

REGULATION OF NITRIC OXIDE TREATMENT IN THE BIOSYNTHESIS OF AROMA VOLATILES IN PEACH FRUIT DURING STORAGE

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ABSTRACT

Background. Nitric oxide (NO) is an important signaling molecule which could regulate different aspects of fruit ripening and senescence. However, the effects of NO treatment on the aroma volatiles of postharvest peaches is rarely reported.

Materials and methods. In the present study, ‘Xiahui 6’ peaches were fumigated with 10 $\mu\text{L}\cdot\text{L}^{-1}$ NO for 3 h, then stored at 20°C for 9 days. Fruit firmness, SSC, TA, ethylene production, the content of fatty acids and aroma volatiles, and related gene expression were evaluated during storage.

Results. Nitric oxide significantly maintained higher fruit firmness, SSC and TA at the end of storage, thus maintaining higher fruit quality. The gene expression of *PpACS1/3*, *PpACO* were significantly depressed in NO-treated peaches, which resulted in the reduction of ethylene production. In addition, NO treatment delayed the decline of C6 volatiles, inhibited the formation of esters and lactones supported by a lower expression level of *PpLOX3/4*, *PpAAT1/2*, *PpACX1/2/3/4*, *PpFAD1/3* and a higher transcript abundance of *PpLOX2*, *PpHPL1*, *PpADH1*. Additionally, the content of PA, SA, OA, LA and LeA reduced in response to NO treatment during the second half of storage, accompanied by a lower mRNA level in *PpFAD1/2/3*.

Conclusions. The effects of NO on peach aroma volatiles during storage were novel among the study of NO in peaches. In this study, NO maintained higher fruit quality, delayed the ripening and senescence of peach fruit after harvest. For aroma volatiles, NO maintained higher levels of C6 volatiles and reduced the content of esters and lactones in peaches, as well as the content of PA, SA, OA and LeA. These results together suggest that the effects of NO on peach aroma volatiles are a result of the regulation of genes related with the biosynthesis of aroma compounds, ethylene and fatty acids.

Keywords: postharvest treatment, aroma volatiles, ethylene biosynthesis, fatty acids, gene expression

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INTRODUCTION

Peach [*Prunus persica* (L.) Batsch] is favored by consumers and cultivated widely around the world, possessing both high edible value and research value for its juicy texture, high nutrient content and pleasant flavor. The flavor is defined as the combination of taste (sweetness, acidity and bitterness) and odor (aroma) (Xi et al., 2012a). Aroma is one of the main properties that affects fruit quality and has gained more and more attention in recent years for its dominant role in consumer preference and acceptance (Bruhn et al., 1991). More than 100 aroma volatiles have been identified in peach fruit, including aldehydes, alcohols, esters, lactones, ketones and terpenes (Aubert and Milhet, 2007).

The majority of these compounds are products of fatty acid (FAs) metabolism, with two pathways, the lipoxygenase (LOX) pathway and β -oxidation pathway, involved in it (Schwab et al., 2008). In peaches, C6 aldehydes, alcohols and esters are derived from the LOX pathway, with LA (18:2) and LeA (18:3) as the main precursors. Lactones, the major contributors of perceived peach aroma, originate from saturated FAs in the β -oxidation pathway (Lavilla et al., 2002). During the process of fruit ripening, C6 aldehydes and alcohols, which impart ‘green-note’ odors, generally decrease, while esters and lactones are perceived as ‘fruit-like’ odors which increase with the maturity of fruit (Aubert et al., 2003). Peach aroma volatiles can be affected by fruit cultivars (Aubert et al., 2019), fruit maturity (Ceccarelli et al., 2019), cultivation measures (Xi et al., 2014) and postharvest treatments (Zhou et al., 2019). However, although the effects of many postharvest treatments on peach aroma volatiles have been studied thoroughly, the effects of NO fumigation on aroma quality in peaches during ripening is rarely reported.

As an important signal molecule, NO plays an important role in various response reactions to biotic and abiotic stresses in plants and induces signal transduction and some related gene expression, which directly or indirectly imparts stress tolerance to plants (Wang et al., 2021). It has been reported that treatment with proper concentrations of NO maintains the quality and extends the shelf life of fruit after harvest (Corpas and Palma, 2018). Zhu et al. (2006) presumed that NO binds to 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase to form an ACC oxidase-NO complex,

which is chelated by ACC to produce an ACC-ACC oxidase-NO complex, leading to a decrease in ethylene production in peach fruit. The study of NO at the proteomic level in peach fruit found that NO could induce the enzymes of the antioxidant system, affect ethylene biosynthesis, repress the degradation of cell walls and inhibit electron transport and oxygen consumption, thus delaying the senescence of peach fruit (Kang et al., 2016). Nitric oxide treatment was also reported to maintain fruit quality by regulating sucrose metabolism during storage (Han et al., 2018). Recent research in peaches during cold storage found that NO treatment could maintain mitochondrial function, alleviate chilling injuries by regulating the metabolism of lipids and cell walls and enhance antioxidant capacity (Song et al., 2021; Wang et al., 2021; Zhao et al., 2021).

However, although the effects of NO on the storage and preservation of postharvest peach fruit have been reported, little is known about its effect on peach aroma quality, which is a very important attribute of peaches during fruit ripening. Thus, the objective of this study was to investigate the effects of postharvest NO treatment on peach aroma volatiles during storage at the levels of physiology, biochemistry and molecules. Firmness, soluble solid contents (SSC), titratable acid (TA), ethylene production, the composition and content of aroma volatiles and FAs and transcript abundance of gene encoding related enzymes in volatile formation were examined in the present study to get a comprehensive insight into the regulation mechanism of NO treatment in peach aroma volatiles during fruit ripening.

MATERIALS AND METHODS

Materials and treatments

Melting-type, mid-season peaches (*Prunus persica* L. Batsch cv. Xiahui 6) were picked from an orchard in Nanjing, Jiangsu Province, China in early July. Only fruit of a uniform size and with no obvious decay on the surface could be selected as experimental materials. For NO treatment, peaches were sealed into boxes and fumigated with 10 mL·L⁻¹ NO with nitrogen (Nitrogen, 99%, Nanjing special gas Factory Co., Ltd., Nanjing, China) as a carrier gas for 3 h according to the method reported by (Han et al., 2018). Control peaches were flushed with pure N₂ for the same time at 20°C. A 1% (w/v) KOH solution was also put into

the boxes to absorb the carbon dioxide produced by the peaches. Both NO-treated and control groups were replicated three times. After treatment, the peaches were ventilated for 2 h at 20°C, then stored at 20 ± 1°C for 0, 1, 3, 5, 7 and 9 days with about 90% relative humidity. At each sampled point, after the measurement of firmness, SSC, TA and ethylene production, the peach flesh was frozen in liquid nitrogen and stored at –80°C in a fridge for further analysis.

Determination of quality and physiology parameters

Fruit firmness was measured using a Fruit Hardness Tester (FHM-1, Takemura Electric Works Ltd., Tokyo, Japan) equipped with an 8.0 mm diameter conical probe head. The probe head was penetrated into the fruit mesocarp at a final penetration depth of 10 mm by removing a 1 mm-thick section of peel. Each fruit was measured twice on opposite sides at the equator. The results were expressed in newtons (N).

The peeled peaches were squeezed into juice for the measurement of SSC and TA using a digital handheld refractometer (Atago, PAL-1, Tokyo, Japan). The results of SSC and TA were expressed as °Brix and percentage (%), respectively.

Three replicates with five fruit of each replicate were used for the measurement of ethylene production according to the method reported by Khan and Singh (2008). The peaches were sealed in 3.18 L gas tight glass jars for 1 h. After that, 1 mL headspace gas was taken up into a syringe and injected into a gas chromatograph (GC, Agilent Technologies 7890A) equipped with an HP-AL/S column (30 m × 0.53 mm × 15 mm, Agilent, USA) and flame ionization detector (FID). 99.9% N₂ was used as a carrier gas with a flow rate of 3 mL·min⁻¹. The temperatures of the oven, inlet and detector were 100°C, 120°C and 200°C, respectively. The ethylene in the samples was identified according to the peak time of ethylene standard gas. The ethylene content in the samples was determined using a standard curve of ethylene standard gas with different concentrations. The results were expressed as nmol kg⁻¹·s⁻¹.

