Effects of Resveratrol on Superficial Scald of 'Dangshansuli' Pears

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Abstract: The effects of resveratrol (Res) on the quality and superficial scald of 'Dangshansuli' pears (*Pyrus bretchnederi* Rehd.) during cold storage (0±0.5°C) and shelf life (20°C) were investigated. The results showed that treatments with 0.1, 1.0, 10 and 100 mg L^{-1} Res significantly delayed the decrease of firmness, soluble solids contents (SSC), juice rate, reduced the relative conductivity, MDA, α-farnesene and conjugated trienes in the cold storage. At the end of 210 d storage, α-farnesene of control, 0.1, 1.0, 10 and 100 mg L^{-1} Res treatments were 14.81, 11.0, 10.49, 9.31 and 11.4 nmol cm^{-2}, respectively. Whereas conjugated trienes were 6.38, 4.17, 4.04, 3.67 and 4.39 nmol cm^{-2} respectively. After 210 d, the scald incidence of control, 0.1, 1.0, 10 and 100 mg L^{-1} Res treatment were 45.5%, 4.3%, 0%, 0% and 7.1%, respectively, and their scald index were 18.2%, 2.9%, 0%, 0% and 3.1%, respectively. After additional 7 d at 20°C, scald incidence successively were 100%, 21.4%, 22.4%, 14.1% and 20.9%, while the scald index were 71.2%, 23.3%, 22.9%, 15.4% and 22.8%, respectively. Res treatments maintained the quality and inhibited superficial scald, and 10 mg L^{-1} Res was the optimum treatment. Thus, it is likely that Res can serve as a potential antioxidant for the preservation and storage of postharvest 'Dangshansuli' pears.
Keywords: Alpha-farnesene; Conjugated trienes; 'Dangshansuli' pears; Resveratrol; Superficial scald

1. Introduction

Superficial scald is one of physiological disorders that occurs in some cultivars of pears and apples after long-term cold storage [1]. And it generally occurs irregular brown or black patches on the skin of the fruit after several months of storage at low temperature and rapidly intensifies when the fruits are transferred to room temperature [2]. Although it does not affect the flesh quality, it seriously influences the fruit appearance and the commercial value [3][4]. As the most popular pear cultivar in China, 'Dangshansuli' pear (*Pyrus bretschneideri* Rehd) is made unsuitable for sale as a fresh commodity because of superficial scald. Subsequently, it causes severe economic losses [5].

Superficial scald is attributed to autoxidation products of α-farnesene [6][7][8][9], including conjugated triene hydroperoxides [10][11][12][13], intermediary free radicals [14][15], and 6-methyl-5-hepten-2-one [6][16][17][18][19][20][21]. At present, the effective chemicals to inhibit superficial scald are diphenylamine (DPA), ethoxyquin and 1-methycyclopropene (1-MCP). Although DPA is the most widespread method in many cultivars [2][22][23][24][25], it is no longer accepted because of the presence of chemical residues and the potential harm to the human body [2][26][27]. Ethoxyquin also is no longer used to inhibit superficial scald for the same reason [1]. 1-MCP as a new ethylene inhibitor, it can effectively inhibit superficial scald [2][12][20][28][29][30]. However, 1-MCP has side effects and even induces disorders [30][31][32][33]. Therefore, it is necessary to develop a new, safe and non-toxic method for inhibiting this postharvest disease.

Resveratrol (3, 5, 4′-trihydroxystilbene, Res) belongs to stilbenoids and consists of two aromatic rings attached by a methylene bridge. It is a polyphenolic phytoalexin widely found in natural plant active monomer and enriched in red wine, black false hellebore, cassia, polydatin, grape, bilberry, peanut, pineapple and other sources [34][35]. It has trans and cis stereo isomeric forms, and the trans form is the more common [36][37]. Studies found that Res has many effects, such as anti-cancer [38][39], antioxidation [40][41], anti-hypertension [42], immunoregulation [43], anti-aging [44] and atherosclerosis prevention [45]. Furthermore, it produces no atoxic side effects [46][47]. Studies have demonstrated that Res can maintain the postharvest quality of fruit and vegetables [48][49]. Besides, Res can increase total phenolics, vitamin C and total carotenoids concentration and the antioxidant capacity of Satsuma mandarin [50]. Res can retain quality of 'El-Bayadi' table grapes after cold storage and shelf life [51]. And Res has positive effect on inhibiting superficial scald of Dangshan pears [52]. So, as a natural antibiotic, Res may have the potential to provide people with a new, economic and safe way to maintain fruit quality, and delay shelf life.

