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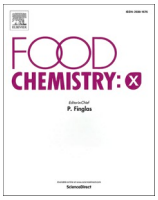

Widely targeted metabolomic analysis reveals effects of yellowing process time on the flavor of vine tea (*Ampelopsis grossedentata*)

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ABSTRACT

The bitter and astringent taste and miscellaneous smell of vine tea prevent its further development. In this study, we used a processing technology that mimics yellow tea to improve the flavor of vine tea and revealed its internal reasons through metabolomics. Sensory evaluation showed the yellowing process for 6–12 h reduced the bitterness and astringency significantly, and enriched the aroma. The improvement of taste was mainly related to the down-regulation of anthocyanins (54.83–97.38%), the hydrolysis of gallated catechins (34.80–47.81%) and flavonol glycosides (18.56–44.96%), and the subsequent accumulation of D-glucose (33.68–78.04%) and gallic acid (220.96–252.09%). For aroma, increase of total volatile metabolite content (23.88–25.44%) and key compounds like geraniol (239.32–275.21%) induced the changes. These results identified the positive effects of yellowing process on improvements in vine tea flavor and the key compounds that contribute to these changes.

1. Introduction

Vine tea (VT, *Ampelopsis grossedentata* (Hand.-Mazz.) W. T. Wang) is usually grown in the valley forest or hillside thicket at an altitude of 200 ~ 1500 m in the southern part of China. The name is derived from its grapevine-like appearance and tea-like consumption method. This non-*camellia* beverage has been a portion of local dietary culture for over 700 years (Zeng et al., 2023). Traditionally, vine tea is considered to have properties, such as clearing away heat, detoxification, relieving sore throat, activating blood circulation, and dissipating blood stasis. Modern pharmacological studies have proved the benefits of vine tea for chronic disease like non-alcoholic hepatitis, type 2 diabetes and so on (Q. Zhang et al., 2021). Owing to its numerous health benefits, vine tea is increasingly being consumed as a functional beverage. However, the astringent taste and miscellaneous smell of vine tea would be off-putting for many first-time consumers, thereby limiting its further promotion as a beverage(M. Zhang, 2022). Consequently, there is a pressing need for research on methods for improving the flavor profile of vine tea.

In China, the types of tea processed include green tea, black tea, oolong tea and, less frequently, white tea, dark tea, and yellow tea. The traditional process of vine tea resembles that of green tea, involving several steps: spreading, fixation, rolling, and drying. However, green tea undergoes no fermentation, preserving a more original flavor. This may benefit the pleasant *camellia* plants, but for vine tea with astringent taste and miscellaneous smell, it may accentuate its disadvantages. Conversely, yellowing is a crucial process for enhancing the sweetness and mellowness of yellow tea, which is achieved by wrapping and piling the fixed tea leaves under high-temperature and high-humidity conditions for a certain duration(Xu et al., 2018). Such process can trigger the breakdown of bitter compounds such as gallated catechins and flavonol glycosides and the accumulation of free amino acids and soluble sugars, thereby increasing the sweetness and decreasing the bitterness and astringency of yellow tea (Wei et al., 2022). The main improvement areas of yellowing treatment coincide with the defects of vine tea. However, there is no research on the application of yellowing process to vine tea. We hypothesize that adopting the yellowing process to vine tea will also ameliorate its flavor.

Metabolomics based on liquid or gas chromatography mass spectrometry (LC-MS or GC–MS) have been widely employed to elucidate the biochemical variations of various food and beverage products, as well as their modifications after different treatments (Gui et al., 2023). Nevertheless, assays relying on targeted metabolites encompass limited substances, whereas non-targeted metabolomics encounters challenges of low sensitivity and inadequate qualitative and quantitative accuracy (Ma et al., 2023; Zhou et al., 2022). Widely targeted metabolomic analysis can be regarded as an innovative method that combines the

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advantages of targeted and non-targeted metabolomics, attains a high-throughput, and demonstrates a high sensitivity and broad coverage (Wang et al., 2021). Previous studies have successfully applied this method on the dynamic changes of other similar researches on tea beverage (Meng et al., 2022). Hence, widely targeted metabolomics is in all probability a suitable technique to investigate the yellowing process to enhance the flavor of vine tea.

The aim of this study was to improve the flavor of vine tea by dull yellow processing technology, and to reveal the material basis behind the change by widely targeted metabolomics. In this study, (i) vine tea was prepared with different yellowing process time of 0 h, 3 h, 6 h, 9 h, 12 h; (ii) flavor characteristics of yellowing vine tea were evaluated by artificial sensory evaluation in terms of taste and odor; (iii) non-volatile and volatile metabolites were identified by LC-MS/MS and GC–MS; (iv) metabolomics studies and rOAV analysis were used to find key compounds causing flavor change. Based on sensory evaluation and widely targeted metabolomics, the datasets for optimizing vine tea processing and provide theoretical reference and objective basis. The results may also have important implications for the food industry, as they provide insights into the factors that contribute to the unique taste and ordor profiles of vine tea and can be used to guide the development of other tea-like beverage.

2. Materials and methods

2.1. Chemicals and regents

LC-grade methanol and acetonitrile were procured from Merck (Darmstadt, Germany), LC-formic acid was obtained from Aladdin (Shanghai, China). The authentic standards employed in the comprehensive metabolomics analysis were supplied by MetWare (Wuhan, China). All other related chemicals were of analytical grade.

2.2. Preparation of vine tea

A total of 5 kg fresh leaves of vine tea samples were procured from Yunfeng Wild plant development Co., LTD (Jiangkou County, Tongren City, Guizhou province, China) on April 10, 2023.All the vine tea samples were collected locally (Fanjing Mountain in Tongren City) by the company and were thoroughly mixed prior to processing. All samples were simultaneously manufactured using a method that imitates the processing of yellow tea, which encompasses withering, fixation, rolling, yellowing, and drying processes, with detailed conditions such as grouping, temperature, humidity and time indicated in Fig. 1. The variable under control was the duration of yellowing, with groups being designated according to their respective yellowing times: 0 h, 3 h, 6 h, 9 h, and 12 h. Among these groups, the one with a yellowing time of 0 h represents the traditional production process of vine tea. Post-manufacture, all samples were sealed and stored at a temperature of 4 °C until the metabolomics investigation was conducted.

2.3. Sensory evaluation

The sensory evaluation was conducted in accordance with the Methodology for sensory evaluation of tea (China National Institute of Standardization (CNIS) GB/T 23776–2018), albeit with minor modifications. A panel of ten professional evaluators, comprising an equal number of males and females aged between 26 and 54, assessed the gustatory and olfactory qualities of the vine tea infusions. According to the Sensory analysis-General guidance for the staff of the sensory evaluation laboratory–Part 1: Staff responsibilities (CNIS GB/T 23470.1–2009) and the customary in our country, a permission to conduct food sensory evaluation research is not required by our institution, though the necessary steps have been taken to protect the rights and interests of the volunteers. All evaluators voluntarily participated in the sensory evaluation when informed of the purpose of the experiment, the use of the information, and the potential risks. The grouping of vine tea was unknown to the evaluator. Each sample, weighed with a precision of 3.00 g, was placed in a standard evaluation cup. Subsequently, 150 mL of hot water, approximately at a temperature of 95 °C, was added to the cup. The infusion was allowed to steep for a duration of 5 min. The evaluators then carried out a descriptive analysis of the aroma and taste characteristics using a 10-point scale. The scale was delineated as follows: 0 = no or imperceptible intensity, 3 = weak intensity, 5 = moderate intensity, 7 = high intensity, and 10 = extremely high intensity. Each expert independently evaluated the samples and documented their scores.

2.4. UPLC-MS/MS analysis of non-volatile metabolites

Every vine tea group products were placed in a lyophilizer (Scientz-100F) for vacuum freeze-drying, then ground to a powder form. Following this, 50 mg of the sample powder was added to 1200 μL of a pre-cooled (−20 °C) 70% methanol mixed. The mixture was then vortexed six times, with each vortexing session lasting for 30 s and occurring every 30 min. Post-vortexing, the mixture was centrifuged at 12000 rpm for 3 min. The supernatant was filtered through a microporous membrane with a pore size of 0.22 μm, sample solution was obtained.

Vine tea sample solution was analyzed by Nexera UHPLC LC-30 A (Shimadzu, Japan) and Triple Quad™ 4500MD (AB SCIEX, USA). The UPLC conditions were set as following: Agilent SB-C18 column (1.8 μm, 2.1 mm × 100 mm); flow rate 0.35 mL/min; column oven temperature 40 °C; injection volume was 2 μL. Mobile phase A: pure water with 0.1% formic acid, mobile phase B: acetonitrile with 0.1% formic acid, gradient program: 0 min, 95% A; 9 min, 5% A; 10 min, 5% A; 10.1 min, 95% A; 13 min, 95% A.

The MS conditions were set as following: source temperature 550 °C; ion spray voltage 5500 V (positive ion mode)/−4500 V (negative ion

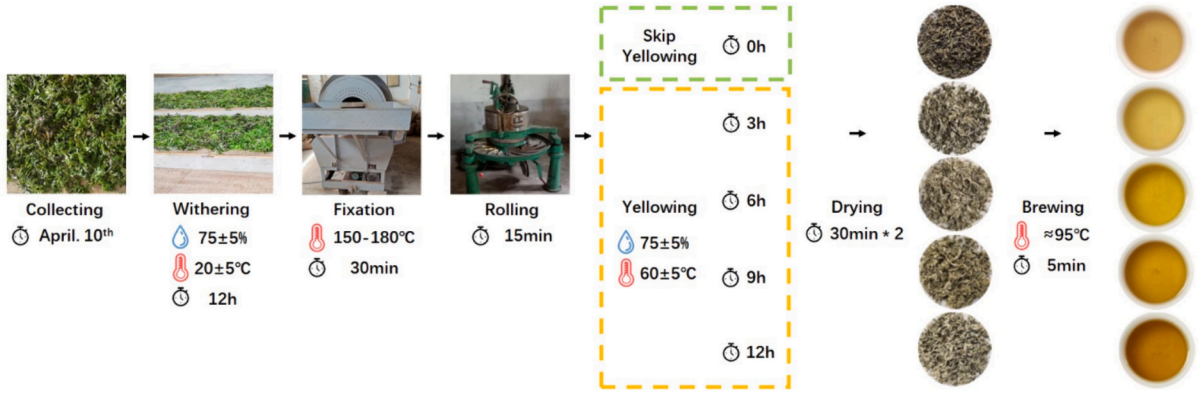

Fig. 1. Schematic diagram of the whole process of vine tea preparation.

mode); ion source gas I, gas II, curtain gaswere set at 50, 60, and 25 psi, respectively; the collision-activated dissociation was high. The triple Quadrupole (QQQ) scans were conducted as Multiple Reaction Monitoring (MRM) experiments with collision gas (nitrogen) set to medium.

