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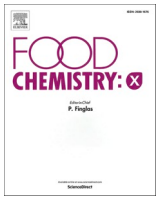

Insight into flavor difference of cherry (*Prunus avium L.*) grown in facility environment and outdoors through metabolomics and correlation analysis

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ABSTRACT

The flavor profiles of cherries cultivated in greenhouse and those grown in open fields show significant variations, however, the underlying flavor-contributing factors remain unidentified. Hence, a joint investigation with widely targeted metabolomics analysis, volatile fingerprint analysis, and descriptive sensory analysis for the Russia 8 and Tieton cherry cultivars was conducted using UPLC-MS/MS and GC × GC-TOFMS to clarify the flavor differences of open-air and greenhouse-grown cherries. The study found that open-air cultivation could lead to the accumulation of non-volatile flavor substances and prompted appearance of higher acidity, astringency, plum-like flavor, and fresh herb notes; most of differential metabolites were significantly positively correlated with astringency, plum-like flavor and bitterness. Through correlation analysis and path analysis, potential flavor components and key important pathways contributing to flavor disparities were provided, and light intensity, soil moisture content, temperature and humidity were inferred as the main factors affecting the flavor profiles of open-air and greenhouse-grown cherries.

1. Introduction

The cherry (*Prunus avium L.*), also referred to as the European cherry, belongs to the plum species within the rosaceae family and is indigenous to Europe and western Asia. The fruit exhibits vibrant hues and a pleasing flavor, earning it the moniker “the jewel of fruits”. It is abundant in sugars, proteins, vitamins, calcium, iron, as well as essential elements such as anthocyanins, proanthocyanidins, lutein, and other polyphenols. This endows it with significant nutritional and medicinal value, rendering it highly esteemed by consumers. (Bland & Oomah, 2019; Mirto et al., 2018; Schmitz-Eiberger & Blanke, 2012). Now, the cherry has emerged as one of the fruits with most market potential and economic value. Currently, China stands as the world’s largest country in terms of cherry cultivation area. In 2019, the cherry cultivation area surpassed 3 million mu, reaching approximately 3.5 million mu, accounting for nearly half of the global cherry cultivation area (Wang, Mei, Wang, & Zeng, 2021). By 2021, China’s sweet cherry cultivation area had expanded to cover 246,000 hm^2, including a greenhouse sweet cherry area of 13,000 hm^2 (Li et al., 2023).

Product quality serves as the foundation of industrial development, and flavor, being one of the crucial indicators for assessing intrinsic product quality, directly determines the excellence and value of agricultural products. Moreover, it stands as the most significant evaluation criterion influencing consumers’ purchasing behaviors (Wang, Wei, Wang, Zhang, & Gong, 2023). Currently, numerous scholars have conducted extensive research on cherry aroma and have discovered that sweet cherries contained over 100 volatile organic compounds (Gonçalves et al., 2022; Zhang, Liu, Li, & Li, 2021; Qiu et al., 2021). In addition to aroma compounds, non-volatile compounds also constitute the primary constituents of flavor in agricultural products. Through targeted and non-targeted metabolomics approaches, several scholars have successfully identified simple sugars, organic acids, polyphenols, and flavonoid components (Cao et al., 2015; Yang et al., 2021), which were reported that they had great contribution to the taste perception (Ikegaya et al., 2019; Fan et al., 2021). Nevertheless, the specific compounds responsible for the flavor profile of cherries remain to be conclusively identified. Flavor substances were easily significantly influenced by environmental conditions, cultivation techniques, and

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genetic variations within different varieties (Bustamante et al., 2021; Chauvin, Whiting, & Ross, 2009; Ivanova, Serdyuk, Kryvonos, Yeremenko, & Tymoshchuk, 2020; Zhang, Jiang, Zhang, & Li, 2023). Facility cultivation enables the diversified production of agricultural products and enhances their value addition, thereby holding significant implications for contemporary agricultural practices. Consistent facility conditions can ensure consistent product quality, which is important for maintaining the quality consistency of agricultural products (Li, Zhao, Lv, & Cai, 2023). However, compared with open-air planting, even if fertilization and watering conditions are the same, the flavor quality of agricultural products under facility conditions may still change due to differences in sunshine, temperature, and humidity between the two planting modes (Zheng, Yang, Wei, & Zhao, 2022). Currently, some scholars reported the changes of aroma substances in cherry under arched cover and open field conditions (Zhang, Jiang, Zhang, & Li, 2023), but the amount of aroma substances detected was small, and the influence of the changes on cherry flavor was not discussed. Therefore, it remains unknown how the flavor quality of cherries cultivated in facilities varies compared to those grown outdoors. So further investigation is needed to determine whether the influence of cherries cultivated in facilities on flavor is positive or negative. And this is also an urgent theoretical need for the development cherry industry and for cherry quality control under facility conditions.

The association between metabolites and sensory traits can provide a large number of potential contributing factors to food flavor differences (Sung, Suh, Chambers, Crane, & Wang, 2019; Zhang et al., 2024), which provides theoretical support for the identification of key flavor difference factors. Some scholars have explored the correlation between the sensory attributes of cherries and various aroma substances and found that the aroma substances were intimately related to the sensory attributes such as sweet orange, lemon, almond, rose, and wine (Qiu et al., 2023). But non-volatile substances were not involved in the correlation. While Tian (Tian, Zheng, Yu, Chen, & Lou, 2023) mentioned that non-volatile substances also played an irreplaceable role in the formation of food flavor. So, it is necessary to consider the correlation between non-volatile with sensory characteristics when studying the difference of cherry flavor.

Widely targeted metabolomics, which combines the comprehensive coverage of non-targeted metabolomics with the precision of targeted metabolomics (Feng, Gao, Jiao, Shi, & Wang, 2020), represents a state-of-the-art approach in this field. By employing this technique, hundreds of metabolites can be qualitatively identified and quantified with high accuracy. It has been successfully applied for the ultrasensitive detection of constituents in various fruits such as *Eriobotrya japonica (Thunb.) Lindl.* and *Litchi chinensis Sonn.* (Yin, Zhang, Wu, Chen, & Deng, 2022; Zou et al., 2020). Two-dimensional gas chromatography/time-of-flight mass spectrometry (GC × GC-TOFMS) offers excellent peak capacity, high sensitivity, exceptional resolution, and fast analytical speed (Zhao et al., 2022), making it particularly valuable for identifying volatile and semi-volatile compounds. Due to these advantages, it has been widely used in flavor analysis of green tea (Zhu et al., 2018) and pears (Zhang et al., 2023). The above two analysis methods can provide detailed information for cherry flavor fingerprint. Therefore, a wide range of methods, including widely targeted metabolomics analysis, GC × GC-TOFMS analysis, and descriptive sensory assessment, were used to elucidate flavor differences and potential contributing factors between greenhouse and open-air cherry varieties in this study. Firstly, the differential metabolites were screened by multivariate statistical analysis. Then the correlation between the differential metabolites and sensory properties was established using pearson correlation coefficient to identify substances highly correlated with sensory properties. The causes of the differences were further studied through the analysis of different metabolite pathways. This study aimed to reveal the cherry flavor differences caused by plant metabolism variations resulting from different planting environments and identify key difference factors through a new comprehensive analysis method. This study provided data support for further research on the flavor difference between open-air and greenhouse-grown cherries. It was of great significance in improving the flavor quality of cherries in facilities and selecting the proper cultivation mode for cherries.

2. Materials and methods

2.1. Materials

The Tieton and Russia 8 cultivars grown in a greenhouse were named TG and RG respectively, and the Tieton and Russia 8 cultivars planted outdoors were named TO and RO. All samples were collected from Weifang city in Shandong Province. For each treatment, samples were carefully selected from five different trees to ensure intact whole fruits for the experiment. And each treatment had 3 replicates. Traditional metrics such as the growing period (measured in days after flowering), external morphology, and skin color were utilized to determine the maturity of each cultivar. The designated harvesting dates for TG and RG were May 5th, while TO and RO were harvested on June 1st. All harvested fruits were promptly preserved at low temperatures and expeditiously transported to the Institute of Quality Standard and Testing Technology for Agro-Products at Shandong Academy of Agricultural Sciences.

2.2. Reagents

Optima™ LC/MS grade methanol was purchased from Fisher Chemical™ (Waltham, Massachusetts, USA). 2-Nonanone (>99 %) was obtained from Dr. Ehrensorfer (Augsburg, Bavaria, Germany). 100 μg/mL 2-nonanone was prepared using methanol prior to its application, and subsequently 10 μg/mL 2-nonanone was diluted with methanol. A mixture of 500 μg/mL C_8–C_{40} *n*-alkanes was purchased from Dr. Ehrenstorfer (Augsburg, Bavaria, Germany), and 2000 μg /mL C_6–C_{10} *n*-alkanes was obtained from AccuStandard (New Haven, CT, USA). Pure water was purchased from Wahaha (Hangzhou, China). (2*E*, 4*E*)-2,4-hexadienal, benzaldehyde, phenylacetaldehyde, benzyl butyrate, (+)-limonene, α-terpineol, sucrose, citric acid, caffeine, and alum were purchased from Merck KGaA (Darmstadt, Hesse-Darmstadt, Germany), and were prepared as reference solution (as detailed in Table S1) with pure water. Trans-*β*-ocimene was urchased from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China).

