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*Research Article*

# *LOX* and *AAT* Genes Affect the Aroma of "Xiaobai" Apricot during Postharvest Storage

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"Xiaobai" apricot is one of the most popular fruits in China. However, during postharvest storage, fruit aroma loss occurs easily. In this study, factors affecting the aroma changes in different ripening stages of "Xiaobai" apricot during postharvest storage were searched. Immature and mature "Xiaobai" apricot samples were collected and monitored for sensory changes during postharvest storage. After 25 days of storage, the aromatic ester and alcohol abundance in mature showed a decrease, related to the loss of characteristic aroma. Immature fruit still tasted sour with an indistinct characteristic aroma, as a result of decreased alcohol and increased acid abundance. The *ATT*, *ADH*, *PDC*, and *LOX* genes were identified, and their expression levels were detected at different ripening stages during storage. The correlation analysis showed that the expression of *LOX* and *ATT* was positively correlated with the variation of aromatic ester ($P < 0.05$), a key factor affecting the apricot aroma during postharvest storage.

## 1. Introduction

Apricot (*Prunus armeniaca* L.) is a popular fruit produced in temperate countries; it possesses many health-promoting effects, including the protection of the liver and heart, antioxidant, and anti-inflammatory [1, 2]. "Xiaobai" apricot is a unique variety of grown in Xinjiang Province. It is mainly planted in Kuqa and Luntai counties in the south of Xinjiang Province [3]. Compared to other varieties, the size of "Xiaobai" fruit is small but the nutritional value is very high [4].

The aroma is an important sensorial factor indicating fruit quality, which also affects the purchasing behavior of consumers and fruit sales [5]. Since the "Xiaobai" apricot's mature stage is concentrated in June [3], special attention must be paid to the retention of characteristic aroma during the process of fruit storage and transportation. However, since the "Xiaobai" apricot is a climacteric fruit, aroma loss occurs easily during storage, even at low temperatures [6], causing severe economic loss. Therefore, it is essential to explore factors affecting aroma during postharvest storage, which may help in developing a strategy to protect the aroma. Volatile compounds in various fruits have been identified by combining headspace solid-phase microextraction with gas chromatography-tandem mass spectrometry (HS-SPME-GC-MS) [7, 8]. Such volatile compounds, including esters, alcohols, aldehydes, ketones, and acids, mainly contribute to the fruit's aroma [7, 9]. Esters and alcohol are important compounds contributing to the aroma of many fruits. For example, esters are associated with the "fruit candy" aroma [10, 11], while aldehydes are responsible for the bitter almond aroma of apricot [12, 13].

Complex biochemical pathways are involved in the formation of volatile compounds in fruit [14]. The lipoxygenase (*LOX*) pathway is an important pathway for volatile compound synthesis [15]. In this pathway, fatty acids are firstly

catabolized into hydroperoxides by *LOX* and then into aldehyde by *pyruvate decarboxylase* (*PDC*); *alcohol dehydrogenase* (*ADH*) removes hydrogen and converts aldehydes to alcohol. Finally, *alcohol acyltransferase* (*AAT*) is responsible for the synthesis of various esters via combining alcohols and acyl-CoAs [16]. These genes are considered the key genes associated with the synthesis of volatile compounds in various fruits [17, 18]. For instance, in "Shine Muscat" grape, *LOX* and *ADH* genes were the limiting factors for the synthesis of the volatile compounds [19], and in jujube, the *LOX* gene was related to the accumulation of (E)-2-hexenal [20]. Also, the high expression of the *LOX* gene was shown to enhance the synthesis of flavoring compounds in tomatoes [21]. *LOX* activity was also shown to affect the formation of the main volatile compound in *Arbutus unedo* L. [22], while *ADH* activity was associated with alterations in the abundance of various aldehydes and alcohols in tomatoes. Therefore, identifying the key enzymes in the *LOX* pathway is essential to understand molecular mechanisms of volatile compound synthesis in "Xiaobai" apricot, which may serve to develop strategies for the extension of the aroma retention. However, the relationship between these genes and volatile compound synthesis during postharvest storage in "Xiaobai" apricot remains unclear.

In this study, we collected immature and mature "Xiaobai" apricot fruit from Luntai City, Xinjiang Province, China. The changes in the sensory and volatile compound profiles during postharvest storage of both immature and mature apricots were analyzed using HS-SPME-GC-MS technology. Furthermore, we identified *LOX*, *PDC*, *ADH*, and *AAT* genes and determined their transcription levels.

## 2. Materials and Methods

*2.1. Sample Collection.* Immature and mature "Xiaobai" apricot samples were harvested in Luntai City, Xinjiang Province, China. The sugar degree of "Xiaobai" apricot samples in immature and mature stages were $7.3 \pm 0.18$ and $10.8 \pm 0.22$ brix. The firmness of apricot samples in immature and mature stages was $21.5 \pm 0.21$ N and $17.5 \pm 0.31$ N, respectively. All samples were precooled for 24 h at 4°C and then stored at 2°C, $90 \pm 5$ rh%. During storage, the sensory changes of "Xiaobai" apricot were determined every 5 days. Fifteen fruits were collected after 0, 5, 10, 15, 20, and 25 days of storage, snap-frozen with liquid nitrogen, and stored at -80°C.

*2.2. Sensory Evaluation.* The acidity, sourness, and skin-flesh separation of "Xiaobai" apricot during storage were assessed by the sensory evaluation method. Sensory evaluation was carried out by an untrained panel (fifty volunteers from Xinjiang Institute of Technology, Xinjiang, China), briefly introducing the evaluation criteria of the sample indicators and giving a score of 9 points, of which 9 points represent the sample's strong aroma, obvious sweetness, heavy sourness, and fruit being very soft, and a score of 1 which means almost no aroma, sweetness, and sourness and high hardness. Before and after the evaluation of one sample, participants were asked to clean their mouths with water [23].

