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Characterization of the aroma and flavor profiles of guava fruit (*Psidium guajava*) during developing by HS-SPME-GC/MS and RNA sequencing

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ABSTRACT

The flavor of guava, an important tropical fruit, is influenced by secondary metabolites. However, the mechanisms and processes underlying flavor formation in guava remain unclear. In this study, dynamic changes in volatile organic compounds (VOCs), sugars, and organic acids in guava peel and flesh across different developmental stages were investigated using headspace solid-phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC–MS) and high-performance liquid chromatography (HPLC). Here, we identified 90 VOCs, three sugars and eight organic acids. The dynamics of VOCs differ between the flesh and peel. The early developmental stages are more critical in influencing the variation of VOCs in the flesh, while VOC changes in peel occur more progressively across the developmental stages. By integrating transcriptomic and metabolomic analyses, we identified several key genes involved in VOC, sugar, and acid metabolism. This is the first study to describe the expression patterns of these genes throughout guava development, providing new insights into guava flavor development.

1. Introduction

Guava (*Psidium guajava* L.), an evergreen plant belonging to the Myrtaceae family, is native to the Americas and thrives in tropical and subtropical regions (Menzel, 1985). This fruit is popularly known as "Apple of Tropics" or "Poor Man's Fruit", owing to its adaptability and exceptional nutraceutical properties (Feng et al., 2021). It also earned a good reputation as a superfruit due to its abundant nutrients, such as various vitamins, dietary fibers, and minerals (Nayak et al., 2019). Beyond its nutritional benefits, guava possesses various pharmacological properties such as antispasmodic and antimicrobial properties, which may be beneficial in the treatment of diarrhoea and dysentery (Barbalho et al., 2012; Joseph and Priya, 2011; Gutiérrez et al., 2008). Besides been consumed fresh, guava is also processed into jam, juice, wine, essential oils, and other industrial products (Narváez-Cuenca et al., 2020).

Plants produce various volatile organic compounds (VOCs) that play crucial roles in attracting pollinators or seeds-dispersing animals, enhancing resistance to pests and diseases, and alleviate abiotic stress (Brilli et al., 2019). VOCs, including esters, terpenoids, flavonoids, isothiocyanate, and allicin, not only shape the sensory properties, but also possess bioactive medicinal components (Aguiar et al., 2021). In edible fruits, VOCs contribute significantly to the characteristic aromas and flavors, thereby influencing consumer preferences and acceptance (Chen and Quek, 2022).

VOCs are gradually synthesized through various metabolic pathways from precursors such as fatty acids, amino acids and carbohydrates (Defilippi et al., 2009). The fatty acid pathway is one of the most important biosynthetic pathways during fruit maturation, leading to the production of many important VOCs, including aldehydes, esters, and alcohols (Granell and Rambla, 2013). For example, the distinctive scent described as "green and leaf-like" is primarily caused by C6 aldehydes and alcohols, which are mainly derived from the oxidation of linoleic acid and linolenic acid pathways (Chen et al., 2021). The typical fruity aroma found in many mature fruits is primarily originates from esters, which are generated through the esterification of acyl-CoAs and alcohols in the lipoxygenase (LOX) pathway (Chen et al., 2020). This metabolic pattern is commonly observed during the ripening process in a variety of fruits, including apples, pears and tomatoes (Wei et al., 2017; Klee, 2010; Song and Bangerth, 2003).

The VOCs in guava have received significant attention due to their potential nutraceutical and economic value. Various VOCs have been

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successfully extracted from guava tissues such as fruit skins, pulps, leaves, bark, roots, and twigs (Guevara et al., 2019). These compounds mainly consist of terpene hydrocarbons, alcohols, esters, aldehydes, ketones, and some miscellaneous compounds (Lee et al., 2011). Current research suggests that the unique aroma of guava fruits is not attributed by a single class of volatile constituents, but rather to the presence of aldehydes, esters, and terpenes (Chen et al., 2007, Cuadrado-Silva et al., 2017).

Considerable research has been conducted on VOCs in guava fruits, encompassing VOCs determination in diverse varieties under various treatment conditions (Mishra et al., 2022). Concurrently, the release of the complete reference genome of guava and the elucidation of cellulose regulatory mechanisms during maturation have greatly facilitated our ability to perform integrated analysis of gene function and metabolomics in guava, thereby broadening our understanding of guava fruit biology (Feng et al., 2021). The composition and content of compounds in guava result in distinct uses for the peel and flesh. The flesh is often consumed fresh, while the peel, which contains antimicrobial and antioxidant compounds, have beneficial pharmacological properties (Angulo-López et al., 2021; Liu et al., 2018). However, the differential composition and alteration of VOCs in fruit peel and pulp across the developmental stages remained largely unknown. In this study, we perform a metabolomic analysis and transcriptome analysis to investigate and elucidate the characteristics of VOCs in the peel and pulp of guava during development. Moreover, we also aim to identify the key genes involved in the regulatory pathways.

2. Materials and methods

2.1. Plant materials and sample preparation

Guava fruits at different developmental stages were sampled from the cultivar "*New Age*" in the garden of Guangdong Ocean University (Zhanjiang, Guangdong province, China). Briefly, samples were collected from young fruits (two weeks after fruit set), expanding fruits (five weeks after fruit set), and mature fruits (eight weeks after fruit set) with three biological replicates (Feng et al., 2021). Each replication comprised at least three fruits which were mixed in equal quantities. The flesh and peel samples of guava were manually separated and stored in liquid nitrogen until use.

2.2. Determination of VOCs

The frozen samples were ground to a powder quickly in liquid nitrogen. A total of 1.5 g of powder samples were placed into a 20 mL headspace extraction bottle (Agilent, Palo Alto, CA, USA) containing 2 mL of NaCl saturated solution to inhibit enzyme reaction.

The VOCs in each sample were identified and quantified by headspace-solid phase microextraction (HS-SPME) with gas chromatography-mass spectrometry (GC–MS) analysis according to previous study (Li et al., 2021; Zhang et al., 2021). The initial column temperature was adjusted to 40°C for 1 min, and raised to 280 °C at a rate of 5 °C/min. Helium was used as the carrier gas and column flow of constant as 1.0 mL/min. Mass spectra were measured and recorded in electron impact (EI) ionization mode at 70 eV. The volatile compounds were determined by comparing spectral similarity with the library NIST 14 and linear retention index and quantified with internal standard method.