2.3. Widely targeted metabolomics analysis

The initial step involved subjecting the four cherry samples to cryogenic freezing using liquid nitrogen, immediately after collection. Subsequently, the samples were processed by a specialized external service provider (Genedenovo Biotechnology Co., Ltd., Guangzhou, China). In brief, the biological samples were subjected to freeze-drying using a Scientz-100F vacuum freeze-dryer (Ningbo Scientz Biotechnology Co., Ltd., Ningbo, China). Subsequently, the dried samples were pulverized using an MM 400 mixer mill (Retsch GmbH, Haan, Germany) equipped with a zirconia bead at a frequency of 30 *Hz* for a cycle duration of 1.5 min. The lyophilized powder (100 mg) was then mixed with 1.2 mL of a 70 % methanol solution, followed by vortexing for 30 s at 30 min intervals for a total of six iterations, and then left in a refrigerator at 4 °C overnight. Then the obtained mixture was centrifuged at 12000 rpm for 10 min, and the liquid supernatant were filtered using 0.22 μm SCAA-104 filter membranes (ANPEL, Shanghai, China) prior to subsequent ultra performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) analysis.

A UPLC system (UPLC, SHIMADZU Nexera X2; Kyoto, Japan) was utilized in conjunction with an MS instrument (Allen-Bradley Applied Biosystems 4500 Q TRAP; Milwaukee, Wisconsin, USA) for the analysis of non-volatile sample extracts. The analytical conditions were configured as follows: an Agilent SB-C18 (1.8 μm, 2.1 mm × 100 mm) (San

Jose, California, USA) was used as the column. The mobile phase consisted of solvent A, which was pure water containing 0.1 % formic acid, and solvent B, which was composed of acetonitrile with 0.1 % formic acid. Sample analysis was conducted using a gradient program that initiated with 95 % A and 5 % B. Within 9 min, a linear gradient transitioned to 5 % A, which was maintained for 1 min. This was followed by an adjustment to a composition of 95 % A and 5.0 % B within 1.1 min, which was maintained for 2.9 min. The flow rate was set at 0.35 mL per min, the column temperature at 40 °C, and the injection volume at 4 μL. The effluent from UPLC was alternately connected to Q TRAP-MS. The UPLC/MS/MS System equipped with an electrospray ionization (ESI) Turbo Ion-Spray interface, and was analyzed in both positive and negative ion modes and managed using Analyst 1.6.3 AB Scitex software (Milwaukee, Wisconsin, USA). The ion source was operated under the following conditions: turbo spray for the ion source, source temperature at 550 °C, ion spray voltage (IS) at 5500 V (positive ion mode) and −4500 V (negative ion mode), and gas settings of ion source gas I (GSI), gas II (GSII), and curtain gas (CUR) at 50, 60, and 25.0 psi, respectively. Collision-activated dissociation (CAD) was set to high. The acquisition of triple quadrupole (QQQ) scans was conducted as multiple reaction monitoring (MRM) experiments, using nitrogen as the collision gas at medium settings. Distinct declustering potential (DP) and collision energy (CE) parameters were established for each MRM transition, with further optimization. A specific set of MRM transitions was monitored for each analysis period, tailored to the elution profiles of metabolites within that timeframe.

2.4. Analysis of volatile compounds

Upon immediate return to the laboratory, the four fresh cherry samples were subjected to analysis. The headspace solid phase microextraction (HS-SPME) method was employed for the extraction of volatile compounds. A Supelco 50/30 μm DVB/CAR/PDMS SPME fiber (Bellefonte, Pennsylvania, USA) was selected based on prior validation and established literature (Zhang et al., 2023). Using the quartic method, each cherry sample was quartered and the coreless flesh was sliced into pieces measuring 0.5 cm × 0.5 cm. Subsequently, a total of 6.0 g of the mixed sample was placed into a 15 mL headspace bottle. Prior to sealing the vials, an internal standard of 5 μL from a solution containing 10 μg/mL of 2-nonanone solution was added. Then the cherry samples were kept at room temperature for 10 min prior to aroma extraction, subsequently placed on a constant-temperature device at 40 °C and extracted for 40 min with solid-phase microextraction device. Finally, the SPME fiber was promptly inserted into the GC injector with a split ratio of 1:1 for desorption at a temperature of 270 °C for two minutes.

The volatile compound analysis was conducted using an Agilent 7890B gas chromatograph (San Jose, California, USA) equipped with a LECO Pegasus 4D—C time-of-flight mass spectrometric detector (Saint Joseph, California, USA). A Shimadzu Rxi-5MS column (Kyoto, Japan) (30 m × 250 μm × 0.25 μm) served as the first-dimension (1D) column, while a Shimadzu Rxi-17Sil MS column (Kyoto, Japan) (2 m × 250 μm × 0.25 μm) functioned as the second-dimension (2D) column. Helium was employed as the carrier gas, flowing steadily at 1.4 mL/min. The front inlet and transfer line temperatures were set to 270 °C and 280 °C, respectively. The temperature programming for the oven was as follows: an initial temperature of 40 °C for 2 min, increasing at a rate of 5 °C/min up to 200 °C, then further elevated to 280 °C at a rate of 20 °C/min and held for 2 min. The secondary oven temperature remained 5 °C higher than the GC oven temperature throughout the chromatographic run. The modulator temperature was set 15 °C higher than the secondary oven temperature. Modulation occurred every 3 s with a 0.6 s hot pulse. The mass spectrometry parameters were set as follows: acquisition delay 60 s, acquisition rate 100 spectra/s, acquisition voltage 1450 V, electron energy −70 V, and ion source temperature 250 °C. Mass spectra were captured within the *m/z* range of 35–550 amu.

2.5. Sensory evaluation

A quantitative descriptive analysis was conducted to assess the flavor profiles of distinct cherry samples, encompassing six aroma attributes (almond, fresh herb, green grass, plum-like, apple-like and woody) and four taste attributes (sweetness, sourness, bitterness and astringency).

A panel of ten expert assessors with professional training was recruited. A 0–5 intensity scale was adopted for rating the flavor attribute. The definition, reference solution, and intensity for each flavor attribute were presented in Table S1. The sensory evaluation took place within 24 h of sample acquisition in an odor-free room maintained at 22 ± 1 °C. For each cultivar, selection process involved randomly choosing between 20 and 50 cherry fruits; samples without cores were quartered approximately 30 min before the experiment. After thorough mixing, a total of ten samples consisting of cut pieces weighing between 15 and 30 g were randomly prepared and presented to evaluators in a randomized sequence. The assessors then rated the samples and recorded the sensory attribute intensities. Sufficient resting periods were provided between each sample to alleviate sensory fatigue. The experiment was conducted three times.

This study was approved by the ethics committee of the Shandong Academy of Agricultural Sciences (Reference number: SAAS-2024-G71).

2.6. Identification of differential metabolites and metabolic pathway analysis

The metabolomics data exhibit high dimensionality and magnitude, necessitating the integration of univariate and multivariate statistical analyses to accurately identify differential metabolites (DMs). In this study, metabolites with a variable importance in projection (VIP) > 1 between cherries grown in greenhouse and outdoors were initially screened using an orthogonal projection to latent structures discriminant analysis (OPLS-DA) (biological replication ≥ 3). Additionally, the fold change (FC) values from univariate analysis were selected to further refine the selection of differential metabolites, focusing on those with fold changes ≥ 2 or ≤ 0.5. Subsequently, biomarkers were subjected to pathway analysis by annotating them using the kyoto encyclopedia of genes and genomes (KEGG) compound database (http://www.kegg.jp/kegg/compound/) and mapping them onto the KEGG pathway database (http://www.kegg.jp/kegg/pathway.html).

2.7. Correlation relationship between sensory evaluation and instrumental analysis

The pearson correlation coefficient is commonly utilized to assess the degree of association between two variables, X and Y, and effectively evaluate the linear relationship between two continuous variables. In this study, the pearson correlation coefficient was employed to examine the link between sensory intensity of cherry flavor and flavor substance content in order to identify significant contributors to flavor.

2.8. Data processing and statistical analysis

The mass spectrometry fragments corresponding to each chromatographic peak were initially searched using NIST2017, and volatile aroma compounds with matching scores exceeding 700 were singled out. Meanwhile, the retention index for each aroma component was calculated using the established formula (Zhang et al., 2023). Then the accuracy of the matched aroma substances was further validated through the retention index. Quantification of aroma constituents was accomplished by assessing peak areas through the internal standard method.

Principal component analysis (PCA) and OPLS-DA were conducted using SIMCA 14.1 to group cherry samples according to the detected volatile components and non-volatile metabolites. Heatmap of correlation analysis were conducted utilizing Tbtools. Volcano Plot were drawn

by SIMCA 14.1 to display differential metabolites. The data underwent variance analysis via SPSS Statistical 19.0, and mean values were compared utilizing Tukey's test at a significance level of $P < 0.05$. Radar plots and histograms were drawn using Originlab 2022.

