05$). Thus, scald development is strongly positively correlated with the endogenous MHO level in 'Dangshansuli' pear peels.

During the 9-day non-MHO post-storage, α -farnesene, conjugated trienes, and MHO – chemical compounds believed to be related to superficial scald – reached their maximum on day 3. These maxima corresponded to a sharp increase in the control's endogenous MHO concentration and scald incidence, which accords with the findings of many other experiments (Mir et al., 1999; Wang and Dilley, 2000a).

In general, while DPA treatment, relative to the control, had little effect on α -farnesene production, it did inhibit endogenous MHO and conjugated trienes production, and scald incidence. This fact implies a positive correlation between conjugated trienes and MHO production, on the one hand, and a positive correlation between both of these factors and scald incidence, on the other. Besides, researches has found that MHO production correlated with α -farnesene and CTs during postharvest storage and shelf-life (Whitaker and Saftner, 2000; Pesis et al., 2014; Farneti et al., 2015). The positive correlation between conjugated trienes and MHO makes sense, for the highly unstable conjugated trienes compounds (Anet, 1969) oxidize into stable MHO (Whitaker and Saftner, 2000): The more conjugated trienes, the more MHO, and vice versa. Because MHO is the more stable compound, it may be considered more closely related to scald development than conjugated trienes and, hence, serve as a measure both of conjugated trienes oxidation and superficial scald. In addition, Farneti et al. (2015) suggested to use MHO as a possible biochemical marker for an early

detection of the superficial scald symptoms. What's more, the ratio between 6-methyl-5-hepten-2-one and 6-methyl-5-hepten-2-ol might serve as an index of superficial scald severity (Pesis et al., 2010).

4.3 Effects of exogenous MHO on superficial scald in 'Dangshansuli' pears

At harvest, treatment with the 1.0 mL L⁻¹ and 2.0 mL L⁻¹ exogenous MHO induced scald, and the severity increased with MHO concentrations. Acetone, however, did not. This showed that the scald was not due to ketone injury per se. However, the 0.5 mL L⁻¹ MHO treatment did not induce peel browning. Thus, it was predicted that the scald would develop when the MHO content in peel accumulated to a certain level.

Rowan et al. (2001) did not find induction of scald when they treated apples with MHO, but found scald symptoms when CTs and CTols were applied. However, after cold storage, the 0.5, 1.0, and 2.0 mL L⁻¹ exogenous MHO treatments all increased scald severity in the previously scalded control pears. Post-cold storage MHO emissions were also higher than post-harvest MHO emissions. Therefore, it is possible that the endogenous MHO concentration in the pear peels was lower before cold storage, but then increased during cold storage. Additionally, peel tissue sensitivity to exogenously applied MHO increased dramatically with the duration of storage. These findings are similar to those of Beaudry and Song (1996) for apples.

DPA and 1-MCP can affect α -farnesene, conjugated trienols, and superficial scald in apples and pears (Arquiza et al., 2005; Rudell et al., 2005; Gapper et al., 2006; Isidoro and Almeida, 2006; Moggia et al., 2010). Lurie and Watkins (2012) reviewed that DPA can cause a wide range of metabolic responses, including decreased ethylene production, and activities

of polyphenol oxidase, peroxidase and lipoxygenase, and an increased contribution of the pentose phosphate pathway to respiration. And its effects on α -farnesene accumulation are variable, however, DPA consistently reduces CTol accumulation (Whitaker, 2000; Arquiza et al., 2005). DPA also markedly reduced MHO production (Mir et al., 1999). 1-MCP inhibit scald by inhibiting ethylene production and thereby α -farnesene production, resulting in less substrate for oxidation (Watkins et al., 2000; Arquiza et al., 2005; Moggia et al., 2010; Yazdani et al., 2011). Both after harvest and after cold storage, the 1.0 mL L⁻¹ exogenous MHO treatment induced scald in pears treated with DPA and 1-MCP. The scald incidence in these pears was essentially the same as in the control. This supports the notion that MHO may be an initiating factor of scald development. Although Ju and Curry (2002) thought that MHO's ineffectiveness in inducing apple scald was due to the use of a closed system, MHO treatment in an open system also induced peel browning. MHO is also highly lipophilic (Song et al., 1998) and it may cause cell membrane damage by altering the regional distribution of phenolic compounds and polyphenol oxidase, resulting in enzymatic browning (Rao et al., 1998), thus leading to scald. And Hou et al. (2013) found that with the increase of exogenous MHO treatment concentration, the contents of MDA, O₂⁻ and H₂O₂ in apple peel increased, PPO activity gradually increased, while SOD, CAT and POD activities decreased, and the endogenous MHO and scald severity increased. It implied that exogenous MHO may reduce the onset of antioxidant enzyme activities to participate in scald development of apple.

