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Metabolomic Profiling of Aroma Compounds in Eriobotrya japonica

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Abstract This study analyzed the composition of aroma-related metabolites in different tissues of loquat. The results show that some aromatic substances, such as benzoates, methoxybenzaldehyde and (2-nitroethyl) benzene, are the main volatile components in flowers. The leaves and roots contain a relatively large amount of flavonoids and phenols, which have certain biological activities. Genomic and transcriptomic analyses have identified genes related to aroma and the synthesis of its precursors, some of which belong to amplified synthetic families and may be associated with the high accumulation of aroma substances and medicinal components in loquat. This study aims to provide a reference for understanding the molecular basis of loquat aroma, flavor formation and medicinal value, and also to offer a scientific basis for subsequent flavor improvement and functional component development.

Keywords Loquat (Eriobotrya japonica); Aroma compounds; Metabolomics; Volatile benzoates; Flavonoids and phenols

1 Introduction

Loquat (*Eriobotrya japonica*) is widely distributed in Asia and the Mediterranean region. It is not only widely consumed because of its delicious fruit, but also valued for its application in traditional medicine (Dhiman et al., 2022). The aroma of loquat fruits is a key quality that influences consumer experience and market value. The diversity and uniqueness of aroma endow loquat with a distinctive flavor, which is of great significance for enhancing economic and nutritional value (Wang, 2021; Kim et al., 2025).

The aroma of loquat comes from a variety of volatile organic compounds (VOCs), including phenylpropanoids, benzoates, aldehydes and alcohols (Kuwahara et al., 2014; Kuwahara and Asano, 2018). The composition and content of these compounds are influenced by factors such as genetic background, variety, developmental stage and environment. Studies have found that different loquat varieties show significant differences in the accumulation of key aroma substances such as phenylpropanes and benzoates, and the related metabolic pathways are usually regulated by multiple genes (Koeduka et al., 2016). In addition, environmental conditions such as climate, soil and cultivation management can also affect the synthesis and accumulation of aroma (Wang, 2021; Zhang et al., 2024). Therefore, the aroma characteristics of loquat are very complex. In-depth research on its metabolic basis and regulatory mechanism is of great significance for variety improvement and quality enhancement.

This study analyzed the aroma compounds in loquat fruits using metabolomics methods, revealing the composition of major metabolites, key aroma components and their changes in different varieties and developmental stages. In addition, by combining genetic and environmental factors, the molecular regulatory mechanisms of aroma traits were also explored. This study aims to provide theoretical basis and data support for the molecular breeding and industrial application of the aroma quality of loquat.

2 Biological Basis of Aroma in Loquat

2.1 Major classes of volatile compounds: esters, aldehydes, terpenoids, alcohols

The aroma of loquat comes from a variety of volatile organic compounds (VOCs), mainly including esters, aldehydes, terpenoids and alcohols. Studies have found that the main aroma components of loquat flowers are benzoic acid substances such as benzaldehyde, p-methoxybenzaldehyde, methyl p-methoxybenzoate and (2-nitroethyl) benzene. These components are present in high quantities in flowers and flower buds and are the



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main source of floral fragrance (Song et al., 2009; Kuwahara et al., 2014; Kuwahara and Asano, 2018). In addition, some terpenoids (such as triterpenoids and sesquiterpenoids) have also been detected in leaves, flowers and roots, many of which have biological activity (Wang, 2021). Alcohols, such as benzyl alcohol and 2-phenylethanol, have also been identified as important aroma components of loquat flowers (Song et al., 2009; Kuwahara and Asano, 2018).

2.2 Biosynthetic pathways underlying aroma compound formation

The biosynthesis of loquat aroma mainly relies on the phenylpropane pathway and the terpene synthesis pathway. Benzoic acids and related phenethyl compounds, with L-phenylalanine as the precursor, are metabolized through the phenylpropane pathway to generate benzaldehyde, benzyl alcohol and 2-phenylethanol, etc. Some compounds, such as (2-nitroethyl) benzene, can be directly derived from Z- and E-2-phenylacetaldehyde oxime (Kuwahara and Asano, 2018). The synthesis of terpene compounds involves the generation of terpene skeletons, monoterpenes and triterpenes. The related terpene synthases are expressed in different tissues of loquat (Wang, 2021). In addition, the formation of esters and aldehydes is closely related to fatty acid metabolism and the activities of related enzymes (Song et al., 2009; Wang, 2021).