3. Results

3.1. Descriptive sensory analysis of cherry cultivars grown in different planting modes

Through descriptive sensory evaluation, it was observed that cherry samples exhibited distinct perception of 6 aroma attributes (almond, fresh herb, green grass, plum-like, apple-like and woody) and 4 taste attributes (sweetness, sourness, bitterness and astringency). Notably, the intensity of sweet, sour, almond, and plum-like flavor attributes was comparatively higher. Furthermore, variations in each flavor attribute were evident across different samples. The radar chart (Fig. 1) facilitated a comprehensive comprehension of these dissimilarities. The Russia 8 cherry exhibited a high level of sweetness, regardless of whether it was cultivated in a greenhouse or in an open field. However, the open-grown cherry exhibited a more intricate flavor profile, characterized by heightened sourness (5.00), pronounced notes of almond (4.07) and plum-like (4.43), subtle hints of lemon-like (0.60), delicate fresh herb (1.97), and certain level of astringency (2.47). But for RG, the attributes of green grass flavor (2.50) and woody flavor (2.47) were more pronounced. For Tieton cherries, the almond (3.03), lemon-like (1.40), green grass (2.50), and apple-like (2.40) flavors of cultivated in greenhouses exhibited higher intensities, whereas the flavors of sourness (4.53), fresh herb (2.47), plum-like (3.47), and woody (2.07) exhibited heightened prominence in TO. Through comparison, it was found that the two kinds of cherries grown in the open air had high acidity, astringency, plum-like flavor and fresh-herb flavor.

3.2. Analysis of non-volatile and volatile metabolite profiles

To gain deeper insights into the flavor disparities between cherries in controlled facility environments and those grown under natural conditions, a comprehensive analysis encompassing widely metabolite profiling of Tieton and Russia 8 cherries were conducted. Additionally, GC × GC-TOFMS was employed to conduct a thorough comparison of the aroma profiles among cherries. The total ion chromatograms of non-volatile metabolites of RO, RG, TO, TG were showed in Fig. S1, Fig. S2 Fig. S3 and Fig. S4, and the total ion chromatograms of volatile aromas of RO, RG, TO, TG were showed in Fig. S5.

In total, 1377 non-volatile metabolites, encompassing 72 amino acids and their derivatives, 219 phenolic acids, 40 nucleotides and their derivatives, 288 flavonoids, 88 lignans and coumarins, 16 quinones, 61 sugar alcohols, 17 tannins, 99 alkaloids, 147 terpenoids, as well as organic acids (62), lipids (161), vitamins and other substances (107) were successfully identified, as detailed in Table S2. Additionally, our analysis identified 199 aroma component which included aldehydes (29), alcohols (25), alkenes (29), esters (22), ketones (18), alkanes (33), aromatics compounds (23) along with other compounds (20), as detailed in Table S3.

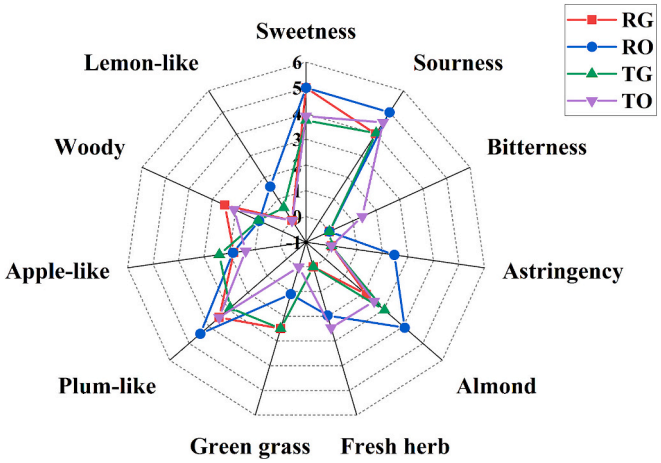

Fig. 1. Radar diagram of the sensory characteristics of cherries.

The principal component analysis revealed a clear distinction between cherries based on the composition of both non-volatile and volatile components, as illustrated in Fig. 2A and B. In the case of non-volatile constituents, the first two principal components (PCs) accounted for 59.2 % of the total variance (PC1 = 33.0 %, PC2 = 26.2 %). For volatile components, the first PC collected 71.8 % of the total variation (PC1 = 43.4 %, PC2 = 28.4 %). The results suggested significant disparities existed in non-volatile and volatile compounds of cherry samples under investigation. Moreover, the initial two principal components from PCA of non-volatile and volatile compounds effectively captured the overall sample differentiation, respectively.

By conducting an analysis of the response of non-volatile substances (Fig. 2C) and the relative percentage content of volatile substances (Fig. 2D), significant disparities in substance composition were observed. In terms of non-volatile components, the content responses of amino acids and their derivatives, flavonoids, phenolic acids, sugar alcohols, organic acids, alkaloids and terpenoids were all above 1×10^8 (Fig. 2C), highlighting their significant role as pivotal flavor contributors in food. This observation is consistent with previous literature sources (Liu et al., 2022; Zou et al., 2020). The content responses of amino acids and their derivatives, terpenoids, alkaloids, organic acids, and lipids in Tieton cherries were observed to be higher than those in Russia 8 cherries, as depicted in Fig. 2C. For the Russia 8 cherry, whether it was planted in the open air or in the greenhouse, the content responses of phenolic acids and flavonoids were between 7.00×10^8–9.30×10^8 and 6.95×10^8–1.20×10^9, respectively, far higher than other kinds of substances. The content responses of amino acids and their derivatives (4.59×10^8), phenolic acids (9.30×10^8), sugar alcohols (2.76×10^8), tannins (1.77×10^7), quinones (6.81×10^7), alkaloids (3.52×10^8), terpenoids (5.22×10^8), lignans and coumarins (2.07×10^8), organic acids (2.38×10^8) and other substances (5.61×10^8) in RO were higher than those in RG. For Tieton cherries, the responses of amino acids and their derivatives, phenolic acids, flavonoids, alkaloids, terpenoids, lipids and other substances were generally high. And the responses of amino acids and their derivatives (5.56×10^8), phenolic acids (8.09×10^8), flavonoids (7.49×10^8), quinones (5.31×10^7), lignans and coumarins (1.60×10^8), sugar alcohols (2.39×10^8), terpenoids (6.42×10^8) and organic acids (3.11×10^8) in TO were higher than those in TG. Hence, it was found that open-air cultivation was beneficial to the accumulation of amino acids and their derivatives, phenolic acids, quinones, lignans and coumarins, tannins, sugars alcohols, terpenoids in fruits (Fig. 2C). Through the sensory evaluation, it was found that the acidity of open-air cherries was higher than that of greenhouse cherries, which was consistent with the changes of organic acid content of cherries (Fig. 2C).

The specific aroma characteristics of the matrix are determined by the ratio between different types of aromas for volatile compounds (Zhang et al., 2023). Therefore, an analysis of the relative percentage changes in aroma compounds were primarily conducted to elucidate the aromatic characteristics of each cherry sample. After analysis, it was discovered that the cherry samples exhibited a significantly higher percentage of aldehydes (62.4 %–69.7 %) compared to other substances (Fig. 2D). In the case of Russia 8 cherries, the relative concentration of aldehydes was higher in RO than that in RG, whereas for Tieton cherries, TG exhibited a slightly higher relative content of aldehydes compared to TO. Aldehydes are known to contribute to aromatic attributes such as almond, green grass, citrus, apple, floral and fat (Guo, Schwab, Ho, Song, & Wan, 2022; Kim, Cha, Shin, & Chun, 2018). The variations in aldehyde content provided a partial explanation for the fluctuations in