2.3. Determination of soluble sugar and organic acid in guava

The soluble sugar and organic acid contents were measured by HPLC (LC-20AT, Shimadzu, Kyoto, Japan) according to the known method with minor modifications (Xiao et al., 2024; Zhou et al., 2023). Briefly, four grams of the sample were ground into powder in liquid nitrogen, then the powder was homogenized with 5 mL of 80 % ethanol and an internal standard solution. The homogenate was incubated at 35°C for 20 min, and then centrifuged at 10,000 × *g* for 5 min. The supernatant was collected and aforementioned procedure was repeated twice for the residue. The supernatants were combined and adjusted to 25 mL, and then filtered through a 0.45 μm filter before analysis. For the determination of soluble sugars, a mobile phase of acetonitrile: water (70:30) was used at a flow rate of 1 mL/min. For the measurement of organic acid, 10 μL sample was injected into a 5 μm ODS C18 column (Waters Corp. USA), and measured in a solvent system of 50 mmol $(NH_4)_2HPO_4$ at a flow rate of 0.5 mL/min. The soluble sugar and organic acid were quantified by comparing the area under peak with the known standards.

2.4. Key genes identification and transcriptome analysis

The gene expression level (fragments per kilobase of transcript per million fragments mapped, FPKM) for each sample was obtained from a previous study (Feng et al., 2021). The pairwise Pearson correlation coefficient (PCC) analysis between gene expression levels (FPKM) and metabolite level was performed to generate the relationship of genes and metabolites. Genes were retained only if the absolute value of PCC greater than 0.9 and FPKM ≥ 1 in any sample. The known genes from the Plant Metabolic Pathway Database (Plant Metabolic Network, PMN database, https://plantcyc.org) were used as query to BLAST against the retained guava genes, and the candidate key genes involving in each metabolic pathways were retrieved with the highest identity (Hawkins et al., 2021).

2.5. Statistics analysis

All the statistics analysis and visualization were performed in R v4.3.1. Principle component analysis (PCA) and orthogonal projection to latent structure-discriminant analysis (OPLS-DA) were carried out in factoextra and metaboAnalystR package in R, respectively (Chong and Xia, 2018). The differences in differentially accumulated VOCs (DAV) were filter out using a threshold of absolute value of LogFC ≥ 1 and P value < 0.05, and visualized in ggvolcano, upsetR, and VennDiagram package, respectively. Heatmaps was carried out by pheatmap package.

3. Results

3.1. Characteristic of flavor-associated metabolites during guava development

A set of volatile compounds contribute to the first sensory flavor and unique characteristics, while sugars and organic acids provide the acceptable of taste for most of fruits. To investigate the flavor-associated metabolic profiling during the different developmental stages in guava, samples from different tissues (flesh and peel) and developmental stages (S1 to S3 representing young fruit, expanding fruit and mature fruit, respectively) were selected for analysis.

A total of 90 different VOCs were identified in all samples and assigned names C1 to C90 (Fig. 1A&B, Tables S1 and S2). These metabolites were classified into nine categories based on their functional groups, including terpenoids, alcohols, aldehydes, alkanes, aromatic hydrocarbons, esters, ketones, phenols, and others (Fig. 1A and Table S2). Among the detected VOCs, terpenoids were the most diverse class, representing 43.33 % (39) of the total compound types. Followed by aldehydes (10), aromatics (9), hydrocarbons (9) and alcohols (8), comprising 11.11 %, 10 %, 10 %, 8.89 % of the total compound types, respectively. Of the total 90 VOCs, 46 compounds were consistently present in all tissues across different developmental stages, constituting the basis of the flavor of guava (Fig. 1B). Furthermore, 29 VOCs were exclusively detected in the peel of guava, suggesting that these compounds may form the foundation of the unique smelling flavor characterizing of guava (Tables S1 and S2). Interestingly, the majority of them are terpenoids, comprising 17 types (accounting for 58.6 %). Followed

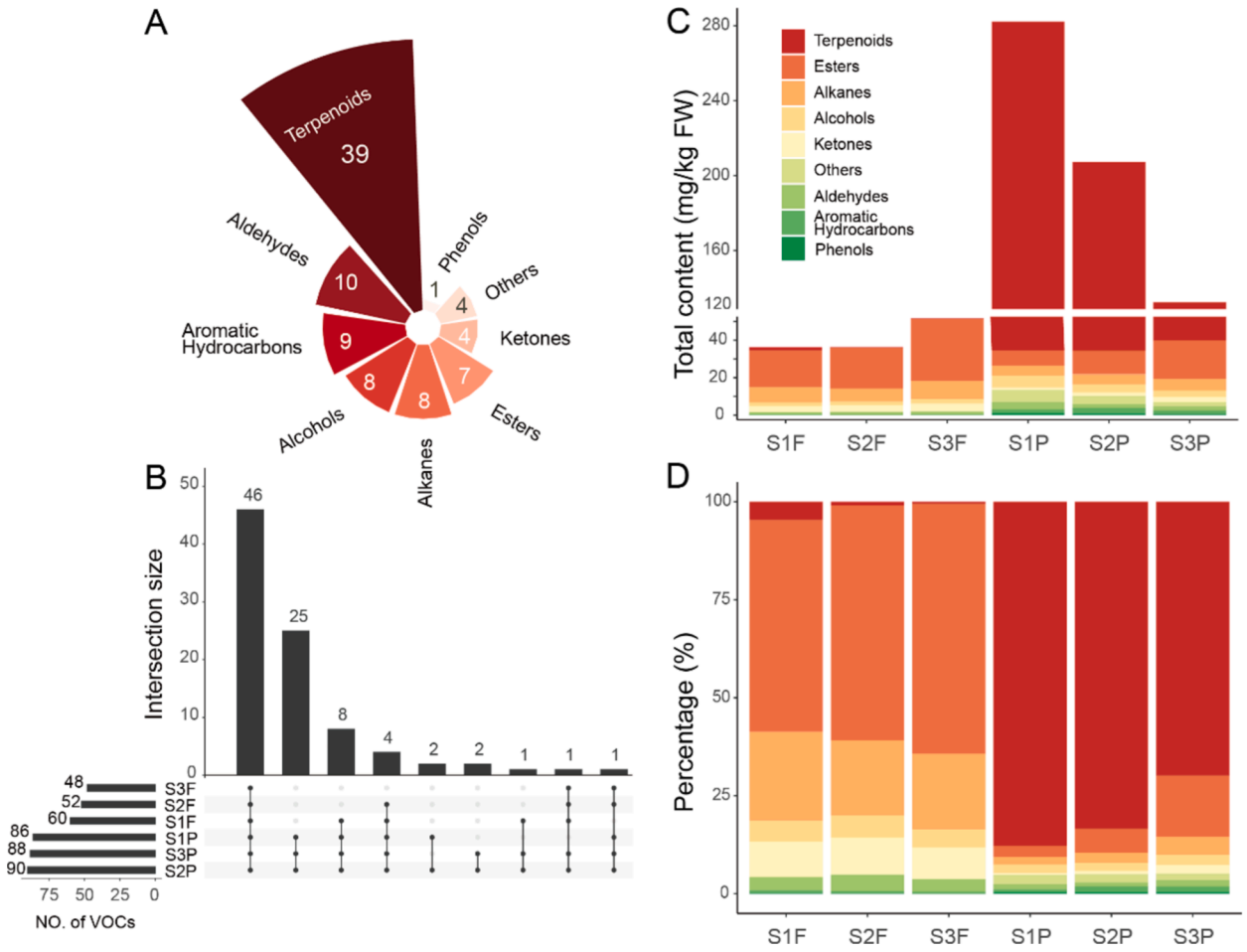

Fig. 1. The volatile compounds in guava. A. The number and classification of VOCs. B. The intersection number of types between different samples. Horizontal bars indicate the number of VOCs in each sample, while the vertical bars indicate the number of shared VOCs across samples. The total content (C) and proportion (D) of these VOCs in each sample.