This study used three approaches (the three phases of the experiment) to demonstrate that MHO may be the actual causal agent of superficial scald in pears. Clearly, further studies

are needed to delineate the exact mechanism and effects by which MHO may directly induce superficial scald, or play a role with other factors in ‘Dangshansuli’ pears.

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References

- Anet E F L J., 1969. Autoxidation of α -farnesene. Australian Journal of Chemistry 22, 2403-2410.
- Anet E F L J., 1972. Superficial scald, a functional disorder of stored apples. IX. Effect of maturity and ventilation. Journal of the Science of Food and Agriculture 23, 763-769.
- Arquiza, J., Hay, A.G., Nock, J.F., Watkins,C.B.,2005.1-Methylcyclopropene interactions with diphenylamine on diphenylamine degradation, α -farnesene and conjugated trienol concentrations, and polyphenol oxidase and peroxidase activities in apple fruit. Journal of Agricultural and Food Chemistry 53, 7565-7570.
- Asanda Mditshwa, Olaniyi A. Fawole, Filicity Vries, Kobus Van Der Merwe, Elke Crouch, Umezuruike Linus Opara,2016. Classification of ‘Granny Smith’ apples with different levels of superficial scald severity based on targeted metabolites and discriminant analysis. Journal of Applied Botany and Food Quality 89, 49-55.
- Brian Farneti, Nicola Busatto, Iuliia Khomenko, Luca Cappellin, Soledad Gutierrez, Francesco Spinelli, Riccardo Velasco,Franco Biasioli, Guglielmo Costa,Fabrizio Costa,2015. Untargeted metabolomics investigation of volatile compounds involved in the development of apple superficial scald by PTR-ToF–MS. Metabolomics 11,341–349.
- Bruce D. Whitaker,2007.Oxidation Products of α -Farnesene Associated with Superficial Scald Development in d’Anjou Pear Fruits Are Conjugated Trienols. Journal of Agricultural and Food Chemistry 55, 3708-3712.
- Bruce D. Whitaker, Max Villalobos-Acuna, Elizabeth J. Mitcham, James P. Mattheis, 2009.Superficial scald

- susceptibility and α -farnesene metabolism in ‘Bartlett’ pears grown in California and Washington. *Postharvest Biology and Technology* 53, 43-50.
- C. Moggia, M. A. Moya-León, M. Pereira, J. A. Yuri and G. A. Lobos, 2010. Effect of DPA and 1-MCP on chemical compounds related to superficial scald of Granny Smith apples. *Spanish Journal of Agricultural Research* 8(1), 178-187.
- Du Z, Bramlage WJ, 1993. A modified hypothesis on the role of conjugated trienes in superficial scald development on stored apples. *Journal of the American Society for Horticultural Science* 118, 807-813.
- Edna Pesis, Ana M. Ibanez, My Lin Phu, Elizabeth J. Mitcham, Susan E. Ebeler, Abhaya M. Dandekar, 2009. Superficial Scald and Bitter Pit Development in Cold-Stored Transgenic Apples Suppressed for Ethylene Biosynthesis. *Journal of Agricultural and Food Chemistry* 57, 2786–2792.
- Edna Pesis, Oleg Feygenberg, Revital Sabban-Amin, Susan E. Ebeler, Elizabeth J. Mitcham, Ruth Ben-Arie, 2014. Low oxygen pre-storage treatment is effective in reducing chilling injuries of deciduous fruit. *International Journal of Postharvest Technology and Innovation* 4, 23-32.
- Edna Pesis, Susan E Ebeler, Sergio Tonetto de Freitas, Malkeet Padda, Elizabeth J Mitcham, 2010. Short anaerobiosis period prior to cold storage alleviates bitter pit and superficial scald in Granny Smith apples. *Journal of the Science of Food and Agriculture* 90, 2114-2123.
- Fan X, Mattheis JP, Blankenship S., 1999. Development of apple superficial scald, soft scald, core flush, and greasiness is reduced by MCP. *Journal of Agricultural and Food Chemistry* 47, 3063-3068.
- GUAN Jun-feng, GAO Man-man, ZHAO Qian, 2014. Effect of 1-MCP on Quality of Different Harvested ‘Wujiuxiang’ Pears during Cold Storage. *Modern Food Science and Technology* 30, 130-135.
- Hou zhen, Hui wei, Xu Xiaoyan, Guo Yurong, 2013. Effects of MHO treatment on the development of superficial scald and reactive oxygen species metabolism in the apple peel. *Acta Botanica Boreali-Occidentalia Sinica* 33, 1183-1189.
- Huelin FE, Coggiola I M., 1970a. Superficial scald, a functional disorder of stored apples V. Oxidation of α -farnesene and its inhibition by diphenylamine. *Journal of the Science of Food and Agriculture* 21, 584-589.
- Huelin FE, Coggiola I M., 1970b. Superficial scald, a functional disorder of stored apples. VII. Effects of applied α -farnesene, temperature, and diphenylamine on scald and the concentration on oxidation of α -farnesene in the fruit. *Journal of the Science of Food and Agriculture* 21, 584-589.
- HUI Wei, NIU Rui-xue, SONG Yao-qiang and LI De-ying, 2011. Inhibitory Effects of 1-MCP and DPA on

