Effects of MAP Treatment on Aroma Compounds and Enzyme Activities in Flat Peach during Storage and Shelf Life

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Abstract. Cold storage is used to delay the senescence of peaches, but it can also lead to internal browning and aroma loss. Modified atmosphere packaging (MAP) has been reported to inhibit the internal browning and prolong the storage time. Four MAP treatments in the present study were set as follows: I: O₂ 1% to 3%, CO₂ 3% to 5%, and N₂ 92% to 96%; II: O₂ 3% to 5%, CO₂ 3% to 5%, and N₂ 90% to 94%; III: O₂ 6% to 8%, CO₂ 3% to 5%, and N₂ 87% to 91%; and control (CK): O₂ 21%, CO₂ 0.03%, and N₂ 79%. The concentration of sugars, acids, aroma compounds, superoxide radical (O₂^{•−}), hydrogen peroxide (H₂O₂), and malondialdehyde (MDA), as well as the activities of enzymes, such as superoxide dismutase (SOD), peroxidase (POD), lipoxygenase (LOX), hydroperoxide lyase (HPL), alcohol dehydrogenase, and alcohol O-acyltransferase (AAT) activities, were investigated. The results revealed that MAP, especially for treatment II, could inhibit the loss of flavors such as sugars, acids, and aroma compounds; maintain higher SOD and POD activities; and inhibit the accumulation of O₂^{•−}, H₂O₂, and MDA during shelf life after storage at low temperature for 30 days. It could also inhibit the LOX and HPL activities at low temperature, but maintain higher LOX and HPL activities during shelf life. These findings indicated that treatment II could prolong the storage time to 30 days and shelf life for 3 days; maintain the higher content of sugars, acids, and aroma compounds; protect the cell membrane from oxidative injury; and inhibit internal browning during cold storage and shelf life.

Cold storage was used to delay ripening and decay of peaches. However, storage of peaches at low temperature also led to the development of chilling injury (CI), such as flesh meatiness, flesh leatheriness, flesh browning, flesh bleeding, loss of sensory-related compounds, and development of sensory-related compounds, limiting the storage time and shelf life of fruits, thereby reducing consumer acceptance and economic value. The content of organic acids and sugars in peach fruits determined the fruit quality, and also had an important influence on the development of fruit flavor and color (Xi et al., 2014). Sugar metabolism and signal transduction pathways were closely related to the senescence of fruits and were vital in fruit flavor, function, storage, and structure (Xu et al., 2016). Acids, which were consumed as respiration substrates and took part in a variety of metabolic processes, were also crucial

in the development of flavor and physiological metabolisms (Wu et al., 2005). Some authors considered that plants required more energy production under chilling stress (Lee et al., 2009). Anaerobic respiration occurred, leading to the fast breakdown of fruit sugars at a specifically reduced level of O₂, which was named as the Pasteur effect (Kader, 2009). Apples stored at 2% O₂ and 1% CO₂ had a higher sugar content compared with the CK, which had CI, indicating that MAP was important in apple sucrose and acid metabolism and could induce sucrose hydrolysis (Zhu et al., 2013).

Recently, aroma has gained increasing attention as another important characteristic of fruit flavor quality. With the development of science and technology, aroma compounds were normally investigated by gas chromatography–mass spectrometry (GC-MS) and other analytical apparatus such as electronic nose (Aubert and Milhet, 2007). At present, more than 100 compounds have been identified from peach fruits. However, only a few ingredients or representative compounds could determine the aroma of peach fruit (Rizzolo et al., 2013). Aubert et al. (2003) reported that hexanal, benzaldehyde, linalool, γ -decalactone, and δ -decalactone were the major aroma compounds, but Sumitani et al. (1994) reported that hexanal was not an

aroma compound. The accumulation of volatile substances was associated with peach maturity, of which the biggest change was esters (Zhang et al., 2011). Long-time storage at low temperature could reduce the loss of aroma, which was the symptom of CI (Jin et al., 2016; Murray et al., 2007). The content of *cis*-2-hexene aldehyde increased, and the content of γ -decalactone decreased significantly when the fruits had CI (Hatanska, 1993). This was similar to the decrease in the γ -decalactone content with the extension of low-temperature storage (James et al., 2005). MAP could decrease the respiratory intensity and ethylene release rate and inhibit the formation of aroma compounds (John et al., 2005; Ortiz et al., 2010; Thewes et al., 2017). The abundance of glucose-6-phosphate dehydrogenase, nicotinamide adenine dinucleotide phosphate (NADP)-dependent isocitrate dehydrogenase, and NADP-dependent malic enzyme was found to decrease in peach fruits stored at 0 °C, and a low temperature of 0 °C might regulate the endogenous H₂O₂ level, increasing the transcriptional level of genes encoding the proteins related to membrane stability (Zhang et al., 2010). The accumulation of reactive oxygen species (ROS) might induce the loss of cell membrane integrity. Protecting cell membrane from oxidative injury was thought to be a major mechanism of resistance to chilling stress, and this resistance was likely to depend on the competence of antioxidant system (Huan et al., 2017). Treatment with 5 mM oxalic acid or 2 mM salicylic acid could enhance the resistance of mango fruits to low temperature, attributed to inhibiting ROS accumulation, delaying H₂O₂ decrease, and inducing higher reducing status of ascorbate and glutathione (Ding et al., 2007).