by aromatic hydrocarbons, alcohols, aldehydes, esters, and other compounds are represented by 4, 3, 2, 1, and 2 types, respectively (Table S1). Furthermore, the number of VOC types remains relatively stable across different developmental stages of the peel, however, there is a significant decrease in the variety of terpenoid compounds in the flesh (Fig. S1, Tables S1 and S2).

The types of VOCs in each sample are highly diverse, notably, their contents also exhibit significant variability. Specifically, compounds such as terpenoids, aldehydes, and aromatic hydrocarbons are found in much higher quantities in the fruit peel than in the flesh (Fig. 1C & D). Furthermore, while the types and contents of VOCs in the peel remain relatively stable across different developmental stages, terpenoids in the flesh exhibit significant variation during development, both in types and content (Fig. 1C & D, Table S2). For instance, from young fruit (S1) to mature fruit (S3) stage, the types of terpenes in the flesh decreased from 21 to 11, with the total content decreasing from 1.67 mg/kg FW (fresh weight) to 0.25 mg/kg FW. In contrast, the total content in the peel decreased from 247.64 mg/kg FW to 92.48 mg/kg FW, while the number of terpenoids remained relatively stable (Fig. 1C & D). The dominant VOCs also vary between tissues. For instance, esters and alkanes were the predominant contents in flesh across different stages, but terpenoids were predominant in peel (Fig. 1C & D).

3.2. Dynamic changes of VOCs during fruit development in guava

To investigate the variation of metabolomic compounds in these samples, the orthogonal partial least squares discriminant analysis (OPLS-DA) and principal component analysis (PCA) was conducted.

The results of PCA analysis based on the VOCs showed that a significant difference between two different tissues (peel and flesh in Fig. 2A), accounting for 39.8 % of the total variance (PC2). However, the majority of the total variance (PC1, 56.3 %) appeared from developmental stages (Fig. 2A). Among these VOCs, compound of C86 (Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)-, (1S-cis)-), C85 (Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-), C56 (Humulene), C87 (Cubenene), C54 (Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1-alpha-,7-beta-,8a-alpha-)]-), C59 (gamma-Muurolene), C61 (Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1-alpha-,4a-beta-,8a-alpha-)-), C79 (D-Limonene), and other terpenes mainly appear in the peel of guava, while C39 (Acetic acid, 2-phenylethyl ester), C24 (Pentadecane), C38 (Octanoic acid, ethyl ester), C36 (Heptanoic acid, ethyl ester), C45 (4-Heptanone, 2,6-dimethyl-), and C1 (1-Hexanol) are found primarily in the flesh. These compounds are the main differences between tissues (arrows in Fig. 2A). The results of PCA analysis based on samples also indicated that the main factor between samples was the different tissue types, accounting for 67.9 % of the total variance (Fig. 2B). Interesting, the significant contribution observed between young fruit peel (S1P) and other samples may be attributed to the abundance of terpenoids in the S1P sample. (Fig. 2B & Fig. 1C).

Based on the variation of metabolite concentration, these metabolites could be divided into five clusters (Cluster I to Cluster V in Fig. 3A). Metabolites in Cluster I and Cluster V are primarily contained alkanes,

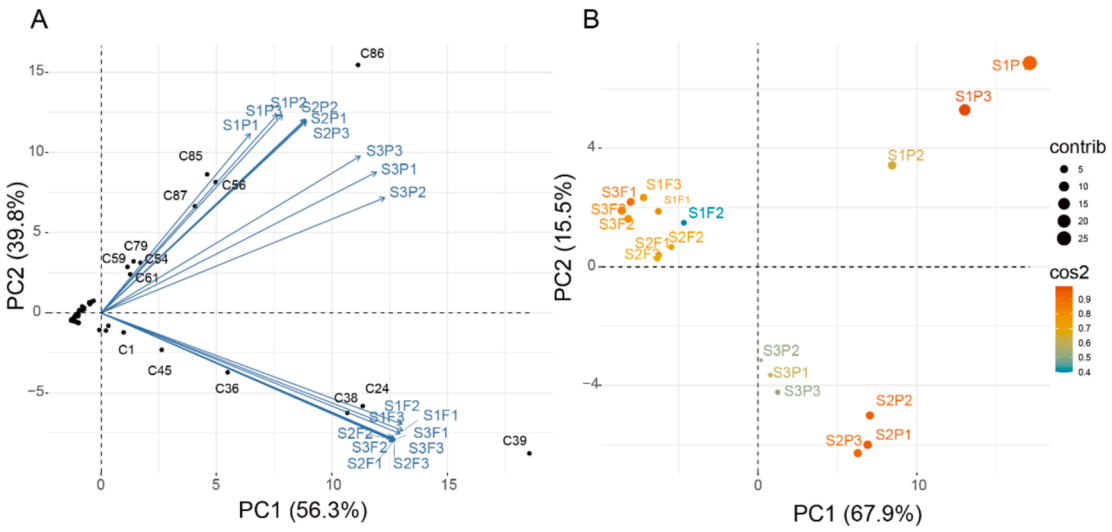

Fig. 2. The principal component analysis (PCA) in guava. A. The PCA results based on VOCs. B. The PCA results based on samples. Contrib indicates their contributions to definition of the principal dimensions, while cos2 corresponding to their quality of representation on the factor map.

ketones, esters and aromatic hydrocarbons, which accumulate preferentially in flesh (Fig. 3A). These compounds may account for the typical volatile flavor of flesh in ripe guava. Metabolites in Cluster III included terpenoids, aldehydes, and aromatic hydrocarbons, which accumulated primarily in the peel of expanding and mature fruit (S2P and S3P). These VOCs could potentially serve as the primary contributors to the distinctive aroma of peel in mature guava (Fig. 3A). Additionally, the metabolites in Group II and IV were predominant accumulated in peel of young fruit and expanding fruit. These VOCs mainly consisted of terpenoids, aromatic hydrocarbons, esters, alcohols, and phenols (Fig. 3A). These compounds may constitute characteristic astringency flavor in immature fruit peel of guava, serving to protect fruit from pests and diseases. The results of K-means analysis further supporting that the varies of VOCs across different tissues and developmental stages could primarily be categorized into five clusters (Fig. 3B).