- Superficial Scald of Dangshansuli Pear. *Agricultural Sciences in China* 10, 1638-1645.
- Jing Zhao, Xingbin Xie, Xiang Shen, Yan Wang, 2016. Effect of sunlight-exposure on antioxidants and antioxidant enzyme activities in 'd'Anjou' pear in relation to superficial scald development. *Food Chemistry* 210, 18-25.
- Ju Z, Curry E A., 2002. Effects of 6-methyl-5-Heptene-2-one vapor on peel browning of 'Delicious' and 'Granny Smith' apples: open vs. closed system. *Postharvest Biology and Technology* 25, 265-272.
- Jordi Giné Bordonaba, Vincent Matthieu-Hurtiger, Pascale Westercamp, Claude Coureau, Eve Dupille, Christian Larrigaudière, 2013. Dynamic changes in conjugated trienols during storage may be employed to predict superficial scald in 'Granny Smith' apples. *LWT - Food Science and Technology* 54, 535-541.
- Fujun Lia, Xinhua Zhang, Baicheng Song, Jiazheng Li, Zhonglin Shang, Junfeng Guan, 2013. Combined effects of 1-MCP and MAP on the fruit quality of pear (*Pyrus bretschneideri* Reld cv. Laiyang) during cold storage. *Scientia Horticulturae* 164, 544-551.
- LI Xiang-li, ZHANG Zi-de, LIU Jing., 2007. Effects of different packages and antioxidants on the skin brown of Whangkeumbae. *Food Research and Development* 28, 140-143.
- Manman Gao, Shuo Zhou, Junfeng Guan, Yingying Zhang, 2015. Effects of 1-methylcyclopropene on superficial scald and related metabolism in 'Wujiuxiang' pear during cold storage. *Journal of Applied Botany and Food Quality* 88, 102-108.
- Mir N, Perez R, Beaudry RM., 1999. A poststorage burst of 6-methyl-5-hepten-2-one (MHO) may be related to superficial scald development in 'Cortland' apples. *Journal of the American Society for Horticultural Science* 124, 173-176.
- Mir NA, Beaudry R., 1999. Effect of superficial scald suppression by diphenylamine application on volatile evolution by stored Cortland apple fruit. *Journal of Agricultural and Food Chemistry* 47, 7-11.
- Navid Yazdani, Kazem Arzani, Younes Mostofi, Maryam Shekarchi, 2011. α -Farnesene and antioxidative enzyme systems in Asian pear (*Pyrus serotina* Rehd.) fruit. *Postharvest Biology and Technology* 59, 227-231.
- Nelson Isidoro, Domingos P.F. Almeida, 2006. α -Farnesene, conjugated trienols, and superficial scald in 'Rocha' pear as affected by 1-methylcyclopropene and diphenylamine. *Postharvest Biology and Technology* 42, 49-56.
- Nigel E. Gapper, Jinhe Bai, Bruce D. Whitaker, 2006. Inhibition of ethylene-induced α -farnesene synthase gene *PcAFSI* expression in 'd'Anjou' pears with 1-MCP reduces synthesis and oxidation of α -farnesene