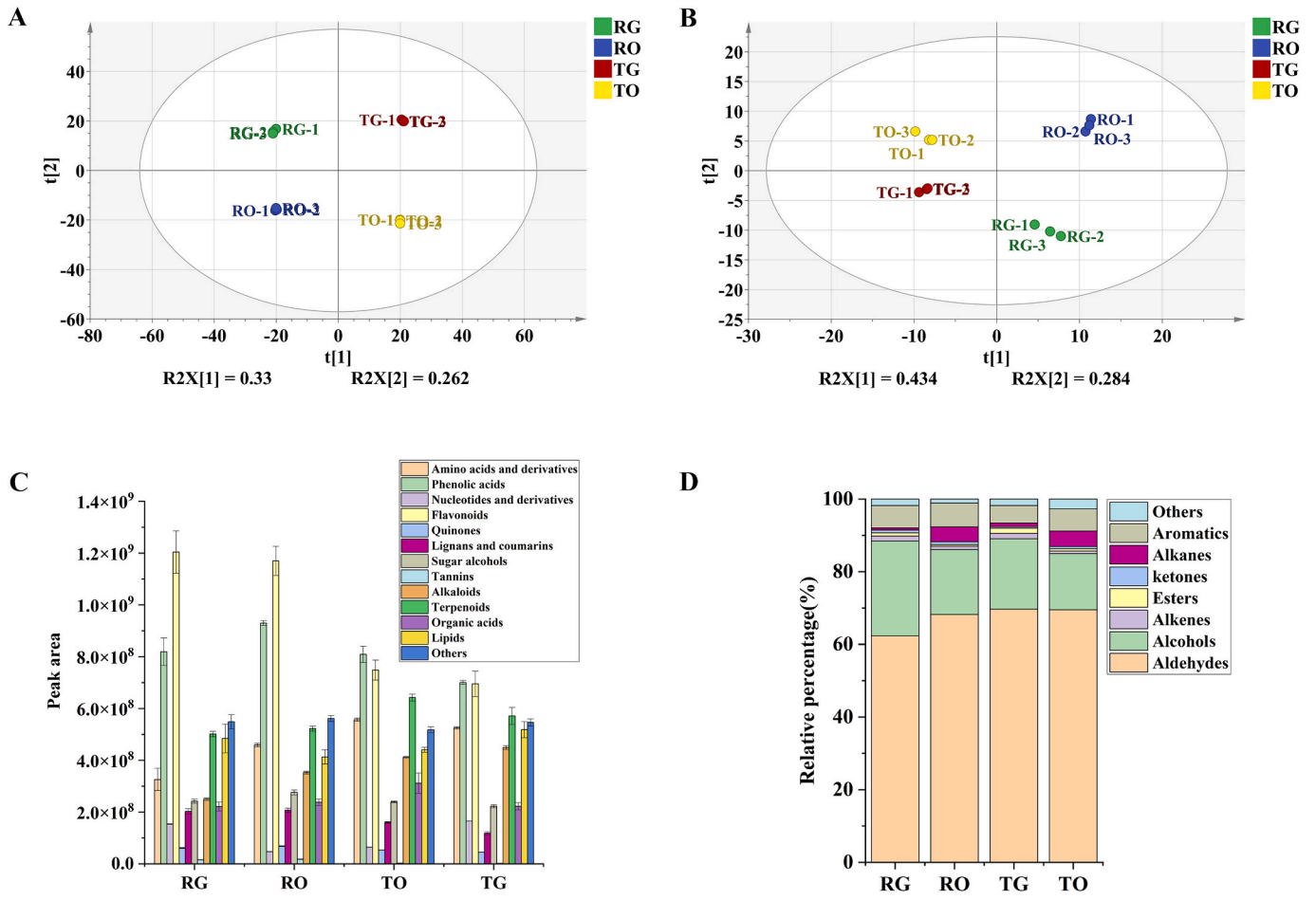

Fig. 2. Analysis of non-volatile and volatile metabolite profiles. A: Principal component analysis of non-volatile compounds in cherry samples; B: Principal component analysis of volatile compounds in cherry samples; C: Response of different types of metabolites in cherry samples; D: Relative content proportions of different volatile compounds in cherry samples.

almond and lemon flavors. Alcohol compounds (15.5 %–26.1 %) exhibiting the second highest relative proportion are characterized by fresh and grass scents (López-López, Sánchez, Cortés-Delgado, Castro, & Montaño, 2018). And its percentage in the greenhouse cherry samples were higher than in the open-air cherries. The ester aroma compounds with sweet and fruity tastes (Qin et al., 2012) accounted for 0.4 %–1.5 %, and the alkenes with floral attributes possessed 0.7 %–1.5 %. The percentage of esters and alkenes in the greenhouse cherry samples was also higher than that in the open-air cherry samples. The relative percentages of ketones (0.4 %–0.8 %), alkanes (0.6 %–4.2 %) and aromatics (4.8 %–6.6 %) in the open-air cherries were higher than those in the greenhouses. The changes of above aldehydes and alcohols were generally consistent with those reported in the literature (Zhang, Jiang, et al., 2023). Although the relative proportions of these various substances aligned with changes in certain flavor attributes, further analysis and investigation were necessary to identify the primary contributors.

3.3. Identification and analysis of differential metabolites between cherry samples from greenhouse and outdoors

Volcano plots were employed to identify metabolites with VIP scores greater than 1 and FC less than 0.5 or greater than 2, as depicted in Fig. 3A-D. A total of 570 differential metabolites were identified and filtered from the Tieton and Russia 8 cherries, representing approximately 41.4 % of all annotated compounds (Fig. S6 Table S1). The number of non-volatile metabolites identified in RO vs RG group was slightly lower than that in TO vs TG group. In RO vs RG group, the up-regulated components (217 species) in RO were much more than the down-regulated components (140 species); Similarly, the number of up-regulated components (234 species) far exceeded that of down-regulated components (139 species) in TO vs TG group (Fig. 3A, B). The results demonstrated that open-field planting was beneficial for the diversity and accumulation of non-volatile chemical substances in cherries. For volatile compounds, a higher number of up-regulated aroma substances (16) was observed compared to down-regulated substances (8) in the RO vs RG group, whereas the TO vs TG group exhibited an opposite trend with the number of up-regulated aroma substances (7) less than that of down-regulated substances (11), as shown in the Fig. 3C and D.

To better understand the change of differential compounds between cherry grown in facility environment and outdoors, all biomarkers were divided into 14 groups, and the up-regulated or down-regulated amounts of different types of compounds were analyzed, as shown in Fig. 3E and F. The substances with higher up-regulation amounts in RO vs RG and TO vs TG groups were phenolic acids (45, 46), flavonoids (46, 80), lignans and coumarins (18, 22), alkaloids (28, 16) and terpenoids (28, 23). The number of down-regulated lipids (30, 41) was relatively high among the identified substances; notably, a significant decrease in flavonoids (37) was also observed in RO vs RG group. In conjunction with Table S2, our analysis revealed that the proportion of differential quinones, amino acids and alkaloids in the total pool of quinones, amino acids and alkaloids exceeded 30 % in Russia 8 cherry, while differential quinones, flavonoids, lignans and coumarins accounted for more than 30 % of its total content in Tieton cherry. With the exception of sugars in

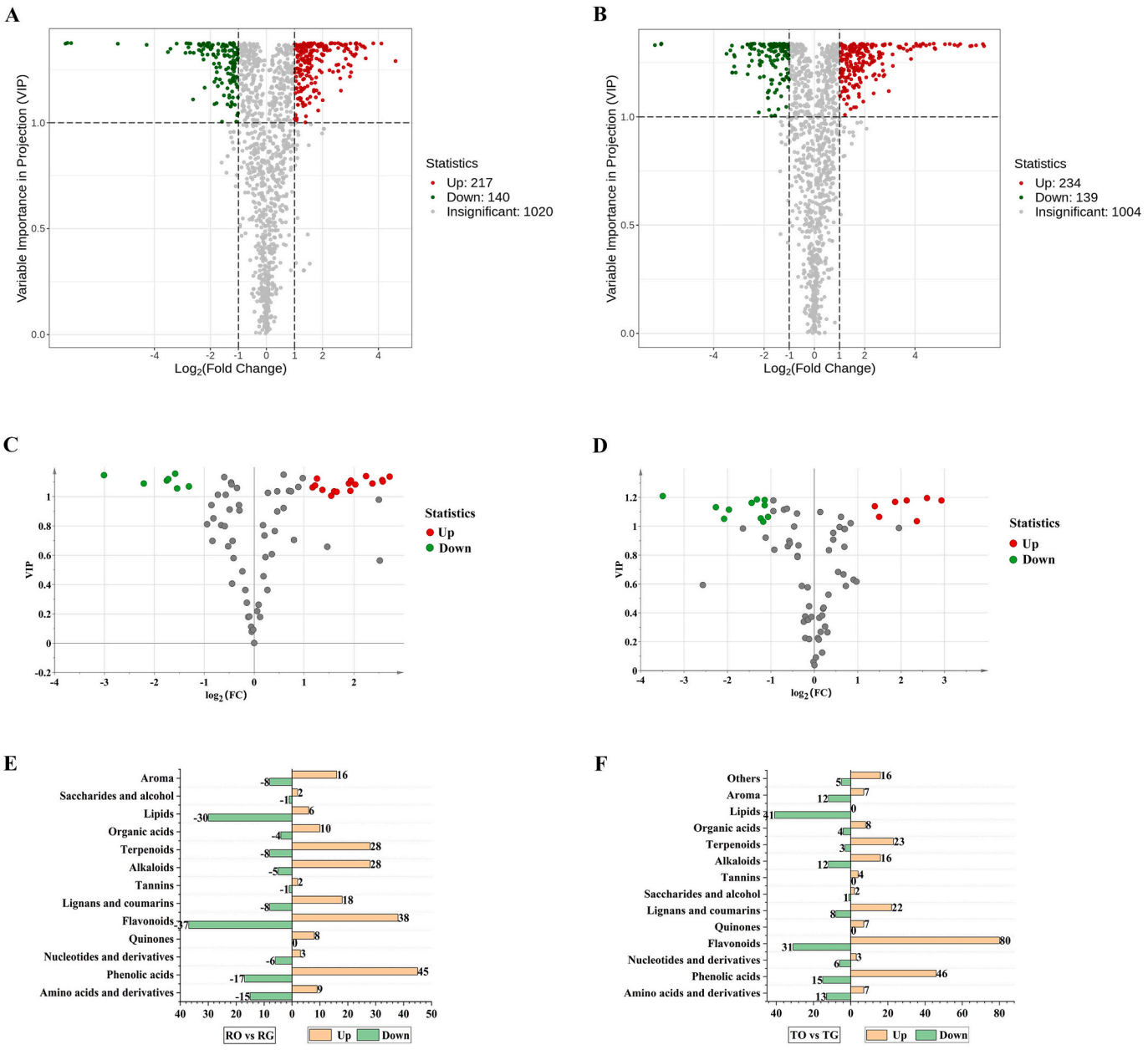

Fig. 3. Volcano map of different metabolites for non-volatile metabolite in RO vs RG group (A), TO vs TG group (B), and volatile metabolite in RO vs RG group (C), TG vs TO group (D); the up-regulated or down-regulated amounts of different types of metabolites in RO vs RG group (E), TO vs TG group (F).

the Russia 8 cherry and quinones in Tieton cherry, all other differential substances exhibit percentages exceeding 17 % of their total content. In conjunction with Table S3, our analysis revealed that the up-regulated volatile compounds in cherry primarily consisted of aldehydes, alkane, aromatic and ketones, whereas the down-regulated substances predominantly still was comprised of aldehydes, along with alcohols, esters, alkenes. It was found that the factors of facility environment and outdoors could promote the change of most substances in cherries. Compared with greenhouse cherries, outdoors cherries were favorable for the accumulation of non-volatile metabolites, as well as aromatic, alkane sand ketones compounds. Whereas, aldehydes were found to be up-regulated in open-air Russia 8 cherry samples, but no significant difference was observed between Tieton cherries from open-air and greenhouse.

