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Metabolomic analysis of quality improvement through refiring in Fenghuang Dancong tea

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ABSTRACT

Processing techniques determine the quality of tea. This study elucidates the impact of refiring process on Fenghuang Dancong Tea (FDT) under controlled experimental conditions by integrating sensory evaluation, non-targeted metabolomics (UPLC-MS/MS), and flavoromics (HS-SPME-GC × GC-TOF-MS). The results indicate that the decrease in polyphenols of the FDT mainly resulted from their transformation through the phenylpropanoid biosynthesis pathway during refiring, thereby reducing the bitterness and astringency of the tea while enhancing its sweetness. Although volatile diversity decreased with higher temperatures, the content of key aroma-contributing hydrocarbons and ketones peaked at 90 °C. Relative odor activity value (ROAV) analysis identified 9 key aroma compounds (ROAV ≥1), with 2-methyl-butanal, (E)-2-nonenal, 2-pentyl-furan, and 2,3-butanedione being the most impactful. This study provided a comprehensive investigation into metabolite changes during the refiring process of FDT, contributing valuable insights for precisely utilizing the refiring technique to improve the quality of tea.

1. Introduction

Fenghuang Dancong Tea (FDT), as one of the representatives of Chinese Oolong tea, is renowned for its unique aroma and flavor, highly esteemed in the tea community (Qin et al., 2023; Wei et al., 2025). It is produced in the Fenghuang Mountain area of Chaozhou City, Guangdong Province, where the distinctive geographical and climatic conditions contribute to its unparalleled tea's flavor. FDT encompasses a variety of fragrance types, including Yashi Xiang, Milan Xiang, Rougui Xiang, Jianghua Xiang, and others. Different tea varieties typically exhibit distinct flavors, which is referred to as "varietal flavor" (Hu et al., 2018). For a single variety, the processing technique is the most direct method affecting the quality of tea. The production process of FDT is meticulous and complex. The initial processing employs traditional procedures, involving withering, shaking, fixation, rolling, and drying to produce the primary tea, also known as "Maocha". On this basis, further sorting is required to remove stems and fallen leaves, selecting more uniformly textured tea leaves to improve their form and quality, and the aroma and flavor of the primary tea are not yet fully developed. For instance, during the shaking stage of FDT, semi-fermentation (rate about 30%–40%) promotes the hydrolysis of glycosidic aroma precursors, generating volatile terpenoids, while high-temperature processes can further facilitate the production and release of these terpenoids (Schwab et al., 2015). Meanwhile, the interaction and transformation of volatile aroma components with low boiling points are the main reasons for the formation of the distinctive "floral and honey" flavor of FDT. Several metabolomic and flavoromic studies have been conducted on FDT, primarily focusing on characterizing its diverse aroma types and the impact of primary processing. For instance, previous studies have successfully identified the key odor-active compounds responsible for the characteristic "floral and honey" aroma, including β-ionone, geraniol, and indole (Chen et al., 2022; Qin et al., 2023). In addition to profiling key aroma compounds, several studies have employed integrated approaches to understand aroma formation. One study revealed that

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upregulation of UDP-glycosyltransferase genes promotes terpene glycoside biosynthesis–a process driven by the subsequent release of these glycosides during tea processing, which is key to FDT's intense and persistent aroma (Zheng et al., 2024). Furthermore, the picking season and altitude of FDT were successfully identified by combining isotope labeling (such as δ^2H and δ^{18}O) with machine learning. This research has very important practical significance (Liu et al., 2025). Despite these advances in understanding primary metabolism and seasonal effects, systematic insights into the processing of FDT, particularly regarding how the refiring process improves its quality, remain limited. On the other hand, the primary tea has a high moisture content of about 7%. During the sorting process, prolonged exposure to air further increases the moisture content of the tea, which is detrimental to the storage stability of the tea.

Therefore, to effectively reduce the moisture content in tea and enhance the quality of FDT, the primary tea after aging needs to undergo a secondary drying process, known as refiring. The main quality indicators of tea include aroma and flavor, which are highly dependent on the support of metabolites. Therefore, the types and contents of metabolites within the tea determine its quality. The process of refiring reduces the moisture content of tea through a second thermal treatment, during which a series of complex and intense thermal chemical reactions occur in the tea leaves, including thermal degradation, Maillard reactions, redox reactions, and isomerization reactions (Ho et al., 2015). This process leads to significant changes in the content and composition of metabolites in the tea, directly affecting its quality. Although extensive research has been conducted on the transformation of metabolites in tea during drying, such as the differential isomerization, degradation, and oxidative polymerization of tea polyphenols during heat treatment, which reduced the bitterness of the tea infusion (Chen et al., 2013); the free fatty acids in tea, including linoleic acid and oleic acid, undergo complex esterification reactions under high-temperature conditions, producing volatile compounds such as alcohols, aldehydes, ketones, and esters, enhancing the aroma of the tea (Zhou et al., 2014). Unlike other types of tea, the refiring process is a unique processing technique for oolong tea, serving as an indispensable and crucial step in the refinement of FDT. However, the mechanisms underlying the generation and transformation of metabolites during the refiring process are not yet fully understood, and still require in-depth investigation.