Hence, the objective of this paper is to investigate the effects of Res on 'Dangshansuli' pears, in order to select the appropriate concentration to inhibit superficial scald and maintain postharvest quality.

2. Materials and methods

2.1 Experimental materials treatments

'Dangshansuli' pears were harvested on the morning of September 25th at a commercial orchard in Pucheng County, Shaanxi Province, China. The pears were selected for the uniformity of weight and shape with carpopodium, no mechanical injury or diseases. And pears were randomly divided into 5 groups, each group had 3 repetitions. Pears were transferred to Pucheng Yongben Fruit Company within the same day. Four groups were treated with 0.1, 1.0, 10 or 100 mg L^{-1} Res for 3 min respectively in the next day after emitting field heat overnight. It is important that Res powder must be dissolved in a little ethylalcohol, and then mixed with water to the corresponding concentrations. The fifth group was untreated using as the control. Then, all pears of 5 groups were stored in cold storage (0±0.5°C, 85%-95% RH) for 210 d. The firmness, soluble solids contents (SSC), juice rate, relative conductivity, malonyldialdehyde (MDA), α-farnesene and conjugated trienes of 10 pears per replicate were measured every 30 d. After 210 d, pears were removed from cold storage and placed at 20°C for 7 d shelf life with measuring scald incidence and scald index on day 210 and day 210+7 respectively.

2.2 Firmness and SSC

Flesh firmness was measured by 10 fruit per replicate using fruit pressure tester (FT-327, Italy). The pears were peeled off the skin of fruit symmetry plane near the equator and poked into 0.8 cm by probe. SSC was measured by pocket refractometer (PAL-1, Japan).

2.3 Juice rate

A certain amount flesh of 10 fruit was cut to ground into homogenous solution, and centrifuged for 20 min at full speed. It was calculated with the following formula:

$$\text{Juice rate} = \text{fruit juice weight/fresh fruit weight} \times 100\%$$

2.4 The relative membrane permeability

The relative membrane permeability was expressed by relative electrolytic leakage. Forty discs of skin tissue (4 discs per pear) were removed from 10 pears using a brass cork borer (8 mm diameter).The discs were rinsed 3 times by redistilled water, then transferred in conical flask with 15 mL redistilled water. Subsequently, they were shaken on the table concentrator for 20 min at 20 °C, and relative electrolytic leakage (P_1) was measured. After that, the samples were boiled for 10 min and relative electrolytic leakage (P_2) measured. The results were calculated:

$$\text{Relative electrolytic leakage} = P_1 / P_2 \times 100\%$$

2.5 MDA content

MDA was measured with 1 g (FW) skin tissues. Skin tissues were removed from 10 pears, put in the ice-bath mortar. A liitle silica and 2 mL 0.05 mol L^{-1} phosphate buffer solution (PBS) at pH 7.8 were added into the mortar and ground to homogenate. The homogenate was transferred into test tube with rinsing by 2-3 mL PBS. Then, 5 mL 0.5% thiobarbituric acid was added in the tube and shook well. Then the extract was boiled for 10 min. Afterwards, the tube was taken out and dipped into the cold bath. It was centrifuged (3000 g) for 15 min after cooling. Whereafter, the volume(V_t) supernatant fluid was measured. Finally, 4 mL supernatant (V_s) was used to measure absorbances at 532, 600 and 450 nm, and 0.5% thiobarbituric acid as control. Calculation formula was as follows:

$$\text{MDA (nmol g}^{-1}) = [6.452 \times (A_{532} - A_{600}) - 0.559 \times A_{450}] \times V_t / (V_s \times FW)$$

2.6 α-Farnesene and conjugated trienes

The contents of α-farnesene and conjugated trienes measured by the improved methods of Anet [14]. Twenty discs of skin tissue were removed from 10 pears (2 discs per pear) in each replicate using a brass cork borer (2.0 cm diameter). The discs were immersed in 10 mL of purified n-hexane and agitated for 2 h. After being filtered, α-farnesene and conjugated trienes were determined. 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 (OD_{232} and E=27740) and conjugated trienes ($OD_{281-290}$ and E=25000) in units of nmol m^{-2}.