3.4. Association analysis of key metabolite profiles and important sensory traits

The volatile and non-volatile substances were categorized into 8 groups for correlation heatmap analysis, revealing a significant association ($p < 0.01$) between numerous substances within each group and sensory attributes. Through comprehensive analysis, a significant positive correlation between numerous non-volatile substances and olfactory sensory attributes was identified (Table S4). From reported articles, it was found non-volatile substances have the ability to either enhance or inhibit the aroma characteristics of aromatic substances, while volatile substances can either enhance or inhibit the taste characteristics of non-volatile substances (Tian et al., 2023; Xiao, Chen, Niu, & Zhu, 2021; Zhang et al., 2024). These findings partially supported the correlation analysis results presented in this paper. Our findings demonstrated the pivotal role of amino acids and their derivatives in modulating bitterness, astringency, and plum-like flavor profiles in Fig. 4. Notably, there

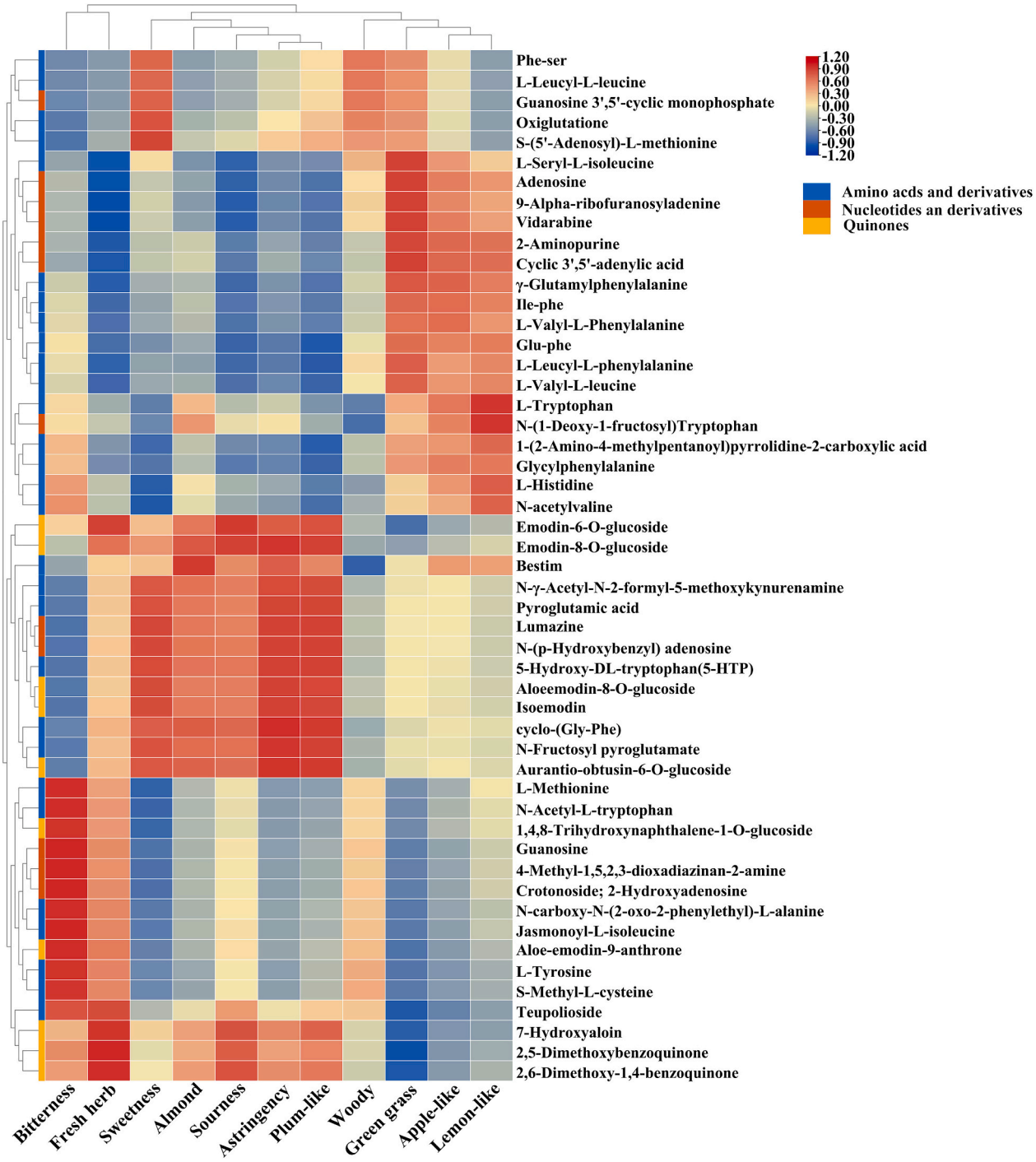

Fig. 4. The correlation analysis of amino acids and derivatives, nucleotides and derivatives, quinones compounds with sensory attributes.

were seven substances containing *N*-acetyl-*L*-tryptophan, *N*-carboxy-N-(2-oxo-2-phenylethyl)-*L*-alanine, jasmonoyl-*L*-isoleucine and others ($p < 0.01$) significantly linked to bitterness taste perception. Several amino acids, namely *L*-histidine, *L*-tryptophan, *N*-fructosyl pyroglutamate, *N*-γ-acetyl-*N*-2-formyl-5-methoxykynurenamine, and cyclo-(Gly-phe), significantly contributed to the astringency as well as the almond and plum-like flavors of the cherry ($p < 0.01$). Additionally, these amino acids also played a role in enhancing sweetness and sourness. Bestim, *N*-acetylvaline, and *L*-tryptophan ($p < 0.01$) contributed to the lemon-like flavor while synergistically contributing to apple-like aroma; γ-glutamylphenylalanine, *L*-valyl-*L*-phenylalanine, and *L*-leucyl-*L*-phenylalanine ($p < 0.01$) contributed to the green grass flavor while synergistically contributing to both apple-like and lemon-like aromas; five compounds containing oxiglutatione, phe-ser, and *S*-(5′-adenosyl)-*L*-methionine ($p < 0.01$) exhibited a significant positive correlation with sweetness. The nucleotides and their derivatives exhibited significant correlations with sensory attributes such as bitterness, astringency, plum-like flavor, and green grass (Fig. 4). Specifically, 9-alpha-ribofuranosyladenine and adenosine ($p < 0.01$) positively correlated with the perception of green grass, synergistically contributing to an apple-like aroma. On the other hand, compounds like 4-methyl-1,5,2,3-dioxadiazinan-2-amine, anthocyanin, and guanosine ($p < 0.01$) played important roles in enhancing bitterness while also synergistically intensifying the fresh herb sensation. Additionally, lumazine and *N*-(p-

hydroxybenzyl) adenosine ($p < 0.01$) were found to be positively associated with astringency and plum-like flavor respectively; these compounds further contributed to the perception of sweetness along with hints of almond and sourness. Although the amount of quinones was relatively small, they exhibited significant associations with various sensory attributes (Fig. 4). 2,5-Dimethoxybenzoquinone, 2,6-dimethoxy-1,4-benzoquinone, 7-hydroxyaloin ($p < 0.01$) were positively correlated with fresh herb attributes; aloeemodin-8-*O*-glucoside and aurantio-obtusin-6-*O*-glucoside ($p < 0.01$) contributed to astringency and plum-like characteristics; while 1,4,8-trihydroxynaphthalene-1-*O*-glucoside and aloe-emodin-9-anthrone ($p < 0.01$) were responsible for bitterness attributes.