Table 1: PCR primers used in this study.

| Gene | Primer sequence | Production size |
|---|---|---|
| *LOX* | ATTCCCAGTGCCTCAAGTGA (5′-3′) | 559 |
| | CACAAACGGTTCAATCACTGC (3′-5′) | |
| *PDC* | CTGTAACTTGGCTGGGATTCC (5′-3′) | 533 |
| | GAGCAGTGTTGTTGTGGTTGA (3′-5′) | |
| *ADH* | GTGGAAAGCGTAGGTGAGG (5′-3′) | 809 |
| | TCCAGCTCCTTATTCATGTACAT (3′-5′) | |
| *AAT* | GGAAGGACCCAACAGAAAGC (5′-3′) | 683 |
| | CATTGCCGTAGAATCCCGAG (3′-5′) | |

*LOX*, *PDC*, *ADH*, and *AAT* represent *lipoxygenase*, *pyruvate decarboxylase*, *alcohol dehydrogenase*, and *alcohol acyltransferase* genes, respectively.

*2.3. Volatile Compound Analysis.* Volatile compounds were analyzed by grounding the fruit into a fine powder using liquid nitrogen; five grams of this powder was mixed with 5 mL of saturated NaCl solution and placed into a 15 mL headspace vial. The internal standards included acetaldehyde, 2-hexenal, benzaldehyde, and $\gamma$-decalactone. Volatile compounds were detected using HS-SPME-GC-MS. The mixed sample was equilibrated for 5 min at 50°C. Fiber (PDMS 100 $\mu$m, SUPELCO, USA) was used to adsorb the headspace volatile compounds. The microextraction was performed at 40°C for 30 min. GC-MS analysis was performed using an HP-5 column (30 m × 0.1 mm inner diameter, 0.33 $\mu$m film thickness: Agilent, Santa Clara, CA) on a Thermo TRACE GC100 system equipped with an FID detector. The oven temperature was maintained at 50°C for 2 min, then increased to 250°C at a rate of 4°C/min, and held for 10 min. The electron impact energy used was 70 eV, the ion source temperature was set at 200°C, and the scans were performed in the range of 29–540 m/z. The mass spectra of the compounds were compared to those available in the NIST2000 database.

*2.4. Determination of LOX, ADH, AAT, and PDC Enzyme Activities*

*2.4.1. LOX Enzyme Activity.* Four milliliters of phosphate-buffered saline (50 mM, pH = 7.2, 4°C) was added to 1.5 g of finely grinded flesh samples; the samples were then ultrasonically treated at 0°C for 30 min and centrifuged at 4°C for 15 min at 15000 × g. Later, the supernatant was collected and used for further analysis.

The reaction mixture included a total volume of 3 mL, including 25 $\mu$L sodium linoleate solution (0.1 M), 2.775 mL acetic acid buffer (100 mM, pH = 5.5), and 0.2 mL supernatant (thick enzyme fluid). After 15 s of enzyme addition, the change in the OD value was recorded within 1 min while the enzyme activity was expressed as $OD_{234}$ $g^{-1}$ FW $min^{-1}$. The LOX enzyme activity was determined at 234 nm at 30°C.

*2.4.2. ADH Enzyme Activity.* Three milliliters of extraction buffer (100 mM MES-TRIS buffer, 2 mM MDTT, 1% PVP,

Table 2: RT-PCR primers used in this study.

| Gene | Primer sequence (5′-3′) | Production size (bp) |
|---|---|---|
| *LOX* | ATGGGGATCAAACAAGTCAAATAAC (5′-3′)<br>GGCTTCAAAGTCCCATCATTTTCT (3′-5′) | 210 |
| *PDC* | TTCTTGAACAAGGCAGTGAAAC (5′-3′)<br>GACTCCACAATCTCAGCACAA (3′-5′) | 214 |
| *ADH* | GGGAGTGGATAGGAGTGTTGA (5′-3′)<br>TACAACTGGCTCACACACTGC (3′-5′) | 202 |
| *AAT* | GGAAGGACCCAACAGAAAGC (5′-3′)<br>ATCCAAAGATGAAGCCTCCACA (3′-5′) | 226 |
| Actin | CATTCTTCGTCTGGACCTTGC (5′-3′)<br>TTGTAGGTAGTCTCATGAATTCC (3′-5′) | 275 |

*LOX*, *PDC*, *ADH*, and *AAT* represent *lipoxygenase*, *pyruvate decarboxylase*, *alcohol dehydrogenase*, and *alcohol acyltransferase* genes, respectively.

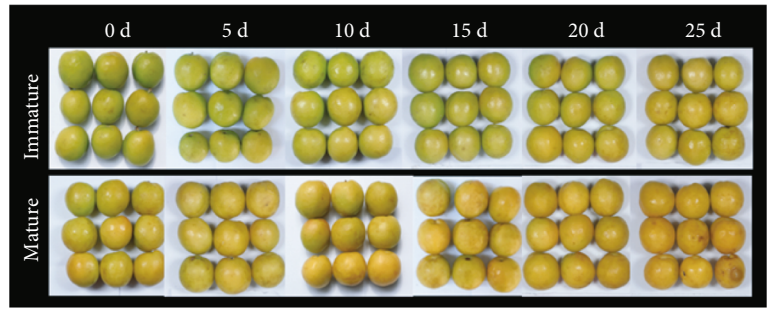


Figure 1: Changes in the appearance of immature and mature "Xiaobai" apricot during postharvest storage.

pH = 6.5, 4°C) were added to 1.5 g of finely grinded flesh samples; the samples were ultrasonically treated at 0°C for 30 min and centrifuged at 4°C for 30 min at 15000 × g. The supernatant was collected and used for further analysis.