To explore the relationship between various VOCs and differences among samples, we conducted an OPLS-DA analysis on samples divided into tissues and developmental stages. The results suggest that tissue is the most important influencing factor throughout the three classification groups, accounting for 67.2 %, 66 %, and 53.9 % of the variance when grouped by sample, developmental stages, and tissue, respectively (Fig. 4A–C).

The variable importance projection (VIP) values of OPLS-DA analysis based on all samples revealed that the C1 (1-Hexanol), C84 (beta-Bisabolene) and C77 (3-Hexen-1-ol, acetate, Z-) were the most important metabolites (Fig. 4D). The OPLS-DA results based on different developmental stages (Fig. 4E) showed that the most important metabolites are C1 (1-Hexanol), C15 (2,4-Heptadienal, (E,E)-), and C40 (*trans*-Geranic acid methyl ester). While these metabolites are C57 (Alloaromadendrene), C41 (2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-), and C74 (D-Carvone) in different tissue groups (Fig. 4D–F). These results suggest that certain alcohols, esters, aromatic hydrocarbons and terpenes, such as C1 (1-Hexanol), C41 (2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-), C15 (2,4-Heptadienal, (E,E)-), C32 (Ethanone, 1-(4-methylphenyl)-), and C84 (beta-Bisabolene), play crucial roles in shaping the flavor profile of guava fruit.

Furthermore, there were slight shifts in the contributions of these VOCs to the flavor of guava across different development stages or tissues. For instance, the compounds that contribute most to the flavor in flesh across different developmental stages shift from C54 (Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1-alpha-,7-beta-,8a-alpha-)]-) to the C79 (D-Limonene, Fig. S2D–E). However, this transition shift from C46 (1-Octen-3-one) to C65 (−alpha-Guaiene) in the peel (Fig. S2J–K). These results also suggesting that terpenes and aromatic hydrocarbons play a significant role in shaping the flavor profile of the guava during development (Fig. S2). In addition, the contribution of each VOC to the flavor of guava varies among different tissues. For instance, during the S1 to S3 stages, the flavor differences between fruit peel and flesh are mainly attributed to C83 ((+)-alpha-Longipinene), C57 (Alloaromadendrene) and C60 (Benzene,1-(1,5-dimethyl-4-hexenyl)-4-methl-) (Fig. S3D-F). Terpenoids, esters, and aromatic hydrocarbons continues to significantly contribute to the flavor differences between the fruit flesh and peel.

The significant differences of DAV are observed between different developmental stages and tissues (Fig. S4). For instance, the number of DAV between different developmental stages within the same tissue decreased significantly across development (Fig. S4D-I). Additionally, it is noteworthy that the DAV between flesh and peel shared 38 core VOCs during different developmental stages (Fig. S5A). These VOCs include alcohols, aromatic hydrocarbons and terpenoids, which potentially underlie the basis of flavor distinctions between flesh and peel in guava. Interesting, the number of DAV in flesh between S1 and S2 is markedly higher than other stages, suggesting that this developmental stage (S1 to S2) may be crucial for the flavor formation or transformation in the flesh (Fig. S5B). Both the number and shared number of DAV in the peel are greater than those number in the flesh (Fig. S5B–C), suggesting that the VOCs changes in the peel during different developmental stages are more significant. Based on this observation, it appears that the S1 to S2 stage may be critical for the flavor formation in the fruit flesh, while the flavor formation in peel is likely a gradual process, with the entire developmental stages being crucial this distinction may be vital in understanding the formation of guava flavor.

3.3. *Dynamic changes of sugar and organic acid during fruit development in guava*

Soluble sugars and organic acids play pivotal roles in shaping the taste profiles of various fruits (Ikegaya et al., 2019; Mikulic-Petkovsek et al., 2012). In this study, we identified three types of sugars and eight types of acids (Fig. 5, Table S3). The total sugars content increased steadily during fruit development in both peel and flesh, while the amount of organic acid decreased gradually (Fig. 5). For instance, the content of quinic acid, fumaric acid and shikimic acid decreases sharply during the ripening process in the peel and flesh (Fig. 5C&D, Table S3). This reduction may be a crucial factor in the decrease of sour and astringent taste during the ripening process, and is also an important