The relationship between phenolic acids and sensory attributes was demonstrated in Fig. S7, indicating a positive correlation with astringency, bitterness, plum-like flavor, and fresh herb notes. Notably, 31 phenolic acids ($p < 0.01$), including gallacetophenone, 2-(formylamino) benzoic acid, 3-*O*-(3,5-dimethoxy-4-hydroxybenzoyl)-4-*O*-caffeoylquinic acid, and kushenin were found to contribute significantly to astringency and plum-like flavor while also influence sweetness, sourness, and almond flavor. A total of 37 compounds ($p < 0.01$) were identified as contributors to bitterness and co-contributors to fresh herb, including 3-methoxybenzoic acid, 1-*O*-(6'-*O*-feruloyl) glucoside-3-*O*-caffeoyl quinic acid, cryptochlorogenic acid (4-*O*-caffeoylquinic acid), and 1-*O*-eudesmoylquinic acid. Simultaneously, a set of 9 compounds ($p < 0.01$), such as 1-*O*-*p*-coumaroyl-*β*-D-glucose, *p*-coumaric acid-4-*O*-glucoside, and kushenin, exhibited significant associations with sourness. In addition, a significant positive was observed correlation between 4-(3,4,5-trihydroxybenzoxy) benzoic acid disinapoyl glucoside, 2-feruloyl-6-(4-hydroxycinnamoyl) glucoside, and 7,8-dihydro-buddlenol B (Threo) ($p < 0.01$) with sweetness. Furthermore, these compounds were found to contribute to the aroma profiles of green grass and woody notes. Methyl hydroxycinnamate, salicyl alcohol, 4,6-(*S*)-hexahydroxydiphenoyl-*β*-D-glucose, and coniferyl alcohol exhibited a significant correlation with lemon-like flavor ($p < 0.01$) (Table S3), while synergistically contributing to the perception of apple-like flavor.

Fig. S8 and Fig. S9 demonstrate that the majority of flavonoids were closely associated with bitterness, astringency, plum-like flavor, lemon-like flavor, and sweetness. Specifically, 58 flavonoids ($p < 0.01$) such as 6-hydroxykaempferol-7, 6-*O*-diglucoside, cyanidin 3-*O*-(6-*O*-*p*-coumaroyl) glucoside, kaempferol-3-*O*-glucoside-7-*O*-rhamnoside and luteolin-7-*O*-rutinoside exhibited positive correlations with bitterness and contributed synergistically to fresh herb and woody notes. Chrysoeriol-7-*O*-glucoside, tamarixetin-3-*O*-rutinoside, nobiletin (5,6,7,8,3′,4′-hexamethoxyflavone), kaempferol-3-*O*-glucuronide, irigenin and 18 other substances ($p < 0.01$) had a positive correlation with lemon-like flavor and demonstrated a certain synergistic contribution to apple-like notes as well as green grass and bitterness characteristics. There were 34 types of flavonoids ($p < 0.01$), including rhynchospermin, centaurein, sakuranin, epicatechin-epiafzelechin, cinchonain Ib and other compounds possessed a positive correlation with astringency and plum-like flavor. Additionally, these flavonoids contributed to sweetness, almond notes, and sourness. Among the remaining substances, there were 30 compounds ($p < 0.01$) that exhibited a positive correlation with sweetness, including 3,4,2′,4′,6′-pentahydroxychalcone-4'-*O*-glucoside, morin-3-*O*-arabinoside, aromadendrin (dihydrokaempferol), isoorientin-7-*O*-glucoside and 3,5,7-trihydroxyflavanone (pinobanksin). These compounds also made significant contributions to the woody and green grass notes.

As depicted in Fig. S10, lignans and coumarins, tannins, along with other compounds such as sugar alcohols, vitamins, ketones, etc., were found to be closely associated with astringency, plum-like flavor and bitterness, as well as fresh herb and green grass notes. Notably, 31 substances ($p < 0.01$) including kadsulignan L, macelignan; anwulignan, isoschisandrin ethyl alcohol, 2-methyl-5-acetonyl-7-hydroxychromone glucoside, deoxyschisandrin exhibited positive correlations with astringency and plum-like flavor, additionally contributing to almond flavor, sweetness, bitterness and sourness. The substances that exhibited a positive correlation with bitterness included noreugenin-7-*O*-glucoside, D-ribose, esculin-6-*O*-quinic acid, geranyl 3-*O*-xylopyranosyl-glucopyranoside, and 27 other substances ($p < 0.01$), and they also contributed to the fresh herb. Twelve compounds ($p < 0.01$), including (6*S*,7*E*,9*R*)-6,9-dihydroxy-4,7-megastigmadin-3-one-9-*O*-xylosyl (1–6) glucoside and epipinoresinol, were found to be positively correlated with sweetness while contributing synergistically to woody and green grass flavors. Additionally, seven compounds ($p < 0.01$) such as leucol, trachelegenin and coniferaldehyde were identified to be positively correlated with lemon-like flavor while simultaneously contributing synergistically to apple-like and green grass flavors. The primary contributors to bitterness were tannins, lignans and coumarins, while the other compounds make a slightly higher contribution to astringency.

As depicted in Fig. S11, alkaloids and terpenoids exhibited a close association with astringency, plum-like flavor, bitterness, lemon-like flavor, and fresh herb notes. Notably, 2,3,23-trihydroxyolean-12-ene-28,29-dioic acid 29-methyl ester (phytolaccagenin), norarjunolic acid, 3-pyridine-methanol-*O*-*β*-D-glucopyranosyl, tetraphyllin A along with 29 other compounds ($p < 0.01$) demonstrated positive correlations with astringency and plum-like flavor while also contributing to sweetness, sourness almond-like nuances and bitterness. Thirty compounds ($p < 0.01$), including 4,5,6-trihydroxy-2-cyclohexen-1-ylideneacetonitrile, 2,3-dihydroxyurs-12-en-28-oic acid methyl ester (corosolic acid methyl ester), and *N*-phenylethylcrinasiadine, exhibited a positive correlation with bitterness while synergistically contributing to sweetness, green grass notes, and fresh herb flavors. The lemon-like flavor was positively associated with thirteen compounds ($p < 0.01$), including indole, methoxyindoleacetic acid, *m*-aminophenylacetylene, and isoquinoline, which also contributed to sweetness, apple-like, woody, and plum-like flavors. Five compounds ($p < 0.01$) such as rubuside J, xanthurenic acid 8-*O*-glucoside, and 3-*O*-*p*-coumaroyloleanolic acid were found to be positively correlated with fresh herb and green grass aromas while contributing to sourness and plum-like flavors. Moreover, ursolaldehyde (3-hydroxyurs-12-en-28-aldehyde) and arclyside A ($p < 0.01$) significantly contributed to sweetness while also synergistically enhancing the woody flavor and green grass notes. Alkaloids were the primary contributors to lemon-like flavor, while terpenoids played a larger role in bitterness than alkaloids, and alkaloids also contributed more to astringency.

The relationship between organic acids and lipids with plum-like flavor, astringency, bitterness, green grass, sweetness, lemon-like flavor, and sours was demonstrated in Fig. S12. Fifteen substances ($p < 0.01$) including mandelic acid-*β*-glucoside, citraconic acid, and 2-amino-3-(1*H*-pyrazol-1-yl) propanoic acid exhibited significant positive correlations with plum-like flavor and astringency while synergistically contributing to sweetness, bitterness, almond flavor and sourness. Thirteen substances ($p < 0.01$), including 2*R*-hydroxy-9*Z*,12*Z*,15*Z*-octadecatrienoic acid, gingerglycolipid B and lysoPC 18:1(2n isomer), were significantly positively correlated with sweetness while synergistically contributing to green grass and woody notes. The levels of lysoPE 18:2(2n isomer), lysoPC 18:2(2n isomer), and 12 other compounds ($p < 0.01$) had a positive correlation with the perception of lemon-like flavor, while also synergistically contributing to the perception of bitterness and apple-like flavor; among them, six substances ($p < 0.01$) including 9-oxo-10*E*,12*Z*-octadecadienoic acid and (9*Z*,11*E*)-13-oxooctadeca-9,11-dienoic acid were significantly positively associated with the sensory attribute of green grass. Furthermore, eleven substances ($p < 0.01$) such as 12,13-epoxy-9-octadecenoic acid, alpha-hydroxylinoleic acid, and hydroxy ricinoleic acid also demonstrated a significant positive correlation with green grass. Six compounds ($p < 0.01$), including 2-isopropylmalic acid and citric acid glucoside, had a significant positive correlation with bitterness and contributed to the fresh herb. The lipids contributed to the lemon-like, sweetness, and green grass flavor, whereas the organic acids contributed to the astringency and bitterness.

The volatile aromatic compounds depicted in Fig. S13 possessed

strong associations with sensory attributes such as plum-like, almond, fresh herb, lemon-like, and green grass flavors. Furthermore, these volatile compounds were closely linked to taste characteristics including bitterness, sourness, astringency, and sweetness. Notably, the presence of 5-hepten-2-one, 6-methyl-, decane, 5-methyl-, 2,5-cyclohexadiene-1,4-dione, 2-hexenoic acid (with significant positive correlations) ($p < 0.01$) contributed to the perception of astringency as well as plum and sourness while also enhancing almond flavor along with fresh herb and sweetness. The compounds benzaldehyde, ethanol, benzene acetaldehyde, 1-ethyl-4-methyl-, and 3 other substances ($p < 0.01$) exhibited a positive correlation with bitterness and fresh herb notes while synergistically contributing to woody and sourness. On the other hand, 2-hexen-1-ol acetate (*Z*)-, acetic acid hexyl ester, trans-2-nonenal, and several other compounds ($p < 0.01$) were positively associated with green grass as well as woody and sweetness. The presence of eight compounds ($p < 0.01$), namely hexanoic acid, methyl ester, trans-2-undecen-1-ol, 4,8,12-tetradecatrienal and 5,9,13-trimethyl-, exhibited a positive correlation with the perception of lemon-like flavor while also