Determination of aroma volatiles during ripening

The extraction, measurement and analysis of the aroma volatiles in the peaches were in line with a previous method with some modifications (Cai et al.,

2018). Briefly, the frozen pulp tissue was ground into a powder under liquid nitrogen, then the powder was transferred to a 30 mL glass vial. Before being sealed, internal standard 2-octanol (10 µL, 8.14 mL·L⁻¹) and 3 mL saturated sodium chloride were added to the vial. Then the sample was mixed and equilibrated using a magnetic stirrer at 40°C for 20 min, after which volatile compounds were obtained through headspace solid-phase microextraction-absorption using a fiber coated with 65 µm of polydimethylsiloxane and divinylbenzene (Supelco, Bellefonte, USA) at 40°C for 30 min. The fiber filled with volatile substances was inserted into the splitless injection port of GC (7890A, Agilent Technologies Inc., California, USA) to desorb at 240°C for 5 min. The oven temperature was firstly maintained at 40°C for 3 min, then increased to 130°C with a rate of 3°C·min⁻¹ and kept for 2 min, before finally being increased to 240°C by 8°C·min⁻¹. High purity helium (99.9%) acted as a carrier gas at a flow rate of 1.0 mL·min⁻¹. 70 eV electron ionization and a full scan mode of 29–540 mass units was used to set mass spectrometry (MS 5975A, Agilent Technologies Inc., California, USA). The Aroma compounds and internal standard were identified through comparison with a database of NIST2008 libraries (the minimum matching requirement was 80%). The relative quantification of these compounds was obtained based on the concentration of the internal standard.

Determination of FAs

The composition and content of FAs in the peaches were assayed according to a previous method (Wang et al., 2017). Briefly, 6 g of frozen flesh tissue was ground into a powder under liquid nitrogen, then 15 mL of hexane was added: isopropanol (3:2, v/v) and 7.5 mL 6.7% sodium sulphate (w/v) into powdered samples for the extraction of total lipids. The total FAs were transformed into corresponding fatty acid methyl ester by adding 3 mL of methanol: toluene: sulphuric acid (88:10:2, v/v/v) to the total lipids phase. The mixture was incubated at 80°C for 1 h, before being naturally cooled for further analysis. 1 mL of heptane was added to the mixture, and it was blended carefully, then the upper phase was collected for analysis in GC (Thermo Fisher Scientific Inc., MA, USA) fitted with an FID and a Sp-2560 column (100×0.25×0.2 µm, Supelco, Bellefonte, USA). The GC conditions were set

as follows: injection temperature, 220°C; initial oven temperature, 140°C for 2 min, before being increased to 220°C at 4°C·min⁻¹ and kept for 10 min. Nitrogen was used as the carrier gas with a flow rate of 1 mL·min⁻¹. Identification and quantification of compounds was confirmed by comparison with FA standards.

Real-time quantitative PCR (RT-qPCR) analysis of genes encoding related enzymes

RNA was extracted from the frozen pulp tissue using a MiniBEST Plant RNA Extraction Kit (TaKaRa Bio

Inc., Kusatsu, Japan) and purified using an RNase-free DNase (TaKaRa Bio Inc., Kusatsu, Japan). The Prime-Script™ RT Master Mix (TaKaRa Bio Inc., Kusatsu, Japan) was used for the synthesis of first-strand cDNA according to the manufacturer's instructions. The primers used in this study were designed by Primer Premier 5 software. Only primers with high specificity and 95–105% amplification efficiency could be used for final PCR analysis. The detailed information of gene-specific primers is shown in Table S1.

Table S1. Primers for real-time PCR

| Gene | Gene accession number | Forward primer (5'–3') | Reverse primer (5'–3') | Product size, bp |
|---------------|-----------------------|--------------------------|--------------------------|------------------|
| <i>PpSAMS</i> | Ppa006841 m | CCATCTTCCATCTCAACCCATCTG | TCCCATCCTCCATAGGTGTCAA | 107 |
| <i>PpACS1</i> | Ppa005017 m | CCCCTA AGGCATTGGACTACGA | CTTGGCATAAAGCAACCACCTG | 198 |
| <i>PpACS2</i> | Ppa005032 m | GACTGGTTGGCGACTTGGATA | GCTCCCTTGGGTCTGAAATCTT | 249 |
| <i>PpACS3</i> | Ppa004774 m | CGAAAGCGAGAGGTGGTAGAGTCA | GATAGTAAGGAGAGGGGACGAGGA | 138 |
| <i>PpACS4</i> | Ppa005521 m | TACTGGACTTGTGTGGACCCTG | TCTTTGAGATTGCGGACTTGAGC | 239 |
| <i>PpACO</i> | Ppa008791 m | TGGACTGGGAAAGCACCTTCTACT | AGGGTTGGGACAAGGAGGGTAGTT | 254 |
| <i>PpFAD1</i> | Prupe.7G076500 | CGCTCCTTCTCCTATGTCTTTT | TGGAGGATTAGACCGACTGTG | 230 |
| <i>PpFAD2</i> | Prupe.7G261900 | GCACCATACGGCACCTCATA | TCTTGAAGCGACTTGTGAGC | 201 |
| <i>PpFAD3</i> | Prupe.2G251200 | CAATACAAGCGCAACAACGA | GGCGACCATAAAAAGAAAAGAG | 241 |
| <i>PpFAD4</i> | Prupe.6G278800 | CGGATTTTCTCTAACCACC | CTCCCTCTAAAGCACGCACT | 111 |
| <i>PpLOX1</i> | Prupe.1G011400 | GTGGACTCACTGGGAGAGGA | GTTGCACGACCATTTCACAC | 125 |
| <i>PpLOX2</i> | Prupe.1G232400 | TCACTACGACAAGCGGAACG | GGTAGGACGGTTTGGCACAT | 160 |
| <i>PpLOX3</i> | Prupe.6G182600 | TCCAGAAACGGCGTCATACT | ACAAGCACAACAGCCAGATTA | 135 |
| <i>PpLOX4</i> | Prupe.4G047800 | CAATACACACCCACACCCTAG | GCCTCCTCAAACCTTTCATCT | 129 |
| <i>PpHPL1</i> | Prupe.3G213800 | ACAAAATGCTTAGTTGGTGCTG | CAATCTTGACAGTGGGGAGG | 112 |
| <i>PpADH1</i> | Prupe.8G212700 | AACGCCCGACTAGTTTGTG | CGATCATTCTCGGCAAATC | 138 |
| <i>PpAAT1</i> | Prupe.5G018200 | TTGGAGAGGTTTGGAGAGGA | AGCCACACAACACAAGACA | 124 |
| <i>PpAAT2</i> | Prupe.5G018000 | ATGTGACGCAGTAGGGTTGG | GTTTCCTGAGGGCTGTAATCT | 251 |
| <i>PpACX1</i> | Prupe.3G127000 | CTCGCCTGACTACAACCAA | CCACCCACAAAAAGAAATG | 210 |
| <i>PpACX2</i> | Prupe.6G279200 | TACCTTTCCTCCCTGCACTT | GGACACCATAACCCTTGAT | 216 |
| <i>PpACX3</i> | Prupe.5G065100 | CAGGGAAGGGAGTGAAAGAA | GCCAAGAAGTGAAGCAAGAT | 227 |
| <i>PpACX4</i> | Prupe.6G181800 | GAGACCCACCAAACCTTCTCC | GCTACTTGTGTATTTCCCATCC | 168 |

PCR analysis was carried out on a Fast Real-Time PCR System (Bio-Rad CFX96, USA) by using SYBR® Premix Ex Taq™ (TaKaRa Bio Inc., Kusatsu, Japan) and primers in a total volume of 20 µL. The thermal cycling conditions were set according to Huan et al. (2016). Translation elongation factor 2 was used as the reference gene for its high expression stability (Tong et al., 2009). The value of the cycle threshold was used to calculate gene expression according to the 'Comparative 2^{-ΔΔCT}' method described by Livak and Schmittgen (2001). RNA extraction and cDNA synthesis were performed in triplicate.

Statistical analysis

All data was calculated using Microsoft Excel 2007 and expressed as means ± standard deviation (SD).

The data was further processed using the analysis of variance (ANOVA) with Duncan's multiple range test at $p < 0.05$ level using SASV8 software (SAS Institute Inc., Cary, NC, USA). All figures were made using Origin Pro 7.5 G (Microcal Software, Northampton, MA, USA).

RESULTS

Changes in quality and physiology parameters

Fruit firmness showed a decreasing trend in both groups, and the NO-treated peaches were firmer than the untreated peaches during the whole storage period (Fig. 1A).

Soluble solid contents (SSC) in both groups increased before day 7, then decreased until the end of storage.