2.5. GC–MS analysis of volatile metabolites

Vine tea samples were pulverized into a fine powder using liquid nitrogen. 500 mg of the powder was then transferred to a 20 mL headspace vial (Agilent, USA), containing NaCl saturated solution, to inhibit any enzyme reaction. The vials were sealed using crimp-top caps with TFE-silicone headspace septa. At the time of solid-phase microextraction (SPME) analysis, each vial was placed in 60 °C for 5 min, then a 120 μm DVB/CWR/PDMS fibre was exposed to the headspace of the sample for 15 min at 60 °C.

The identification and quantification of volatile organic compounds VOCs was carried out using an Agilent Model 8890 GC and a 7000D mass spectrometer (Agilent, USA), equipped with a 30 m × 0.25 mm × 0.25 μm DB-5MS (5% phenyl-polymethylsiloxane) capillary column. After sampling, desorption of the VOCs from the fibre coating was carried out in the injection port of the GC apparatus at 250 °C for 5 min in the splitless mode. Helium severed as the carrier gas at a linear velocity of 1.2 mL/min. The injector temperature was kept at 250 °C. The oven temperature was programmed from 40 °C (3.5 min), increasing at 10 °C/min to 100 °C, at 7 °C/min to 180 °C, at 25 °C/min to 280 °C, held for 5 min. Mass spectra was recorded in electron impact (EI) ionisation mode at 70 eV. The quadrupole mass detector, ion source and transfer line temperatures were set at 150, 230 and 280 °C, respectively. The MS was selected ion monitoring (SIM) mode was used for the identification and quantification of analytes.

2.6. Calculation of relative content and rOAV value

The quantification of volatile components was performed indirectly using the internal standard method with 3-hexanone. The formula employed is as follows:

$$X_i = V_s \times C_s / M \times I_i / I_s \times 10^{-3}$$

X_i is the content of compound i in the test sample (μg/g), V_s is the volume of the internal standard added (μL), C_s is the concentration of the internal standard (μg/mL), M is the amount of sample to be measured (g), I_s is the peak area of the internal standard; I_i is the peak area of compound i in the sample to be measured.

Relative odor activity value (rOAV) was utilized to identify key flavor compounds in complex samples, based on the sensory thresholds of the compounds (Zhu, Wang, Xiao, & Niu, 2018). This method aids in elucidating the contribution of each aroma compound to the overall aroma characteristics of the sample. The rOAV was calculated using the following formula:

$$rOAV_i = C_i / T_i$$

where $rOAV_i$ is the relative odor activity value of compound i, C_i is the relative content of compound (μg/g); T_i is the Threshold of the compound (Threshold, μg/g). The threshold values, T_i were obtained from reputable databases: The Good Scents Company (http://www.thegoodscentscompany.com), Flavor Ingredient Library (https://www.femaflavor.org/flavor-library), LRI & Odor (http://www.odour.org.uk/odour/index.html), and Food Flavor Lab (http://foodflavorlab.cn/#/home).

2.7. Metabolomics analysis

The liquid prime number was then analyzed statistically in the form of peak area. Unsupervised Principal Component Analysis (PCA) was was performed using the prcomp function in R (www.r-project.org). Prior to the PCA, the data was scaled to unit variance. The Hierarchical Cluster Analysis (HCA) results for samples and metabolites were visualized as heatmaps with dendrograms. Pearson Correlation Coefficients (PCC) between samples were calculated using the cor function in R and visualized as heatmaps. Both HCA and PCC were implemented using the ComplexHeatmap package in R. For the two-group analysis, differential metabolites were identified based on Variable Importance in Projection (VIP > 1) and absolute Log2 Fold Change ($|Log2FC| \geq 1.0$). VIP values were extracted from the Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) result, which also included score plots and permutation plots, generated using the MetaboAnalystR package in *R. prior* to OPLS-DA, the data underwent log transformation (log2) and mean centering. Identified metabolites were annotated and mapped using the KEGG database (www.kegg.jp). Pathways with significantly regulated metabolites were then subjected to Metabolite Sets Enrichment Analysis (MSEA), with significance determined by *p*-values from a hypergeometric test. The radar map, venn diagram, and K-means analysis were generated using the free online platform Metware Cloud (cloud.metware.cn). Alluvial diagrams were drawn through RAW Graphs 2.0 (app.rawgraphs.io).

2.8. Data analysis

The analyses were performed in triplicate and the data were presented as mean ± SD. GraphPad Prism 8.0.2 (California, USA) was used for data analysis. One-way ANOVA and multiple comparisons were applied to test the group differences, with $p < 0.05$ as the significance level.

3. Results and discussion

3.1. Characteristics of the vine tea yellow process

The state of vine tea leaves and soups are shown in Fig. 1. The unyellowed vine tea consisted of black-green, tightly rolled leaves and thin stems. The yellowed groups had a looser texture, possibly due to the yellowing process after rolling, which caused the leaves to re-expand in a humid and hot environment. The leaf color became lighter after yellowing, showing a faint yellow at 6 h group, and a clear yellow at 9 h and 12 h groups. However, the 12 h group had white solids on the leaves, obscuring the yellow color from afar. As the content of flavonoids in vine tea is up to >40%, and the original cell structure is damaged after processing, and the flavonoid components are precipitated to form “white frost-like solids” on the leaves. And this phenomenon is also a unique feature of vine tea (Shi, Wan, Wang, Wang, & Fang, 2022). The 0 h group had no frost-like solids, while the 3 h, 6 h, and 9 h groups had a small amount, and the 12 h group had a large amount. The 0 h group soup of vine tea seemed dark and dull, but it appeared brighter after yellowing. The soup was lighter than the 0 h group in the early yellowing stage, but it darkened with increasing yellowing time and turned yellowish-brown at the end. We hypothesized that this yellowish-brown color change may be caused by non-enzymatic browning reactions between reducing sugars and substances containing free amino groups, such as amino acids, or by changes in the content of colored flavonoid aglycones.

3.2. Sensory evaluation of vine tea

According to the previous research method of vine tea quality with slightly modification, the appearance and color of vine tea were described in words, while 2 groups of 12 entries were set up to score the olfactory and taste characteristics of them respectively (M. Zhang, 2022). As is shown in Fig. 2 D, the aroma profile of vine tea is generally mild, with the most intense scent described as ‘grass’, scoring no >5 on a 10-point intensity scale. This ‘grass’ scent is the dominant aroma characteristic of vine tea prior to yellowing, and its intensity decreases

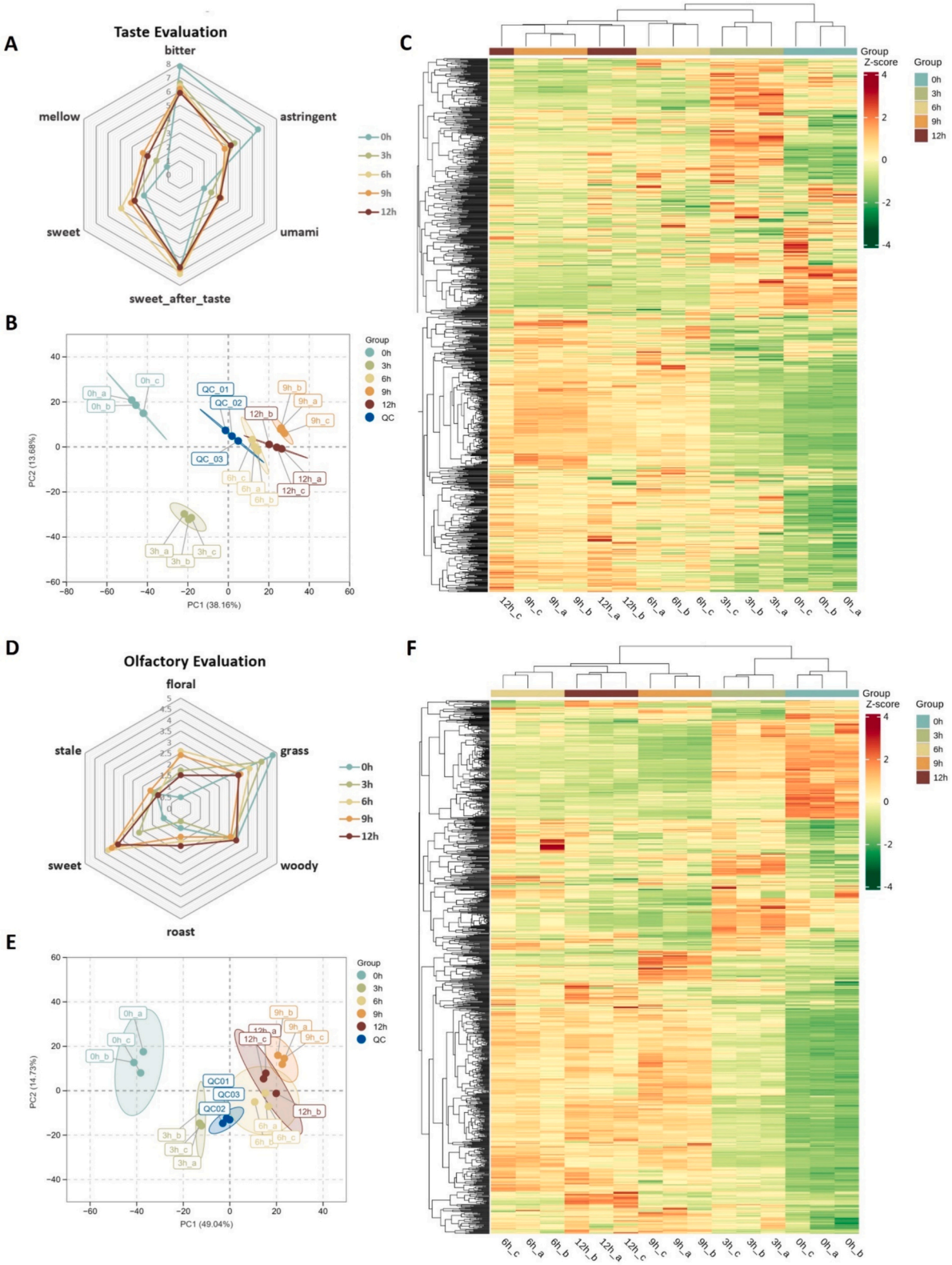

Fig. 2. The flavor changes at the yellowing time of vine tea were generally consistent with the results of compound clustering: 6 h, 9 h, 12 h had great improvement (A) Taste evaluation of vine tea with different yellowing time; (B) PCA plot of non-volatile metabolites in vine tea; (PC1 and PC2 represent the first and second principal components, respectively, and the percentages indicate the explained variance by these principal components for the dataset.) (C) HCA heat map of all non-volatile metabolites from group 0 h, 3 h, 6 h, 9 h, 12 h; (The horizontal axis represents the sample names, while the vertical axis provides specific metabolite information; Red-green blocks are the colors filled with different values obtained after standardized treatment with different relative contents.) (D) Olfactory evaluation of vine tea with different yellowing time; (E) PCA plot of volatile metabolites in vine tea; (F) HCA heat map of all volatile metabolites from group 0 h, 3 h, 6 h, 9 h, 12 h. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