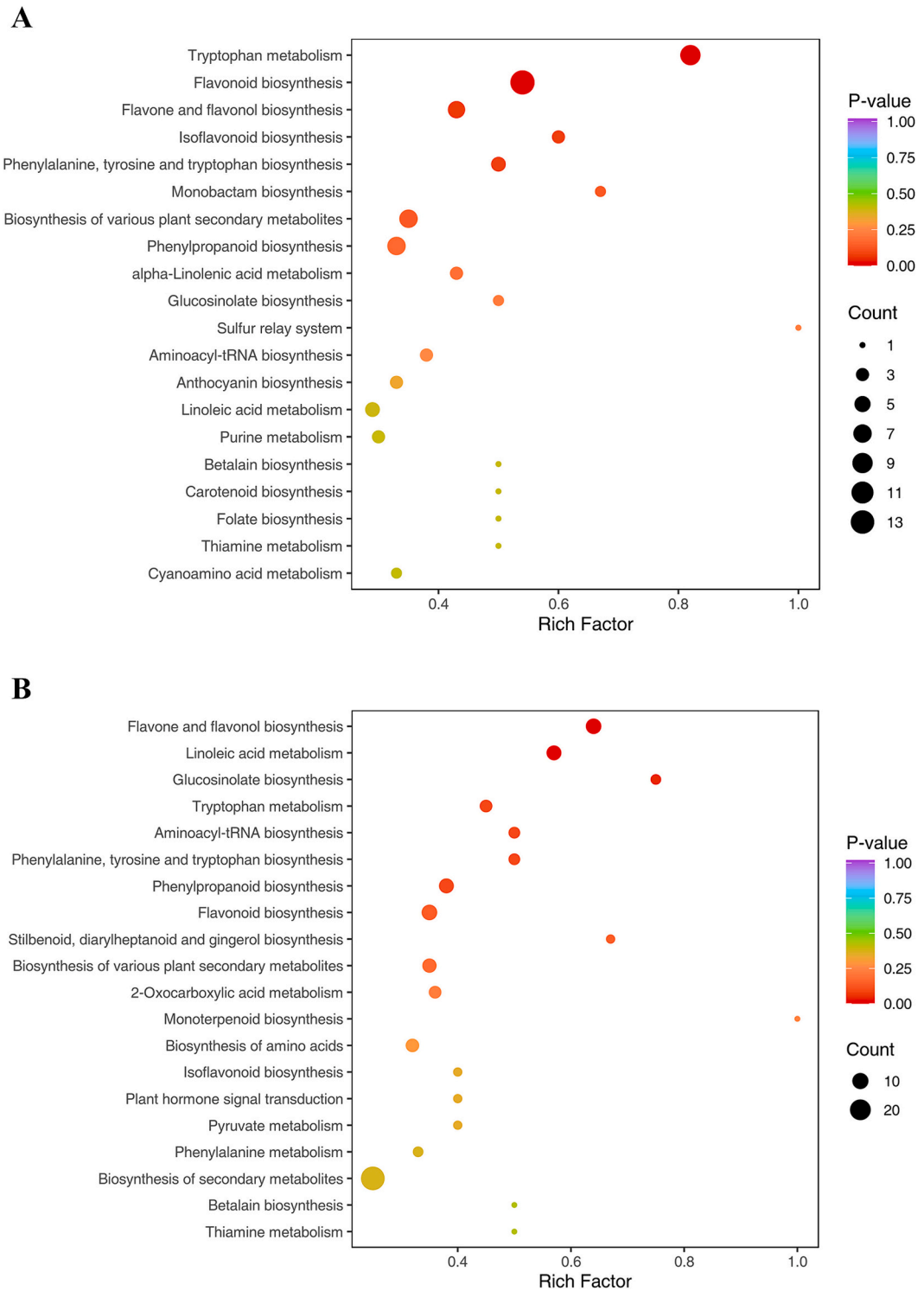

Fig. 5. KEGG enrichment map of differential metabolites for RO vs RG group and TO vs TG group.

contributing to the apple-like flavor as well as imparting bitterness and green grass notes. Moreover, five compounds ($p < 0.01$) including decanal and heptanal demonstrated a positive association with most flavors except for bitterness and woody characteristics.

Through correlation analysis, a significant positive correlation was observed between a large number of substances in each group and the sensory characteristics of astringency, plum-like flavor, bitterness. Additionally, these substances synergistically contributed to sourness perception, almond-like flavor, and fresh herb aroma. A subset of compounds also played a role in lemon-like flavor perception while contributing synergistically to apple flavor and green grass aroma. Furthermore, numerous substances belonging to phenolic acids, flavonoids, alkaloids, terpenoids, lipids, lignans and coumarins demonstrated positive correlations with the sensory characteristics.

3.5. Metabolic pathway analysis of cherry cultivars grown in different planting environments

The majority of the metabolites were assigned to the category of "metabolism" while only a limited number were associated with other systemic information categories, such as "environmental information processing" and "genetic information processing" (Fig. S14 and Fig. S15). Through enrichment analysis, tryptophan metabolism and flavonoid biosynthesis pathways was identified a pivotal role in shaping the flavor variations observed in Russia 8 cherries from greenhouse and open-air. ($p < 0.05$) (Fig. 5A). In addition, some other pathways, such as flavone and flavonol biosynthesis, isoflavonoid biosynthesis, phenylalanine, tyrosine and tryptophan biosynthesis, also played an important role. Fig. 6A summarized the biomarkers in these metabolic pathways. As shown in Fig. 6A, primary metabolites such as lipids and nucleotides, as well as secondary metabolites like certain flavonoids and phenolic acids, were significantly down-regulated in greenhouse cherry. Conversely, most secondary metabolites such as flavonoids, phenolic acids, and alkaloids were significantly up-regulated in open-air cherry, consistent with the trend shown in Fig. 3E. In the RO vs RG group, 9 compounds were identified in the tryptophan metabolism pathway, comprising 2 phenolic acids, 4 alkaloids, 1 organic acid, and 2 amino acids and their derivatives. All of these compounds exhibited up-regulation except for anthranilic acid (Fig. 6A). A total of 14 metabolites participated in the flavonoid biosynthesis pathway, including 1 phenolic acid and 13 flavonoids. Notably, all of these metabolites showed down-regulation except for trans-5-O (*p*-coumaroyl) shikimate, naringenin-7-*O*-glucoside (Prunin), and apigenin-8-*C*-glucoside (vitexin) (Fig. 6A). Additionally, 9-oxo-10*E*,12*Z*-octadecadienoic acid, 13-KODE; (9*Z*,11*E*)-13-oxooctadeca-9,11-dienoic acid, 9,10,13-trihydroxy-11-octadecenoic acid and 12,13-epoxy-9-octadecenoic acid in linoleic acid metabolism, adenosine, cyclic 3′,5′-adenylic acid and guanosine 3′,5′-cyclic monophosphate in purine metabolism exhibited down-regulation; apigenin-6-*C*-glucoside (Isovitexin), apigenin-7-*O*-glucoside(Cosmosiin), biochanin A-7-*O*-glucoside (Sissotrin) and genistein-7-*O*-glucoside (Genistin) in flavone and flavonol biosynthesis and isoflavonoid biosynthesis were up-regulated (Fig. 6A).

For Tieton cherries, the results showed that the pathways of flavone and flavonol biosynthesis, linoleic acid metabolism and glucosinolate biosynthesis metabolic were active ($p < 0.05$) (Fig. 5B). In addition, some other pathways, such as tryptophan metabolism, phenylalanine, tyrosine and tryptophan biosynthesis, phenylpropanoid biosynthesis also played an important role in the flavor difference between Tieton cherries grown in facility environment and outdoors. Fig. 6B presented a summary of the metabolic variances between outdoor-grown and greenhouse-grown Tieton cherries in these metabolic pathways. As depicted in Fig. 6B, there were evident down-regulations of primary metabolites like lipid substances, amino acid substances, as well as some secondary metabolites containing certain phenolic acids, flavonoids, lignin, coumarins, and alkaloids in greenhouse-grown Tieton cherries. Conversely, most of secondary metabolites such as flavonoids, organic acids, phenolic acids, and alkaloids were significantly up-regulated in outdoor-grown Tieton cherries, which aligned with the trend illustrated in Fig. 3F. In the TO vs TG group, flavone and flavonol biosynthesis involved nine types of flavonoids, all of which exhibited up-regulation except for quercetin (Fig. 6B); linoleic acid metabolism encompassed eight lipids, all of which displayed down-regulation (Fig. 6B). Additionally, 5-*O*-*p*-coumaroylquinic acid, syringin, and trans-5-*O*-(*p*-coumaroyl) shikimate in phenylpropanoid biosynthesis, 2-isopropylmalic acid, 2-propylmalic acid, isocitric acid, and quinic acid in phenylalanine, tyrosine and tryptophan biosynthesis, biosynthesis of amino acids and pyruvate metabolism were all up-regulated.