2.3 Genetic and developmental regulation of aroma traits

Aroma traits are regulated by multiple genes, involving the combined effects of structural genes and transcription factors. Transcriptome and genomic studies have shown that structural genes related to phenylpropane metabolism (such as *F5H*, *BGH3B*) are regulated by transcription factors such as MYB, bHLH and NAC, which affect the accumulation of aroma precursors and secondary metabolites by regulating enzyme expression (Wang, 2021; Zhang et al., 2024). In addition, the family amplification of terpene synthesis-related genes contributes to the massive accumulation of aroma substances in loquats (Wang, 2021). During the fruit development process, the accumulation of aroma metabolites is closely related to fruit ripening and tissue differentiation. The aroma components and contents vary significantly among different varieties and development stages (Wang, 2021; Zhang et al., 2024).

3 Metabolomic Approaches for Aroma Profiling

3.1 Analytical platforms: GC - MS, LC - MS, and NMR-based metabolomics

In the study of aroma metabolism of loquat and related plants, commonly used analytical platforms include gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), and nuclear magnetic resonance (NMR). GC-MS is suitable for detecting volatile and semi-volatile aroma components and can efficiently separate and identify complex organic compounds (Ye et al., 2025). LC-MS is more suitable for detecting non-volatile metabolites with high polarity and thermal instability, and is often used for metabolite profile analysis of tissues such as loquat leaves, flowers and roots (Wang, 2021; Ali et al., 2022). NMR is less applied in aroma analysis, but it has the advantages of being non-destructive, quantitative and structurally analytical, and can serve as a supplement (Wang, 2021).

3.2 Data processing, annotation, and compound identification workflows

The data processing flow of metabolomics generally includes the preprocessing of raw data (peak extraction, denoising, alignment), multivariate statistical analysis (such as principal component analysis, OPLS-DA), and compound annotation identification. Researchers often compare the detected metabolites with databases (such as KEGG) and conduct structural identification in combination with reference standards or fragment information (Wang, 2021; Ali et al., 2022). In loquat research, by using LC-MS/MS in combination with the KEGG database, hundreds of metabolites can be annotated and some of them can be classified into specific biosynthetic pathways (Wang, 2021). In addition, chemometrics methods (such as OAV, OPLS-DA) are also often used to screen key aroma components, helping to distinguish samples and conduct traceability (Ye et al., 2025).

3.3 Targeted vs. untargeted metabolomic strategies

Targeted metabolomics is mainly used for quantitative analysis of known aroma compounds and is often used to verify changes in specific metabolic pathways or key components (Ho et al., 2020; Ali et al., 2022). Non-targeted metabolomics focuses more on the comprehensive detection of all metabolites in the sample, which is suitable for



discovering new aroma components and analyzing the overall metabolic network. In the study of loquat aroma, non-targeted LC-MS/MS methods have been used to systematically analyze the metabolite composition of different tissues and varieties, revealing the diversity and tissue specificity of aroma components (Wang, 2021).

4 Integration of Metabolomics with Other Omics

4.1 Transcriptomics and gene-metabolite correlations

The combined analysis of transcriptomics and metabolomics revealed the synthetic genes of metabolites related to the aroma of loquat. Researchers identified candidate genes related to fruit color and flavor through genome-wide association study (GWAS) and transcriptome sequencing. The results indicated that the structural genes of glucose metabolism and phenylpropane pathways were closely related to the contents of aroma substances (such as phenolic acids and flavonoids) (Wang, 2021; Zhang et al., 2024). Weighted gene co-expression network analysis (WGCNA) further screened out some key genes and transcription factors regulating phenylpropane metabolism (such as MYB, bHLH, NAC), whose expression patterns were highly consistent with the accumulation of major aroma components (Figure 1) (Zhang et al., 2024). In addition, the study also speculated that certain β -glucosidase genes are involved in the release of volatile phenolic substances, thereby affecting aroma expression (Zhang et al., 2024).