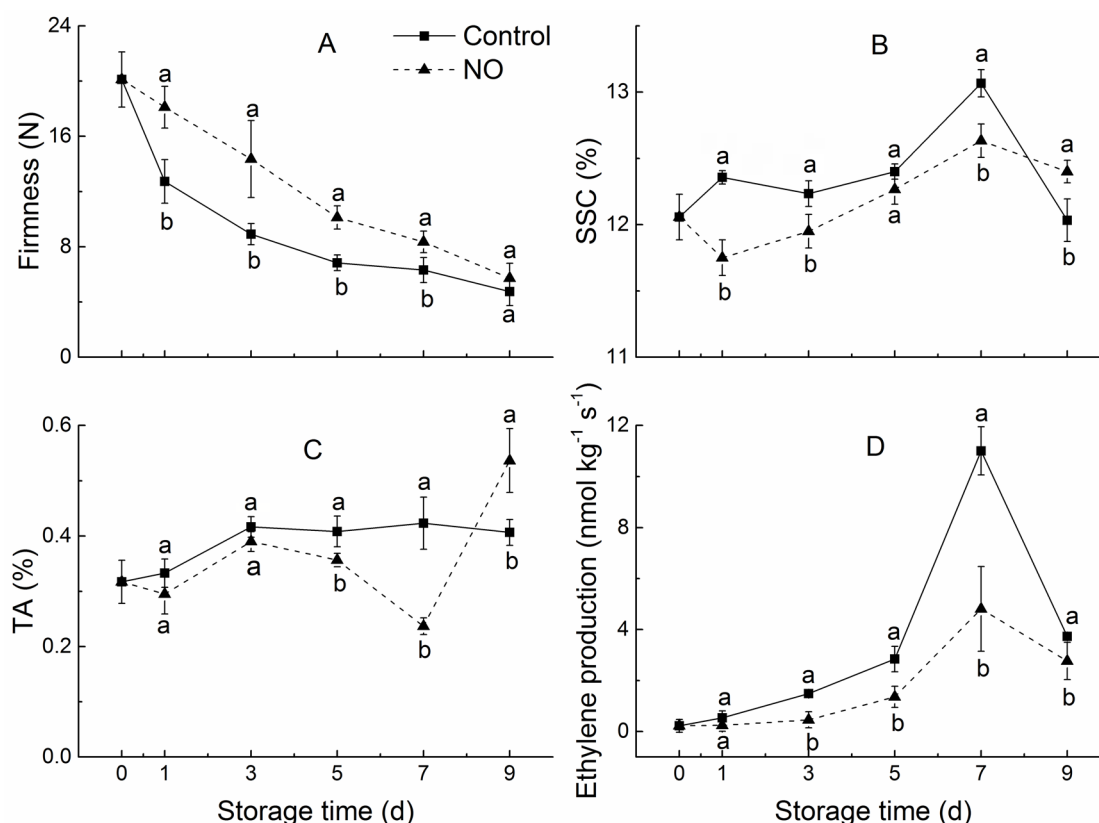


Fig. 1. Change of **A** – firmness, N, **B** – soluble solid contents, SSC, %, **C** – titratable acid, TA, %, and **D** – ethylene production, nmol kg⁻¹·s⁻¹, in peaches during 20°C storage. All the values are expressed as means ±SD of three replicates. The different normal letters indicate significant differences at 0.05 level (Duncan's test) and the same letter means no significant difference between Control and NO-treated peaches

The NO treatment resulted in lower SSC before day 9 and higher SSC on day 9 than the control peaches (Fig. 1B). Similarly, TA was inhibited by the NO treatment before day 9, although no significant difference was found between the two groups before day 5 (Fig. 1C).

Ethylene production in both the treated and control peaches reached the peak on day 7, and NO observably restrained the ethylene level and was about 1.28-fold lower than the untreated peaches on day 7 (Fig. 1D).

Changes in aroma volatiles

In total, 22 aroma volatiles were identified using SPME-GC-MS, including 5 aldehydes, 2 alcohols, 3 esters, 5 lactones, 4 terpenes and 3 ketones (Table 1). Of these aroma compounds, only volatiles derived from FA metabolism (3 C6 aldehydes, 2 alcohols, 3 esters and 5 lactones) were further analyzed by related gene expression. Overall, C6 aldehydes and alcohols decreased and lactones increased, while esters

Table 1. Changes of aroma volatiles in peach fruit treated with NO during storage at 20°C

| Aroma volatiles | Groups | Concentration of measured components, ng·g ⁻¹ FW | | | | | |
|-------------------------------|---------|---|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| | | 0d | 1d | 3d | 5d | 7d | 9d |
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Aldehydes | | | | | | | |
| Hexanal | Control | 532.01 ±22.78 | 547.41 ±35.50 ^a | 408.42 ±48.47 ^b | 225.65 ±84.04 ^b | 125.25 ±34 ^b | 41.22 ±2.87 ^b |
| | NO | 532.01 ±22.78 | 563.26 ±19.54 ^a | 732.06 ±51.56 ^a | 558.92 ±31.01 ^a | 202.33 ±31.36 ^a | 64.53 ±4.25 ^a |
| 2-Hexenal, (E)- | Control | 2.52 ±0.17 | 2.07 ±0.23 ^a | 1.64 ±0.39 ^b | 1.08 ±0.33 ^b | 1.72 ±0.19 ^a | 0.98 ±0.07 ^a |
| | NO | 2.52 ±0.17 | 1.87 ±0.26 ^a | 2.64 ±0.18 ^a | 1.82 ±0.12 ^a | 1.89 ±0.12 ^a | 0.87 ±0.32 ^a |
| 2-Hexenal | Control | 439.31 ±12.05 | 320.61 ±31.50 ^b | 296.00 ±52.88 ^b | 242.10 ±39.55 ^a | 252.21 ±17 ^b | 194.56 ±8.62 ^b |
| | NO | 439.31 ±12.05 | 398.04 ±16.14 ^a | 523.70 ±51.47 ^a | 306.00 ±33.84 ^a | 311.63 ±28.28 ^a | 241.41 ±3.14 ^a |
| Benzaldehyde | Control | 46.17 ±4.78 | 67.99 ±9.06 ^b | 94.83 ±4.41 ^b | 169.29 ±40.38 ^b | 173.97 ±8.91 ^a | 162.66 ±4.99 ^b |
| | NO | 46.17 ±4.78 | 138.53 ±9.55 ^a | 230.70 ±17.86 ^a | 227.93 ±35.52 ^a | 193.71 ±17.68 ^a | 200.48 ±8.52 ^a |
| Nonanal | Control | 2.94 ±0.33 | 2.31 ±0.10 ^a | 3.46 ±0.47 ^b | 2.77 ±0.18 ^a | 1.75 ±0.27 ^a | 1.91 ±0.11 ^a |
| | NO | 2.94 ±0.33 | 3.02 ±0.83 ^a | 5.60 ±0.46 ^a | 2.18 ±0.56 ^a | 1.99 ±0.19 ^a | 1.50 ±0.03 ^b |
| Alcohols | | | | | | | |
| 1-Hexanol | Control | 143.36 ±9.38 | 105.18 ±12.17 ^a | 96.29 ±10.07 ^b | 76.52 ±6.00 ^b | 73.83 ±13.59 ^a | 67.92 ±6.66 ^a |
| | NO | 143.36 ±9.38 | 106.48 ±12.40 ^a | 132.48 ±10.52 ^a | 97.38 ±7.76 ^a | 90.60 ±13.40 ^a | 73.95 ±5.12 ^a |
| 1-Hexanol, 2-ethyl- | Control | 6.66 ±0.21 | 4.90 ±0.03 ^a | 4.03 ±0.11 ^b | 4.46 ±0.65 ^a | 2.60 ±0.12 ^b | 2.63 ±0.29 ^a |
| | NO | 6.66 ±0.21 | 5.16 ±0.62 ^a | 6.22 ±0.84 ^a | 3.61 ±0.29 ^a | 3.06 ±0.45 ^a | 2.17 ±0.62 ^a |
| Esters | | | | | | | |
| 3-Hexen-1-ol, acetate, (Z)- | Control | 117.17 ±4.77 | 114.06 ±7.09 ^a | 184.93 ±7.93 ^a | 138.58 ±9.22 ^a | 125.79 ±7 ^a | 100.21 ±1.72 ^a |
| | NO | 117.17 ±4.77 | 94.63 ±6.75 ^b | 117.14 ±10.79 ^b | 114.66 ±7.95 ^b | 120.05 ±2.85 ^a | 101.40 ±11.47 ^a |
| Acetic acid, hexyl ester | Control | 74.32 ±2.50 | 46.07 ±4.56 ^a | 72.70 ±7.62 ^a | 61.26 ±4.85 ^a | 40.05 ±6.1 ^a | 19.85 ±2.57 ^a |
| | NO | 74.32 ±2.50 | 48.36 ±2.99 ^a | 55.81 ±6.52 ^b | 53.80 ±6.05 ^a | 35.79 ±5.45 ^a | 16.73 ±0.68 ^b |
| 2-Hexen-1-ol, acetate, (Z/E)- | Control | 45.16 ±1.70 | 28.00 ±2.20 ^a | 35.06 ±1.70 ^a | 30.70 ±1.91 ^a | 30.91 ±2.9 ^a | 21.67 ±0.84 ^a |
| | NO | 45.16 ±1.70 | 23.37 ±1.39 ^b | 25.37 ±1.74 ^b | 26.78 ±1.23 ^b | 28.08 ±6.07 ^a | 20.30 ±2.98 ^a |