MAP (5% O₂ and 5% CO₂) storage maintained better membrane integrity of peach fruits compared with the CK. MAP (10% O₂ and 10% CO₂) could maintain a higher content of aroma compounds than that stored at a low temperature (Wang et al., 2005). LOX, AAT, HPL, and alcohol dehydrogenase (ADH) were likely to be control points in the biosynthesis of aroma compounds through the LOX system, whose substrate specificity determined the aroma composition of many plant products despite the specific action of LOX (Thewes et al., 2017). The activity of LOX, an O₂-requiring enzyme, was slowed down by MAP conditions, which might limit the availability of straight-chain precursors of aroma compounds. However, the other three enzymes were not equally sensitive to O₂ concentration (Lara et al., 2003). Therefore, mandarins accumulated larger amounts of acetaldehyde and ethanol after harvest compared with grapefruit because of the higher activity of ADH in juice vesicles and lower permeability of their peel to gases (Shi et al., 2007).

However, the effect of MAP on the content of flavor compounds and enzyme activities in flat peach during storage and shelf life has not been investigated much. Hence, no detailed information is

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available about how the contents of sugars, acids, esters, lactones, and alcohols and enzyme activities change under different MAP conditions. The present novel study explored the relationship between

MAP and the formation of flavor compounds, selecting appropriate gas parameters to provide theoretical and technical support for further scientific research and practice.

Materials and Methods

Plant materials

Flat peach fruits (*Prunus persica* L. cv. Yulu) were obtained from the peach orchard,

Fig. 1. Changes in sucrose, glucose, fructose, and sorbitol content at low temperature.

Fig. 2. Changes in sucrose, glucose, fructose, and sorbitol content during shelf life.

Fig. 3. Changes in the contents of quinic acid, malic acid, and citric acid at low temperature.

the base of Shanghai Academy of Agricultural Sciences located in Jinshan, Shanghai, China, on 22 July 2016. Maturity was assessed on a sample of fruits that were similar in size and skin color, and had no mechanical damage. Subsequently, the fruits were immediately moved back to the experimental laboratory at the Shanghai Academy of Agricultural Sciences within 30 min, precooled at 4 °C for 24 h, and stored at (0 ± 1) °C.

Experimental design

The air inside the bag was vacuumed using a machine and the bag was filled with O₂, CO₂, and N₂ gas at different concentrations (DE Special Gas Co., Ltd., Jinan, China). The size and thickness of the bag were 0.06 × 40 × 25 cm³ and 0.06 mm, respectively. Different treatments were set as follows: I: O₂ 1% to 3%, CO₂ 3% to 5%, and N₂ 92% to 96%; II: O₂ 3% to 5%, CO₂ 3% to 5%, and N₂ 90% to 94%; III: O₂ 6% to 8%, CO₂ 3% to 5%, and N₂ 87% to 91%; and CK: O₂ 21%, CO₂ 0.03%, and N₂ 79%. A total of 1200 fruits were randomly divided into four groups of 300 fruits each in the field. Of these, 20 fruits/group every 5 d were cut into

smaller pieces (2 × 1.5 cm²), mixed, frozen in liquid N₂ at -70 °C, and stored in triplicates for determining the contents of sugars, acids, and aroma compounds. The experiments were repeated for 3 years and a similar result was obtained.

Measurements

Sugar and acid content. Each sample was ground in liquid N₂, and 5 mL of extract was prepared by adding anhydrous ethanol and 0.4% metaphosphoric acid at 80:20 v/v to 0.5 g of the sample in a centrifuge tube, soaked for 24 h, and centrifuged for 10 min at 10,000 g_n. Subsequently, 1 g of the liquid supernatant was concentrated, and redissolved in 0.5 mL ultrapure water for determining sucrose and malic acid contents (mg.g⁻¹) using an Agilent 1100 high-pressure liquid chromatography system (Agilent Technologies, Santa Clara, CA). For sucrose content, a CARBOSep CHO-620 capillary column (10 μm × 6 mm × 250 mm; Transgenomic, Inc., New Haven, CT) and a differential refraction detector were used. The column temperature was 80 °C and 15 μL of sample was injected. The mobile phase was ultrapure water. For malic acid content, a ZORBAX Eclipse

XDB-C185 capillary column (10 μm × 4.6 mm × 250 mm; Agilent Technologies) and an ultraviolet detector were used. The detector was set at λ = 190 nm. The mobile phase was 0.02 M KH₂PO₄ (pH = 4.1).

Content and types of aroma compounds. The powder (5 g) was placed in a headspace extraction bottle and homogenized with 5 mL of saturated aqueous NaCl solution for 5 min. The internal standard (Sigma Fluka Inc., St Louis, MI) was added into the mixture and extracted for 2 h at 4 °C. Agilent 7890A-5975C GC-MS (Agilent Inc.) with a capillary column (DB-WAX, 30 m × 0.25 mm × 0.25 μm; Agilent Inc.) was used to determine the content of aroma compounds. The flow of helium gas was 1 mL.min⁻¹. The column temperature was kept at 40 °C for 5 min, then raised progressively to 250 °C at an increasing rate of 5 °C.min⁻¹, and finally kept at 250 °C for 5 min. The flame ionization detector was maintained at 260 °C for the injection temperature at 250 °C. Twenty different aroma compounds were identified, and their contents (μg.kg⁻¹) were determined according to their pure standards.