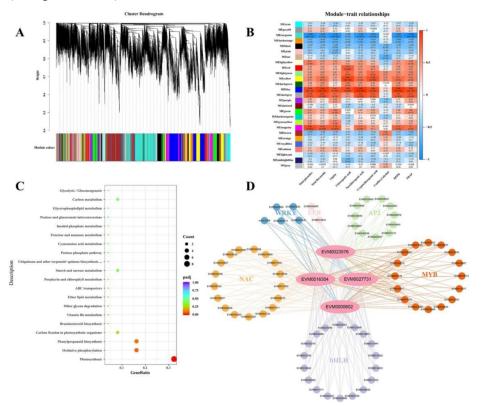


Figure 1 Network analysis dendrogram by WGCNA (Adopted from Zhang et al., 2024)

Image caption: (A) Module hierarchical clustering tree diagram. (B) Heat map of key physiological indicators and inter-module correlations. (C) KEGG enrichment map of hub genes in the blue module. (D) Co-expression network of structural genes in phenylpropanoid biosynthesis pathway and transcription factors (Adopted from Zhang et al., 2024)

4.2 Proteomics in aroma biosynthetic enzyme characterization

Proteomics provides direct evidence for the study of enzymes related to aroma synthesis. Through the combined analysis of proteomics and metabolomics, the enzymes that catalyze the transformation of key aroma precursors can be located. For example, O-methyltransferase (EjOMT1) is highly expressed in loquat flowers. It can catalyze the methylation of guaiacol-type benzoic acid substances and directly affect the formation of flower fragrance (Koeduka et al., 2016). Proteomics also reveals the changes in the abundance of aroma synthase in different tissues or developmental stages, providing a molecular basis for understanding the dynamic accumulation of aroma substances (Koeduka et al., 2016; Wang, 2021).



4.3 Systems biology approaches for network-level understanding

Systems biology methods, by integrating multi-omics data, can construct an aroma metabolism network to understand the formation mechanism of loquat aroma as a whole. KEGG annotation and metabolic pathway enrichment analysis indicated that metabolic pathways such as phenylpropane, flavonoids, and terpenoids constituted the gene-enzyme-metabolite network related to the aroma of loquat. Network analysis not only reveals key metabolic nodes and regulatory factors, but also predicts new functional genes and potential regulatory mechanisms. For instance, the expansion of gene families is often associated with the accumulation of high-abundance aroma substances. The results of systems biology provide theoretical references for the improvement of the aroma quality of loquat and molecular breeding (Wang, 2021).

5 Environmental and Postharvest Influences on Aroma Profiles

5.1 Effects of cultivation conditions: soil, climate, altitude

The aroma components of loquats can be affected by the environment. Soil type, climatic conditions (such as temperature and precipitation), and altitude all affect the accumulation of sugar, organic acids, and volatile organic compounds (VOCs) in fruits, thereby altering their flavor and aroma. For instance, an appropriate supply of minerals (such as spraying exogenous boron) can significantly increase the soluble sugar content in fruits, reduce the level of organic acids, and improve the sugar-acid ratio and flavor quality. Changes in cultivation conditions can also affect the activity of related enzymes and gene expression, thereby regulating the synthesis and degradation of aroma precursor substances (Ali et al., 2022).

5.2 Postharvest handling, storage, and ripening regulation

Post-harvest treatment and storage methods can also alter the aroma of loquats. During the normal temperature shelf life, some esters and ketones in the fruit (such as ethyl acetate, methyl 3-methylbutyrate, dimethyl ketone) will gradually decrease, while some aldehydes and furans (such as (E) -2-heptenal, heptanal, 2-pentylfuran) will increase, resulting in changes in the overall aroma. Although low-temperature storage can extend the shelf life, it is prone to cold damage and lignification, which affects the flavor and commercial value. By adopting methods such as modified atmosphere packaging, low-temperature regulation, heat treatment or edible coating, the deterioration of quality can be slowed down to a certain extent and the aroma and nutrition can be maintained (Huang et al., 2023; Shah et al., 2023; Zhao, 2024).

5.3 Epigenetic and stress-related modulation of aroma metabolism

Environmental stress (such as high temperature, mechanical damage and pathogen infection) and epigenetic regulation may also affect the aroma metabolism of loquats. Stress can induce changes in the expression of metabolic pathway genes, thereby altering the synthesis and degradation of volatile aroma substances. At present, there are few epigenetic studies on the aroma metabolism of loquat. However, existing studies have found that some post-harvest treatment methods (such as heat treatment and controlled atmosphere storage) can indirectly affect the accumulation of aroma substances by regulating the activities of related enzymes and gene expressions (Ali et al., 2022; Shah et al., 2023). Further research on the role of epigenetic mechanisms such as DNA methylation and histone modification in aroma regulation is still needed in the future.