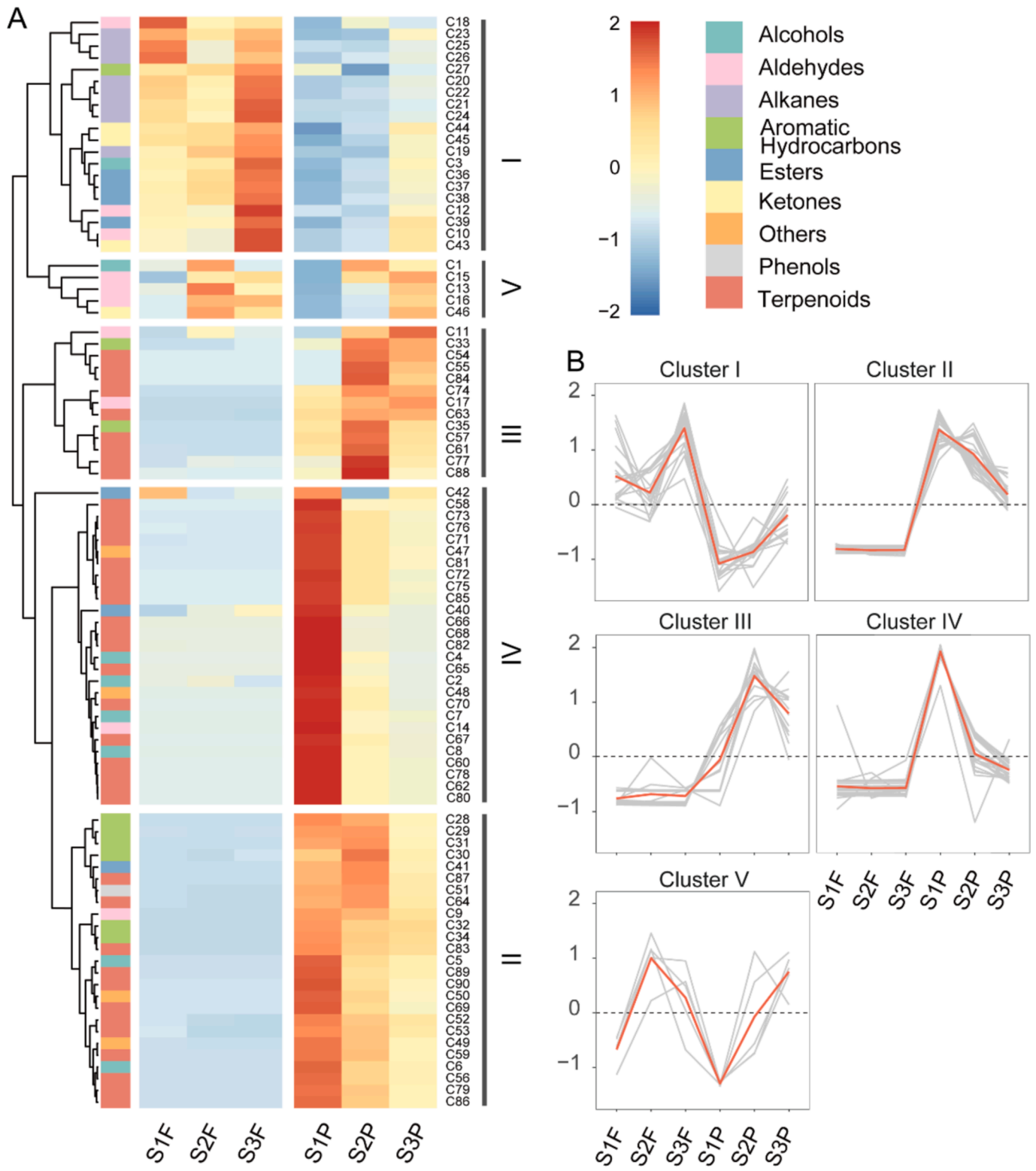

Fig. 3. The heatmap and cluster analysis of VOCs. A. The heatmap of the VOCs. Red and blue indicate relative higher and lower concentration of metabolites, respectively. B. The K-means cluster analysis of the VOCs. The x-axis represents different samples, while the y-axis represents the normalized value. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

feature of dynamic changes in taste during development stages in guava. Among these compounds, four types of organic acids (oxalic acid, quinic acid, malic acid, and shikimic acid) and all three types of sugars (fructose, glucose, and sucrose) were consistently found across all samples (Table S3). Furthermore, it was observed that fumaric acid accumulates in both the peel and flesh of guava at the mature stage (S3), while succinate is consistently present in the flesh but accumulates solely in the peel at young fruit stage and gradually diminishes as the fruit develops (Fig. 5, Table S2).

Similar to the results observed in the analysis of VOCs, the

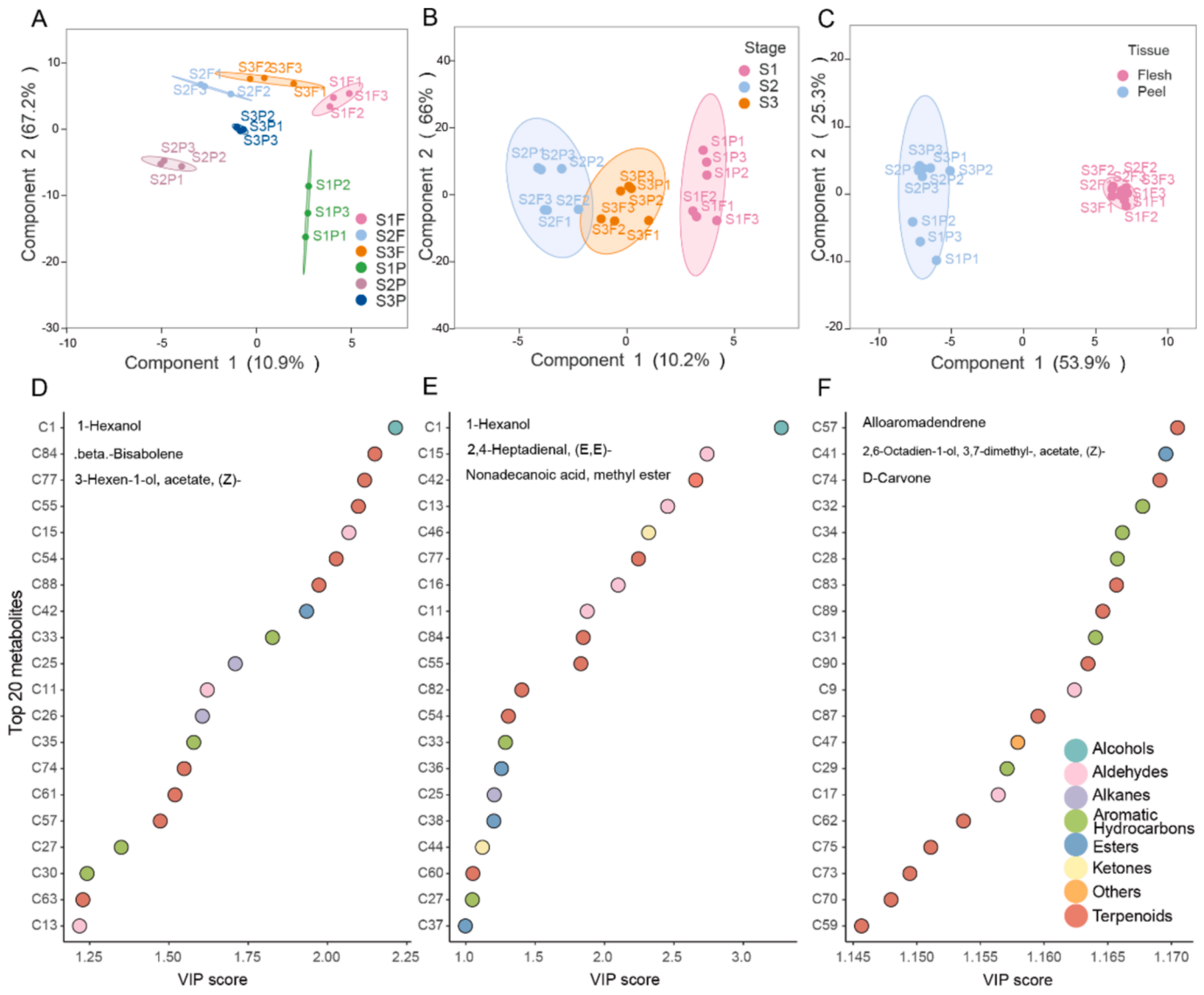

Fig. 4. The orthogonal projection to latent structure-discriminant analysis (OPLS-DA) results of the VOCs. The OPLS-DA results of VOCs based on the total VOCs (A), developmental stages (B), and different tissues (C), respectively. D-F. The variable importance projection (VIP) of the top 20 important metabolites based on the results of A-C, respectively.

contributions of each component within sugars and organic acids vary across developmental stages and tissues. For example, shikimic acid and fumaric acid stand out as the most important compounds across different developmental stages (Fig. 6E), while succinate and malic acid are the most important components between peel and flesh in guava (Fig. 6F). The OPLS-DA analysis results, grouped by developmental stages (Fig. 6B, Component 1, 52.7 %) and different tissues (Fig. 6C, Component 2, 57.6 %), collectively suggest that the developmental stage is a key factor influencing the grouping. These findings are consistent with the flavor transformation of guava fruit during ripening, wherein the fruit is acidic and astringent before ripening and becomes sweet gradually during maturation.