2.7 Superficial scald

Each treatment was randomly surveyed 50 fruit. The fruit which have been surveyed scald incidence was taken to calculate scald index. Superficial scald was classified into 4 grades according to the percentage of scald areas in the whole surface of pear [53]. Grade: (0) no scald; (1) 0-25% scalded surface; (2) 25-50% scalded surface; (3) the scalded surface >50%. Results were calculated by the formulas:

Scald incidence = Number of scalded pears / Total number of pears × 100 %

Scald index=Σ (Scald fruit number × Grade)/ (Total fruit number × The highest grade) ×100%

2.8 Statistical analysis

All measurements were conducted with three replicates, Data were evaluated with Variance Analysis (L.S.D. test at $p<0.05$) using SPSS 20.0 software. All figures were produced with Origin 6.1.

3. Results

3.1 Effects of Res on the firmness

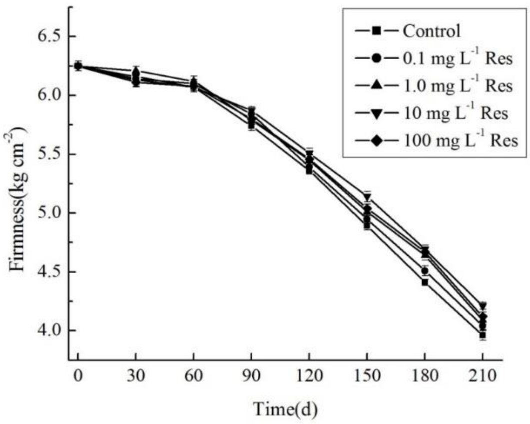

Fig. 1 Effects of Res on firmness of 'Dangshansuli' pears during cold storage. Bars indicate S.D. of means.

Fig.1 shows that the firmness of 'Dangshansuli' pears presented a general trend of descending during cold storage. Compared with the control, Res delayed the decrease of flesh firmness and better maintained the firmness, in which the firmness of 10 mg L^{-1} Res treatment was significantly higher than that of other treatments ($p<0.05$). After 210 d, the firmness of the control, 0.1, 1.0, 10 and 100 mg L^{-1} treatments were 3.96, 4.04, 4.09, 4.21 or 4.12 kg cm^{-2}, respectively. And there were highly significant differences between treatments and control ($p<0.05$).

3.2 Effects of Res on SSC

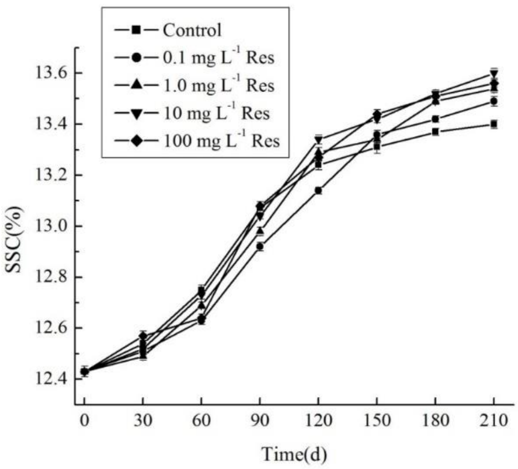

Fig. 2 Effects of Res on SSC of 'Dangshansuli' pears during cold storage. Bars indicate S.D. of means.

Fig.2 shows that SSC of 'Dangshansuli' pears presented an increasing trend in the cold storage. After storage, SSC of the control, 0.1, 1.0, 10 and 100 mg L^{-1} Res were 13.4%, 13.49%, 13.54%, 13.6%, 13.56%, respectively. And the SSC of all treatments were higher ($p<0.05$) than that of the control, but there were no difference ($p>0.05$) among treatments.