- and delays development of superficial scald. *Postharvest Biology and Technology* 41, 225-233.
- Rao MV, Watkins CB, Brown SK, Weeden NF, 1998. Active oxygen species metabolism in 'White Angel'×'Rome Beauty' apple selections resistant and susceptible to superficial scald. *Journal of the American Society for Horticultural Science* 123, 299-304.
- Revital Sabban-Amin, Oleg Feygenberg, Eduard Belausov, Edna Pesis, 2011. Low oxygen and 1-MCP pretreatments delay superficial scald development by reducing reactive oxygen species (ROS) accumulation in stored 'Granny Smith' apples. *Postharvest Biology and Technology* 62, 295-304.
- Rowan, D. D., Hunt, M. B., Fielder, S., Norris, J., Sherburn, M. S., 2001. Conjugated triene oxidation products of α -farnesene induce symptoms of superficial scald on stored apples. *Journal of Agricultural and Food Chemistry* 49, 2780-2787.
- Rudell, D.R., Mattheis, J.P., Fellman, J.K., 2005. Relationship of superficial scald development and α -farnesene oxidation to reactions of diphenylamine and diphenylamine derivatives in cv Granny Smith apple peel. *Journal of Agricultural and Food Chemistry* 53, 8382-8389.
- Rui Guerra, Ines V. Garde, Maria D. Antunes, Jorge Marques da Silva, Rosario Antunes, Ana M. Cavaco, 2012. A possibility for non-invasive diagnosis of superficial scald in 'Rocha' pear based on chlorophyll a fluorescence, colorimetry, and the relation between α -farnesene and conjugated trienols. *Scientia Horticulturae* 134, 127-138.
- Seok-Kyu Jung, Chris B. Watkins, 2008. Superficial scald control after delayed treatment of apple fruit with diphenylamine (DPA) and 1-methylcyclopropene (1-MCP). *Postharvest Biology and Technology* 50, 45-52.
- Song J, Beaudry RM., 1996. Rethinking apple scald: New hypothesis on the causal reason for development of scald in apples. *HortScience* 31, 605.
- Song J, Gardner BD, Holland JF, Beaudry RM., 1997. Rapid analysis of volatile flavor compounds in apple fruit using SPME and GC/time-of-flight mass spectrometry. *Journal of Agricultural and Food Chemistry* 45, 1801-1807.
- Song J, L Fan, R M Beaudry, 1998. Application of solid phase microextraction and gas chromatography/time-of-flight mass spectrometry for rapid analysis of flavor volatiles in tomato and strawberry fruit. *Journal of Agricultural and Food Chemistry* 46, 3721-3726.
- Watkins CB, Bramlage WJ, Cregoe BA., 1995. Superficial scald of 'Granny Smith' apples is expressed as a typical chilling injury. *Journal of the American Society for Horticultural Science* 120, 88-94.

- Whitaker B.D, Saftner RA., 2000. Temperature-dependent autoxidation of conjugated trienols from apple peel yields 6-methyl-5-hepten-2-one, a volatile implicated in induction of scald. *Journal of Agricultural and Food Chemistry* 48, 2040-2043.
- Whitaker, B. D., 2004. Oxidative stress and superficial scald of apple fruit. *HortScience* 39, 933-937.
- Yazdani, N., Arzani, K., Mostofi, Y., Shekarchi, M., 2011. α -Farnesene and antioxidative enzyme systems in Asian pear (*Pyrus serotina* Rehd.) fruit. *Postharvest Biology and Technology* 59, 227-231.
- Zanella A., 2003. Control of apple superficial scald and ripening-a comparison between 1-methylcyclopropene and diphenylamine postharvest treatments, initial low oxygen stress and ultra low oxygen storage. *Postharvest Biology and Technology* 27, 69-78.
- Zhenyong Wang, David R. Dilley, 2000a. Hypobaric storage removes scald-related volatiles during the low temperature induction of superficial scald of apples. *Postharvest Biology and Technology* 18, 191-99.
- Zhenyong Wang, David R. Dilley, 2000b. Initial low oxygen stress controls superficial scald of apples. *Postharvest Biology and Technology* 18, 201–213.
- Zhi-qiang. Li and Liang-ju Wang., 2009. Effect of 1-methylcyclopropene on Ripening and Superficial Scald of Japanese Pear (*Pyrus pyrifolia* Nakai, cv. Akemizu) Fruit at Two Temperatures. *Food Science and Technology Research* 15, 483–490.
- Zhou S T, Wang K F, Zhang X N, Hu X S, Li G F, Dong S Y, Zhang F C., 1984. Preliminary study of superficial scald mechanism in ‘Yali’ pear. *Journal of Beijing Agricultural University* 10(1), 55-59.
- Zhu Meiyun, Li Xiaoyue, Liang Lisong, Ma Qinghua, Wang Guixi, 2015. Effect of Different Postharvest Treatments on Quality of ‘Abate’ Pear During Shelf Life After Cold Storage. *Chinese Agricultural Science Bulletin* 31, 273-278.