Table 1 – cont.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|-------------------|---------|---|-------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Lactones | | | | | | | | |
| γ-Hexalactone | Control | | 1.39 ±0.10 | 1.59 ±0.01 ^a | 3.57 ±0.20 ^a | 5.08 ±0.53 ^a | 6.24 ±0.09 ^b | 7.25 ±0.41 ^a |
| | NO | | 1.39 ±0.10 | 1.88 ±0.42 ^a | 2.53 ±0.40 ^b | 3.96 ±0.48 ^b | 6.58 ±0.09 ^a | 7.47 ±0.09 ^a |
| γ-Octalactone | Control | | ND | ND | 1.60 ±0.11 ^a | 4.59 ±0.12 ^a | 0.36 ±0.11 ^b | 2.05 ±0.11 ^a |
| | NO | | ND | ND | 0.97 ±0.18 ^b | 1.48 ±0.26 ^b | 1.93 ±0.16 ^a | 2.13 ±0.03 ^a |
| γ-Decalactone | Control | | 2.28 ±0.37 | 10.35 ±0.39 ^a | 55.05 ±3.28 ^a | 49.71 ±1.97 ^a | 44.57 ±3 ^a | 34.38 ±2.36 ^a |
| | NO | | 2.28 ±0.37 | 11.13 ±2.92 ^a | 34.18 ±2.83 ^b | 40.64 ±3.77 ^b | 34.93 ±1.44 ^b | 29.47 ±0.77 ^b |
| δ-Decalactone | Control | | ND | 2.88 ±0.13 ^a | 12.10 ±0.65 ^a | 8.16 ±0.84 ^a | 7.4 ±1 ^a | 4.92 ±0.44 ^a |
| | NO | | ND | 3.55 ±1.13 ^a | 7.64 ±0.78 ^b | 5.45 ±0.19 ^b | 7.81 ±0.71 ^a | 3.92 ±0.08 ^b |
| δ-Dodecalactone | Control | | ND | ND | 0.97 ±0.10 ^a | 1.01 ±0.09 ^a | 2.36 ±0.20 ^a | 1.86 ±0.18 ^a |
| | NO | | ND | ND | 0.43 ±0.38 ^b | 1.02 ±0.15 ^a | 1.20 ±0.24 ^b | 1.46 ±0.03 ^b |
| Terpenes | | | | | | | | |
| 1R-.alpha.-Pinene | Control | | ND | ND | ND | 0.84 ±0.03 ^a | 0.41 ±0.10 ^b | 0.72 ±0.12 ^a |
| | NO | | ND | ND | ND | 0.63 ±0.14 ^b | 0.70 ±0.13 ^a | 0.84 ±0.15 ^a |
| D-Limonene | Control | | 2.77 ±0.23 | 2.68 ±0.11 ^a | 3.74 ±0.52 ^a | 2.87 ±0.72 ^a | 2.54 ±0.25 ^a | 1.89 ±0.31 ^a |
| | NO | | 2.77 ±0.23 | 2.97 ±0.29 ^a | 2.33 ±0.23 ^b | 2.69 ±0.38 ^a | 2.66 ±0.90 ^a | 1.48 ±0.12 ^a |
| Linalool | Control | | 24.74 ±0.23 | 61.09 ±0.78 ^a | 90.41 ±2.77 ^a | 65.43 ±5.89 ^a | 47.09 ±1 ^a | 24.45 ±1.84 ^a |
| | NO | | 24.74 ±0.23 | 50.06 ±7.68 ^b | 68.99 ±4.00 ^b | 33.73 ±8.80 ^b | 21.27 ±0.94 ^b | 25.64 ±0.35 ^a |
| D-Camphor | Control | | 11.38 ±0.30 | 11.09 ±0.25 ^a | 25.50 ±1.40 ^a | 14.56 ±1.63 ^a | 11.85 ±0.34 ^a | 9.88 ±1.23 ^a |
| | NO | | 11.38 ±0.30 | 12.91 ±3.44 ^a | 18.66 ±1.66 ^b | 13.12 ±1.95 ^a | 9.68 ±1.14 ^b | 9.25 ±0.65 ^a |
| Ketones | | | | | | | | |
| Dihydro-β-ionone | Control | | 0.63 ±0.02 | 5.43 ±1.03 ^a | 10.79 ±0.34 | 4.89 ±2.28 ^a | 3.05 ±0.09 ^a | 3.90 ±0.79 ^a |
| | NO | | 0.63 ±0.02 | 2.52 ±0.01 ^b | 7.58 ±0.51 | 5.50 ±0.01 ^a | 2.57 ±0.13 ^b | 3.14 ±0.94 ^a |
| β-Ionone | Control | | ND | 0.87 ±0.41 ^a | 1.06 ±0.13 ^a | 1.33 ±0.14 ^a | 1.07 ±0.24 ^a | 0.46 ±0.19 ^a |
| | NO | | ND | ND | ND | 0.73 ±0.05 ^b | 0.86 ±0.06 ^a | 0.41 ±0.05 ^a |
| Geranylacetone | Control | | 1.10 ±0.13 | ND | ND | 0.97 ±0.04 ^a | 0.64 ±0.09 ^a | 1.73 ±0.47 ^a |
| | NO | | 1.10 ±0.13 | ND | 0.77 ±0.09 | 0.88 ±0.03 ^b | 0.50 ±0.12 ^a | 1.27 ±0.13 ^a |

Data were expressed as means ±SD of three replicates. Different letters within the same column mean a significant difference ($p < 0.05$) between control and NO, and the same letter indicates no significant difference. ND – not detected.

changed a little throughout the whole storage period after comparing the abundance of these compounds at harvest (day 0) and the end of storage (day 9).

C6 aldehydes consisted of hexanal, 2-hexenal and E-2-hexenal, with hexanal and 2-hexenal accounting

for 99% of the total C6 aldehydes. In the NO-treated peaches, the content of total C6 aldehydes showed a sharp increase on day 3, followed by a successive decreasing trend, with significantly higher levels than the control peaches found during the whole storage period

except on day 1. The changes in the trends of hexanal and 2-hexenal were similar with the total C6 aldehydes. While the level of E-2-hexenal fluctuated during the whole storage period, and NO induced a higher content than the control peaches on days 3 and 5. 1-Hexenol was the dominant C6 alcohol detected in the present study, presenting a decreasing change trend during 20°C storage. Observably higher levels of 1-hexenol were found in the peaches treated with NO on days 3 and 5 compared with the untreated peaches. No significant difference was found in esters between the two groups during the whole storage period, with the exception of days 3 and 5, when extremely lower levels were found in the NO-treated peaches. Five lactones, including γ -hexalactone, γ -octalactone, γ -decalactone, δ -decalactone and γ -dodecalactone were obtained in the present study, with γ -decalactone the most abundant lactone in the ‘Xiahui 6’ peaches. In general,

these lactones all showed increases but not the same change trends during the whole storage period. The peak of total lactones was significantly postponed by NO treatment, and NO also suppressed the abundance of total lactones after the first day of 20°C storage.