3.3 Effects of Res on juice rate

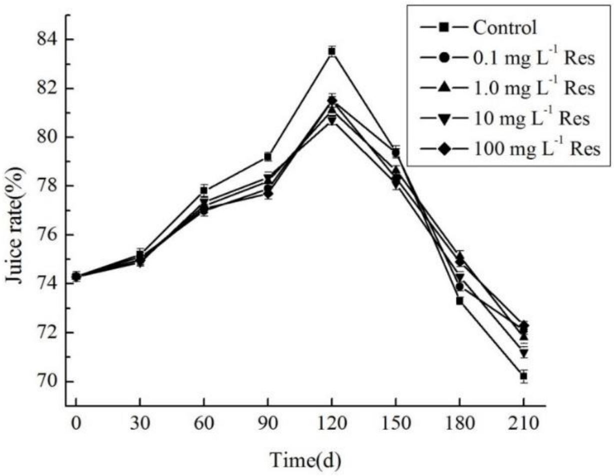

Fig. 3 Effects of Res on juice rate of 'Dangshansuli' pears during cold storage. Bars indicate S.D. of means.

As shown in Fig.3, the juice rate increased to the maximum on day 120, then declined gradually. From 0 to 120 d, the juice rate of control was significantly higher than that of the Res treatments ($p<0.05$). For example, the control juice rate was 83.5% at 120th d, while pears treated with 0.1, 1.0, 10 and 100 mg L^{-1} Res were 81.5%, 81.1%, 80.7% and 81.5%, respectively. Stored up to 210 d, the juice rate of control was relatively lower. Among the different concentrations of Res treatments, the juice rate of 10 mg L^{-1} Res treatment was higher than that of the other treatments ($p>0.05$).

3.4 Effects of Res on the relative membrane permeability

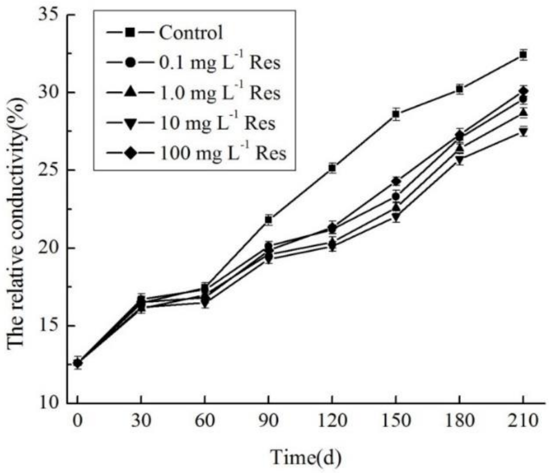

Fig. 4 Effects of Res on the membrane permeability in the peel of 'Dangshansuli' pears during cold storage. Bars indicate S.D. of means.

As shown in Fig.4, the relative conductivity in the peel of 'Dangshansuli' pears constantly increased during cold storage. No significant differences ($p>0.05$) occurred between control and each treatment in the earlier storage. However, from day 90 on, the data of control were higher than each treatment all the while ($p<0.05$). Among treatments, the relative conductivity of treatment with 10 mg L^{-1} was lower than treatment with 0.1, 1.0 or 100 mg L^{-1} ($p<0.05$). After 210 d, the relative conductivity of treatment with 10 mg L^{-1} Res was 27.5%, while treatments with 0.1, 1.0 or 100 mg L^{-1} were 29.6%, 28.7% and 30.1%, respectively.

3.5 Effects of Res on MDA

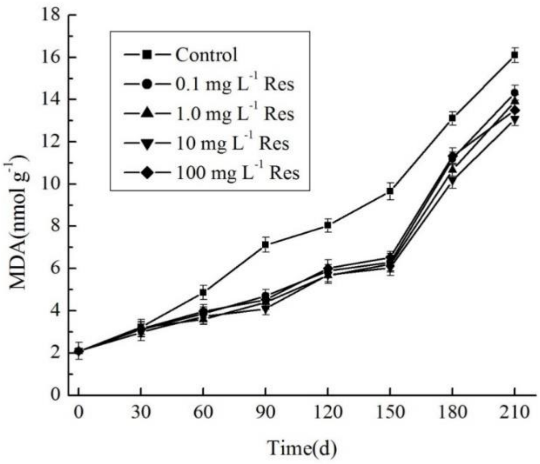

Fig. 5 Effects of Res on MDA in the peel of 'Dangshansuli' pears during cold storage. Bars indicate S.D. of means.