SOD activity. The SOD activity was determined photochemically using the method of Huan (Huan et al., 2016) as the unit of SOD activity that caused a 50% inhibition of nitroblue tetrazolium; the unit was U.kg⁻¹.

POD enzyme activity. The POD enzyme activity was expressed as the optical density (OD) change of 0.01 for one unit of enzyme activity at 470 nm with a spectrophotometer (Beckman Inc., California, CT); the unit was U.kg⁻¹.min⁻¹. The reaction system was as follows:

- A: 0.1 mol.L⁻¹ PBS (pH 7.8) and 0.1 mM EDTA;
- B: 0.01 mol.L⁻¹ H₂O₂;
- C: 1% guaiacol.

Determination of POD activity :

- 2.5 mL of A + 0.2 mL of C + 0.3 mL of B + 1 mL of supernatant

Blank : 2.5 mL of A + 0.2 mL of

- C + 0.3 mL of B + 1 mL of PBS(pH 7.8)

The mixture was allowed to react for 3 min at 4 °C.

Contents of superoxide radical and hydrogen peroxide. The contents were measured according to the method of Xu with slight modifications. The sample (3 g) was homogenized in 5 mL of 50 mmol.L⁻¹ sodium phosphate buffer (pH 7.8) and centrifuged at 10,000 g_n for 10 min at 4 °C. The incubation mixture containing 2 mL of supernatant and 1 mL of hydroxylammonium chloride was set into a water bath for 1 h at 25 °C. Subsequently, 1 mL of sulfanilic acid and 1 mL of α-naphthyl amine were added to the incubation mixture separately. The mixture was allowed to react at 25 °C for 20 min.

Fig. 4. Changes in the contents of quinic acid, malic acid, and citric acid during shelf life.

The absorbance was read at 530 nm using a spectrophotometer (Beckman Inc.). A standard curve with NaNO_2 was used to calculate the superoxide radical (O_2^-) content, which was expressed as micromoles 1 g of fresh weight.

Furthermore, 2.5 g of the sample was homogenized in 5 mL of chilled 100% acetone and centrifuged at $10,000 g_n$ for 10 min at 4 °C. The incubation mixture contained 5 mL of 20% TiCl_4 , 0.2 mL of ammonium hydroxide, and 2 mL of supernatant. The mixture was allowed to react, producing peroxide. Subsequently, the mixture was centrifuged at $10,000 g_n$ for 10 min. The supernatant was removed and peroxide was dissolved in 5 mL of 1 mmol·L⁻¹ H_2SO_4 , which was centrifuged again. Finally, the absorbance was read at 410 nm. The hydrogen peroxide (H_2O_2) content was expressed as micromoles 1 g of fresh weight.

MDA content. Next, 2.5 g of the sample was homogenized in 10 mL of chilled 5% trichloroacetic acid, which was ground and centrifuged at $10,000 g_n$ for 10 min at 4 °C. The incubation mixture contained 2 mL of

0.67% (w/v) thiobarbituric acid and 2 mL of supernatant. It was boiled at 100 °C for 30 min, and the absorbance was read at 450, 532, and 600 nm using a spectrophotometer (Beckman Inc.).

$$\text{MDA concentration}(\mu\text{mol}) = 6.45(A_{532} - A_{600}) - 0.56A_{450}.$$

LOX activity. Next, 2.5 g of sample was homogenized in 5 mL of 50 mM sodium phosphate buffer (pH 7.0) and centrifuged at $10,000 g_n$ for 20 min at 4 °C. The supernatant comprised the enzyme extract. Subsequently, 2.7 mL of sodium phosphate buffer (pH 7.0) was kept in a water bath at 30 °C for 30 min and then mixed with 40 μL of 0.1 mol·L⁻¹ linoleic acid. Finally, 1 mL of supernatant was added to the mixture. The absorbance was read at 234 nm using a spectrophotometer (Beckman Inc.). The LOX activity was expressed as OD change of 0.001 for one unit of enzyme activity at 234 nm; the unit was $\text{U}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$.

HPL activity. Next, 1.5 g of sample was homogenized in 2 mL of chilled extracting solution and centrifuged at $10,000 g_n$ for 15 min

at 4 °C, which contained 150 mmol·L⁻¹ 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid (HEPES)-KOH (pH 8.0), 250 mmol·L⁻¹ sorbitol, 10 mmol·L⁻¹ EDTA, 10 mmol·L⁻¹ MgCl_2 , 1% glycerinum, 4% polyvinylpyrrolidone, and 0.1 mmol·L⁻¹ phenylmethylsulfonyl fluoride. The reaction system was as follows:

A: 10 mL distilled water, 200 μL 10 mmol·L⁻¹ linoleic acid, and 400 μL LOX (0.1 g·L⁻¹ borate buffer, pH 9.0), bathed at 30 °C for 2 h before use;

B: 150 mmol·L⁻¹ HEPES-KOH (pH 8.0), 2.5 mmol·L⁻¹ sorbitol, 10 mmol·L⁻¹ EDTA, and 10 mmol·L⁻¹ MgCl_2 ;

C: 1.6 mmol·L⁻¹ NADH;

D: ADH (0.05 g·L⁻¹ of borate buffer, pH 8.6);

E: supernatant.