3.4. *Key genes involve in metabolism of VOCs, sugars and organic acids*

To investigate the genes involved in metabolic pathways of VOCs, sugars, and organic acids in guava, we analyzed the metabolic pathways by combining the results of transcriptome analyses. By comparing the relationship between gene expression levels and metabolites, as well as the results of BLAST, we identified several key genes involved in these pathways (Table S4).

Sucrose is the principal component of sugars and serves as the substrate for sugar and organic acid metabolite pathways. The sucrose content in both peel and flesh increases rapidly during the ripening process (Fig. 5). Additionally, the expression levels of two sucrose invertase genes (*Pgu01978* and *Pgu21122*) are also increased during ripening (Fig. 7), which may play important roles in the biosynthesis of fructose and glucose. Additionally, five hexokinase genes (*Pgu04371*, *Pgu25345*, *Pgu05223*, *Pgu12547*, and *Pgu15941*) and two fructokinase genes (*Pgu18445* and *Pgu03622*) were also identified as potentially playing a crucial role in sugar metabolism (Fig. 7).

Throughout the development of guava, quinic acid exhibits the highest content in all tissues and developmental stages, with accumulation primarily occurring in the early stages (Fig. 5). This finding is consistent with observations in citrus, kiwifruit and peach (Su et al., 2022; Marsh et al., 2009; Albertini et al., 2006). The reason may be attributed to its antioxidant properties and protecting fruit from insect damage (Liu et al., 2022). Quinic acid is typically a byproduct of the shikimic acid pathway, meaning that it is usually produced alongside shikimic acid. Seven genes have been identified that may be related to

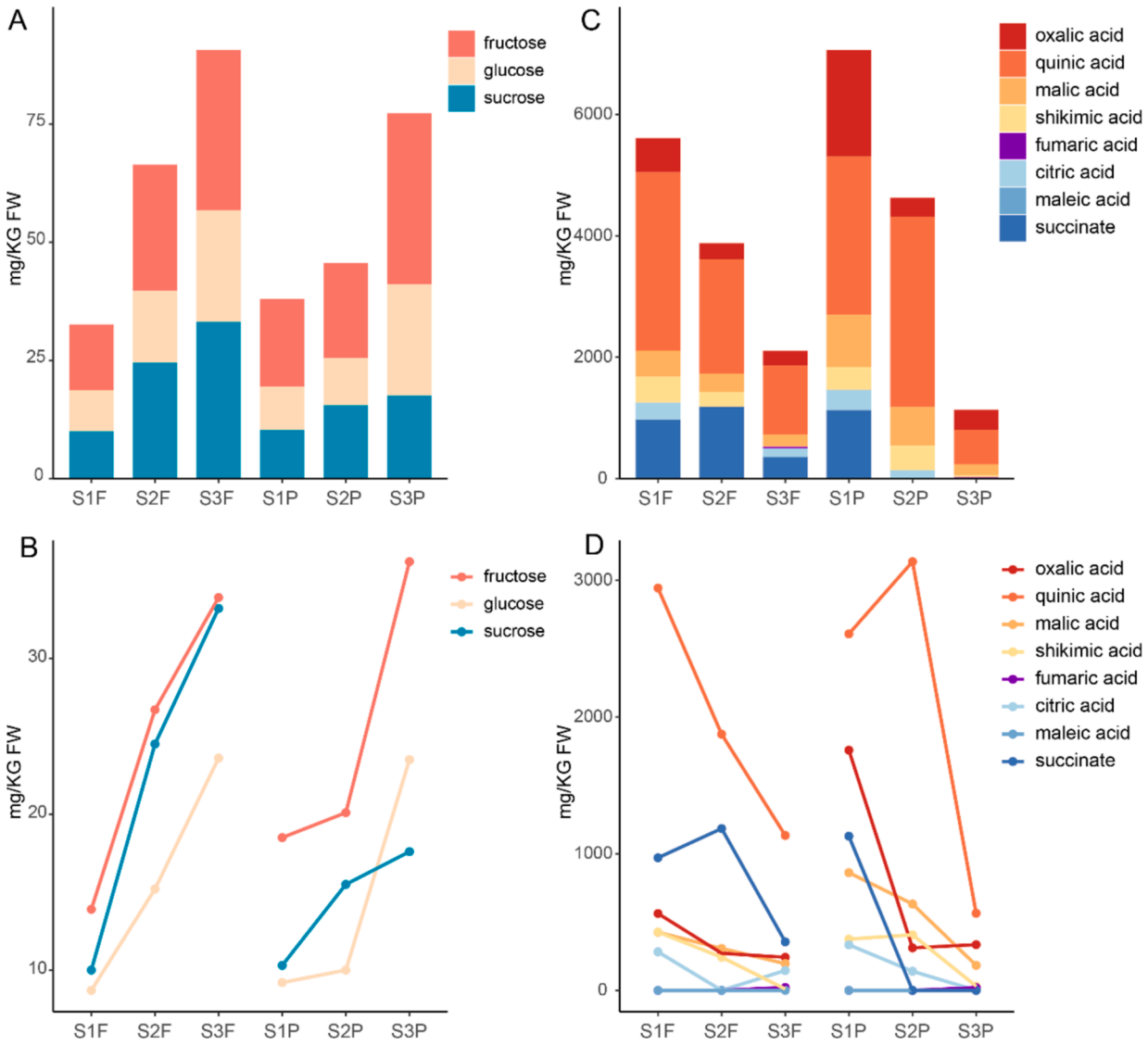

Fig. 5. The sugar and organic acid in guava. The contents of sugars (A) and variation (B) in different samples. The contents of organic acid (C) and variation (D) in different samples. The x-axis indicates different samples, and y-axis indicates the contents in fresh weight.

the biosynthesis of both quinic acid and shikimic acid. Among the seven genes, four genes (*Pgu17831*, *Pgu02719, Pgu22116*, and *Pgu17900*) are primarily expressed during the early developmental stages in both peel and flesh. The remaining three genes (*Pgu17874*, *Pgu15970*, and *Pgu01100*) are predominantly expressed during the late developmental stage in both peel and flesh (Fig. 7). As the levels of quinic acid and shikimic acid sharply decrease during the developmental stages, the first four genes are likely involved in quinic acid and shikimic acid biosynthesis, while the other three genes may be related to its metabolism or degradation. Besides, it is noteworthy that the peel contains higher levels of oxalic acid and malic acid, while the flesh is rich in succinate (Fig. 5). These acids, including fumaric acid and citric acid, are products of the tricarboxylic acid (TCA) cycle. Here, we identified 11 genes related to the biosynthesis of organic acid in TCA cycle. Nine of them (*Pgu22828*, *Pgu01070*, *Pgu06035*, *Pgu19407*, *Pgu12005*, *Pgu11987*, *Pgu23194*, *Pgu19961*, and *Pgu24986*) were mainly expressed during the ripening stage (S3) in both peel and flesh, suggesting that the ripening stage is the most active metabolism period in both peel and flesh. The other two genes (*Pgu15126* and *Pgu19633*) showed predominant expression during the S2 stage in flesh (Fig. 7).