significantly from 4.8 to 3.1 post-yellowing. In contrast, floral and sweet fragrances are absent in unyellowed vine tea but emerge with weak intensity after yellowing. The roasted and woody aromas remained at a low level throughout. Improper handling or prolonged exposure to high temperature and humidity during the yellowing process can lead to excessive oxidation of aroma substances, resulting in an unpleasant odor. To monitor this, a 'stale' category was added, which maintained a low rating between 1.2 and 1.6. In contrast to the convergence observed in olfactory evaluation, the taste scores were more distinctive (Fig. 2 A). The raw bitterness and astringency of vine tea are extremely pronounced, scoring 7.8 and 6.5 respectively. This is a primary factor limiting its potential consumer base. After yellowing, these two indices show a significant decline, averaging around 6.2 and 4.2 depending on the duration of yellowing. 'Sweet-after-taste' is an important evaluation criterion for high-grade tea and a characteristic feature of vine tea (Chong et al., 2019). It is a complex sensation: initially, the infusion tastes bitter, but over time, this bitterness is gradually supplanted by sweetness. After swallowing, a sweet and moist sensation can be felt at the base of the tongue and throat. The 'sweet-after-taste' score remained high (between 6.7 and 7.2) throughout the yellowing process.

In general, yellowing alleviates the bitter and astringent taste of the vine tea and enriched the aroma. Yellowing of 3 h failed to achieve the expected effect; 6 h, 9 h and 12 h had a good performance in most indexes; Of the three, 6 h and 9 h group had slight differences compared to 12 h. Based on these indices, it can be tentatively concluded that yellowing for at least 6 h optimizes vine tea's flavor to the greatest extent.

3.3. Analysis of non-volatile metabolites during the processing of VT

3.3.1. Overview of the non-volatile metabolite

A total of 1013 non-volatile components including 12 categories were tentatively identified, including 353 amino acids and derivatives, 279 phenolic acids, 269 flavonoids, 165 lipids, 145 alkaloids, 120 organic acids, 76 nucleotides and derivatives, 60 terpenoids, 48 lignans and coumarins, 36 tannins, 10 quinones, 2 steroids, and 237 other metabolites. The overlapping display of the total ion current (TIC) graphs analyzed by ESSM and a multi-peak detection plot of the metabolites in the multiple reaction monitoring mode (MRM) are shown in (Fig. S1 A-D). A high overlap ratio of TIC curves was observed for the variant quality control (QC) samples, suggesting that the test results are repeatable and reliable.

To obtain an overview of the difference and stability of data in each group, a non-supervised metrology principal component analysis (PCA) tool was conducted (Fig. 2 B). The distribution within each group is compact, with sufficient separation between groups. The QC groups are centrally located, indicating that the analysis results are reliable and stable. Notably, the PCA results roughly divided all five groups into three categories according to the size of spacing: the 0 h group without yellowing process, the 3 h group with short yellowing time, and the 6 h, 9 h, 12 h groups with medium to long yellowing time. Similar results were observed in the visual heatmap through hierarchical clustering analysis (HCA) (Fig. 2 C), where every three biological repeats clustered together. Specifically, the 6 h, 9 h, and 12 h groups were relatively close while the remaining two groups were individually clustered. This situation is consistent with the results of sensory evaluation. These results suggest that the first six hours of the yellowing process induce the most significant metabolite changes, with smaller changes occurring in the subsequent six hours.

3.3.2. Screening for differential non-volatile metabolites

To gain a deeper understanding of the impact of the yellowing process on vine tea metabolism, the orthogonal partial least squares discriminant analysis (OPLS-DA) model was employed to compare the metabolic characteristics of different samples. Specifically, metabolites at the 3 h, 6 h, 9 h, and 12 h stages were compared with those in the 0 h group. The results are shown in Fig. 3 A-D and Table S1. R^2X and R^2Y represent the explanatory rate of the model for the X and Y matrices, respectively, and Q^2 represents the predictive power of the model. In all the four models, R^2X are higher than 0.5, while R^2Y and Q^2 are higher than 0.9, which indicated stability and reliability of the model.

The analysis of volcano plots was further applied to visualize the differences in the metabolites. In the comparison between the 3 h, 6 h, 9 h, and 12 h groups and the 0 h group, 76, 184, 236, 201 up-regulated (VIP $>$ 1.0, P $<$ 0.05 and FC $<$ 0.5) metabolites and 22, 67, 69, 54 down-regulated (VIP $>$ 1.0, P $<$ 0.05 and FC $>$ 2.0) metabolites were found (Fig. 3 *E*-H), respectively. The trends of metabolites in different categories of groups are shown in Fig. 3 I-L. In terms of categories most pertinent to flavor, amino acids and derivatives, lipids, and phenolic acids generally exhibited an upward trend. While flavonoids—the main components of vine tea—had >20 up-regulated metabolites and slightly lower down-regulated metabolites. The main differential metabolites are shown in Table S2.

3.3.3. Multi-angle analysis of differential non-volatile metabolites

A Venn diagram was constructed based on the differential metabolite data (Fig. 4A). Among these, 47 differential metabolites were observed in the comparison of all four groups simultaneously, indicating a common trend of change during the yellowing process. The most significant overlap of 162 metabolites was found in the comparisons involving the later three groups (0 h vs 6 h, 0 h vs 9 h, 0 h vs 12 h), demonstrating the relative stability of the chemical composition during the middle and late stages of yellowing once again.

K-means is a popular clustering algorithms known for its fast and simple (Raykov, Boukouvalas, Baig, & Little, 2016). During the yellowing process, the differential metabolites primarily exhibited eight trends as depicted in Fig. 4 B. Among them, sub class 1, 2 and 7 contains metabolites that were higher in late stage of yellowing than in middle and early stages, while sub class 4 and 5 contains opposites ones. The mutation nodes of sub class 3, 6 and 8 are clustered at 3 h, which were likely to be intermediate products in the yellowing process and cannot exist for a long time, which were not the subject to be explored in this paper. Thus five trends were circled in Fig. 4 B, which were highly positively or negatively correlated with the results of sensory evaluation, and the subsequent discussion will focus on these metabolites.

Annotation and enrichment analysis of differential metabolites were performed between 0 h and 3 h, 6 h, 9 h, 12 h using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Fig. 4 C–D and Fig. S3A–B). The latter two groups did not change much and they are showed in the supplementary material. Among the top 20 metabolic pathways in the four groups shown according to *P*-values, pathways such as flavone and flavonol biosynthesis, flavonoids biosynthesis, anthocyanin biosynthesis, linoleic acid metabolism, glutathione biosynthesis, purine metabolism, cutin, suberine and wax biosynthesis appeared multiple times, indicating the main areas of influence of yellowing. In addition to the early stage of yellowing (0 h vs 3 h), where the change was not obvious, anthocyanin biosynthesis and linoleic acid metabolism consistently appeared in the top two in the other three comparisons. The most obvious up-regulation pathway was linolenic acid metabolism. Linolenic acid is a precursor to various flavor substances, such as 1-octen-3-ol and 3-octanone, by forming different hydroperoxides followed by homolysis (Wu et al., 2021). The up-regulation of linolenic acid pathway provided the basis for improving vine tea aroma. In addition, flavonoid-related pathways like flavone and flavonol biosynthesis, flavonoids biosynthesis, anthocyanin biosynthesis and isoflavonoids biosynthesis, appeared several times in the enrichment analysis with different trends. Considering that flavonoids are the most characteristic group of compounds in vine tea: their content is higher than 30% (Q. Zhang et al., 2021), we would focus on the changes of flavonoid components in the yellowing process in the following studies.