The reaction mixture included a total volume of 3 mL, including 2.4 mL MES-TRIS buffer (pH = 6.5), 0.15 mL 1.5 mM NADH, 0.15 mL 80 mM acetaldehyde, and 0.3 mL supernatant. After 15 s of enzyme addition, the change in OD value was recorded within 1 min while the enzyme activity was expressed as $OD_{340}\ g^{-1}$ FW $min^{-1}$. The *ADH* enzyme activity was determined at 340 nm at 30°C.

*2.4.3. AAT Enzyme Activity.* Sixteen milliliters of extraction buffer (100 mM $K_3PO_4$, 2.5 g PVPP, pH = 6.5, 4°C) was added to 8 g of finely grinded flesh samples; the samples were ultrasonically treated at 0°C for 30 min and centrifuged at 4°C for 30 min at 15000 × g. The supernatant was collected and used for further analysis.

The reaction mixture included a total volume of 3 mL, including 2.25 mL 100 mM $K_3PO_4$, 0.3 mL 10 mM LDTNB, 0.03 mL 1 M $MgCl_2$, 0.06 mL 20 mM isopentanol, 0.06 mL 50 mM acetyl-CoA, and 0.3 mL supernatant. The change in OD value was recorded within 1 min while the enzyme activity was expressed as $OD_{234}\ g^{-1}$ FW $min^{-1}$. The *AAT* enzyme activity was determined at 340 nm at 30°C.

*2.4.4. PDC Enzyme Activity.* Five milliliters of extraction buffer (2 mM DTT, 4% PVPP (*w*/*v*), pH = 6.5, 4°C) was added to 5 g of finely grinded flesh samples; the samples were ultrasonically treated at 0°C for 30 min and centrifuged at 4°C for 30 min at 15000 × g. The supernatant was collected and used for further analysis.

The reaction mixture included a total volume of 2.6 mL, including 1.5 mL 100 mM MES, 0.2 mL 5 mM thiamine pyrophosphate, 0.2 mL 50 mM $MgCl_2$, 100 $\mu$L 1.6 mM NADH, 0.2 mL ethanol dehydrogenase, 200 $\mu$L 50 mM pyruvic acid, and 0.2 mL supernatant. After 15 s of enzyme addition, the change in OD value was recorded within 5 min while the enzyme activity was expressed as $OD_{340}\ g^{-1}$ FW $min^{-1}$. The *PDC* enzyme activity was determined at 340 nm at 30°C.

*2.5. RNA Extraction and cDNA Synthesis.* The total RNA was extracted from 1 g of sample by the Plant Total RNA Isolation Kit (Sangon Biotech, Shanghai, China) following the manufacturer's protocol. The RNA was reversely transcribed into cDNA using PrimeScript RT reagent kit (Takara, Dalian, China) following the manufacturer's protocol. The cDNA was used as the template for PCR and real-time quantitative PCR (RT-qPCR) analysis. PCR was performed using the "Xiaobai" apricot cDNA as the PCR template.

*2.6. Identifying and Sequencing LOX, PDC, ADH, and AAT Genes.* The primers used to amplify the target gene fragment are shown in Table 1. The PCR reaction volume was 25 $\mu$L containing 12.5 $\mu$L Taq PCR Master Mix (Sangon Biotech,

Table 3: Sensory score of "Xiaobai" apricot during storage.

| Ripening stage | Storage day | Aroma ($M \pm SD$) | Sweetness ($M \pm SD$) | Sourness ($M \pm SD$) | Firmness ($M \pm SD$) |
|---|---|---|---|---|---|
| Immature sample | 0 | $1.1 \pm 0.3$ | $1.5 \pm 0.1$ | $8.2 \pm 0.2$ | $2.2 \pm 0.2$ |
| | 5 | $2.0 \pm 0.2^{a}$ | $1.8 \pm 0.3^{a}$ | $8.0 \pm 0.1^{a}$ | $2.5 \pm 0.3^{a}$ |
| | 10 | $2.2 \pm 0.1^{a}$ | $2.6 \pm 0.6^{b}$ | $7.8 \pm 0.2^{a}$ | $4.8 \pm 0.4^{b}$ |
| | 15 | $3.8 \pm 0.6^{b}$ | $3.7 \pm 0.1^{c}$ | $5.7 \pm 0.5^{b}$ | $5.7 \pm 0.1^{c}$ |
| | 20 | $4.8 \pm 0.5^{c}$ | $4.1 \pm 0.2^{c}$ | $5.1 \pm 0.1^{c}$ | $6.0 \pm 0.3^{c}$ |
| | 25 | $5.4 \pm 0.4^{d}$ | $6.0 \pm 0.2^{d}$ | $3.6 \pm 0.5^{d}$ | $7.1 \pm 0.2^{d}$ |
| Mature sample | 0 | $8.5 \pm 0.6$ | $7.8 \pm 0.2$ | $1.8 \pm 0.4$ | $6.2 \pm 0.3$ |
| | 5 | $7.0 \pm 0.2^{a}$ | $7.5 \pm 0.4^{a}$ | $1.6 \pm 0.3^{a}$ | $6.5 \pm 0.5^{a}$ |
| | 10 | $5.6 \pm 0.5^{b}$ | $6.2 \pm 0.3^{b}$ | $1.4 \pm 0.6^{a}$ | $6.9 \pm 0.3^{b}$ |
| | 15 | $3.3 \pm 0.4^{c}$ | $5.8 \pm 0.1^{bc}$ | $1.9 \pm 0.2^{b}$ | $7.1 \pm 0.1^{b}$ |
| | 20 | $1.6 \pm 0.1^{d}$ | $2.3 \pm 0.4^{d}$ | $1.8 \pm 0.4^{ab}$ | $7.8 \pm 0.4^{c}$ |
| | 25 | $1.5 \pm 0.3^{d}$ | $2.1 \pm 0.3^{d}$ | $1.7 \pm 0.5^{ab}$ | $8.5 \pm 0.4^{d}$ |