Determination of HPL activity :

2 mL of B + 0.75 mL of A

+0.15 mL of C + 0.1 mL of D + 1 mL of E

Blank : 2 mL of B + 0.75 mL of

A + 0.15 mL of C + 0.1 mL of

D + 1 mL of PBS(pH 8.0)

The mixture was allowed to react for 3 min at 4 °C.

ADH activity. Next, 1.5 g of sample was homogenized in 5 mL of chilled extracting solution and centrifuged at $10,000 g_n$ for 15 min at 4 °C, which contained 1 mol·L⁻¹ Tris-HCl, 5 mmol·L⁻¹ dithiothreitol, 1 mM EDTA, and 1% (w/v) Polyclar AT (PVP), pH 7.4. The reaction system was as follows: 1 mL supernatant was added into 2.85 mL enzyme reaction liquid (150 mmol·L⁻¹ Tris and 0.3 mmol·L⁻¹ NAD, pH 8.0), mixed, and then added to 30 μL 95% ethanol. The absorbance was read at 340 nm using a spectrophotometer (Beckman Inc.). The ADH activity was expressed as OD change of 0.01 every minute for one unit of enzyme activity at 340 nm; the unit was $\text{U}\cdot\text{kg}^{-1}$.

AAT activity. A 2-g sample was homogenized in 5 mL of chilled extracting solution and centrifuged at $10,000 g_n$ for 20 min at 4 °C, which contained 0.1 mol·L⁻¹ phosphate buffer, 1 mmol·L⁻¹ EDTA, and 0.1% (w/v) Triton X-100, pH 8.0. The reaction system was as follows:

A: 2.5 mL MgCl_2 solution (0.1 mol·L⁻¹ phosphate buffer, 5 mmol·L⁻¹ MgCl_2 , pH 8.0);

B: 150 μL acetyl coenzyme A solution (0.1 mol·L⁻¹ phosphate buffer, 2.5 mmol·L⁻¹ acetyl coenzyme A, pH 8.0);

C: 50 μL butanol solution (0.1 mol·L⁻¹ phosphate buffer, 0.2 mol·L⁻¹ butanol, pH 8.0);

D: 0.2 mL supernatant was mixed and water bathed for 15 min at 35 °C. Subsequently, 100 μL 10 mmol·L⁻¹ DTNB was

Fig. 5. Changes in the content of aroma compounds at low temperature.

added to it and placed at room temperature for 10 min. The absorbance was read at 412 nm using a spectrophotometer (Beckman Inc.). The AAT activity was expressed as OD change of 0.01 for one unit of enzyme activity at 412 nm; the unit was $\text{mmol} \cdot \text{g}^{-1} \cdot \text{min}^{-1}$.

Statistical analyses

All data were expressed as mean $\pm$ SD. The statistical analyses were performed using the SPSS software version 17 for Windows (SPSS Inc., Chicago, IL). The treatments were compared using the Duncan's new multiple range test after the analysis of variance. A P value less than 0.05 was considered as significant.

Results

Effects of MAP treatment on sugar content during storage and shelf life. As illustrated in Fig. 1, the sucrose content of CK decreased during storage. The effects of treatments II and III were stable and significantly higher than that of CK from 15 to 30 d ($P < 0.05$). The glucose content of CK decreased, but the content of different treatments increased and was significantly higher

($P \leq 0.05$) than that of CK during storage. The fructose content of CK decreased during storage. The content of treatments II ($7.98 \text{ g} \cdot \text{kg}^{-1}$) and III ($7.17 \text{ g} \cdot \text{kg}^{-1}$) was significantly higher than that of CK ($5.60 \text{ g} \cdot \text{kg}^{-1}$) ($P < 0.05$) at 30 d and stable during storage. The sorbitol content of CK decreased, whereas the content of different treatments was stable during storage. The sorbitol content of treatment II was significantly higher than that of CK ($P < 0.05$) from 15 to 30 d. At 3 d of shelf life (Fig. 2), the sucrose and fructose contents of treatments II (121.34 and $9.73 \text{ g} \cdot \text{kg}^{-1}$) and III (118.51 and $9.76 \text{ g} \cdot \text{kg}^{-1}$) were significantly higher ($P < 0.05$) than those of CK (104.97 and $6.46 \text{ g} \cdot \text{kg}^{-1}$). The glucose and sorbitol contents of different treatments were also significantly higher ($P < 0.05$) than those of CK. No significant decrease in sucrose and glucose contents of treatments II and III was observed, whose fructose and sorbitol contents increased significantly during the shelf life. The aforementioned observations exhibited that treatments II and III could inhibit the decrease in sugar content during storage and shelf life.