The composition and content of FAs

Five FAs, including palmitic acid (PA 16:0), stearic acid (SA 18:0), oleic acid (OA 18:1), linoleic acid (LA 18:2) and linolenic acid (LeA 18:3) were identified in the present study (Fig. 2), in consistence with a previous report (Izzo et al., 1995). Linoleic acid – LA and LeA were the most abundant FAs during fruit ripening, followed by PA and SA, while the abundance of OA was the least. Palmitic acid – PA and SA showed the same change profile in both groups, decreasing from day 0 to day 3, then increasing till the end of storage (Fig. 2A, 2B). Nitric oxide – NO significantly

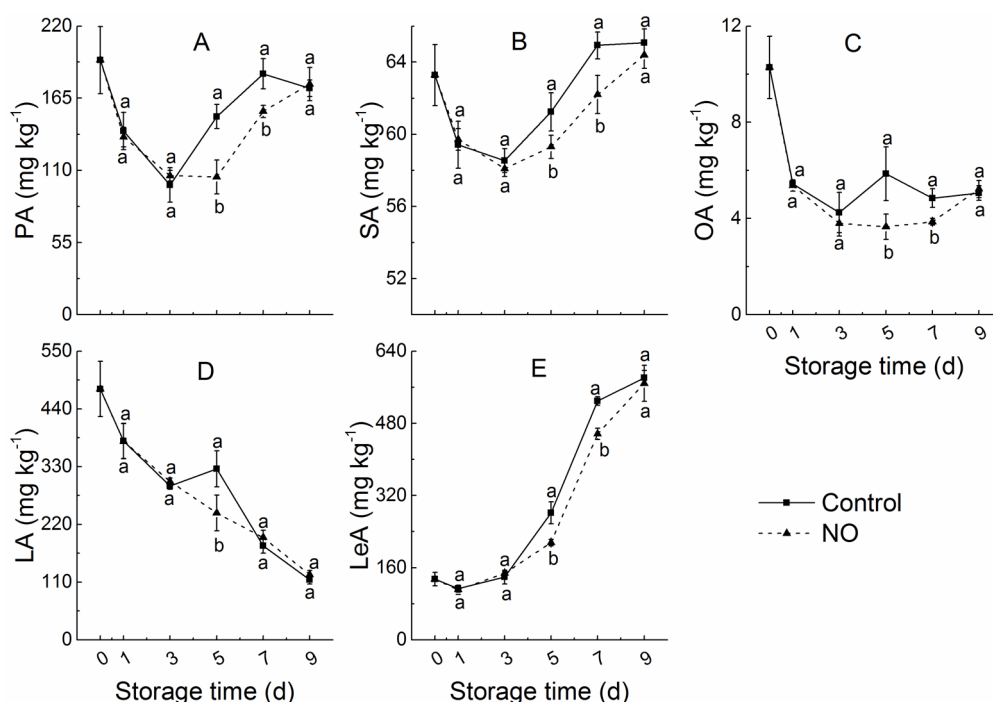


Fig. 2. Change of **A** – palmitic acid (PA 16:0), $\text{mg}\cdot\text{kg}^{-1}$, **B** – stearic acid (SA 18:0), $\text{mg}\cdot\text{kg}^{-1}$, **C** – oleic acid (OA 18:1), $\text{mg}\cdot\text{kg}^{-1}$, **D** – linoleic acid (LA 18:2), $\text{mg}\cdot\text{kg}^{-1}$, and **E** – linolenic acid (LeA 18:3), $\text{mg}\cdot\text{kg}^{-1}$, in peaches during 20°C storage. All the values are expressed as means \pm SD of three replicates. Significant differences at 0.05 level (Duncan’s test) between NO-treated and control group are indicated with different letters. There are no significant differences between Control and NO-treated peaches whose letters are same

inhibited the abundance of PA and SA from day 3 to day 7. Similarly, the content of OA kept decreasing until day 3, followed by a slight increase in both groups, with lower levels found in the NO-treated peaches on day 5 and day 7 (Fig. 2C). Linoleic acid – LA exhibited a practically straight downward trend, and no significant difference was found between the treated and untreated peaches, except for on day 5, when a significantly higher level was found in the control peaches (Fig. 2D). By contrast, the level of LeA in both groups showed a tendency to constantly accumulate throughout the storage period, being 3.22-fold higher on day 9 than the harvest day (day 0; Fig. 2E). Nitric oxide – NO obviously reduced the content of LeA from day 3 to day 7 compared with the control peaches.

Changes in gene expression related to ethylene biosynthesis

There are three key enzymes that regulate ethylene biosynthesis, namely s-adenosylmethionine synthetase (SAMS), 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS) and ACC oxidase (ACO). The

transcript abundance of these genes on day 0 is shown in Figure 3A. No significant difference in the expression of *PpSAMS* can be found between the treated and control groups throughout storage (Fig. 4). The expression level of *PpACS3* was the most abundant gene among the four ACS genes on day 0. *PpACS1* in the control peaches changed a little during the whole storage period, while NO treatment downregulated its expression from day 1 to day 7. The mRNA abundance of *PpACS2* exhibited a slightly decreasing trend in two groups during the first half of storage, then increased to the level of the harvest day. NO upregulated the expression of *PpACS2* on day 3, and downregulated its level from day 5 to day 7 compared with the control peaches. Similar to ethylene production, *PpACS3* in the peaches treated with NO was significantly lower than the control peaches from day 3 to day 7. A significant lower *PpACS4* level was found in the NO-treated than in the control peaches on day 1 and day 3. *PpACO* showed a gradual increasing trend during the whole storage period with significantly lower transcript levels found in the NO-treated peaches from day 5 to day 9.

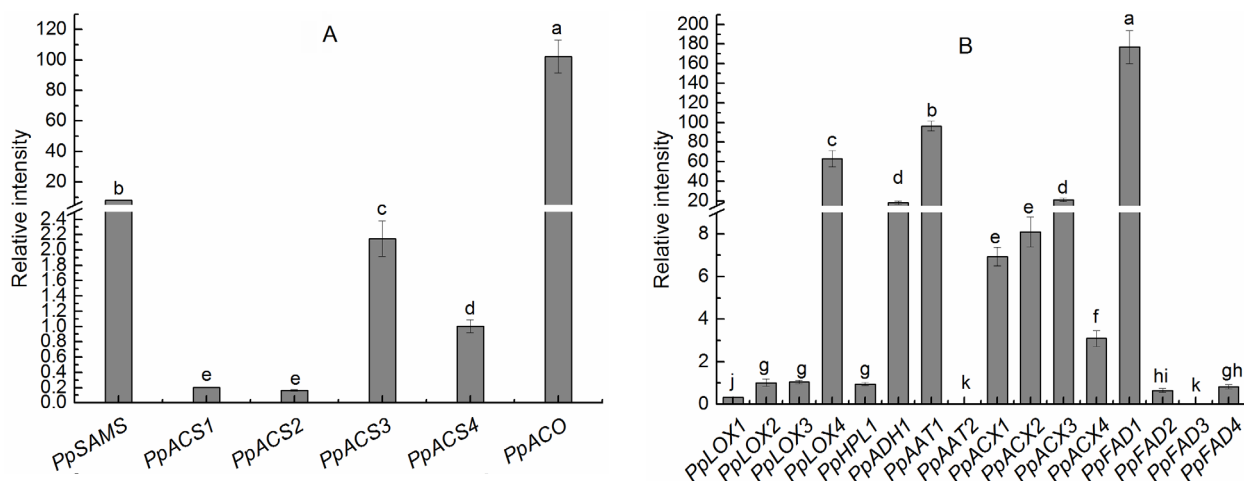


Fig. 3. A. Relative expression of ethylene synthesis genes at harvest (day 0). Transcript levels are normalized with respect to translation elongation factor 2 and are expressed relative to the values of *PpACS4*, which are set to 1. B. Relative expression of genes related with aroma compound synthesis at harvest (day 0). Transcript levels are normalized with respect to translation elongation factor 2 and are expressed relative to the values of *PpLOX2*, which are set to 1. The different normal letters indicate significant differences at 0.05 level (Duncan's test), and the same letter means no significant differences between different gene expression on day 0