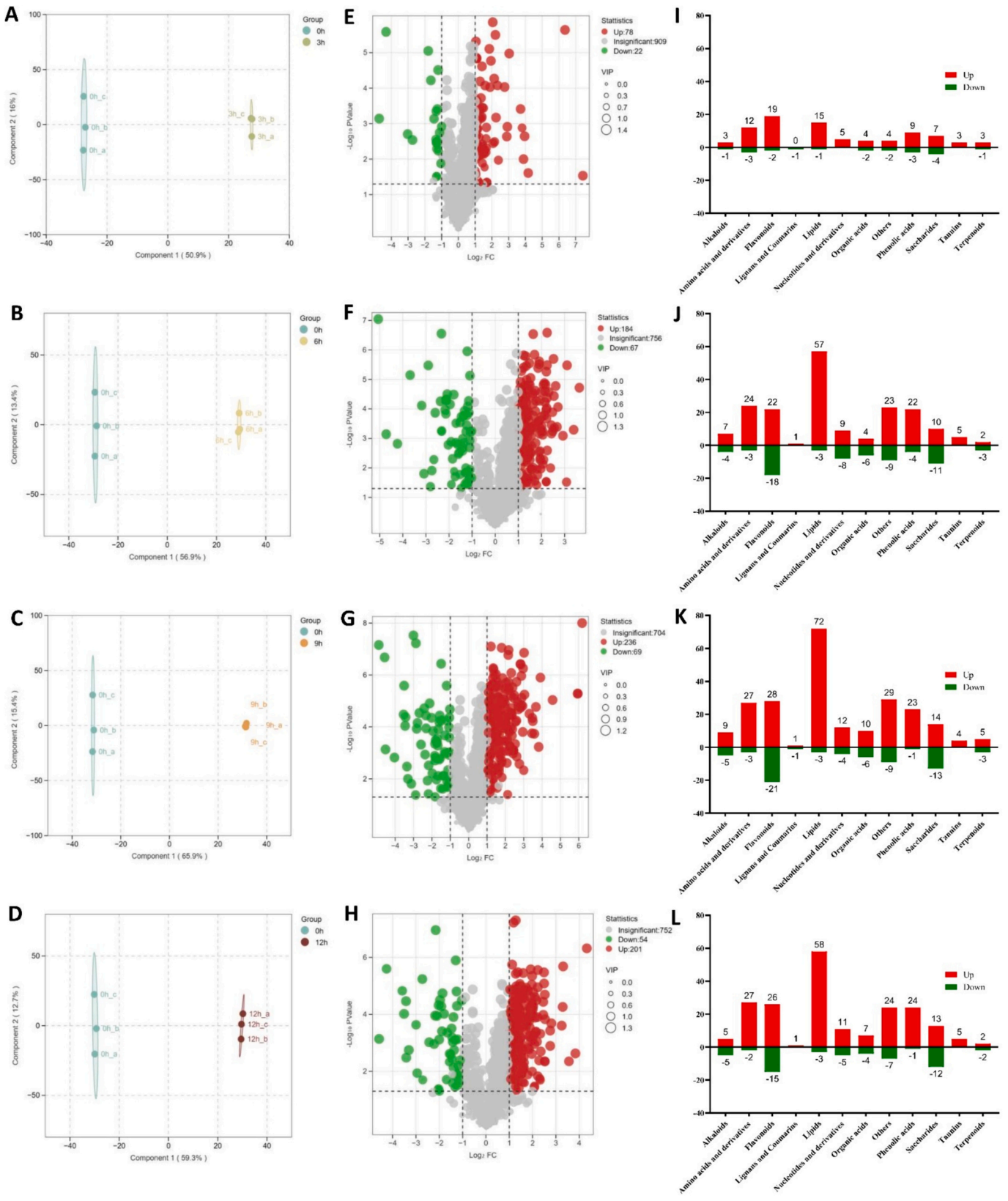

Fig. 3. Screening and classification of differential non-volatile metabolites (A-D) OPLS-DA score plot of non-volatile metabolites between 0 h vs 3 h, 0 h vs 6 h, 0 h vs 9 h and 0 h vs 12 h; (The abscissa represents the predicted principal component, the ordinate represents the orthogonal principal component, and the percentage indicates the explained variance by that component for the dataset); (E-H) Volcano plot of non-volatile metabolites between 0 h vs 3 h, 0 h vs 6 h, 0 h vs 9 h and 0 h vs 12 h; (I-L) Different metabolites in 3 h, 6 h, 9 h, 12 h vs 0 h; (The ordinate and the numbers above the bar chart indicate the number of up-regulated and down-regulated compounds.)

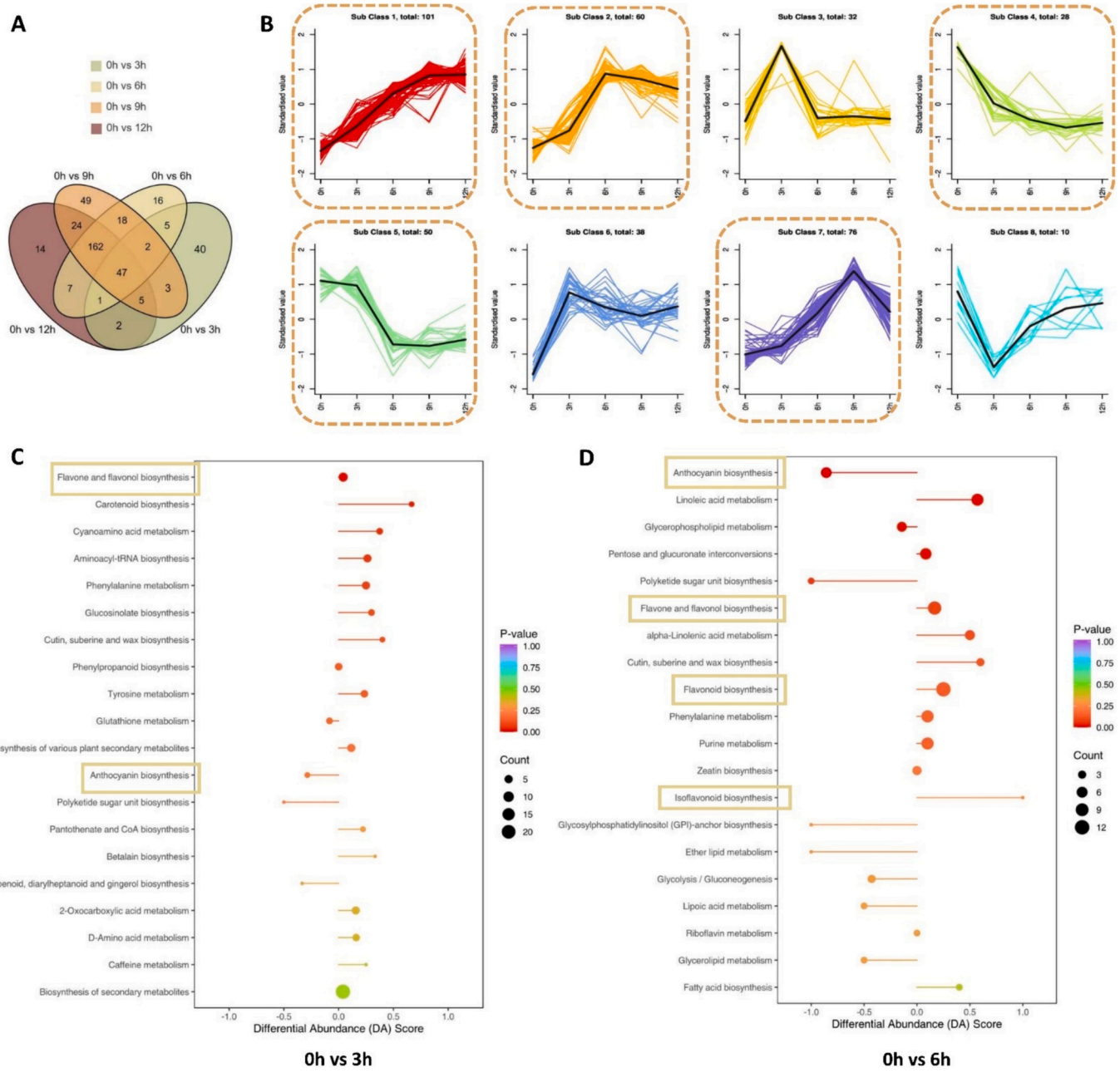

Fig. 4. Multi-angle analysis of differential non-volatile metabolites (A) Venn diagram of differential non-volatile metabolites between 0 h vs 3 h, 0 h vs 6 h, 0 h vs 9 h and 0 h vs 12 h (The numbers in the overlapping and non-overlapping parts of the circles represent the count of differential metabolites shared between comparison groups and unique to each comparison group, respectively); (B) K-Means cluster of differential metabolites in vine tea (The horizontal axis represents the sample group; the vertical axis represents the standardized relative metabolite content; 'Sub class' represents the number of metabolites within each category exhibiting the same trend; 'total X' refers to the count of metabolites representing this category); (C) KEGG enrichment analysis of differential non-volatile metabolites in 0 h vs 3 h; (D) KEGG enrichment analysis of differential non-volatile metabolites in 0 h vs 6 h; (The ordinate represents the differential pathway name (sorted by *P*-value), and the abscissa represents the differential abundance score (DA Score). DA Score reflects the overall change of all metabolites in the metabolic pathway. A score of 1 indicates that the expression trend of all identified metabolites in the pathway is up-regulated, and a score of −1 indicates the opposite. The dots distributed on the left side of the axis and the longer the line segment, the more inclined the overall expression of the pathway is to be down-regulated; the right side is the opposite. The size of the dot reflects the number of metabolites, and the color of the line segment and dot reflects the P-value).

3.3.4. Dynamic changes of the differential non-volatile metabolites

3.3.4.1. Flavonoids and their hydrolyzed products. Flavonoids are secondary metabolites with three rings (C6-C3-C6) as the basic skeleton, and often accumulate in vacuoles of plant cells in the form of glycosides. They are the most typical secondary metabolites in vine tea (Zeng et al., 2023). Flavonoids are the main source of bitterness, astringency, and yellow color in plant beverages. Exactly, bitterness and astringency were also the biggest change in sensory evaluation. Due to the importance of flavonoids in vine tea, and several related metabolic pathways of flavonoids were shown in KEGG enrichment analysis, pathways of flavonoid biosynthesis, flavone and flavonol biosynthesis, anthocyanin biosynthesis were combined in order to visualize the metabolic network of flavonoids in vine tea during yellowing process (Fig. 5). All the enriched flavonoids with up-regulated down-regulated or no significant change are shown in the figure. In addition, the flavonoid combination