Effects of MAP treatment on acid content during storage and shelf life. Acid was the

metabolic substrate of all activities, and these activities continued in fruits after harvest. The acid content changes in flat peach during different treatments are illustrated in Fig. 3. The malic acid content of treatments II and III was significantly higher than that of CK ($P < 0.05$) from 10 to 30 d. The malic acid content of treatments II and III was 1.16 and $1.24 \text{ g} \cdot \text{kg}^{-1}$, respectively, at 30 d and was significantly higher than that of CK ($0.82 \text{ g} \cdot \text{kg}^{-1}$) ($P < 0.05$). The citric acid content of CK decreased sharply, whereas the contents of treatments II and III remained higher. The citric acid content of treatment II was $0.71 \text{ g} \cdot \text{kg}^{-1}$ at 30 d, which was higher than that of treatments I and III (0.57 – $0.59 \text{ g} \cdot \text{kg}^{-1}$). The content of CK ($0.27 \text{ g} \cdot \text{kg}^{-1}$) was the lowest. The quinic acid content of all treatments decreased and was significantly lower than that of CK ($P < 0.05$) from 10 to 25 d. No regular changes in the quinic acid content of treatment III were observed. During shelf life, the malic acid and citric acid contents of CK increased sharply, which might be related to the anaerobic respiration during senescence and softening (Fig. 4). Treatments II and III could inhibit the decrease in malic acid and citric acid contents. The quinic acid

Fig. 5. (Continued)

acid content of all treatments had no significant changes. Moreover, no significant differences in malic and citric acid contents were found between different treatments and CK. The citric acid content of treatments II and III was 0.65 and 0.56 g.kg⁻¹, respectively, and lower than that of CK (0.88 g.kg⁻¹). These findings exhibited that treatments II and III could inhibit the consumption and degradation of malic acid and citric acid at low temperature, and also inhibit the accumulation by the anaerobic respiration during senescence and softening.

Effects of MAP treatment on the content of aroma compounds during storage and shelf life. Lactones and aldehydes were named “green aroma,” the esters and terpenoids were named “fruit aroma,” and lactones were named “sweet aroma.” More than 200 volatile compounds were identified, but only 19 compounds were considered as aroma compounds and detected during the development of “Yulu” flat peach: five esters (ethyl acetate, hexyl acetate, butyl acetate, *cis*-3-hexenyl acetate, and diisobutyl phthalate), six lactones (γ -decalactone, δ -decalactone, γ -hexalactone, γ -heptalactone, γ -octalactone, and γ -dodecalactone), three alcohols (1-hexanol, *cis*-3-hexen-1-ol, and *trans*-2-hexen-1-ol), three

aldehydes (hexanal, benzaldehyde, and nonanal), and two terpenoids (S-linalool and *trans*- β -ionone). The results are illustrated in Fig. 5. Four major esters were detected and investigated in the present study. A higher content of ethyl acetate (25.79 μ g.kg⁻¹) and hexyl acetate (24.66 μ g.kg⁻¹) and a low content of butyl acetate (0.32 μ g.kg⁻¹) and *cis*-3-hexenyl acetate (3.91 μ g.kg⁻¹) were found when the fruits were picked. The contents of hexyl acetate and *cis*-3-hexenyl acetate of different treatments decreased, but the contents of ethyl acetate and butyl acetate increased during storage at low temperature. The content of hexyl acetate, ethyl acetate, and *cis*-3-hexenyl acetate of treatment II was higher ($P < 0.05$) than that of CK from 20 to 30 d. Six major lactones were detected and investigated. A higher content of γ -hexalactone (220.92 μ g.kg⁻¹); a lower content of γ -heptalactone (6.976 μ g.kg⁻¹), γ -octalactone (3.73 μ g.kg⁻¹), γ -decalactone (8.99 μ g.kg⁻¹), and δ -decalactone (5.15 μ g.kg⁻¹); and the lowest content of γ -dodecalactone (0.669 μ g.kg⁻¹) were found when the peaches were picked near the height of their maturity. The γ -hexalactone content of treatment II decreased at the early stage and then increased at a later stage,

which was significantly higher than that of CK ($P < 0.05$). The contents of γ -octalactone, γ -decalactone, γ -heptalactone, and δ -decalactone decreased from 0 to 20 d and then increased from 20 to 30 d; the contents of treatments II and III were significantly higher ($P < 0.05$) than those of CK ($P < 0.05$). The γ -dodecalactone content of all treatments was extremely low with no significant ($P > 0.05$) changes during storage. The content of *trans*- β -ionone of treatment II increased and was significantly higher ($P < 0.05$) than that of CK; the content of the other treatments decreased to 0. The linalool content of CK increased during storage, whereas the content of treatments II and III decreased to zero. Four C6 compounds were detected and described as the major compounds in immature peaches with a “grassy” flavor; however, their levels decreased with maturity. The present study demonstrated a higher content of 1-hexanol (298.11 μ g.kg⁻¹) and *cis*-3-hexen-1-ol (65.08 μ g.kg⁻¹) and a lower content of *trans*-2-hexen-1-ol (2.26 μ g.kg⁻¹) when the fruits were picked near the height of their maturity. The content of 1-hexanol and *cis*-3-hexen-1-ol decreased during storage; the content of CK was higher ($P < 0.05$) than that of different treatments from 20 to 30 d. The

Fig. 5. (Continued)

content of *trans*-2-hexen-1-ol of different treatments was constant, with no significant difference ($P > 0.05$). The content of benzaldehyde and hexanal ($146.60 \mu\text{g}\cdot\text{kg}^{-1}$) was extremely high at the time of picking, but the nonanal content was extremely low. The benzaldehyde content of treatments II and III was higher than that of CK ($P < 0.05$) from 5 to 30 d. The hexanal content of different treatments decreased sharply from 0 to 10 d and was only $1\text{--}5 \mu\text{g}\cdot\text{kg}^{-1}$ at 10 d. The content of hexanal and nonanal of treatment II was close to zero from 10 to 30 d and significantly lower than that of CK ($P < 0.05$). The aroma compounds of CK were lost during shelf life after storage at low temperature for 30 d (Fig. 6), indicating that the fruits had CI. Treatments II and III could accelerate the formation and volatilization of esters and lactones, and also inhibit the accumulation of harmful substances (e.g., alcohol and benzaldehyde) during shelf life.