An essential metabolic pathway for the production of fruit scent volatiles is fatty acid metabolism. The majority of plant volatiles are generally derived from saturated and unsaturated fatty acids and fatty acids-derived metabolites (such as alcohols, ketones, esters, and aldehydes), and are formed mainly in three pathways: α-oxidation, β-oxidation and lipoxygenase (LOX) pathway (Schwab et al., 2008). Aldehydes and alcohols produced by the fatty acid metabolism pathway typically impart a “fresh green” flavor to fruits and play important roles in defense against pests (Matsui, 2006). Our findings revealed 18 genes related to the fatty acid metabolites, which can be categorized into four groups. The genes in the first group (*Pgu18373, Pgu10525, Pgu02753,* and *Pgu18240*) are predominantly expressed in the early stage (S1) in both fresh and peel. The genes in the second group (*Pgu02790*, *Pgu00842* and *Pgu16942*) are highly expressed at the S2 stage in peel. The genes in the third group (*Pgu09564*, *Pgu18241*, *Pgu05524*, *Pgu18363*, *Pgu15902*, *Pgu02712*, *Pgu02657*, *Pgu15809*, and *Pgu00276*) are mainly expressed in the mature stage (S3) in both peel and flesh, while the genes in the fourth group (*Pgu13051* and *Pgu18615*) are primarily expressed during the ripening stage (S3) in flesh and young fruit stage (S1) in peel, respectively (Fig. 7). This finding is consistent with the earlier observation that the majority of genes involved TCA cycle exhibit high expression levels during the maturation stage. Similarly, lipid metabolism is also most active during this period.

Terpenoids, the largest family of natural products with over 80,000

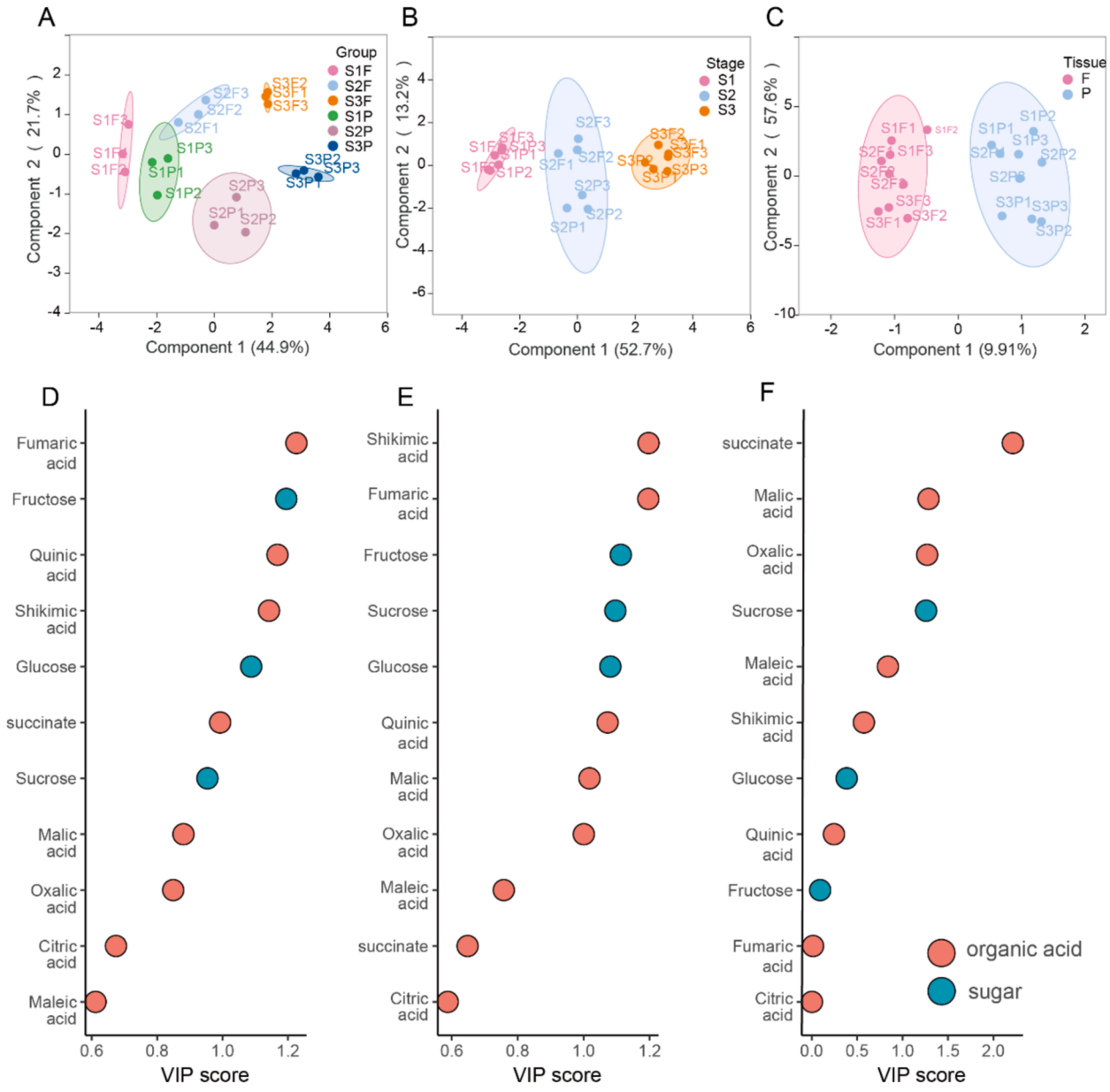

Fig. 6. The OPLS-DA results of sugars and organic acids. The OPLS-DA results of sugars and organic acids based on the total samples (A), developmental stages (B), and different tissues (C), respectively. D-F: The VIP score of these components important metabolites based on the results of A-C, respectively.