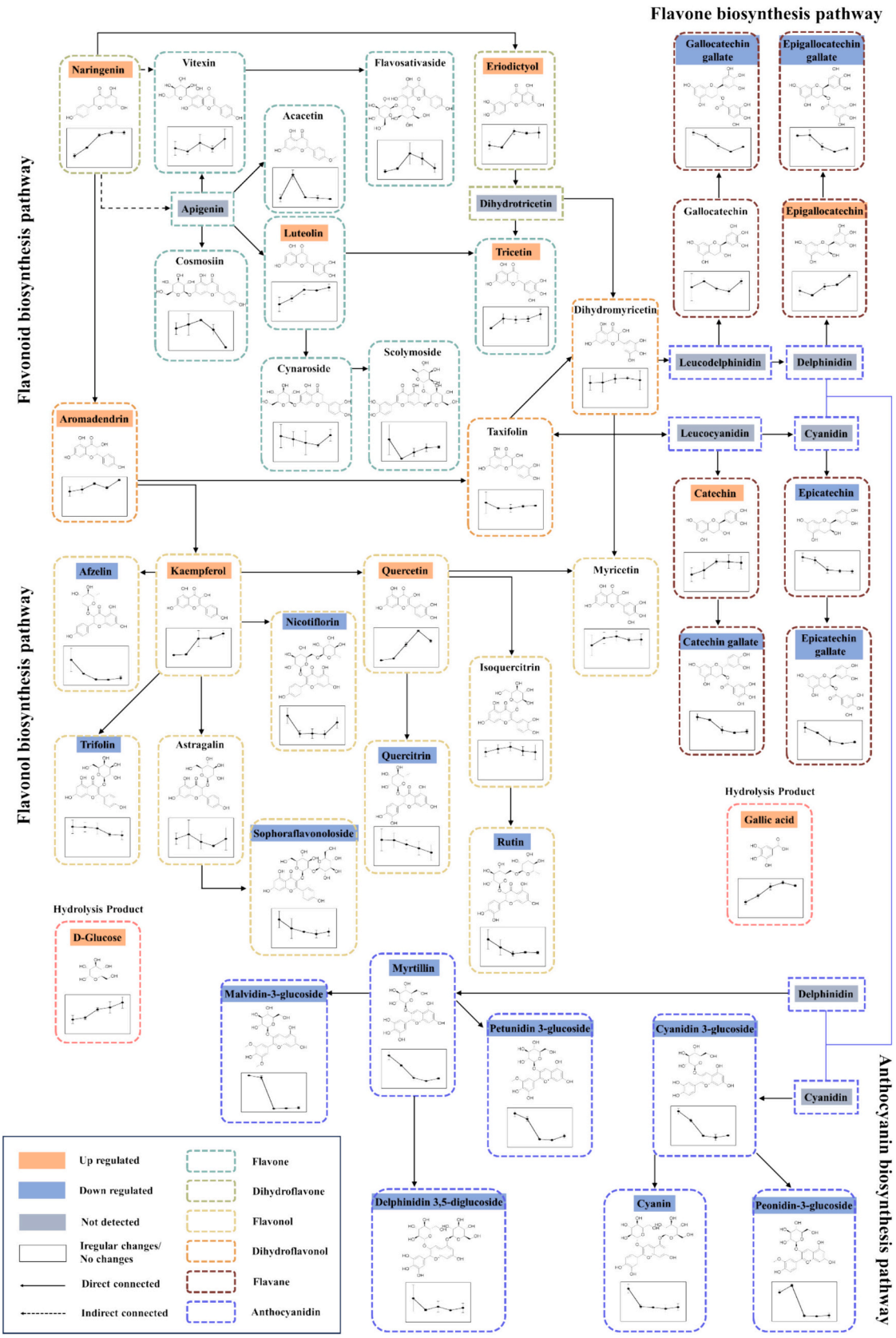

Fig. 5. Metabolic network of flavonoids in vine tea during yellowing process (The line chart from left to right shows the change in metabolite content from 0 h to 12 h).

through differential compounds and K-means two-step screening was displayed in the heat map (Fig. 6), including 9 anthocyanins, 1 biflavones, 7 catechins, 7 flavones, 6 flavanonolses, 14 flavonols and 4 other flavonoids, as a supplement to the metabolic network map.

In the upstream flavonoid synthesis pathway, we observed the increase of Naringenin and Eriodictyol content, while the change of flavones was not obvious, and generally had a high bar value. However, for the flavonol synthesis pathway, we found that while upstream kaempferol and quercetin increased, their downstream afzelin, trifolin and querectin showed opposite changes. In this region, the up-regulated are aglycones, while the down-regulated are flavonol glycosides (18.56–44.96%). The same changing trends can also be observed in Fig. 6, with butin, naringenin, morin increased and butin-7-O-glucoside, naringenin-4'-O-glucoside, morin- 3-O-arabinoside decreased. The normal synthesis of various flavonol glycosides depends on the participation of the corresponding glycosyltransferase, and these enzymes in the process of fixation and yellowing with temperature treatment, even if not completely inactivated, their efficiency is greatly reduced. The high temperature and high humidity environment brought by yellowing is conducive to the hydrolysis of flavonoid glycosides(Wei et al., 2023). Therefore, the most reasonable explanation is that yellowing inhibits the continued synthesis of flavonol glycosides and promotes their decomposition. D-glucose is one of the products of hydrolysis, and the rise of its content (33.68–78.04%) undoubtedly supports this inference. An

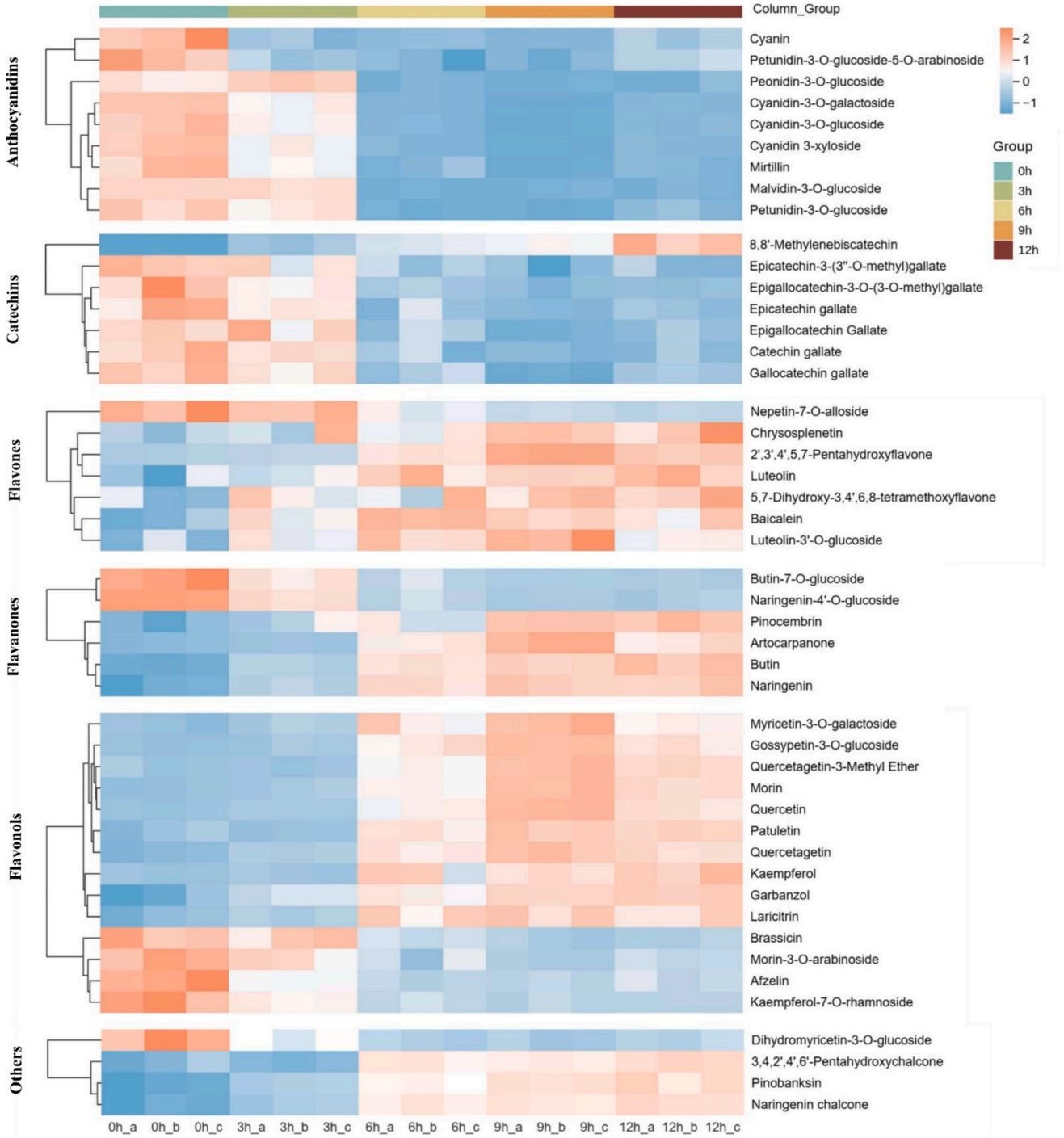

Fig. 6. HCA heat map of important differential flavonoids from vine tea yellowing process.

experiment based on black tea found that flavonol glycosides generally brought about stronger astringency than other phenolic acids or flavonoids (Scharbert & Hofmann, 2005). The accumulation of soluble sugars in this process further turns bitter into sweetness.

A similar phenomenon was observed in flavanols, where we saw a decrease in catechins gallate, epicatechins gallate, gallocatechin gallate, epigallocatechin gallate, epigallocatechin-3-O-(3-*O*-methyl) gallate, epicatechin-3-(3''-*O*-methyl) gallate and an increase in catechin, epigallocatechin gallic acid, methyl gallate and 3-*O*-methylgallic acid. This indicated that flavanols normally exist in the form of gallic ester in the tea, and the hydrolysis of these substances under high temperature and high humidity conditions leads to the decrease of their content and the up-regulation of gallic acid (220.96–252.09%) and methyl gallic acid (234.83–280.33%) in the middle and late stage of yellowing. An in vitro assay described the astringency order as epicatechin gallate > epigallocatechin gallate > gallocatechin gallate > catechin gallate > epigallocatechin > epicatechin > gallocatechin > catechin, which testified the trans from gallate-type catechins to non-gallate-type catechins was beneficial to taste (C. T. Liu & Tzen, 2022). Gallic acid produced from the hydrolysis of flavanols has been associated with the enhanced umami taste and sweet of green tea infusion (Kaneko, Kumazawa, Masuda, Henze, & Hofmann, 2006). Although gallic acid itself contributes to bitterness and astringency due to its multiple phenolic hydroxyl groups, this effect may be less pronounced than before hydrolysis. Furthermore, gallic acid itself contributed positively to sweetness in non-high-sweetness solutions (Che, Yang, Li, & Hu, 2022). Thus, the increase in the content of similar compounds may explain the change in the taste of vine tea.

The structure of anthocyanins determines their instability, and the content of it decreased sharply (54.83–97.38%) at the third or sixth hour of yellowing. According to Deng et al. (2022), anthocyanin content decreases when degradation dominates, causing leaf color to fade. This was consistent with the observation that the color of vine tea leaves changes from dark to light. Anthocyanins themselves had a bitter taste. A study based on green tea 'Longjing 43' verified the relationship between positive regulation of anthocyanin biosynthesis pathway and negative impact on sensory evaluation, which is a 'mirror image' of our results (Jia et al., 2020).

Additionally, the most important flavonoids in vine tea, dihydromyricetin and myricetin did not appear significant difference in the yellowing process. Dihydromyricetin and myricetin are often considered to be the material basis for various health benefits of vine tea, including lowering blood sugar and lipids, protecting the liver and anti-inflammatory effects (Q. Zhang et al., 2021). Therefore, the yellowing method may not compromise the potential of vine tea as a health tea drink.