Effects of MAP treatment on SOD and POD activities and the contents of O_2^- , H_2O_2 , and MDA during storage and shelf life. As illustrated in Fig. 7, the SOD activity of different treatments, especially for treatment II, was higher than that of CK during storage,

which decreased sharply from 0 to 5 d and slowly from 5 to 30 d. The POD activity of CK decreased during storage. The POD activity of treatment II decreased from 0 to 10 d, increased from 10 to 25 d, and then decreased from 25 to 30 d. The POD activity of treatment II ($63.53 \text{ U}\cdot\text{kg}^{-1}$) was higher than that of CK ($29.83 \text{ U}\cdot\text{kg}^{-1}$) and other treatments ($36.95\text{--}46.42 \text{ U}\cdot\text{kg}^{-1}$) at 30 d. The O_2^- production rate of CK increased sharply and was higher than that of other treatments during storage. The O_2^- production rate of treatments I and III was higher than that of CK and treatment II from 0 to 15 d. The O_2^- production rate of treatments II ($15.66 \text{ mmol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) and III ($16.01 \text{ mmol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) was lower than that of other treatments ($21.13\text{--}26.36 \text{ mmol}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$) at 30 d. The H_2O_2 content of different treatments increased during storage; the content of treatments II and IV was lower than that of CK. The H_2O_2 content of treatment II was lower (0.20 OD/kg) than that of other treatments ($0.22\text{--}0.25 \text{ OD/kg}$) at 30 d. The MDA content of different treatments increased slowly from 0 to 15 d and sharply from 15 to 30 d; the content of treatment II was lower than that of other treatments from 15 to 30 d. The MDA content

of treatment II ($2.88 \text{ mmol}\cdot\text{kg}^{-1}$) was the lowest among different treatments ($3.31\text{--}4.84 \text{ mmol}\cdot\text{kg}^{-1}$) at 30 d. The SOD and POD activities decreased, and the O_2^- production rate, H_2O_2 content, and MDA content of different treatments increased irreversibly during shelf life (Fig. 8). The SOD and POD activities of treatment II were significantly higher than those of CK. The O_2^- production rate, H_2O_2 content, and MDA content of treatment II were the lowest during different treatments at 3 d. The aforementioned findings demonstrated that treatment II could maintain higher SOD and POD activities and inhibit the accumulation of hazardous substances, such as O_2^- , H_2O_2 , and MDA, thereby keeping the membrane integrity.

Effects of MAP treatment on LOX, HPL, ADH, and AAT activities during storage and shelf life. As illustrated in Fig. 9, the LOX activity peak of CK appeared at 10 d and the peak value was $136.8 \text{ U}\cdot\text{kg}^{-1}$. Treatment III could delay the appearance of LOX activity peak (appeared at 15 d) and decrease the peak value ($76.27 \text{ U}\cdot\text{kg}^{-1}$); treatment II could inhibit the LOX activity at an early stage (0–15 d) and maintain higher LOX activity at a later stage (15–30 d). The AAT activity of different

Fig. 6. Changes in the content of aroma compounds during shelf life.

treatments decreased during storage and the peak appeared at 10 d, which was significantly higher than that of CK from 20 to 30 d. No significant differences were found during different MAP treatments from 15 to 30 d. At last, the AAT activity of treatment II ($6.42 \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$) was higher than that of CK ($5.61 \mu\text{mol}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$) at 30 d. The HPL activity of CK decreased sharply from 10 to 30 d, whereas the activity of different MAP treatments decreased from 0 to 10 d and then increased; the HPL activity of treatment II was higher than that of CK. The ADH activity of different treatments decreased during storage, especially from 15 to 30 d; no significant difference was found between different MAP treatments and CK. The LOX activity increased, HPL activity decreased, and ADH and AAT activities of treatment III did not significantly change during the shelf life (Fig. 10). The LOX activity of treatment III ($56.12 \text{ U}\cdot\text{kg}^{-1}$) was higher than that of other treatments ($20.26\text{--}21.82 \text{ U}\cdot\text{kg}^{-1}$); the lowest was that of CK ($18.48 \text{ U}\cdot\text{kg}^{-1}$). The HPL activity of different treatments ($79.96\text{--}88.13 \text{ U}\cdot\text{kg}^{-1}$)

was higher than that of CK ($60.08 \text{ U}\cdot\text{kg}^{-1}$). No significant difference was found in ADH and AAT activities between treatments I and III and CK. The aforementioned findings revealed that treatment II could inhibit the LOX and HPL activities at an early stage and maintain higher LOX and HPL activities at a later stage. It could also maintain higher LOX activity during shelf life, which might induce the formation of esters and lactones after ripening.