6 Case Study: Metabolomic Profiling of Aroma Compounds in *Eriobotrya japonica* 6.1 Research background and objectives

Loquat is not only an important fruit tree, but also its flowers, leaves and roots hold significant positions in traditional medicine due to their rich content of active ingredients. Aroma is a key factor in evaluating the quality of loquat fruits and flowers and determining consumer acceptance. However, at present, the metabolic characteristics and molecular basis of aroma compounds have not been systematically revealed. The objective of this study case is to comprehensively analyze the aroma-related metabolites in different tissues of loquat using metabolomics methods, understand their composition and distribution, as well as their effects on sensory quality, thereby providing a theoretical basis for the improvement of aroma quality and the development of functional components of loquat (Kuwahara et al., 2014; Kuwahara and Asano, 2018; Wang, 2021).



6.2 Methodology: sampling, analytical platform, data analysis

This case study selected the flowers, leaves and roots of representative varieties such as 'Big Five-pointed Star'. Metabolite detection utilized high-throughput platforms such as UKC-ESI-MS /MS and GC-MS. The analysis of aroma components combined GC-O, odor activity value (OAV) assessment and chemometrics methods. Data processing included multivariate statistical analyses such as PCA and OPLS-DA to reveal the differences in aroma metabolism among different tissues and varieties, as well as their relationships with sensory characteristics (Kuwahara et al., 2014; Kuwahara and Asano, 2018; Wang, 2021).

6.3 Key findings: identified aroma compounds and their contribution to sensory quality

A total of 577 metabolites were detected by metabolomics, including 98 phenolic acids, 95 flavonoids, 28 terpenoids, as well as various phenylpropanoids and glycosides (Wang, 2021). Among the aroma substances, the main volatile components of the flower are (2-nitroethyl) benzene, p-methoxybenzaldehyde and methyl p-methoxybenzoate. These benzoic acid compounds endow loquat flowers with a unique sweet aroma (Kuwahara et al., 2014; Kuwahara and Asano, 2018). In addition, phenylpropanoids (such as chlorogenic acid, p-coumaric acid), flavonoids (such as quercetin, kaunferol), and terpenoids (such as oleanolic acid, ursolic acid) are abundant in different tissues and are closely related to physiological activities such as antioxidation and anti-inflammation (Wang, 2021; Zhang et al., 2024; Kim et al., 2025).

The distribution of aroma substances in different tissues is significantly different: the content of benzoic acid and phenylpropanoids in flowers is relatively high; The leaves and roots are mainly composed of flavonoids, phenolic acids and triterpenoids (Wang, 2021). Some substances (such as (2-nitroethyl) benzene) have only been detected in flowers and are the main source of floral fragrance (Kuwahara et al., 2014; Kuwahara and Asano, 2018). Genomic and transcriptomic studies have also identified some key structural genes and transcription factors, which are involved in the biosynthesis of aroma substances and provide a molecular basis for understanding their metabolic regulatory mechanisms (Koeduka et al., 2016; Wang, 2021; Zhang et al., 2024).

7 Challenges and Limitations

7.1 Difficulties in capturing low-abundance volatiles

Although the commonly used metabolomics methods at present (such as LC-MS/MS, UKC-ESI-MS /MS) can detect hundreds of metabolites, their sensitivity to low-abundance and highly volatile aroma components is still insufficient. The traditional phytochemical methods are inefficient and have limited throughput, which also makes it difficult to comprehensively detect and accurately quantify some key aroma substances, thereby affecting the integrity and accuracy of the aroma spectrum (Wang, 2021).

7.2 Complexity in linking metabolites to sensory perception

Although research has been able to identify many aroma-related metabolites, it is still difficult to directly correlate these substances with actual sensory experiences, such as aroma types and intensities. The content of some metabolites varies significantly among different tissues, but their contribution to the overall aroma remains unclear. In addition, the physiological functions and sensory thresholds of many metabolites have not been systematically studied, so there is still a 'black box" between metabolomics data and sensory evaluation (Wang, 2021).