In conclusion, the changes of flavonoids in vine tea during the yellowing process are summarized as follows: First, there is general hydrolysis of flavonol glycosides located in the downstream, which leads to a general decrease in flavonol glycosides content and an increase in the contents of glucose and aglycons. Second, similar hydrolysis occurred in gallated catechins, and the content of gallated catechins decreased while the content of the hydrolysate gallic acid increased. Last, there was a general decline in anthocyanins. These changes were a reasonable explanation for the decrease in bitterness and astringency and the increase in sweetness and sweet-after-taste after yellowing.

3.3.4.2. Amino acids and their derivatives. Ardö (2006) reported that both pleasant and unpleasant aroma compounds are produced from anabolism and catabolism of amino acids, which are important for foods. As depicted in the Fig. 7, glutathione (reduced form) had the highest content in the traditional production process of vine tea (VT). N-substituted 1-amino-1-deoxyketoses, such as N-(1-deoxy-1-fructosyl) glutamic acid, N-(1-deoxy-1-fructosyl) leucine, N-(1-deoxy-1-fructosyl) phenylalanine, and N-(1-deoxy-1-fructosyl) tryptophan, began to increase substantially at the 6th hour, peaked at the 9th hour, and declined slightly at the 12th hour. During the initial stage of the Maillard reaction, amino acids readily react with monose to form N-substituted 1-

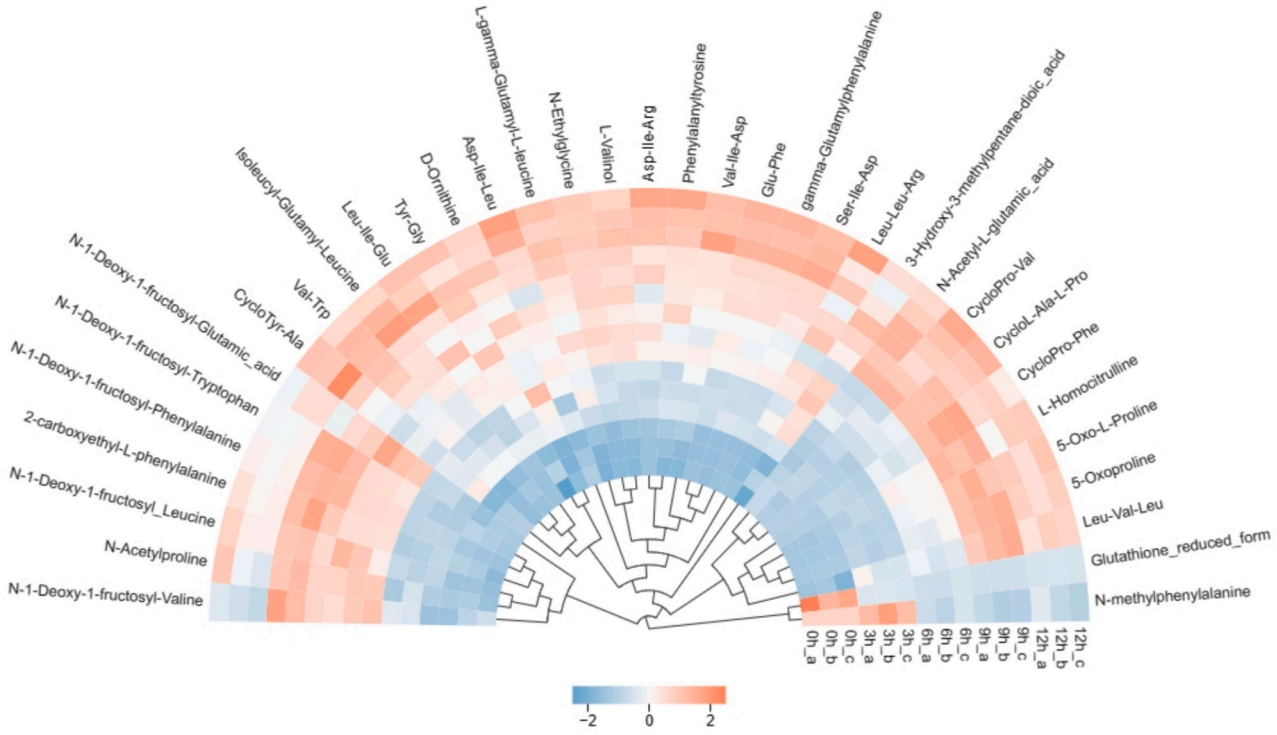

Fig. 7. HCA heat map of important differential amino acids and their derivatives from vine tea yellowing process (Horizontal is the sample name, vertical is the metabolite information, on the left of the figure is the metabolite cluster line, Red-green blocks are the colors filled with different values obtained after standardized treatment with different relative contents). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

amino-1-deoxyketoses (Amadori compounds). Thermal processing of food can produce large amounts of amadori compounds (Andruszkiewicz, D'Souza, Corno, & Kuhnert, 2020). They play a key role in the formation of taste, flavor, and color. Moreover, the Maillard reaction products from fructose-amino acid mixture showed decent UV-absorbance and browning intensity (Hwang, Kim, Woo, Lee, & Jeong, 2011). This was consistent with the observed change in color of vine tea after yellowing process. Additionally, a large number of dipeptides, tripeptides, cyclic peptides, as well as amino acid methylation, acetylation, and oxidation products increased with the yellowing time. Umami is thought to be initiated by binding tastants to G-protein-coupled receptors in taste cells. Although not exclusively, dipeptides and tripeptides with terminal Glu or Asp are often considered umami (X. Yu, Zhang, Miao, Li, & Liu, 2017). γ-Glutamyl peptides are a kind of small peptides with significant kokumi taste and functional attributes because of the presence of γ-glutamyl residues at the N-terminal. A recent study found that the formation of γ-glutamyl peptides is the key to lower bitterness and improve the overall taste of Pea (Yang et al., 2024). The increase of oligopeptides with terminal Glu or Asp and γ-glutamyl peptides provided a potential explanation for the change of VT umami taste.

3.3.4.3. Lipids. Lipids, essential components in plants, play a crucial role in the structure of cell membranes and various physiological activities. In our comparison of vine tea groups, we identified 80 distinct lipid metabolites, including 34 free fatty acids, 14 glycerol esters, 16 (lyso-)phosphatidylcholines (LPC), 15 (lyso-)phosphatidylethanolamines (LPE), and three other lipids (Fig. S3). The majority of lipids exhibited varying degrees of increase after the yellowing process, With the exception of choline alfoscerate, (9Z,11E,13E,15Z)-4-Oxo-9,11,13,15-octadecatetraenoic acid, and (10Z,14E,16E)-10,14,16-octadecatrien-12-ynoic acid. Free fatty acids (FFAs), products of lipid hydrolysis, include 22 unsaturated fatty acids (UFAs) such as linolenic acid and 12 saturated fatty acids (SFAs) such as cetostearic acid. These FFAs have a chain length varying from 12 to 20 carbon atoms. The accumulation of FFAs has also been observed in other mildly fermented beverages and the oxidation of UFAs generates various aroma flavor contributors such as aldehydes, ketones, alcohols, esters, and aliphatic compounds (Li et al., 2023). Glycerol esters are the products of esterification of glycerol and fatty acids. More than half of the glycerides detected are also connected to one or two glucoses by glucoside bonds. Combined with the results of sensory evaluation, it appears that prolonging the yellowing time indiscriminately may not be an ideal choice. LPC and LPE are classified as lyso-glycerophospholipids and are also the most dominant lipid class in tea leaves (M. Y. Liu et al., 2019). Glycerophospholipids are dominant components on cell membranes and play critical roles in maintaining membrane stability, fluidity, and integrity. Lyso-glycerophospholipids are products of glycerophospholipids losing the fatty acid under the actions of phospholipases (Shadyro, Samovich, & Edimecheva, 2019). Based on the same logic that occurs in the processing of other foods or beverages, degradation of the membrane and/or organelle membrane occured under the high temperature and humidity conditions of the yellowing process. Consequently, more flavor substances and nutrients may be released from vacuoles or other subcellular chambers.

3.3.4.4. Other potential flavor contributors. Phenolic acids, recognized as crucial secondary metabolites in plants, play a significant role in the flavor and color of plant beverages. They can directly influence astringency through stable binding with salivary protein (Liu et al., 2023). In our examination of 32 different phenolic acid substances, pyrogallol and 2,4,6-trihydroxybenzoic acid were found to be down-regulated during the yellowing process. The upregulated compounds included eight caffeic acid derivatives. A study has shown that caffeic acid and chlorogenic acid inhibit the bitter taste of green tea infusion at proper concentrations (0–0.2 mM) (Chen et al., 2022), leading us to believe that they can play a similar role in vine tea. The accumulation of phenolic acids and organic acids will lead to a decrease in pH value, which means that we should not keep the yellowing process for a long time, perhaps 6-9 h is the best choice. Finally, the increased content of xylitol and arabitol may have a positive effect on sweetness; Nucleotides and their derivatives generally have different degrees of umami (Kinnamon, 2009). These changes may also have a certain impact on the improvement of the overall taste of vine tea.

3.4. Analysis of volatile metabolites during the processing of VT

3.4.1. Overview of the volatile metabolites during the processing of VT

A total of 949 volatile components spanning 11 categories were tentatively identified. These categories encompassed 26 alcohols, 69 aldehydes, 69 amines, 26 aromatics, 59 esters, 79 hydrocarbons, 152 heterocyclic compounds, 79 ketones, 25 phenols, 180 terpenoids, and 187 other volatile metabolites (Fig. 8 A). The Total Ion Chromatogram (TIC) of the Quality Control (QC) samples demonstrated significant overlap, thereby affirming the stability and reliability of the detection method (Fig. S4). Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were conducted as per Section 3.3.1, with the results depicted in Fig. 2 E and Fig. 2 F. In alignment with the results of non-volatile metabolites, five distinct groups of volatile components were identified. Similar to the results of LC, the differences among the groups at the 6-h, 9-h, and 12-h marks were minimal.

The metabolites detected were semi-quantitatively analyzed using 3-hexanone as the internal standard, and their contents were calculated (Fig. 8 B). The main volatile components of vine tea were aldehydes, heterocyclic compounds, and terpenoids, which accounted for 20.75%–24.07%, 15.28%–22.29%, and 24.15%–30.02% of the total, respectively. In contrast, although the absolute number of unclassified compounds and hydrocarbons were large as 180 and 79, the proportion is extremely low. Compared to traditional manufacturing processes, yellowing, especially yellowing for >6 h, significantly increased the total amount of volatile compounds for 23.88%–25.44%. These increases were mainly caused by the increase of alcohols, aldehydes, aromatics, esters, heterocyclic compounds, and ketones. In contrast, phenols decreased for 31.18%–41.97% and terpenoids decreased for 18.27%–22.02%.