Discussion

This novel study explored the relationship between MAP and the formation of flavor compounds, selecting appropriate gas parameters, to provide certain theoretical and technical support for scientific research and practice. The present study found that MAP treatments, especially treatment II, could maintain higher SOD and POD activities; inhibit the accumulation of harmful substances, such as O_2^- , H_2O_2 , and MDA; inhibit the LOX and HPL

activities at the early stage and maintain higher LOX and HPL activities at later storage; and maintain higher LOX activity during shelf life. Therefore, it could inhibit the loss of flavors such as sugars, acids, and aroma compounds.

Effects of MAP treatment on LOX, HPL, ADH, and AAT activities during low-temperature storage and shelf life. LOX, AAT, HPL, and ADH activities were likely to be the control points in the biosynthesis of aroma compounds through the LOX system, whose substrate specificity determined the aroma composition of many plant products despite the specific action of LOX (Thewes et al., 2017). The LOX activity of different treatments declined during storage from 0 to 5 d, which might be related to self-protection in the low-temperature environment (Thewes et al., 2017). The activity of LOX, an O_2 -requiring enzyme, reduced under MAP conditions, which might have an important role in limiting the availability of straight-chain precursors of aroma compounds (Lara et al., 2003). Treatments II and III could delay the

Fig. 6. (Continued)

appearance of LOX activity peak and decrease the LOX activity, indicating that appropriate MAP conditions could inhibit the senescence process and the formation of esters and lactones (Figs. 5 and 9). The LOX activity decreased sharply from 15 to 30 d, which might be related to ROS accumulation and protection of cell membrane from oxidative injury of resistance to chilling stress; appropriate MAP conditions could inhibit this process (Huan et al., 2017). The LOX activity increased when the fruits were moved from MAP conditions to the normal atmosphere and was higher than that of other treatments, indicating that the formation of esters and lactones was related to not only the LOX activity but also temperature and O_2 concentration. HPL was likely to be another control point in the biosynthesis of aroma compounds through the LOX system, whose substrate specificity determined aroma composition of many plant products despite the specific action of LOX (Wang et al., 2016). The HPL activity of treatments II and III increased and was higher than that of CK from 10 to 30 d and during shelf life; the HPL

activity decreased sharply from 20 to 30 d. The production of butyl acetate, ethyl acetate, and lactones increased during long-term MAP storage (Fig. 5), in contrast to relatively low AAT activity (Fig. 9), which decreased significantly with longer chilling periods, indicating that the AAT activity was not so important for the formation of esters and lactones. No significant changes were observed in the AAT activity of different treatments when the fruits were transferred from MAP to shelf life; the activity of treatments II and III was higher than that of CK. The ADH activity decreased sequentially. The alcohol and hexanal content increased sharply from 20 to 30 d, which might be the major harmful substances produced during fruit senescence because it was related to the downregulation of ADH activity (Jim et al., 2002). Mandarins accumulate larger amounts of acetaldehyde and ethanol after harvest than grapefruit because of higher ADH activity in the juice vesicles and lower permeability of their peel to gases (Shi et al., 2007). No significant differences were observed in ADH activity during different

treatments, which was similar to the result that the hexanal content of different treatments was not significantly different during low-temperature storage and shelf life. No significant effect of low O_2 concentration on ADH activity was observed, probably because ADH is not an O_2 -requiring enzyme.

Effects of MAP treatment on SOD, POD, O_2^- , H_2O_2 , and MDA during low-temperature storage and shelf life. ROS accumulation might induce the loss of cell membrane integrity, which became macroscopically visible through the enzymatic oxidation of phenolic compounds to brown-colored polymers (Franck et al., 2007). The MAP treatment could maintain higher SOD and POD activities and inhibit the accumulation of harmful substances, such as O_2^- , H_2O_2 , and MDA in the present study, which was related to the protection of cell membrane from oxidative injury and thought to be a major mechanism of resistance to chilling stress. This resistance was likely to depend on the competence of antioxidant system (Babalar et al., 2018). The result was similar to the finding that MAP (5% O_2 and 5% CO_2) storage maintained better

Fig. 6. (Continued)

membrane integrity of peach fruits compared with the CK (Wang et al., 2005). Moreover, MAP treatment could regulate the endogenous H_2O_2 level, activating the transcriptional level of genes encoding the proteins related to membrane stability (Zhang et al., 2010), because O_2^- and H_2O_2 acted as potential signaling molecules in the middle stage of fruit development, and only H_2O_2 might function as the main toxic molecule to stimulate lipid peroxidation and oxidative stress in the late stage of fruit ripening (Huan et al., 2016).