The above results indicated that cherry plants grown in open air exhibited a stronger secondary metabolism, while those grown in a greenhouse showed increased accumulation of primary metabolites such as lipids. Generally, phytochemicals play a crucial role in plant defense against pathogens, pests, herbivores, ultraviolet rays, oxidative stress and other harmful substances (Lattanzio, Kroon, Quideau, & Treutter, 2008; Vermerris & Nicholson, 2006). The content of phytochemicals in plants is influenced by factors including variety, geographical location, weather conditions, daylight duration, temperature fluctuations, soil composition and moisture levels (Gutiérrez-Grijalva et al., 2018). The quality, intensity, and duration of light, particularly ultraviolet (UV) light, play a crucial role in the biosynthesis and accumulation of secondary metabolites. It is widely accepted that UV light induces the synthesis of phenolic compounds such as flavonoids by reallocating carbon allocation from growth to defense (Blokhina, 2003). In this study, a significant number of flavonoids were found to be up-regulated in open-air Russia 8 cherries and Tieton cherries. These metabolites mapped onto flavonoid biosynthesis, flavone and flavonol biosynthesis and isoflavonoid biosynthesis pathways which can be impacted by ultraviolet light or light (Blokhina, 2003), and it suggested the importance of light. Furthermore, the up-regulation of lipids and nucleotides underscored the influence of light deprivation on primary metabolite accumulation. Apart from light, differences in temperature, humidity between greenhouse and open-air cherries, as well as soil moisture inside and outside the shed also contribute to variations of metabolites. Typically, the greenhouse humidity is maintained at approximately 50 %, and the evaporation rate in open air exceeds that in the shed, resulting in lower soil moisture content in the open area. Previous research has indicated that moderate drought can enhance organic acid, sugar, and soluble solid levels in cherries, thereby improving their taste and flavor (Yang, 2016). As shown in Fig. 1 and Fig. 2C, the sugar and acid levels of cherries grown outdoors were higher than those grown in the shed, with significantly greater acidity observed during sensory evaluation. And key organic acids in tyrosine and tryptophan biosynthesis, biosynthesis of amino acids and pyruvate metabolism were up-regulated in open-air cherries. Therefore, it was hypothesized that a substantial portion of the flavor disparity between these two types of cherries stemmed from the adverse effects of moderate drought (low soil water content). Low temperature stress inhibits plant growth; however, an appropriate temperature increase can expedite fruit development (Shen, 2023). In this study, the temperature of cherries in greenhouses is generally controlled at 20–25 °C, which is generally higher than the growing temperature of cherries in the open air. From the results we found that the nucleic acids, accelerating tissue growth rate of plants (Liu et al., 2022), of cherries in greenhouses were higher. Furthermore, the content of fatty acids in greenhouse cherries were high, and it is known that free fatty acids are the contributors to membrane lipid structure synthesis, and their up-regulation generally leads to increased cell division intensity (Liu et al., 2022). Both of them indicated that the growth rate of greenhouse cherries was higher than that of open-air cherries. In conclusion, the difference of flavor between open-air cherry and greenhouse cherry was influenced by light intensity, soil moisture content, temperature and humidity, and the relationships varied by the cherry varieties.

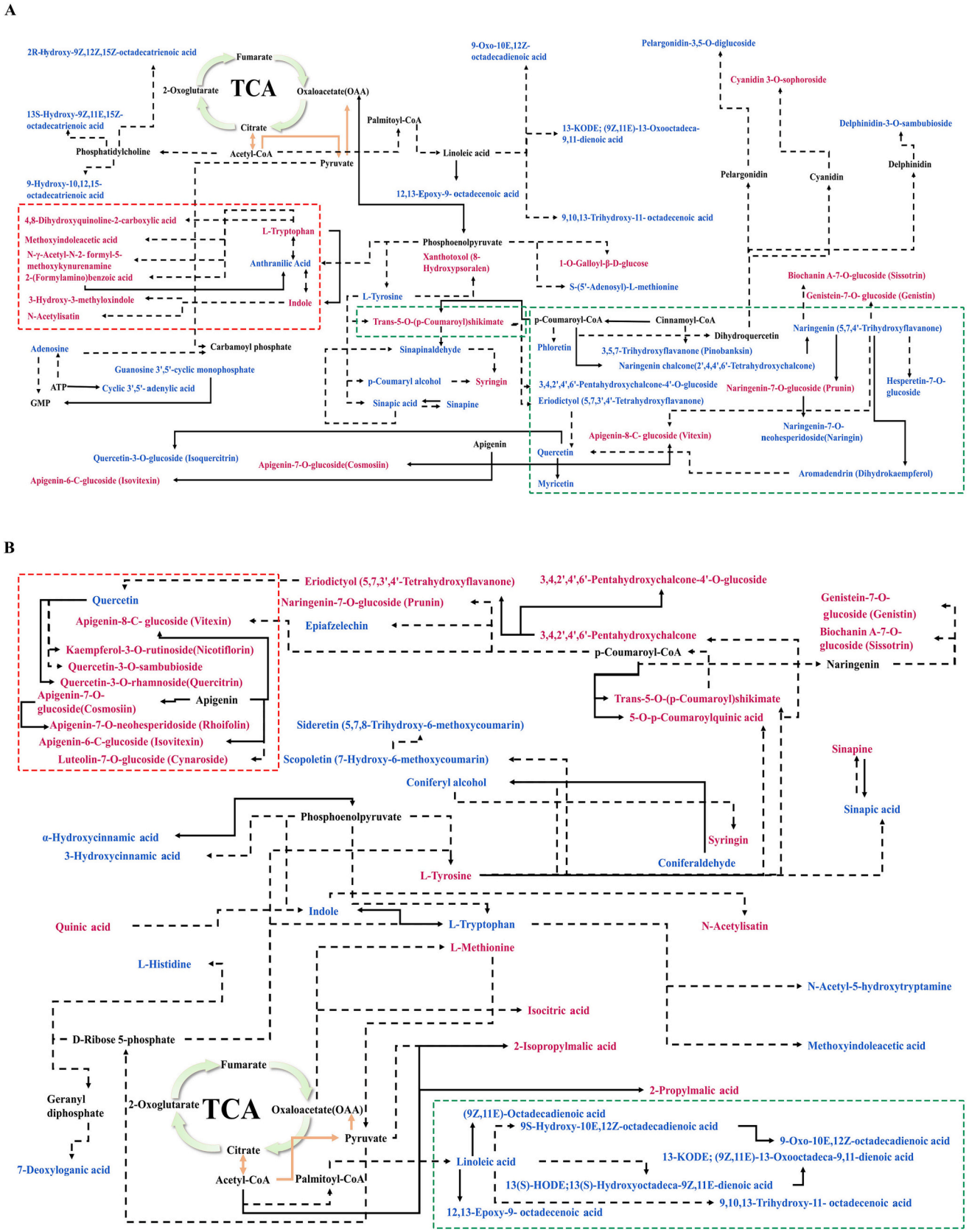

Fig. 6. Changes in the levels of metabolites between RO and RG sherries (A) and between TO and TG sherries (B) shown in a metabolic diagram. Notes: the magenta and cyan fonts display the up- and downregulated metabolites, respectively; the pathway in the red box represents tryptophan metabolism pathway, while the pathway in the green box represents flavonoid biosynthesis pathway in Fig. 6A; the pathways depicted in the red and green boxes represent flavone and flavonol biosynthesis, and linoleic acid metabolism, respectively, as shown in Fig. 6. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4. Conclusions

Comprehensive widely metabolomics analysis and volatile fingerprinting revealed a diverse array of metabolites in cherries, with significant variations in content and types among different varieties and planting environments. The difference of open-field cultivation and greenhouse conditions could induce alterations in the composition of a majority of metabolites in cherries. Compared to facility cherries, open-field cultivation favored the accumulation of non-volatile flavor compounds as well as aromatics, alkanes and ketones compounds and exhibited higher acidity, astringency, plum-like flavor, and fresh herb notes. Numerous differential metabolites including phenolic acids, flavonoids, alkaloids, terpenoids, lipids, lignans and coumarins closely associated with sensory traits. And most differential metabolites were significantly positively correlated with astringency, plum-like flavor and bitterness, and synergistic with sourness, almond flavor and fresh herb aroma. Through path analysis, it was found that the most important pathways explaining the flavor variations for Russia 8 cherries and Tieton cherries from different planting conditions. By analyzing key compounds along the pathways, light intensity, soil moisture content, temperature and humidity were inferred as the major factors for the flavor difference of open-air and greenhouse-grown cherries. This study provides valuable data support for further investigations into the factors influencing flavor disparities between open-field cherries and greenhouse cherries, and is highly significant for enhancing cherry flavor quality and selecting appropriate cultivation methods.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101802.

Ethical statement

The exemption of sensory evaluation in this study was approved by ethics committee (Reference number: SAAS-2024-G71). This studay followed appropriate protocols for protecting the rights and privacy of all participants during the execution of the research. There was no coercion involved in participation, and full disclosure of study requirements and potential risks were provided. Additionally, written or verbal consent were obtained from all participants, and it was ensured that participant data would not be released without their knowledge, and participants had the ability to withdraw from the study at any time.

CRediT authorship contribution statement

Wenjun Zhang: Writing – original draft, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Dongzi Zhu:** Resources, Project administration. **Jiangsheng Mao:** Software, Formal analysis. **Hongxia Du:** Formal analysis. **Hongwei Qin:** Validation. **Jiawei Wang:** Validation. **Chao Zhu:** Formal analysis. **Mengmeng Yan:** Validation, Funding acquisition. **Bo Bai:** Resources, Data curation.

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.

Data availability

The authors do not have permission to share data.

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