3.4.2. rOAV analysis and odor correlation analysis

However, not all volatile components had a significant effect on odor, which was evaluated using relative odor activity value (rOAV) analysis. rOAV was the ratio of the concentrations to the odor threshold in water. Volatile compounds with an rOAV above 1 were considered as aroma-active compounds that contributed to the overall aroma of a sample (S. Li et al., 2022). Table 1 lists the top 30 potential characteristic volatile compounds of vine tea flavor profile (sort by rOAV), and the rest can be found in the supplementary material Table S3. To better understand the correspondence between odors and ingredients, alluvial plots were used to visualize them. The plot of 0 h vs 6 h are shown as an example in the main body and the rest in the supplementary material (Fig. 8 and Fig. S5). The top 30 up-regulated and down-regulated metabolites, the top 10 odors descriptions with the highest frequency of occurrence, and their changing trends were connected. The alluvial stripes reflected the absolute Log2 Fold Change.

The description of green is often found in odor studies of various green tea beverages (Zhai, Zhang, Granvogl, Ho, & Wan, 2022), reflecting the similarities between the traditional process of rattan tea and green tea. Green odor was associated with down-regulated metabolites including 2-mercapto-propanoic acid ethyl ester, 2-pentyl-Furan, (Z)-6-Nonenal, (Z)-4-Heptenal, (E,E)-3,5-Octadien-2-one, (E,Z)-2,6-Nonadienal, (E,E)-2,4-Nonadienal, (2*S*,4*R*)-4-Methyl-2-(2-methylprop-1-en-1-yl)tetrahydro-2H-pyran, which is the This result was consistent with the decline in scores for grass entries in sensory evaluations.

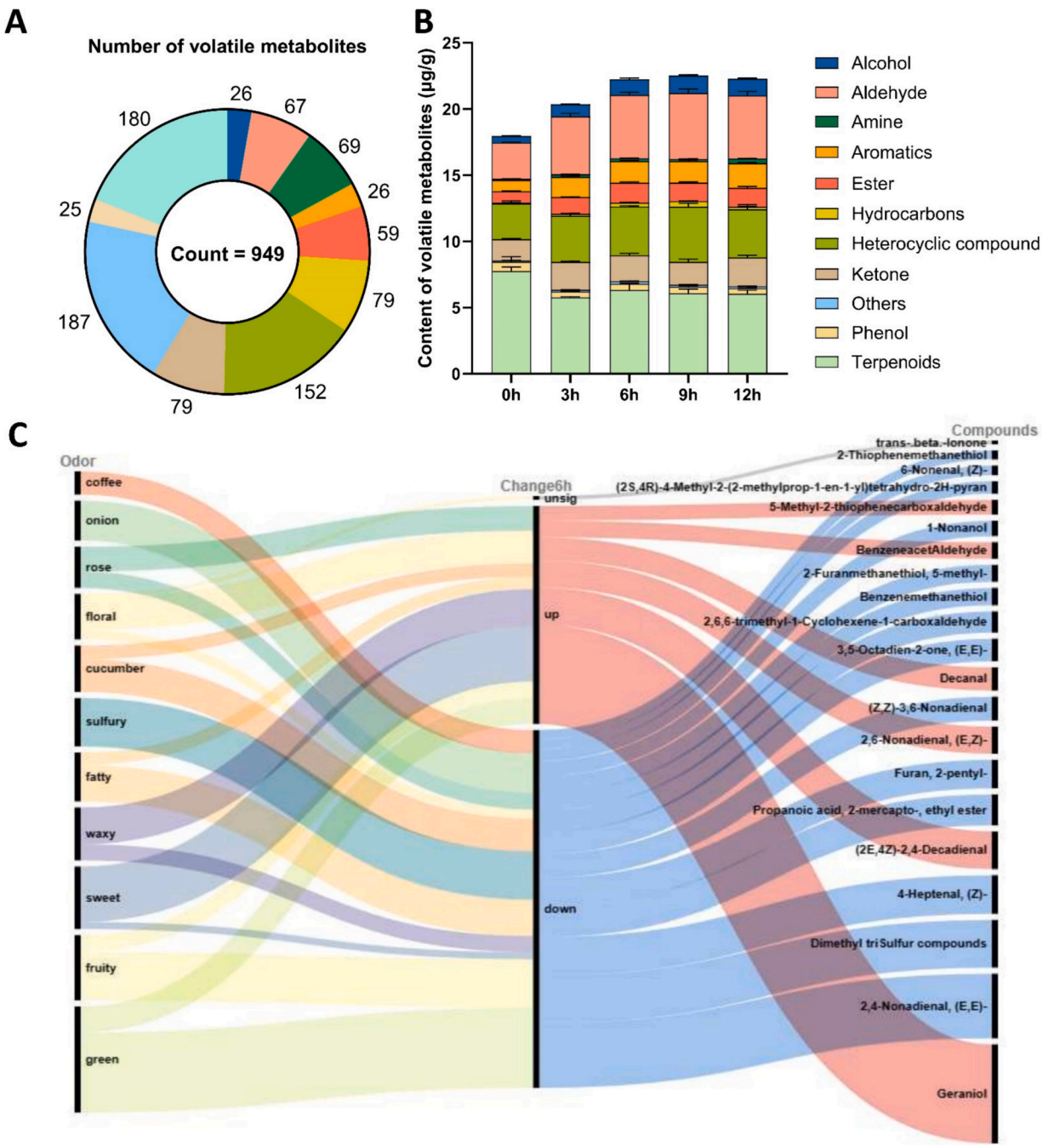

Fig. 8. Analysis of volatile metabolites (A) Numbers of volatile metabolites detected in vine tea; (B) Content of volatile metabolites in vine tea; (C) Alluvial plot between aroma and differential volatile metabolites in group 0 h vs 6 h.

Notably, the smell descriptors for rattan tea include sulfur, cucumber, and onion, which is rare in other tea beverages. The compounds responsible for these odors include (Z)-6-Nonenal, (E,Z)-2,6-Nonadienal, (E,E)-2,4-Nonadienal, (Z,Z)-3,6-Nonadienal, benzenemethanethiol, 2-mercapto-Propanoic acid ethyl ester, 5-methyl-2-Furanmethanethiol, and dimethyl triSulfur compounds. These compounds are not present in high amounts on their own, but generally have extremely low thresholds, making their odor noticeable in rattan tea, for example, benzenemethanethiol (0.0000035) and dimethyl triSulfur compounds (0.000008). These rare and sharp odors may be the reason why the smell of rattan tea is unacceptable to many consumers, and they showed a general decline after yellowing. Another smell that can bring about an unpleasant tea drinking experience is 'fatty', which is dominated by 4-Heptenal, (Z)-1-Nonanol, (Z,Z)-3,6-Nonadienal, and (2E,4Z)-2,4-Decadienal.

Geraniol was the most important up-regulated metabolite detected, with an increase between 239.32% and 275.21%. It is also the characteristic aroma components of the yellowing process, dominates the production of sweet, floral, fruity, rose, waxy, citrus scents. It is generally recognized that the non-enzymatic hydrolysis of glycoside precursors released geraniol under hot and humid conditions during sealed yellowing (Dong et al., 2023). Such explanation of degradation was consistent with our results of non-volatile components in section 3.3.4.1. According to the results of sensory evaluation, yellowing improved both sweet and floral odors. In addition to geraniol, decanal, benzeneacet aldehyde, 1-Nonanol, and (2*S*,4*R*)-4-Methyl-2-(2-methylprop-1-en-1-yl) tetrahydro-2H-pyran etc. had varying degrees of upward regulation. Together, they constituted the sweet and flavor smell of vine tea after

Table 1
Top 30 volatile aroma-active compounds in vine tea.