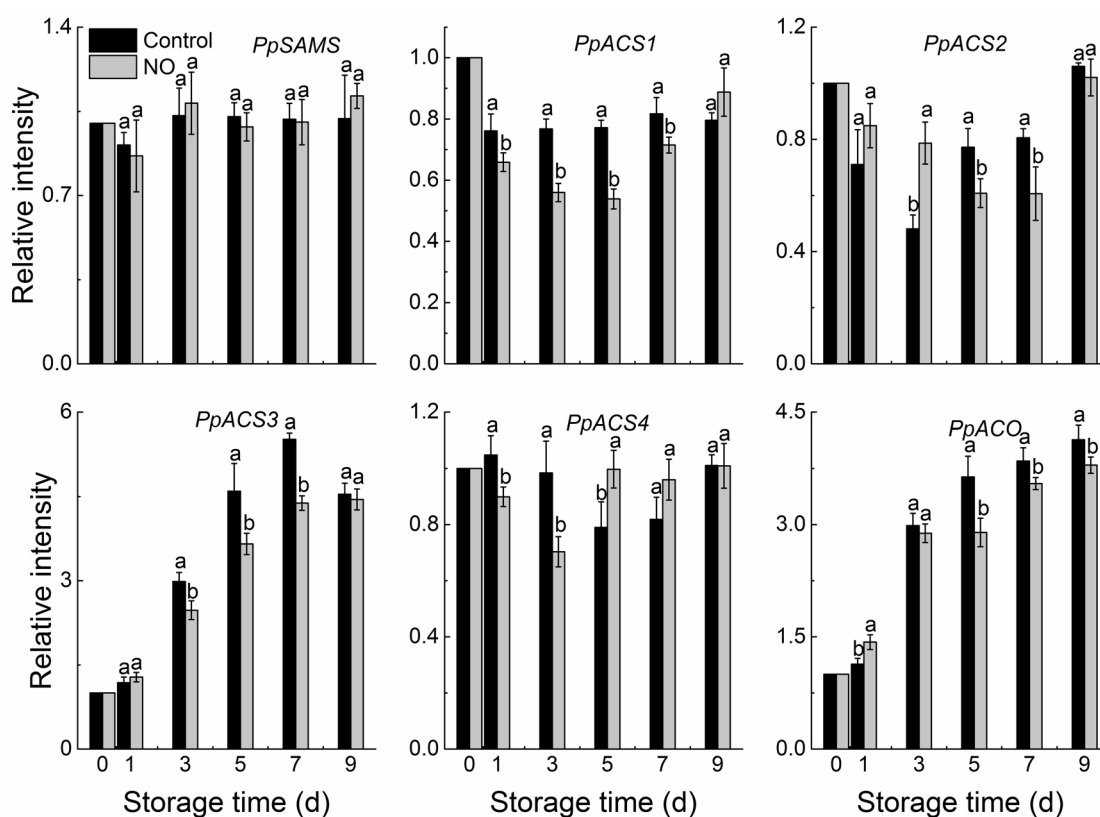


Fig. 4. The expression of genes related with ethylene biosynthesis. A ratio relative to the harvest day (0 d) was used as expression levels of each gene. All the values are expressed as means \pm SD of three replicates. The different letters showed significant differences at 0.05 level based on Duncan's test; no differences were detected whose letters are the same

Changes in gene expression related to aroma compounds and FA biosynthesis

Key enzymes involved in the LOX pathway and β -oxidation mainly include LOX, hydroperoxide lyase (HPL), alcohol dehydrogenase (ADH), alcohol acyl-transferase (AAT) and acyl-CoA oxidase (ACX). The transcript abundance of these genes on day 0 is shown in Figure 3B. The transcript abundance of *PpLOX1* and *PpLOX2* decreased to a lower level in both groups compared with the harvest day (Fig. 5). The expression of *PpLOX2* was faintly higher in the NO-treated peaches during storage. In general, a slight increase then a decreasing change trend in *PpLOX3* was found in both groups, with the NO treatment observably postponing its peak arrival for 2 days and inhibiting its expression by 28% when comparing the peak value between the two groups. In general, the expression

level of *PpLOX4* in both groups increased during the first five days of storage, then decreased until the end of storage. Nitric oxide – NO slightly but significantly restrained the expression of *PpLOX4*, except on day 9. Overall, *PpHPL1* showed a decreasing trend during storage, and its transcript abundance was upregulated by the NO treatment compared with the control peaches. After a decrease on day 1, the expression level of *PpADH1* increased slightly during the remaining storage period. The NO treatment promoted its expression on days 3, 5 and 9. *PpAAT1* showed no obvious change during storage, with higher transcript abundance being found in the control peaches on days 1, 5 and 9. *PpAAT2* in the control peaches increased in the first three days of storage then decreased. NO significantly inhibited the mRNA level of *PpAAT2* before day 7. The expression of *PpACX1* and *PpACX2* decreased at

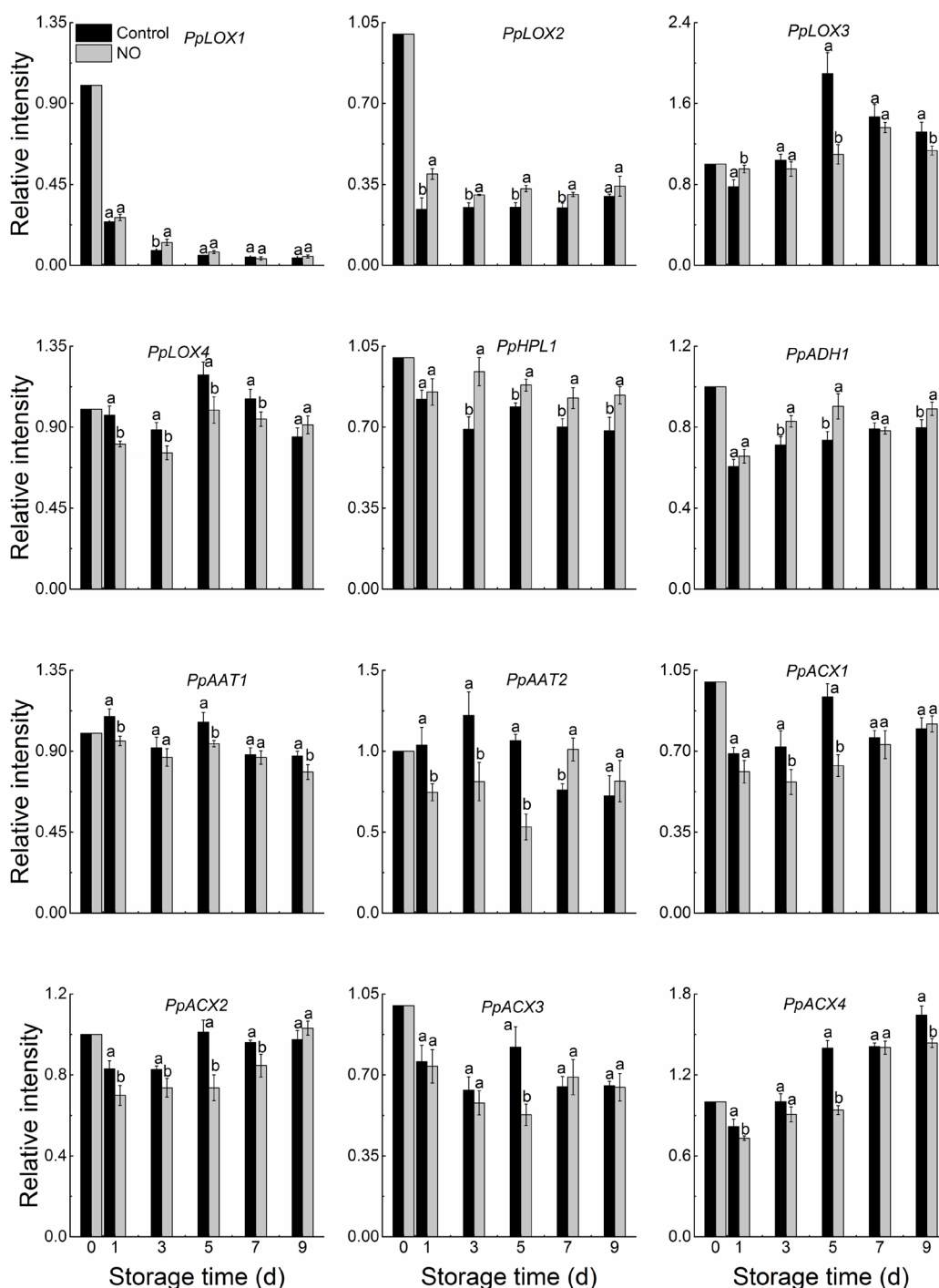


Fig. 5. The expression of genes related with aroma biosynthesis. The expression level of each gene was obtained using a ratio relative to the harvest day (0 d). All the values are expressed as means \pm SD of three replicates. Significant differences between the two groups at the same sampling time were showed using different letters at 0.05 level based on Duncan's test; the same letter means no significant differences between NO-treated and control peaches