A growing trend of MDA content during cold storage is shown in Fig.5. The content of MDA in each treatment was dramaticlly lower ($p<0.05$) than the control ($p<0.05$). After 210 d storage, the contents of MDA treated with 0.1, 1.0, 10 or 100 mg L^{-1} Res were 14.32, 13.9, 13.08 and 13.5 nmol g^{-1}, respectively, whereas 16.1 nmol g^{-1} in the control. Among treatments, fruit treated with 10 mg L^{-1} Res revealed a lower MDA during whole storage, while the effect of 100 mg L^{-1} Res was highest, but no significant differences ($p>0.05$) were observed. The contents of MDA of pears treated with 0.1 and 1.0 mg L^{-1} Res ranged from the results of 10 to 100 mg L^{-1} Res treatments.

3.6 Effects of Res on α-farnesene

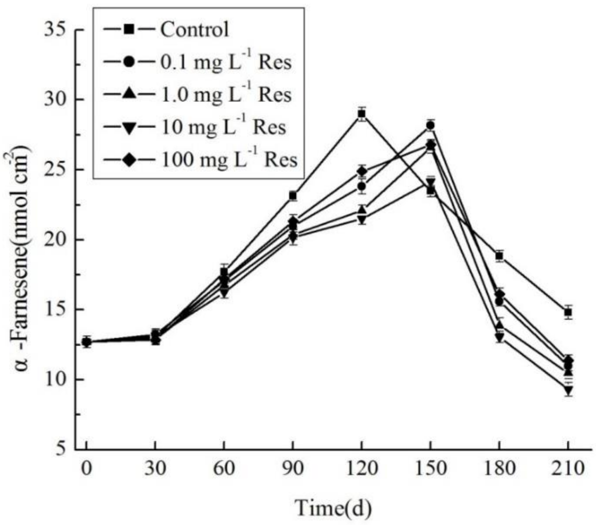

Fig. 6 Effects of Res on α-farnesene in the peel of 'Dangshansuli' pears during cold storage. Bars indicate S.D. of means.

Fig.6 shows the contents of α-farnesene continually increased to the maximum in the earlier storage period, then it declined gradually later. At the beginning of storage, α-farnesene in the control increased and reached its peak value on day 120, consequently decreased. The contents of α-farnesene in pears treated with 0.1, 1.0, 10 or 100 mg L^{-1} Res reached its maximum on day 150, which were 28.16, 26.63, 24.14 and 26.79 nmol cm^{-2}, respectively. What's more, α-farnesene in 10 mg L^{-1} Res treatment maintained minimum all the time, its value was 9.31 nmol cm^{-2} which was significantly lower ($p<0.05$) than 14.81 nmol cm^{-2} of the control after 210 d, whereas the values of 0.1, 1.0 and 100 mg L^{-1} Res treatments were 11.0, 10.49 and 11.4 nmol cm^{-2}, respectively.

3.7 Effects of Res on conjugated trienes

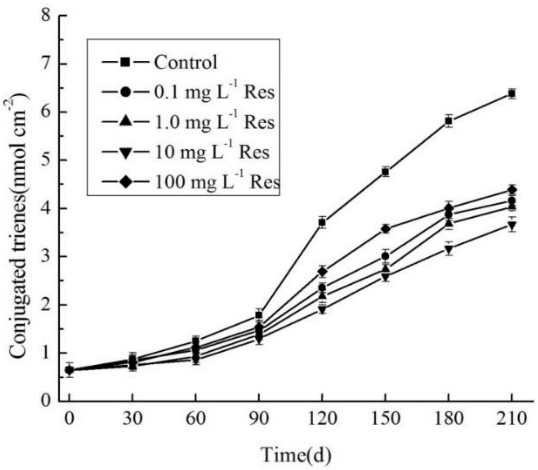

Fig.7 Effects of Res on conjugated trienes in the peel of 'Dangshansuli'pears during cold storage. Bars indicate S.D. of means.