$M \pm SD$: mean ± standard deviation, $n = 3$. $^{a,b,c,d}$Different treatment effects (same storage time) for $P < 0.05$.

Shanghai, China), 0.5 $\mu$L primers, 1 $\mu$L extracted cDNA (10 ng), and 10.5 $\mu$L $ddH_2O$. The amplification conditions were as follows: 5 min at 94°C, followed by 30 cycles for 30 min at 94°C, 30 s at 52°C, and 1 min at 72°C. The PCR production was performed using the Sanger sequencing. The neighbor-joining phylogenetic tree was computed by MEGA X.

*2.7. Determination of Gene Transcription Level by RT-PCR.* The primers used in RT-PCR are shown in Table 2. RT-PCR was performed on LightCycler®96 RT-PCR equipment (Roche, Basel, Switzerland). Each RT-PCR was performed in a total volume of 25.0 $\mu$L containing 12.5 $\mu$L SYBR Green Supermix (SYBR *Premix ExTaq* II, Takara, Dalian, China), 0.4 $\mu$L primers, 1.0 $\mu$L cDNA template, and 13.2 $\mu$L $ddH_2O$. The conditions for amplification and calibration curve construction were as follows: preheating at 95°C for 10 min; 45 cycles of 95°C for 10 s, 52°C for 10 s, and then 72°C for 10 s; and an increase of 4.4°C every 10 s from 65°C to 95°C for melting curve analysis to confirm the specificity of the amplification. The relative expression levels of target genes were quantified by the $2^{-\Delta\Delta CT}$ method using *Actin* gene as an internal control.

*2.8. Statistical Analysis.* Statistical analyses were performed by SPSS Statistics 20 (IBM, Armonk, NY). Independent sample $T$ test was used to calculate the significance of difference. Linear regression analysis was used to calculate the Pearson correlation coefficients ($R$). The statistical significance was evaluated by $P$ value. $P < 0.05$ represents the statistical significance. The Pearson correlation network was performed by Gephi (version 0.9.2).

## 3. Results and Discussion

*3.1. The Sensory Changes in "Xiaobai" Apricot during Postharvest Storage.* The immature "Xiaobai" apricot sample appeared completely green in color, which turned yellow-green after 10 days of storage and yellow and white after 25 days of storage (Figure 1). The color of the mature apricot sample was yellow-green, which was close to that of the immature apricot after 20 days of storage. After 25 days of storage, it changed color to light yellow, with parts of the samples starting to brown.

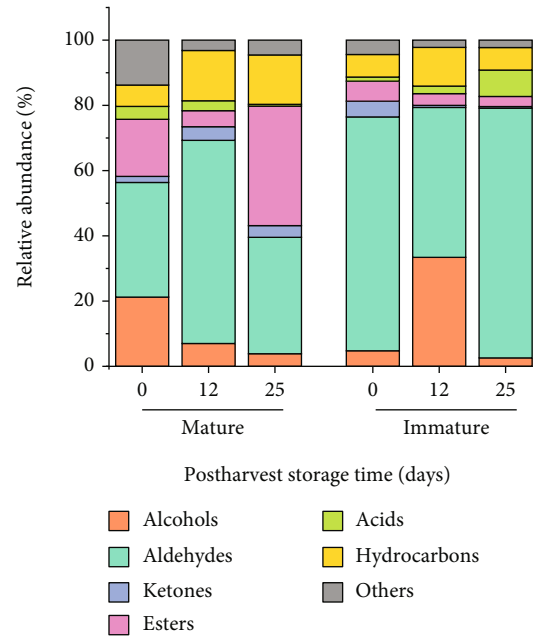


Figure 2: Volatile compound profiles of mature and immature "Xiaobai" apricot after 0, 12, and 25 days of postharvest storage. Volatile compounds with an abundance of less than 1% are combined and shown as "others."

The immature apricot showed a sour taste, with a light aroma (Table 3). After 10 days of storage, the apricot tasted sweet and the aroma is not obvious. After 25 days, the sweetness was enhanced. However, the sour taste was still there, with a light aroma. Mature fruit showed a distinct sweetness and almost no sourness; they also possessed a strong aroma. Sweetness and aroma intensity decreased significantly during storage. The immature apricots were very firm, and the flesh could not be separated easily. However, after 10 days