members, play crucial roles in various aspects of plant defense and hormone regulation. Additionally, they hold significant importance in the flavor and fragrance industry, as well as in pharmaceuticals (Christianson, 2017; Schwab et al., 2008). In plants, terpenoids are primarily synthesized through two core pathways: methylerythritol phosphate (MEP) pathway, which is initiated from triose phosphate and pyruvate in the plastid, as well as the mevalonate (MVA) pathway that initiated from acetyl-CoA in the cytoplasm (Bergman et al., 2019). Here, we identified seven genes (*Pgu10930*, *Pgu19036*, *Pgu24386*, *Pgu06507*, *Pgu10569*, *Pgu06199*, and *Pgu06632*) that may be involved in the MEP pathway. Most of these genes are highly expressed in mature tissues (S3F and S3P), whereas *Pgu10930* and *Pgu06199* are primarily expressed in young fruit and expanding fruit stage (S1F and S2F), respectively (Fig. 7). Additionally, seven genes (*Pgu23803*, *Pgu25502*, *Pgu12437*, *Pgu15231*, *Pgu22534*, *Pgu19338*, and *Pgu14100*) were identified in mevalonate (MVA) pathway. Among these genes, two genes (*Pgu23803* and *Pgu25502*) are mainly expressed in the young flesh and peel, three genes (*Pgu15231*, *Pgu22534*, and *Pgu19338*) are predominantly expressed in the expanding peel (S2P), and *Pgu14100* is mainly expressed in the peel while *Pgu12437* was expressed in all samples except for the expanding stage (S2F and S2P). Furthermore, five genes (*Pgu17620*, *Pgu24052*, *Pgu24986*, *Pgu07131*, and *Pgu12910*) related to the terpenoids synthesis were identified. All of these genes were highly expressed in the peel, especially in the mature stage of peel (S3P). This is consistent with the previous finding of a higher variety and content of terpenes in the peel.

4. Conclusion

Aroma and taste are crucial characteristics of fruit flavour and quality, and are the key factors influencing the acceptability and commercial value of fruit. In this study, we conducted a systematic investigation of VOCs, sugars, and organic acids both in the peel and flesh of guava at different developmental stages. In this study, a total of 90 VOCs, eight organic acids, and three sugars were detected. We investigated the dynamic changes of VOCs across different tissues and

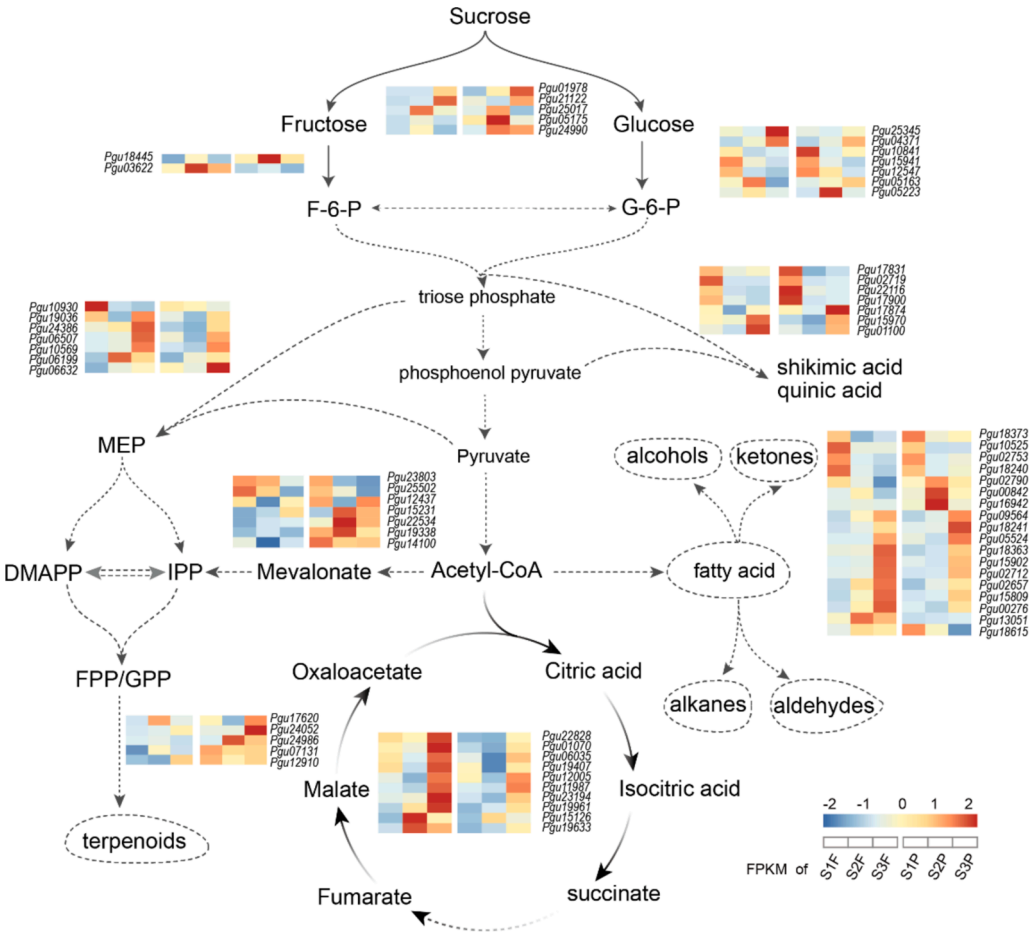

Fig. 7. The simplified model of the sugar, organic acid and VOCs metabolic pathway. Grids in heatmap indicate the gene expression levels in each sample.

developmental stages, suggesting that the S1 to S2 period is critical for the flavor formation of VOCs in the fruit flesh, whereas the formation of VOCs in the peel occurs progressively throughout the entire developmental stages. Additionally, we identified some key genes involved in crucial steps of these metabolic pathways, which is important for further understanding of the flavor changes during guava development. Overall, the flavor of guava is primarily composed of VOCs, sugars, and organic acids, and variations of these compounds at different developmental stages of both the fruit peel and pulp constitute the foundation of guava flavor. Furthermore, the developmental stage has a stronger impact on shaping the flavor of peel compared to the flesh in guava, which may be a key factor in the formation of aroma and flavor.

5. Authorship contribution statement

C.F. designed this study, J.Z. and Y.Z. conducted the data analysis and wrote the manuscript. S.Z., E.Y., Z.L. and T.X. performed the sample collection, metabolites pretreatment and determinations.

CRediT authorship contribution statement

Jie Zhang: Writing – review & editing, Writing – original draft, Visualization, Software, Investigation. **Yi Zhang:** Methodology, Formal analysis, Data curation. **Shuaiyu Zou:** Methodology, Formal analysis. **Endian Yang:** Resources, Formal analysis. **Ziyi Lei:** Writing – review & editing. **Tingting Xu:** Writing – review & editing. **Chen Feng:** Project administration, Funding acquisition, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochms.2024.100228.

Data availability

Data will be made available on request.

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