Fig.7 shows that the contents of conjugated trienes in the peels of 'Dangshansuli' pears presented a rising trend during whole storage. And conjugated trienes in each treatment changed little within earlier 90 d, then gradually increased after that, even continued to rise after 210 d of cold storage. The contents of conjugated trienes in the control were higher ($p<0.05$) than that of each treatment, the growth was fastest and its value was 6.38 nmol cm^{-2} at the end of the storage. Whereas conjugated trienes of the pears treated with 0.1, 1.0, 10 or 100 mg L^{-1} Res were 4.17, 4.04, 3.67, 4.39 nmol cm^{-2}, respectively. And, 10 mg L^{-1} Res treatment had a better role in inhibiting the increase of conjugated trienes.

3.8 Effects of Res on superficial scald

Tab.1 Effects of Res on superficial scald in 'Dangshansuli' pears after 210 d storage at 0±0.5°C and additional 7 d shelf life at 20°C

Treatments	Control		0.1 mg L^{-1}		1.0 mg L^{-1}		10 mg L^{-1}		100 mg L^{-1}	
	210 d	210+7 d	210 d	210+7 d	210 d	210+7 d	210 d	210+7 d	210 d	210+7 d
Scald incidence(%)	45.5 b	100 a	4.3 f	21.4 c	0 g	22.4 c	0 g	14.1 d	7.1 e	20.9 c
Scald index(%)	18.2 c	71.2 a	2.9 e	23.3 b	0 f	22.9 b	0 f	15.4 d	3.1 e	22.8 b

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 $p<0.05$.

Tab.1 illustrates that the scald incidence of the control was 45.5% after 210 d storage, whereas the treatments with 0.1, 1.0, 10 and 100 mg L^{-1} Res were 4.3%, 0%, 0% and 7.1%, respectively, and the differences between control and treatments were significant ($p<0.05$). And pears treated with 0.1 and 100 mg L^{-1} Res occurred remarkable difference ($p<0.05$). Then observed at 20°C, the control fruit depicted a rapid growth, but the treatments only had a certain extent of scald. Scald incidence of the control was 100% after 7 d at 20°C. However, the incidences of treatments with 0.1, 1.0, 10 and 100 mg L^{-1} Res were 21.4%, 22.4%, 14.1% and 20.9%, respectively. And 10 mg L^{-1} Res treatment was lower than the others ($p<0.05$).

The scald index of the control was 18.2% at the end of storage, while the treatments with 0.1 and 100 mg L^{-1} Res were 2.9% and 3.1%, respectively. But there were no scald occurred in 1.0 mg L^{-1} Res treatment and 10 mg L^{-1} Res treatment. Removed from storage and placed 7 d at 20℃, the scald index in both control and treatments substantially increased, and the control's expanded most rapidly to 71.2%. Each treatment somewhat occurred, were significantly lower than the control ($p<0.05$). However, the treatment with 10 mg L^{-1} Res had a minimum index, whereas the other 3 treatments did not occur differences ($p>0.05$).

4. Discussion

Res treatment can extend the shelf life of fruits, and reduce the loss of water (namely maintained firmness) without affecting nutrition contents [49]. The present study indicates that Res delayed and prolonged the softening of 'Dangshansuli' pears. Juice rate reflects the degree of softening to some extent. Fig.3 shows that

Res treatments inhibited the decrease of juice rate. SSC is an important factor for the flavor of fruit. The decline ranges of SSC in Res treatments were smaller than the control during cold storage. Comprehensively, the results indicate that 10 mg L^{-1} Res has the optimal effect on the quality of 'Dangshansuli' pears during cold storage.

Although the physiological mechanisms leading to superficial scald is not yet completely elucidated, scald development is closely related to α-farnesene and conjugated trienes [2][11][13]. It has been indicated that Res can decrease α-farnesene and conjugated trienes of 'Dangshansu' pear [52]. In this paper, Res treatments significantly decreased α-farnesene and conjugated trienes, delayed their peak values, remarkably reduced scald incidence and scald index ($p<0.05$). It indicats that Res plays a great part in inhibiting superficial scald in 'Dangshansuli' pears.