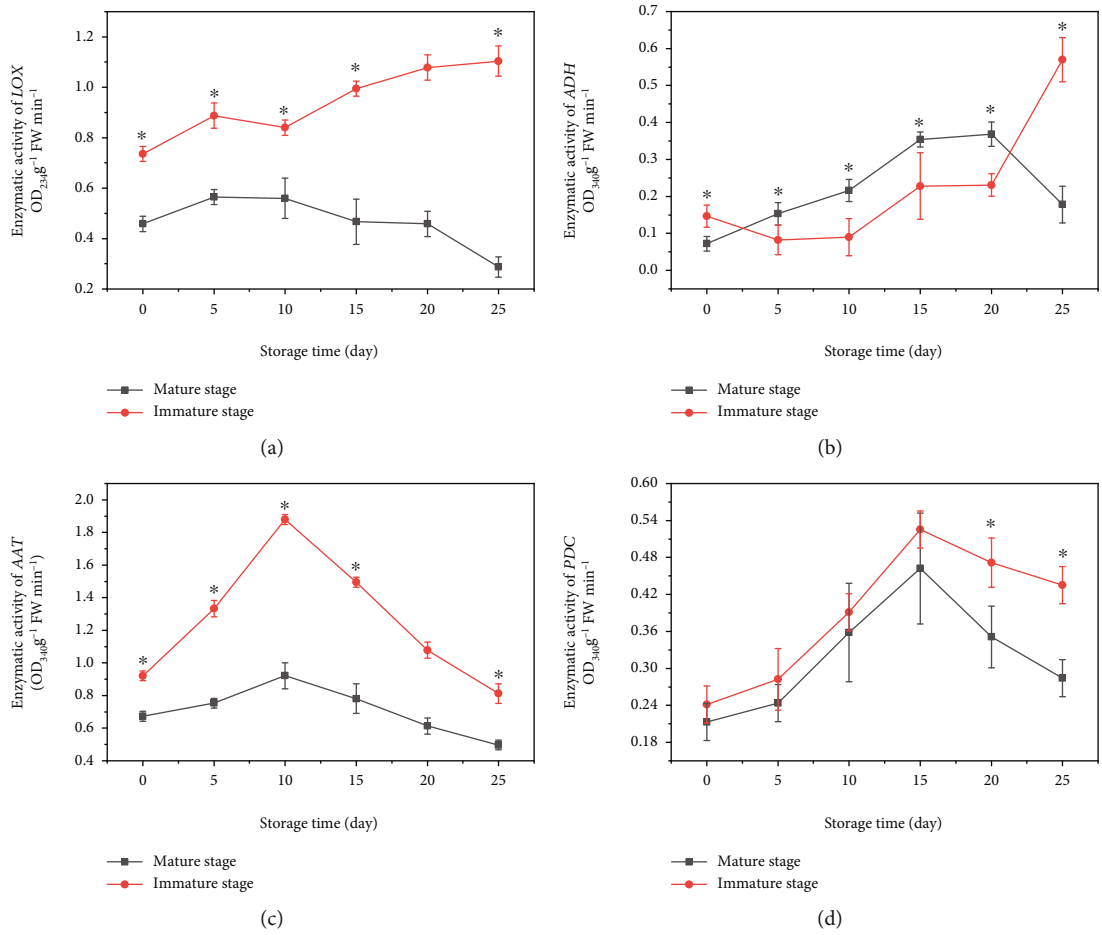


Figure 3: Determination of *LOX* (a), *ADH* (b), *AAT* (c), and *PDC* (d) enzyme activities during the storage of "Xiaobai" apricot harvested at different stages. *LOX*, *PDC*, *ADH*, and *AAT* represent *lipoxygenase*, *pyruvate decarboxylase*, *alcohol dehydrogenase*, and *alcohol acyltransferase* genes, respectively. * represent $P < 0.05$.

of storage, the flesh and stone could be separated easily, while after 25 days of storage, the fruit became soft.

*3.2. Volatile Compound Analysis in "Xiaobai" Apricot during Postharvest Storage.* In immature "Xiaobai" apricot, a total of 42 volatile compounds were identified, which increased to 49 after 25 days of storage. In the mature "Xiaobai" apricot, 40 volatile compounds were identified, which decreased to 33 after 25 days of storage. Ester, alcohol, aldehyde, ketone, acid, and hydrocarbon were the dominant volatile compounds (average relative abundance > 1%) in both immature and mature "Xiaobai" apricots. The volatile compound profile showed noticeable changes during storage (Figure 2). The alcohol content of immature apricot increased from 4.8% to 33.4%, while the aldehyde content decreased from 71.7% to 45.9% after 12 days, which might have contributed to the light aroma after 12 days. However, after 25 days, the alcohol content decreased to 2.6% while acid and aldehyde contents increased to 8.1% and 76.6%, respectively. Meanwhile, the ester content decreased to less than 10%, which was associated with the sour taste and inconspicuous aroma.

During storage, the alcohol content of mature apricot decreased from 21.2% to 3.9%, while the acid content decreased from 4.0% to 0.6%, which was not good for aroma retention. Also, the content of esters decreased (17.6% to 4.9%) after 12 days of storage. However, after 25 days, the ester content increased to 36.6%, including 35.3% of methyl salicylate, accounting for 96.4% of the total ester content, which is described to have an odor of wintergreen or mint [24]. The increase of methyl salicylate may be one of the key factors for the unpleasant odor of "Xiaobai" apricot after 25 days of storage. During the entire postharvest storage, a decrease in aromatic esters was observed, which along the decrease in alcohols may have caused fading of the mature apricot aroma during postharvest storage.