Compounds	Class	Formula	CAS	Odor	Threshold	0 h	3 h	6 h	9 h	12 h
trans-.beta.-Ionone	Terpenoids	C13H20O	79–77-6	dry, powdery, floral, woody, orris	0.0002	3957.25 ± 57.23	3378.23 ± 43.31	5310.67 ± 219.05	4665.92 ± 396.79	4936.46 ± 474.36
Benzenemethanethiol	Sulfur compounds	C7H8S	100–53-8	sharp, alliaceous, onion, sulfury, garlic, horseradish, minty, coffee	0.0000035	5360.62 ± 477.35	4441.99 ± 1256.12	3409.54 ± 267.79	2874.3 ± 313.48	2955.56 ± 508.93
2,6-Nonadienal, (E,*Z*)-	Aldehyde	C9H14O	557–48-2	cucumber, green	0.00001	1411.19 ± 37.89	3511.76 ± 3.81	4063.53 ± 466.45	1786.47 ± 97.94	2365.97 ± 54.02
1-Octen-3-one	Ketone	C8H14O	4312-99-6	mushroom	0.000005	1315.8 ± 184.37	2480.2 ± 95.63	2300.23 ± 116.62	2633.76 ± 189.39	2734.1 ± 577.2
4-Heptenal, (Z)-	Aldehyde	C7H12O	6728-31-0	oily, fatty, green, dairy, milky, creamy	0.000025	3960.22 ± 153.09	2521.44 ± 74.32	874.81 ± 38.61	513.25 ± 11.1	806.55 ± 43.83
6-Nonenal, (Z)-	Aldehyde	C9H16O	2277-19-2	green, cucumber, melon, cantaloupe, honeydew, waxy, vegetable, orris, violet, leafy	0.00014	2243.42 ± 283.04	1485.03 ± 302.35	1539.73 ± 289.43	1832.59 ± 47.82	1396.73 ± 367.59
Cyclohexanone, 2,2,6-trimethyl-	Ketone	C9H16O	2408-37-9	pungent, thujone, labdanum, honey, cistus	0.0001	1499.75 ± 55.08	1633.87 ± 17.1	1720.37 ± 103.4	1981.13 ± 99.52	1316.4 ± 190.41
3,5-Octadien-2-one, (E,E)-	Ketone	C8H12O	30,086–02-3	fruity, green, grassy	0.0005	2712.39 ± 47.96	2121.64 ± 50.02	1126.04 ± 50.23	1044.28 ± 133.5	977.06 ± 115.19
(Z,Z)-3,6-Nonadienal	Aldehyde	C9H14O	21,944–83-2	fatty, soapy, cucumber	0.00005	1924.15 ± 233.77	778.36 ± 163.96	757.16 ± 127.57	705.43 ± 31.62	665.08 ± 47.68
Dodecanenitrile	Nitrogen compounds	C12H23N	2437-25-4	citrus, orange, peel, metallic, spicy	0.00009	684.55 ± 48.06	843.53 ± 31.15	614.71 ± 63.65	528.09 ± 30.12	419.67 ± 122.13
2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (*R*)-	Heterocyclic compound	C11H16O2	17,092–92-1	musky, coumarin	0.0021	765.44 ± 120.51	365.04 ± 20.1	169.29 ± 25.25	108.57 ± 17.14	188.92 ± 28.3
Dimethyl triSulfur compounds	Sulfur compounds	C2H6S3	3658-80-8	sulfury, cooked onion, savory, meaty	0.000008	683.78 ± 138.65	369.02 ± 8.41	105.92 ± 14.39	139.71 ± 14.3	111.68 ± 11.57
2,4-Nonadienal, (E,E)-	Aldehyde	C9H14O	5910-87-2	fatty, melon, waxy, green, violet, leafy, cucumber, tropical, fruity, chicken	0.00016	610.42 ± 13.98	231.84 ± 2.17	172.57 ± 9.66	168.75 ± 8.66	136.83 ± 17.34
2-Thiophenemethanethiol	Heterocyclic compound	C5H6S2	6258-63-5	roasted, coffee, fishy	0.00004	399.2 ± 24.81	239.15 ± 4.01	198.28 ± 7.98	241.34 ± 14.11	150.92 ± 15.18
Geraniol	Terpenoids	C10H18O	106–24-1	sweet, floral, fruity, rose, waxy, citrus	0.0066	90.35 ± 5.86	163.23 ± 5.13	328.76 ± 34.92	339.57 ± 26.6	306.58 ± 39.79
p-Cresol	Phenol	C7H8O	106–44-5	phenol, narcissus, animalic, mimosa	0.00024	321.58 ± 9.46	133.34 ± 1.79	223.93 ± 12.9	233.37 ± 24.61	188.37 ± 7.75
2(5H)-Furanone, 5-ethyl-	Heterocyclic compound	C6H8O2	2407-43-4	spice	0.0097	145.64 ± 9.32	121.03 ± 7.21	149.05 ± 3.84	167.98 ± 16.69	138.86 ± 28.87
Decanal	Aldehyde	C10H20O	112–31-2	sweet, aldehydic, waxy, orange peel, citrus, floral	0.0001	70.11 ± 24.59	137.48 ± 7.29	128.39 ± 11.56	121.23 ± 5.19	125.27 ± 11
5-Methyl-2-thiophenecarboxaldehyde	Heterocyclic compound	C6H6OS	13,679–70-4	sweet, almond, cherry, furfural, woody, acetophenone	0.001	43.48 ± 4.52	90.59 ± 3.4	137.79 ± 14.32	181.09 ± 12.4	109.46 ± 9.8
2,6,6-trimethyl-1-Cyclohexene-1-carboxaldehyde	Terpenoids	C10H16O	432–25-7	tropical, saffron, herbal, clean, rose, sweet, tobacco, damascone, fruity	0.003	156.1 ± 2.45	119.35 ± 1.07	89.83 ± 5.92	89.86 ± 6.39	73.51 ± 8.64
BenzeneacetAldehyde	Aldehyde	C8H8O	122–78-1	floral, honey, rose, cherry	0.0063	60.05 ± 3.09	109.46 ± 4.94	115.05 ± 6.59	114.59 ± 10.56	112.44 ± 16.86
Furan, 2-pentyl-	Heterocyclic compound	C9H14O	3777–69-3	fruity, green, earthy, beany, vegetable, metallic	0.006	130.52 ± 6.9	70.97 ± 4.59	42.88 ± 3.07	40.37 ± 3.77	51.04 ± 4.6
(2E,4Z)-2,4-Decadienal	Aldehyde	C10H16O	25,152–83-4	fried, fatty, geranium, green, waxy	0.00007	24.58 ± 1.51	38.19 ± 2.71	65.52 ± 7.67	95.44 ± 4.07	67.23 ± 8.22
Propanoic acid, 2-mercapto-, ethyl ester	Alcohol	C5H10O2S	19,788–49-9	sulfury, meaty, green, onion	0.001	93.29 ± 5.88	68.55 ± 4.19	41.61 ± 12.76	44.31 ± 4.17	40 ± 1.52
2-Acetyl-1,4,5,6-tetrahydropyridine	Heterocyclic compound	C7H11NO	25,343–57-1	creamy, bread	0.001	94.67 ± 3.87	69.12 ± 1.66	42.02 ± 1.89	32.17 ± 1.1	38.54 ± 1.08
2-Ethoxy-3-methylpyrazine	Heterocyclic compound	C7H10N2O	32,737–14-7	hazelnut, roasted, almond, pineapple, earthy	0.0008	65.75 ± 2.26	44.76 ± 2.21	52.63 ± 2.38	42.88 ± 2.87	48.99 ± 4.21
(2*S*,4*R*)-4-Methyl-2-(2-methylprop-1-en-1-yl)tetrahydro-2H-pyran	Terpenoids	C10H18O	3033-23-6	rose, cortex, green, floral, geranium, powdery, metallic	0.0002	61.96 ± 4.11	49.53 ± 0.97	44.87 ± 3.92	54.45 ± 4.18	38.23 ± 4.82
2-Furanmethanethiol, 5-methyl-	Heterocyclic compound	C6H8OS	59,303–05-8	sulfury, roasted, coffee	0.00005	84.12 ± 9.01	38.48 ± 5.72	43.49 ± 7.4	31.81 ± 5.8	36.02 ± 1.73
1-Nonanol	Alcohol	C9H20O	143–08-8	fresh, clean, fatty, floral, rose, orange, dusty, wet, oily	0.0053	58.45 ± 11.7	44.45 ± 4.48	39.27 ± 0.48	44.27 ± 1.01	36.82 ± 1.84
1,3-Cyclohexadiene-1-carboxaldehyde, 2,6,6-trimethyl-	Terpenoids	C10H14O	116–26-7	fresh, herbal, phenol, metallic, rosemary, tobacco, spicy	0.003	18.21 ± 0.54	38 ± 0.3	47.22 ± 2.28	39.58 ± 2.72	38.47 ± 5.38

yellowing.

Besides the substances discussed above, other components with high odor activity values were trans-β-ionone, 1-octen-3-one, 2,2,6-trimethyl-cyclohexanone and p-Cresol. β-ionone was a common component in tea and similar drinks, and was the 9,10 and 9′,10′ cleavage product of β-carotene by carotenoid cleavage dioxygenase (Paparella, Shaltiel-Harpaza, & Ibdah, 2021; J. Yu, Liu, Zhang, Luo, & Zeng, 2021). After yellowing, β-ionone showed an upward trend, but the range of change was small (17.89%–34.19%). It might bring floral, woody and orris notes to the vine tea, playing a rich aroma role. 1-octen-3-one smells like fresh mushroom The changes in its content made the aroma of vine tea richer, more unique, and more layered. Notably, 2,2,6-trimethyl-cyclohexanone and p-Cresol correspond to the smell of the pungent thujone, and phenol or narcissus off-flavor (Delcros et al., 2023), respectively. Their decrease may enhance the acceptability of vine tea.

In summary, after yellowing, the total amount of volatile components of vine tea increased, making the aroma more obvious. 'Green' related metabolites went down and 'sweet and flavor' related metabolites went up, making vine tea smell more similar to yellow tea than green tea, which demonstrated the effectiveness of the improved process. During yellowing, off-flavors such as sulfur, cucumber, onion, thujone and phenol also decreased at the same time, which will be conducive to consumer acceptance of this beverage.

4. Conclusions and perspectives

This study aimed to improve the taste of vine tea by comparing four different yellowing treatments. A processing technology that mimics yellow tea was used to improve the flavor of vine tea and revealed its internal reasons through metabolomics for the first time. The results of sensory evaluation showed that the yellowing process for at least 6 h reduced the bitterness and astringency to the greatest extent and enriched the aroma. Widely targeted metabolomics was applied by UPLC-MS-MS and GC–MS method, and 1013 non-volatile metabolites and 949 volatile metabolites were identified. For non-volatile metabolites, yellowing process significantly altered the composition of flavonoids, and increased the content of lipids and amino acids derivatives and in vine tea. The improvement of bitter and astringent taste was mainly related to the down-regulation of anthocyanins, the hydrolysis of gallated catechins and flavonol glycosides, and the subsequent accumulation of soluble sugar and gallic acid. For aroma, the improvement was related to increase of total volatile metabolite content, the up-regulation of geraniol, decanal, 1-Nonanol (2E,4Z)-2,4-Decadienal and other compounds, and down-regulation of (Z)-6-Nonenal, (E,Z)-2,6-Nonadienal, (E,E)-2,4-Nonadienal, (Z,Z)-3,6-Nonadienal, benzenemethanethiol, and other compounds. These findings provide a basis for optimizing vine tea processing as well as revealed the flavor changes and material basis of vine tea after yellowing process, and provided theoretical and empirical evidence for optimizing the processing of vine tea. However, the lack of a mechanism to evaluate non-volatile ingredients and the inability to assess the synergistic effects of multiple compounds on taste and smell were limitations of this study. Future research will focus on the formation and transformation mechanism of the characteristic components of flavonoids in vine tea.

Author contribution

Shunyao Qi designed research, processed data, and wrote the manuscript text. Tiexin Zeng analyzed the data and wrote the manuscript. Peiling Wu conducted and performed analysis of the main experiments. Le Sun and Zhengqi Dong revised the manuscript. Lijia Xu and Peigen Xiao revised the manuscript, and provided funding to support the study. All authors read and approved the final version of the manuscript.

Ethical statement

Eethical approval is not required by national laws in this field. To protect the rights and privacy of all participants, in sensory evaluation, participants gave informed consent via the statement "I am aware that my responses are will be counted and may be published, and I agree to participate in this survey" where an affirmative reply was required to enter the survey. The products tested were safe for consumption. We fully disclose the requirements and potential risks of the study. They were able to withdraw from the survey at any time without giving a reason.

CRediT authorship contribution statement

Shunyao Qi: Writing – original draft, Software, Methodology, Conceptualization. **Tiexin Zeng:** Visualization, Validation, Data curation. **Peiling Wu:** Methodology, Formal analysis. **Le Sun:** Writing – review & editing, Resources. **Zhengqi Dong:** Resources, Investigation, Conceptualization. **Lijia Xu:** Supervision, Funding acquisition. **Peigen Xiao:** Project administration.

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

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101446.

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