Cell membrane structures of plants under stress conditions (such as senescence) are damaged first, revealing the increase of membrane permeability. Cell membrane integrity may be reflected by the size of the membrane structure. Electrolyte leakage can significantly increase during storage of 'Granny Smith' apples that are more susceptible to scald development [54]. Conjugated trienes damages cell membranes, which leads to browning of skin cells [55]. CT_{281} accumulation during storage is related to cell damage that leads to the browning of the fruit [9]. As one of the products of membrane lipid peroxidation, MDA also is used to measure cell membrane damage. MDA accumulation is positively correlated with scald development [56]. And, 1-MCP and DPA can decrease the electrolyte leakage related scald [5][30]. POD activity can be increased by trans-resveratrol compares to the control of 'El-Bayadi' table grapes after storage and shelf life [51]. Antioxidant enzymes have important roles in improving antioxidant capacity and eliminating reactive oxygen species, thus, reduce injury of cell membrane and enzymatic browning, finally delay senescence and prevent scald [57]. Hence, the effects of Res inhibiting scald development may have something to do with the membrane stability.

Res effectively inhibited superficial scald in 'Dangshansuli' pears, on the one hand, because of its oxidation resistance. It has been reported that Res can delay the shelf life of tomato, apples and avocadoes [49]. A study reported that calcium apparently inhibited the accumulation of MDA in 'Yali' pear, which contributing to maintain the integrity of the membrane structure, thus delayed its senescence [58]. Treatments with Res effectively restrained membrane lipid peroxidation in the peel of 'Dangshansuli' pears in the process of senescence, better maintained the integrity of peel and reduced the loss of water, hence, maintained the flesh firmness during storage period. This study reveales that Res decreases the membrane permeability and MDA in the peel, and reduces injury severity of cell membrane, thus delays maturation and senescence of 'Dangshansuli' pears. Moreover, it may be that Res inhibits or delays the occurrence of superficial scald through effectively avoiding the destructions of phenolic compounds inside the organization and PPO in regional distribution. Because the study demonstrated that reduced PPO expression occurred with inhibited scald development [59]. On the other hand, it may be related to the antifungal properties of Res. As a natural pesticide, Res can not only inhibit the growth of microbial communities, also can prevent mechanical damage which caused by microbial penetration into the peel. Therefore, it maintains a higher degree of integrity of peel [49]. There is a paper found that 'Granny smith' apples treated with vegetable oil or purified oil formed a layer of grease on the peel surface, which diminished the contact area between peel and air, consequently, the rate of superficial scald was decreased significantly [60]. Thirdly, Res treatments delayed the peak values of α-farnesene and reduced its contents, what's more, the content of α-farnesene in treatment with 10 mg L^{-1} Res was lowest all the time in cold storage. Meanwhile, it remarkably decreased conjugated trienes, and the effect of treatment with 10 mg L^{-1} Res was best. In this experiment, all treatments with different concentrations of Res significantly inhibited the occurrence of superficial scald during storage and shelf life. In addition, the morbidity of treatment with 10 mg L^{-1} Res is lowest. Maybe, Res inhibits the synthesis of α-farnesene and then decreases the content of conjugated trienes, subsequently, reduces superficial scald. Besides, superficial scald is also attributed to intermediary free radicals [14][15], and Res is a natural antioxidant. Hence, Res might play a role in inhibiting scald by scavenging free radicals. Clearly, further studies are needed to delineate the exact mechanism and effects by which Res maintains quality and inhibits scald of 'Dangshanauli' pears.

5. Conclusions

The present study shows that Res can maintain the postharvest quality and inhibit superficial scald of 'Dangshansuli' pears during cold storage and shelf period. And, the results indicate that 10 mg L^{-1} Res has the optimal effects. As an antioxidant with safe and non-toxic effects, Res is likely to be applied to the preservation and storage of postharvest 'Dangshansuli' pears.

Acknowledgements

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