*3.3. Enzymatic Activities of LOX, ADH, AAT, and PDC Enzymes during Postharvest Storage.* During the earlier stages of storage (0–10 days), *LOX* activity showed the same pattern in both mature and immature apricots (Figure 3(a)). It slowly increased between 0 and 5 days and stabilized between 5 and 10 days. After 10 days, the dynamics of *LOX* enzyme activity reversed between mature and

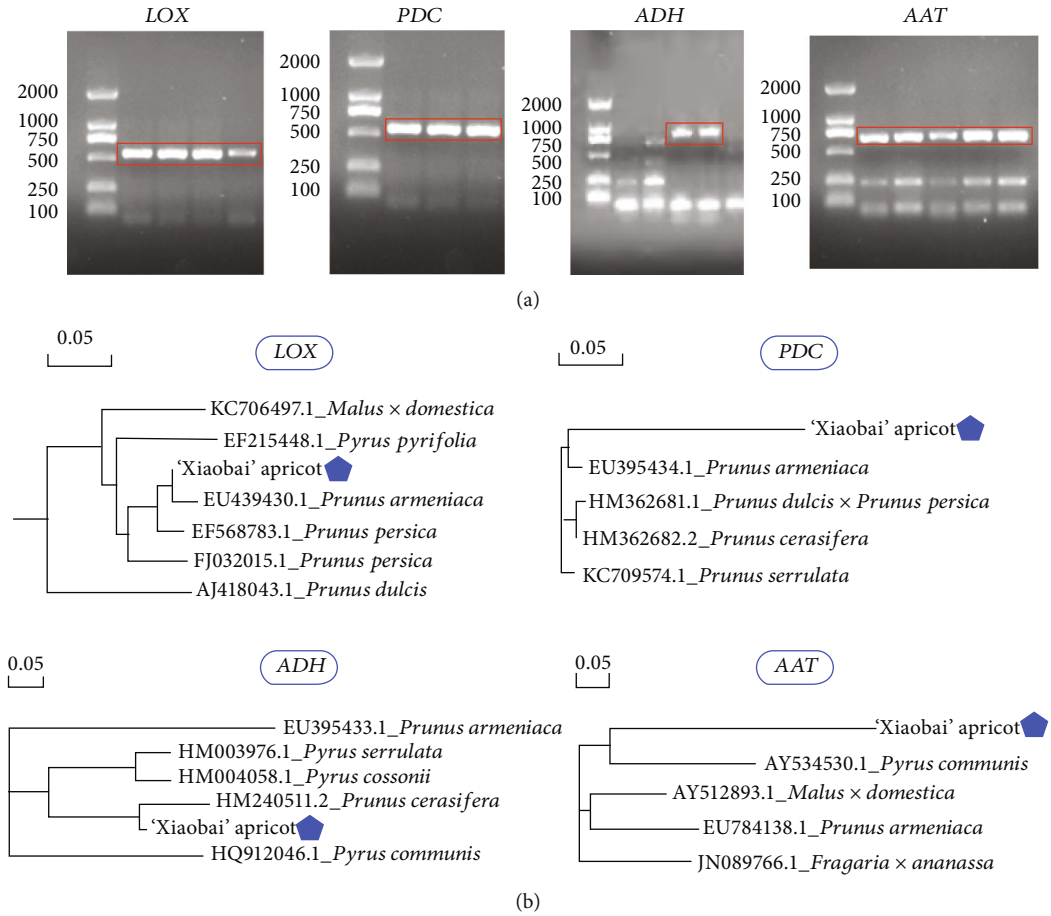


Figure 4: Sequence analysis of genes related to volatile compound biosynthesis. (a) PCR result of *LOX*, *PDC*, *ADH*, and *AAT* genes. The target PCR products are shown in red boxes. (b) Phylogenetic trees of *LOX*, *PDC*, *ADH*, and *AAT* genes. MAGE X was used to infer phylogenetic trees based on default parameters. *LOX*, *PDC*, *ADH*, and *AAT* represent *lipoxygenase*, *pyruvate decarboxylase*, *alcohol dehydrogenase*, and *alcohol acyltransferase* genes, respectively.

immature apricots. It increased from $0.84 \pm 0.03$ to $1.10 \pm 0.06\,OD_{234}\ g^{-1}$ FW $min^{-1}$ during postharvest of immature apricot, while in mature apricot, it decreased from $0.56 \pm 0.08$ to $0.29 \pm 0.01\,OD_{234}\ g^{-1}$ FW $min^{-1}$. During the whole storage time, the LOX activity in the immature apricot remained higher than that of the mature apricot ($P < 0.05$).

The *ADH* enzyme activity of mature apricot was higher than immature apricot except on day 0 and day 25 (Figure 3(b)). During the earlier stages of storage (0–10 days), the *ADH* enzyme activity showed a decrease ($0.15 \pm 0.03$ to $0.09 \pm 0.05\,OD_{340}\ g^{-1}$ FW $min^{-1}$) in immature fruit but an increase ($0.07 \pm 0.02$ to $0.22 \pm 0.03\,OD_{340}\ g^{-1}$ FW $min^{-1}$) in mature fruit. During the later stages of storage (10–25 days), *ADH* enzyme activity significantly increased from $0.09 \pm 0.05$ to $0.57 \pm 0.06\,OD_{340}\ g^{-1}$ FW $min^{-1}$ in immature fruit. After 20 days, the *ADH* enzyme activity in mature fruit gradually increased and peaked at $0.37 \pm 0.03\,OD_{340}\ g^{-1}$ FW $min^{-1}$, followed by a decrease to $0.18 \pm 0.05\,OD_{340}\ g^{-1}$ FW $min^{-1}$. These results suggested that *ADH* was most active during the later stages of ripening that was consistent with the previous study reporting [25]. Previous studies have shown that *LOX* activity is critical to the synthesis of lipids in apples [26]. In peach, the *PpAAT1*, *PpLOX1*, and *PpLOX3* are coincidentally closely associated with ester and lactone synthesis [27], which may be a potential factor for the increased ester content of immature "Xiaobai" apricot after 25 days of storage.