7.3 Gaps in functional validation of candidate genes

The combined analysis of the genome and metabolome has been able to screen out some candidate genes related to aroma synthesis, but the specific functions and regulatory mechanisms of these genes are mostly still lacking in experimental evidence. Association analysis alone cannot confirm the true function of genes, and the lack of verification methods such as transgenic or gene knockout also limits our in-depth understanding of the aroma synthesis network (Wang, 2021).

8 Future Perspectives

8.1 Advancements in high-resolution metabolomics and real-time aroma analysis

With the development of high-resolution mass spectrometry (such as UPLC-ESI-MS/MS) and multi-omics technologies, the detection sensitivity and coverage range of loquat aroma compounds have been significantly

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improved. The latest research has identified hundreds of metabolites in tissues such as leaves, flowers and roots, including phenolic acids, flavonoids and terpenoids, laying the foundation for a comprehensive study of aroma components (Wang, 2021; Kim et al., 2025). Meanwhile, real-time aroma analysis techniques (such as GC-MS and electronic nose) have also begun to be applied, which can achieve dynamic monitoring of aroma components and reveal the variation patterns of aroma in different times and Spaces (Kuwahara et al., 2014; Kuwahara and Asano, 2018; Ye et al., 2025). In the future, if high-throughput, real-time and spatially resolved metabolomics can be combined, it will greatly promote the systematic research on the aroma of loquat.

8.2 Integration of metabolomics into breeding programs for flavor improvement

The combination of metabolomics and genomics provides new ideas for analyzing the genetic basis of flavor traits in loquats and conducting molecular breeding. Through genome-wide association study (GWAS) combined with metabolome data, researchers have identified some candidate genes related to flavor and found that pathways such as glucose metabolism, phenols, and terpenoids play important roles in flavor (Wang, 2021; Zhang et al., 2024; Kim et al., 2025). In the future, metabolomics is expected to become an important tool for flavor improvement breeding, helping to screen superior genotypes and accelerate the development of high-quality new varieties.

8.3 Applications of machine learning and big data analytics in aroma prediction

With the continuous accumulation of aroma metabolome data, machine learning and big data methods have shown great potential in aroma prediction and flavor analysis. It is now possible to effectively distinguish the aroma differences among different varieties, tissues and origins through methods such as chemometrics, principal component analysis and OPLS-DA (Zhang et al., 2024; Ye et al., 2025). In the future, algorithms such as deep learning are expected to achieve high-precision prediction of aroma components, intelligent mining of flavor-related genes, and the establishment of system models for aroma regulation. These methods will provide important support for the precise improvement of the flavor and quality of loquats and their industrial application.

9 Conclusion

Metabolomics studies have found that different tissues of loquat (leaves, flowers, roots) contain rich aroma-related metabolites, including phenylpropanoids, flavonoids, terpenoids and glycosides, etc. Studies have shown that the main aroma components of loquat flowers are (2-nitroethyl) benzene, p-methoxybenzaldehyde and methyl p-methoxybenzoate. These substances have not been detected in the leaves and exhibit obvious organ-specificity. The activity level of the phenylpropanin metabolic pathway and the expression changes of related structural genes directly affect the flavor differences among different varieties and tissues. High-throughput metabolomics detected a total of 577 metabolites, including 98 phenolic acids, 95 flavonoids and 28 terpenoids. Some aroma precursor substances, such as phenylalanine, can be converted into volatile benzoic acid aroma substances through specific enzymatic reactions.

Metabolomics can not only comprehensively detect and quantify aroma components, but also be combined with genomic and transcriptomic data to identify key genes that control aroma synthesis, providing references for molecular breeding and quality improvement. For instance, metabolomics combined with genome-wide association study (GWAS) has identified candidate genes related to pulp color, sugar metabolism and aroma. The research also revealed the differences in the accumulation of aroma components among different varieties and tissues, providing a scientific basis for screening high-quality aroma varieties and developing functional foods.

In the future, the combination of metabolomics and molecular breeding techniques is expected to achieve precise regulation of the aroma and nutritional quality of loquats, promoting the breeding of new varieties that meet consumer demands. At the same time, conducting systematic research on the aroma and functional components of the entire loquat plant (including by-products such as leaves, flowers, and roots) can also promote the high-value utilization of resources and the extension of the industrial chain, and drive the sustainable development and utilization of loquats.

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Conflict of Interest Disclosure

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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