Effects of MAP treatment on aroma compounds during low-temperature storage and shelf life. MAP treatment could inhibit the respiratory intensity and ethylene release rate and then inhibit the formation of aroma compounds (John et al., 2005; Ortiz et al., 2010; Thewes et al., 2017). The γ -hexalactone, γ -decalactone, and δ -decalactone contents of treatments II and III were significantly higher than those of CK in the present study, which were extremely important for the formation of critical peach aroma (Braga and Belo, 2016). The aforementioned finding revealed that MAP treatment could inhibit the loss of aroma and CI (Hatanska, 1993), which was similar to the finding of James (James et al.,

1990) that the γ -decalactone content of peach fruits with CI decreased sharply, but was different from the result that MAP treatment could inhibit the formation of volatile compounds (John et al., 2005). Both acetate and hexyl acetate, especially hexyl acetate, were considered as key odorants influencing the flavor quality of peach fruits (Eduardo et al., 2010). Hexyl acetate may be the key factor affecting aroma (Jin et al., 2016) and MAP could inhibit the loss of aroma (Ortiz et al., 2010). Treatments II and III could significantly inhibit the decrease in hexyl acetate and cis-3-hexenyl acetate in flat peach during low-temperature storage in the present study. The amount of linalool was higher especially before harvest, which was supposed to be one of the major compounds in mature peaches and nectarines (Rizzolo et al., 2013). The amount of CK and treatment II increased during storage, but the amount of other treatments decreased to close to 0. Previous studies reported that C6 compounds were also major compounds when the fruits matured (Wang et al., 2009). The content of 1-hexanol and hexanal decreased during low-temperature storage, and the 1-hexanol content of CK was higher than that of MAP-treated fruits from 20 to 30 d in the present

study, which might be the major harmful compounds produced during fruit senescence (Jim et al., 2002). The benzaldehyde content of treatments II and III was higher than that of CK from 5 to 30 d, which was harmful to the fruits during senescence (Aubert et al., 2003). During the shelf life, the lactone, ester, and *trans*- β -ionone contents of MAP treatment increased, indicating that MAP treatment could inhibit CI and maintain the flavor of peach (Ortiz et al., 2010). The content of benzaldehyde increased sharply, which might be related to stress reaction after the fruit was injured (Aubert et al., 2003).

Effects of MAP treatment on sugar and acid content during low-temperature storage and shelf life. Fruits required more energy under chilling stress (Lee et al., 2009). The appropriate concentration of oxygen and carbon dioxide could significantly inhibit the decrease in sucrose, fructose, glucose, and sorbitol content from 15 to 30 d, especially the glucose, in the present study. The result indicated that MAP treatment could inhibit the fruit sugar metabolism and maintain the inherent flavor well (Xi et al., 2014; Zhu et al., 2013). The sugar content of treatment I was significantly lower than that of other treatments and CK during storage for

Fig. 7. Changes in superoxide dismutase (SOD) and peroxidase (POD) activities and the contents of O_2^- , H_2O_2 , and malondialdehyde (MDA) at low temperature.

Fig. 8. Changes in superoxide dismutase (SOD) and peroxidase (POD) activities and the contents of O_2^- , H_2O_2 , and malondialdehyde (MDA) during shelf life.

Fig. 9. Changes in lipoxygenase (LOX), hydroperoxide lyase (HPL), alcohol dehydrogenase (ADH), and alcohol *O*-acyltransferase (AAT) activities at low temperature.

Fig. 10. Changes in lipoxygenase (LOX), hydroperoxide lyase (HPL), alcohol dehydrogenase (ADH), and alcohol *O*-acyltransferase (AAT) activities during shelf life. All the values are expressed as means $\pm$ SE of three replicates. The different letters indicate significant differences among different treatment and control groups at each time point ($P < 0.05$). No letters indicate that differences were not detected.

0–20 d, which might be related to the Pasteur effect (Kader, 2009). Appropriate concentrations of oxygen and carbon dioxide could also significantly inhibit the decrease in malic acid and citric acid content, which was consistent with the report that fruits stored at appropriate concentrations of oxygen and carbon dioxide had a higher acid content compared with CK (Liu et al., 2016). The malic acid and citric acid contents of treatment I increased sharply during shelf life, indicating that the fruits underwent anaerobic

respiration (Kader, 2009) and GABA accumulation (Lum et al., 2016). It was similar to ethanol accumulation in the apple fruits or its release into the atmosphere at a specifically reduced level of O_2 (Veltman et al., 2003).

The present study found that the long-term storage of flat peach at low temperature could induce the loss of flavors such as sugars, acids, esters, and lactones, as well as browning. Treatment II could inhibit the LOX and HPL activities at the early stage and maintain higher LOX and HPL activities

at a later stage, consistent with the changes in the content of aroma compounds. It could also maintain higher LOX activity during shelf life, which might induce the formation of esters and lactones after ripening. Treatment II also could maintain higher SOD and POD activities and inhibit the accumulation of harmful substances, such as O_2^- , H_2O_2 , and MDA, which was related to the protection of cell membrane from oxidative injury and thought to be a major mechanism of resistance to chilling stress and

the loss of aroma compounds, sugar, and acid content. The present study could provide certain theoretical and technical support for scientific research and peach storage technology. However, the effect of enzymes on the development of flavor in peach needs further investigation.

Conclusions

MAP, especially for treatment II, could inhibit the loss of flavors such as sugars, acids, and aroma compounds (especially γ -hexalactone, ethyl acetate, and butyl acetate content); maintain higher SOD and POD activities; and inhibit the accumulation of O_2^- , H_2O_2 , and MDA during shelf life after storage at low temperature for 30 d. It could also inhibit the LOX and HPL activities at low temperature, but maintain higher LOX and HPL activities during shelf life. These findings indicated that treatment II could prolong the storage time to 30 d and shelf life for 3 d; maintain the higher content of sugars, acids, and aroma compounds; protect the cell membrane from oxidative injury; and inhibit internal browning during cold storage and shelf life.

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