The *AAT* enzyme activity increased during earlier stages of storage (0–10 days) and decreased in 10–25 days in both mature and immature "Xiaobai" apricots (Figure 3(c)). Notably, the *AAT* enzyme activity of the immature fruit was higher than that of mature ones during the whole storage process ($P < 0.05$). The *PDC* enzyme activity increased in 0–15 days in both mature and immature fruits (Figure 3(d)). Meanwhile, the *PDC* enzyme activity was not significant between mature and immature fruits. After 15 days, the *PDC* enzyme activity decreased in both mature and immature fruits. The decreased extent in storage of mature fruit was higher than that of immature fruit ($P < 0.05$). The *PDC* enzymes were found to play an important role in the accumulation of acetaldehyde and alcohol at ripening and postharvest in persimmon, a key factor involved in removing the astringency of persimmons [28], while the correlation between *ADH* enzyme activity and the accumulation of acetaldehyde and alcohol was weak

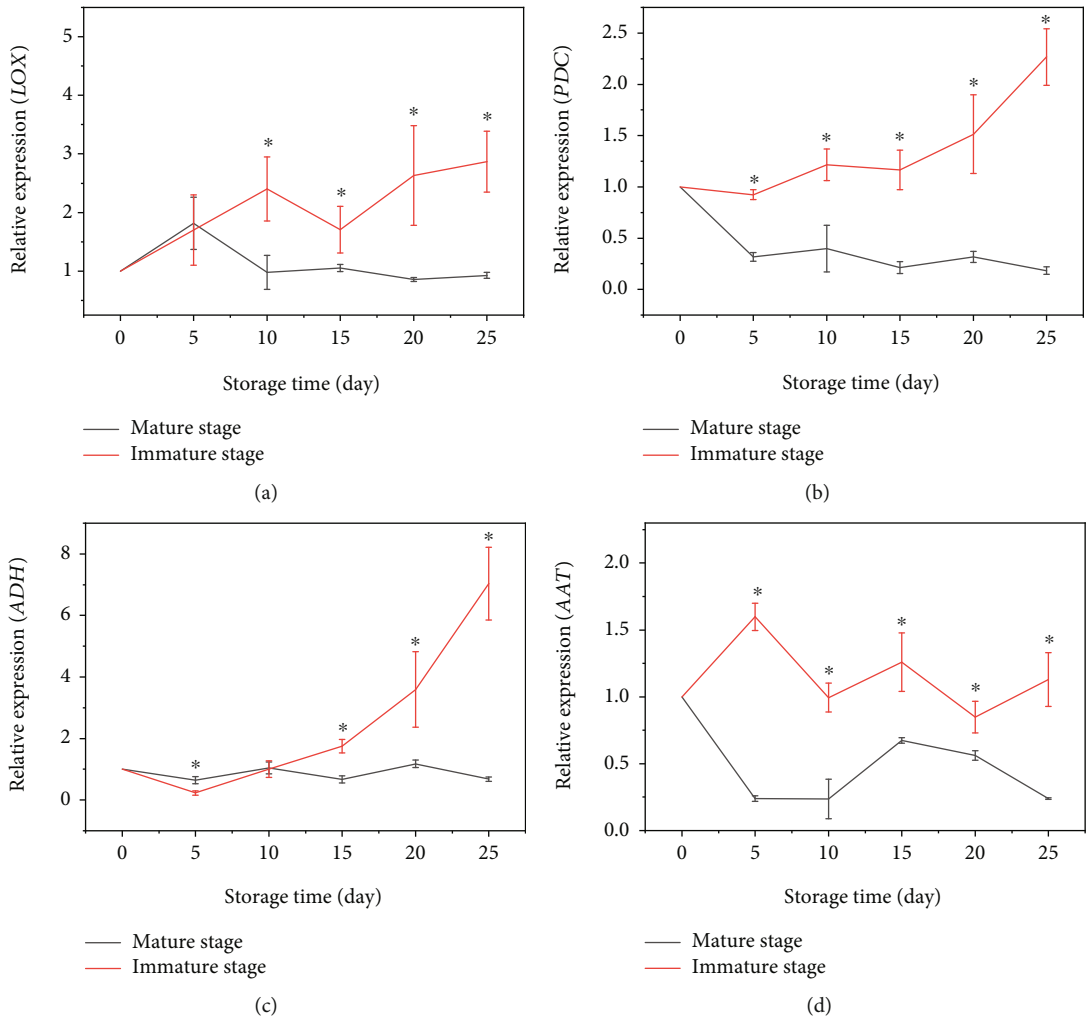


Figure 5: The expression patterns of *LOX* (a), *PDC* (b), *ADH* (c), and *AAT* (d) genes during the storage of "Xiaobai" apricot harvested at different stages. *LOX*, *PDC*, *ADH*, and *AAT* represent *lipoxygenase*, *pyruvate decarboxylase*, *alcohol dehydrogenase*, and *alcohol acyltransferase* genes, respectively. * represents $P < 0.05$.

[29]. The increase in *DkPDC* level of persimmon fruit can promote the accumulation of acetaldehyde, which is conducive to fruit deacidification [30].

*3.4. Sequence Analysis of Genes Related to the Biosynthesis of Volatile Compounds.* In the *LOX* pathway, *LOX*, *PDC*, *ADH*, and *AAT* genes were reported to be related to volatile compound synthesis [15]. The DNA fragments were successfully and specifically amplified, and the results are shown in Figure 4(a). Furthermore, the four fragments were sequenced. The size of the *LOX*, *PDC*, *ADH*, and *AAT* genes were 559, 533, 809, and 683 bp, respectively.