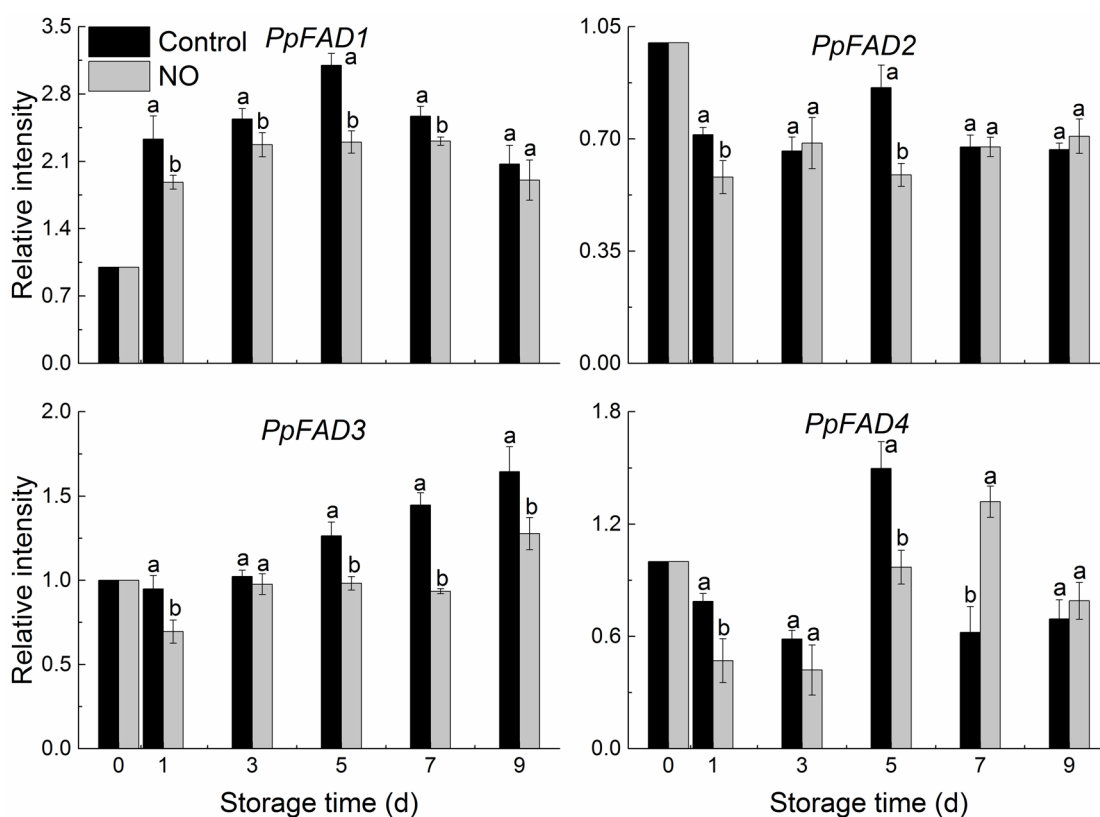


Fig. 6. The gene expression of fatty acid desaturase (FAD). A ratio relative to the harvest day (0 d) was used as expression levels for each gene. All the values are expressed as means \pm SD of three replicates. The different letters show significant differences at 0.05 level based on Duncan's test; no differences were detected whose letters are the same

the beginning of storage, and then slightly increased (Fig. 5). Downregulation in the expression level of the two genes was found in the NO-treated group in most of the storage period. Compared with day 0, *PpACX3* showed a decreasing change trend during storage. No significant difference in the level of *PpACX3* can be found between the two groups, except for on day 5, when NO downregulated its expression by 36%. After a slight decrease, *PpACX4* kept increasing during the remaining storage period, and the NO treatment significantly decreased its level on days 1, 5 and 9 compared with the control peaches.

Four fatty acid desaturase (FAD) genes were considered in the present study (Fig. 6). The transcript abundance of these genes on day 0 is shown in Figure 3B. The mRNA abundance of *PpFAD1* in both groups firstly increased to its highest level, followed

by a decrease. An upregulation in *PpFAD1* was found in the control and the NO-treated peaches throughout the whole storage period. By contrast, the expression of *PpFAD2* showed lower levels during storage than on the harvest day, and NO reduced its level on day 1 and day 5 compared with the control peaches. Generally, *PpFAD3* increased after harvest with a higher transcript level found in the control peaches. *PpFAD4* in the treated and control peaches decreased from day 0 to day 3, then increased during the middle stage of storage, before finally decreasing till the end of storage. NO evidently inhibited its expression on day 1 and day 5 and upregulated its level on day 7.

PCA and heatmap analysis for full data

The principal component analysis (PCA) and hierarchical cluster analysis (HCA) were performed to

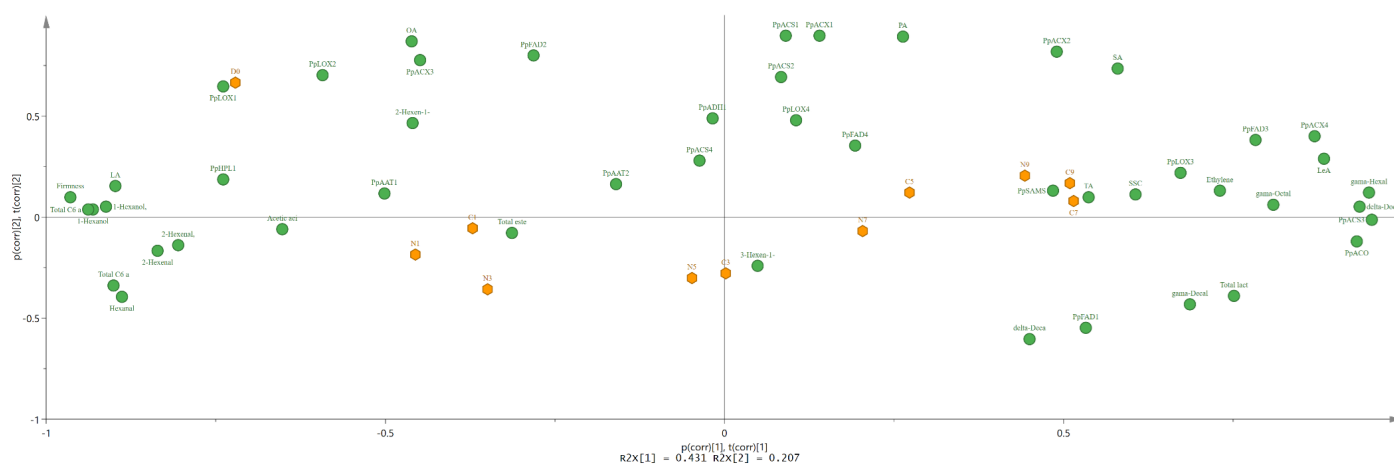


Fig. 7. The principal component analysis of the first two principal components for full data during the whole storage period in peach fruit (C, Control and N, NO treatment) at room temperature. PC1 and PC2 accounted for 43.1% and 20.7% of the variation, respectively. An orange regular hexagon represents peach samples, N1 – samples on day 1 after NO treatment; C1 – samples on day 1 of control fruit, et cetera. A green circle represents indicators of measurement

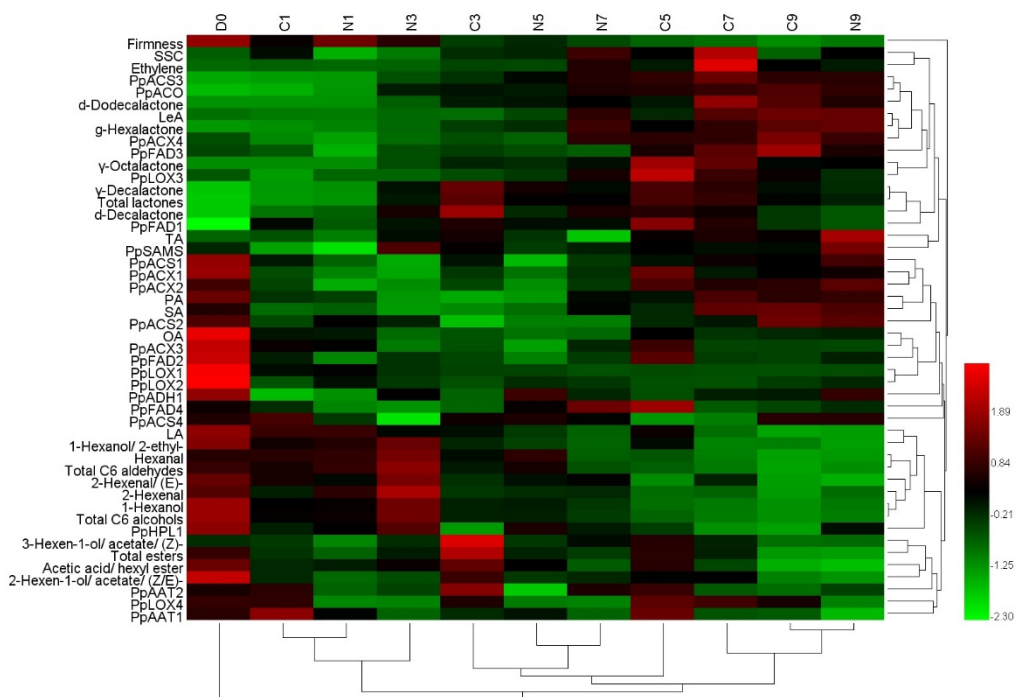


Fig. 8. Hierarchical cluster analysis of full data during the whole storage period in peach fruit (C, Control and N, NO treatment) at room temperature for 9 days. The data were the means of three replicates and were processed with Z-score normalization and hierarchically clustered using Spearman distance