A phylogenetic analysis was performed (Figure 4(b)), and the *LOX*, *PDC*, *ADH*, and *AAT* genes were aligned with EU439430.1 (*LOX*, *Prunus armeniaca* L.), EU395434.1 (*PDC*, *Prunus armeniaca* L.), HM240511.2 (*ADH*, *Prunus cerasifera* Her.), and AY534530.1 (*AAT*, *Pyrus communis* L.) and found similarities of 95%, 99%, 98%, and 99%, respectively, suggesting that these isolated gene fragments corresponded to the target genes.

*3.5. Expression Levels of LOX, AAT, PDC, and ADH Genes during Postharvest Storage.* The expression levels of *LOX*, *AAT*, *PDC*, and *ADH* during storage of "Xiaobai" apricot harvested at different stages are shown in Figure 5. Overall, the expression of these genes was upregulated in the immature apricot (average relative expression level > 1) and downregulated in mature stage apricot (average relative expression level < 1), except for specific days. For example, at 10 days, the expression levels of *AAT* were downregulated ($0.24 \pm 0.15$) in mature apricot.

*LOX* and *ADH* showed a significant correlation ($R > 0.7$, $P < 0.05$). These results suggested that the dynamic changes in the gene expression levels were in line with the activities of corresponding enzymes ($P < 0.05$), indicating that these enzyme activities were controlled by the expression of the identified genes.

Correlation analysis is a useful method to reveal the potential relationship between genes and volatile compounds [31]. The Pearson correlation analysis between gene expression level and the variations in the relative abundance

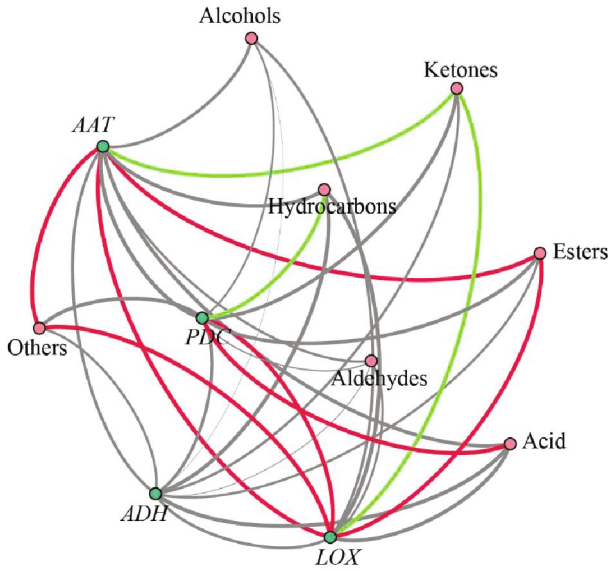


Figure 6: The correlation network between gene expression levels and variations in the relative abundance of volatile compounds. The green circle represents genes, while the red circles indicate volatile compounds. Light red line represents a significantly positive correlation, while the light green line shows a negative correlation. The gray line represents a not significant correlation. *LOX*, *PDC*, *ADH*, and *AAT* represent *lipoxygenase*, *pyruvate decarboxylase*, *alcohol dehydrogenase*, and *alcohol acyltransferase* genes, respectively.

of volatile compounds (compared to 0 days) during postharvest storage (Figure 6) showed that *LOX* was positively correlated with *AAT* ($R = 0.99$, $P < 0.05$) and *AAT* ($R = 0.96$, $P < 0.05$), which suggested a coexpression pattern. *PDC* showed a significant correlation to acids ($R = 0.98$, $P < 0.05$) and hydrocarbons ($R = -0.99$, $P < 0.05$). Meanwhile, *AAT* and *LOX* were positively correlated with aromatic esters ($R > 0.9$, $P < 0.05$) but negatively with ketones ($R < -0.9$, $P < 0.05$). Previous studies reported that *AAT* and *LOX* played roles in the synthesis of volatile compounds in other fruits. For example, *LOX* affected the accumulation of multiple straight-chain esters in melons [18], while the *AAT* gene was involved in the final enzymatic step of the biosynthesis of all esters [32]. Hence, these enzymes are proven to be related to ester compound biosynthesis [33, 34]. Overall, our results indicated that *AAT* and *LOX* genes were related to the synthesis of aromatic esters during the postharvest storage of "Xiaobai" apricot.

## 4. Conclusion

The sensory changes in different stages of "Xiaobai" apricot during postharvest storage were characterized. The loss of aroma after 25 days was related to the decrease of aromatic esters. In the immature "Xiaobai" apricot, the sour taste and inconspicuous aroma during late storage could be attributed to the decrease in alcohols and increase in acid abundance. Based on the correlation network analysis between gene expression level and variation in the volatile abundance, *LOX* and *ATT* genes were believed to play a key role in affecting the synthesis of aromatic esters. In conclusion, the factors affecting aroma in apricot of different ripening stages were found at the gene expression level, shedding light into the mechanism of apricot flavor formation during postharvest storage.

## Data Availability

Data will be made available on request.

## Additional Points

*Novelty Impact Statement*. This work characterized the sensory changes in different stages of "Xiaobai" apricot during postharvest storage. The changes of characteristic aroma were linked with volatile compound profiles. Further, we identified volatile compound synthesis-related genes. Based on the correlation network analysis, we identified the key genes affecting the synthesis of the volatile compounds.

## Conflicts of Interest

We declare no conflicts of interest.

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