provide an overview of the whole dataset. PC1 and PC2 accounted for 63.8% of the total variable when quality and physiology parameters, aroma volatiles, the composition and content of FAs and gene expression related to biosynthesis of ethylene, aroma compounds and FAs were selected to characterize the peach fruit during the whole storage period. The postharvest ripening and senescence process could roughly be divided into four groups (D0; C1, N1 and N3; C3, C5 and N5, N7; C7, C9 and N9) based on PCA and HCA (Fig. 7, 8). D0 was clustered with high values of firmness, aldehydes, alcohols, LA, OA, *PpLOX1*, *PpLOX2*, *PpACX3*, *PpHPL1* and *PpFAD2*, which indicated that these indexes play dominant roles in unripe fruit. C1, N1 and N3 were clustered in the second group, together with *PpAATI/2* and total ester content. The third group was characterized by PA, ethylene biosynthesis genes (*PpACS1/2/4*) and genes related to aroma biosynthesis (*PpLOX4*, *PpFAD4*, *PpACX1* and *PpADH1*). With the occurrence of climacteric properties, the peach fruit entered into a stage of senescence manifested by the higher levels of TA, SSC, SA, LeA, aroma lactones and mRNA abundance of *PpLOX3*, *PpFAD3*, *PpACX4*, *PpACS3* and *PpACO*. These results suggested that NO could delay fruit ripening and senescence for at least two days at room temperature storage.

DISCUSSION

In the present study, NO treatment significantly retarded the decline of fruit firmness during storage with the exception of day 9 and maintained higher contents of SSC and TA at the end of storage (Fig. 1A–1C), indicating that NO could retain higher peach fruit quality during postharvest storage. A recent review also summarized examples of the beneficial effects of exogenous NO on different fruit cultivars (Corpas and Palma, 2018).

The peach is a climacteric fruit with a respiration and ethylene burst occurring during its ripening. It has been widely accepted that ethylene is a key promoter of leaf, flower and fruit senescence, while NO plays an opposite role (Freschi, 2013). Here, ethylene production was observably inhibited by 10 $\mu\text{L}\cdot\text{L}^{-1}$ NO treatment (Fig. 1D), which suggested that NO could delay fruit senescence by inhibiting ethylene biosynthesis. The crosstalk between NO and ethylene during fruit ripening has previously been demonstrated

(Mukherjee, 2018). In the ethylene biosynthesis pathway, ACS is responsible for the synthesis from *s*-adenosylmethionine to ACC, which was considered as the rate-limiting step for ethylene formation. ACO is a key enzyme that catalyzes the conversion of ACC to ethylene. In peach fruit, Zhu et al. (2006) found that the activity of ACS and ACO was inhibited by NO treatment, leading to a decrease in ethylene production. In this study, no significant difference in *PpSAMS* was found between the two groups, while NO inhibited the expression of *PpACS* and *PpACO*, which is consistent with the above reports (Fig. 4).

Aroma compounds in fruit are under the positive regulation of ethylene, which has been reported in peach (Ortiz et al., 2010), apple (Wang et al., 2018) and avocado (Garcia-Rojas et al., 2016) etc. In consistency with these reports, higher levels of C6 aldehydes and alcohols, as well as lower levels of esters and lactones were found in NO-treated peaches accompanied by significantly lower ethylene production (Table 1; Fig. 1D). These results suggest that the effect of NO on peach aroma volatiles may be a result of decreased ethylene production. To figure out the underlying molecular mechanisms of aroma biosynthesis, gene expression of key enzymes was taken into consideration. In the LOX pathway, LOX firstly catalyzes unsaturated FAs (e.g. LA and LeA) to form hydroperoxides which can be cleaved into C6 aldehydes by HPL. C6 aldehydes can be further converted to form the corresponding C6 alcohols by ADH, followed by the conversion from alcohols to esters by AAT action (Schwab et al., 2008). Compared with the harvest day, *PpLOX1* and *PpLOX2* in both groups decreased, and NO induced higher levels of *PpLOX2*, which is similar to the change trends of C6 volatiles (Fig. 5). *PpLOX3* and *PpLOX4* showed similar change trends with ethylene production and NO significantly inhibited their level, as well as the content of peach-like volatiles (esters, lactones) and ethylene production, which may suggest that the two genes are regulated by ethylene and closely correlated with the biosynthesis of esters and lactones. Lipoxygenase – LOX activity and gene expression have been reported to closely correlate with the production of C6 aldehydes, alcohols, esters and lactones (Zhang et al., 2010). Nitric oxide – NO treatment retained higher *PpHPL1* and *PpADH1* than the control peaches, which is in line

with C6 volatile compounds. It has been reported that both AAT gene expression and alcohols are important for ester biosynthesis, which are regulated by alcohol supply (González-Agüero et al., 2009; Ortiz et al., 2009). However, higher alcohols and lower *PpAAT* were found in the NO-treated peaches, accompanied by lower ester levels. This may be explained by lower *PpAAT* in the NO-treated peaches, which controls the biosynthesis of esters despite a higher alcohol content. In the β -oxidation pathway, ACX is the first and rate-limiting enzyme and is associated with lactone formation in peach fruit (Arent et al., 2008). Xi et al. (2012b) proposed that ACX activity with C16-CoA and *PpACX1* with C10~C16-CoA are responsible for the biosynthesis of higher lactone contents. In our study, although the four ACX genes all showed higher level in the control peaches, only *PpACX4* showed obvious increases compared with the level on harvest day, which is in accordance with Xi's report because *PpACX4* here shared 78.45% identity with *AtACX2*, which encodes long chain ACX (>C12).

It has been reported that the compositions and contents of FAs, especially LA (18:2) and LeA (18:3), directly affect the profiles of aroma volatiles in peach fruit (Xi et al., 2012a), kiwifruit (Zhang et al., 2009), tomato (Ties and Barringer, 2012) and pear (Qin et al., 2014) etc. In the present study, LA displayed a degressive trend, while LeA kept increasing throughout storage, which suggests that a conversion from 18:2 to 18:3 existed. A similar phenomenon was also found in previous research (Cai et al., 2019). LeA in the group of NO treatment was lower than that in the control peaches from day 3 to day 9, which matches the change trend of lactones, indicating that LeA may be responsible for the biosynthesis of peach lactones. The desaturation of FAs is catalyzed by FAD, which is related with FA composition in plants (Wang et al., 2016). A blast search using mRNA CDS showed that *PpFAD1* and *PpFAD2* had 73.97%, 78.34% homology with *AtFAD2* and *AtFAD6* respectively, which are responsible for the formation of 18:2 from 18:1 (<http://www.arabidopsis.org/index.jsp>; <https://www.rosaceae.org/blast>). Here, *PpFAD1* increased with the maturity of the fruit, while *PpFAD2* decreased compared with the level on harvest day. Gene expression of both was lower in the NO-treated peaches, indicating that NO inhibited the biosynthesis of 18:2 from

18:1. A lower content of OA also confirmed this conjecture. *PpFAD3*, *PpFAD4* shared 72.67% and 78.36% identity with *AtFAD3* and *AtFAD8* respectively, which has enzyme activity for the formation of 18:3 from 18:2 (<http://www.arabidopsis.org/index.jsp>; <https://www.rosaceae.org/blast>). In the present study, the change trends of *PpFAD3* were similar with LeA, with higher levels found in the control peaches, suggesting that this gene may be responsible for the biosynthesis of LeA in peaches. *PpFAD4* showed a fluctuating but generally decreasing change trend compared with the level on day 0, which may suggest that this gene decreased with fruit ripening. Similar results were also found in other research (Wang et al., 2016).

CONCLUSION

In summary, postharvest NO treatment with a dose of 10 $\mu\text{L}\cdot\text{L}^{-1}$ significantly maintained higher fruit quality by delaying the decline of fruit firmness during storage, except on day 9, and by retaining higher SSC and TA content at the end of storage. NO treatment also reduced ethylene production by inhibiting ACS and ACO gene expression, therefore delaying the ripening and senescence of the peach fruit during storage. For aroma volatiles, NO maintained higher levels of C6 volatiles, including aldehydes and alcohols, and reduced the content of esters and lactones in the peaches, along with the content of PA, SA, OA and LeA, mostly by regulation of FAD and the genes involved in the LOX and β -oxidation pathways. These results together suggest that the effects of NO on peach aroma volatiles are a result of the regulation of genes related with the biosynthesis of aroma compounds, ethylene and fatty acids. This study may provide theoretical reference for agricultural food (fruit, vegetables et al.) preservation, thus reducing the losses for farmers during perishable agricultural food production. Difficulties may also exist in the implementation of NO treatment due to the high price of NO and huge volume and production of agricultural food.

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