Although dynamic metabolite changes during earlier manufacturing stages (e.g., withering, shaking, fixation, rolling, and drying) have been extensively characterized (Chen et al., 2020; Hao et al., 2024), systematic insights into the compositional dynamics of both volatile and non-volatile metabolites throughout the entire refiring process remain limited. We hypothesized that refiring significantly reshapes key metabolite composition, thereby modulating the sensory properties and overall quality of oolong tea. To verify the conjecture and fill the research gap, we conducted a comprehensive dynamic profiling of metabolite changes during FDT refiring. In contrast to previous studies relying on a single analytical technique, we employed an integrated approach that combined sensory evaluation with UPLC-MS/MS and GC × GC-TOF-MS, supported by multivariate chemometric analyses. This integrative platform enables unbiased and high-resolution detection of a broad spectrum of metabolites, facilitating the establishment of robust correlations between chemical composition and sensory perception. The findings from this work are expected to elucidate the underlying mechanisms by which refiring influences tea quality and to provide a scientific foundation for precision processing and quality standardization in high-grade oolong tea.

2. Materials and methods

2.1. Chemicals and reagents

Formic acid (≥ 99.9%) was supplied by Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China), Merck (Darmstadt, Germany) supplied chromatographically pure methanol and acetonitrile. Rutin (≥98%), anhydrous glucose (≥98%) and glutamic acid (≥98%)were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Stannous chloride, folinol, alkaline lead acetate, ninhydrin, anthrone, metaphosphoric acid, ammonium molybdate, oxalic acid, Na_2CO_3, KH_2PO_4 and ethanol were purchased from Aladdin Biological Co., Ltd. (Shanghai, China). N-Hexyl-d13 Alcohol was obtained from C/D/N Isotopes Inc. (Quebec, Canada). n-Alkanes standard mixture was procured from Merck (Darmstadt, Germany), and n-Hexane was supplied by Yonghua Chemical Co., Ltd. (Shanghai, China).

2.2. Preparation of tea samples

Twenty kilograms of "Yashixiang" (*Camellia sinensis* (L.) O. Kuntze var. *sinensis*) fresh tea leaves were harvested in March 2025 from Senfeng Tea Company Limited's tea plantation in Fenghuang mountain (116° 40′ E; 23° 58′ N, altitude: ~700 m), Cahzhou city, Guangdong Province, China. After plucking, the leaves were withered under the sunlight at ambient temperature of 28–30 °C and relative air humidity of 56% for 5 h until the moisture content dropped to 65%. Then, the all samples were moved indoors and stand for 8 h to dissipate excess heat. Following the withering process, the leaves were subjected to five cycles of shaking and standing. The duration of shaking was 10, 8, 6, 4, and 2 min, and the interval of each was 30 min. Subsequently, fixation by a machine (WX100, Hongshneg Machinery Co., Ltd., China) with temperature of 110 °C for 30 min. Fixated tea samples were rolled and dried at 100 °C for 4 h with the moisture content dropped to 8%, the machine used tea drying machine (model 6CCB-981ZD, Zhejiang Yinqiu Machinery Co., Ltd). The optimized sample was dried in a vacuum drier at 25 °C for two months to aging. To minimize biological variation and isolate the specific effects of refiring temperature, the entire experiment was conducted using a single, homogeneous batch of Yashi Xiang tea leaves. After aging, the treated samples were randomly and evenly divided into four groups. Three groups were subjected to a 1 h refiring treatment at 80, 90, and 100 °C, respectively, while the remaining group served as the untreated control. These temperatures and the duration were selected to reflect common industrial practice while establishing a thermal gradient for systematic scientific investigation. This duration was verified by preliminary trials to be optimal for inducing discernible chemical transformations. All collected tea samples (labelled as T80, T90, T100 and Control) were divided into three parts and frozen in liquid nitrogen immediately and stored at −80 °C until use. The entire process flow was detailed in Fig. 1, prepare three copies of each sample to ensure the reliability and repeatability of the results. Yashi Xiang Dancong tea refers to a group of region-specific cultivars historically selected from single ancient tea trees in Fenghuang Mountain. All raw tea materials in this study originated from this taxonomic background.

2.3. Sensory evaluation

The sensory quality of each FDT sample was assessed in accordance with the GB/T 23776–2018 Chinese National Standard. Five sensory attributes were evaluated in this study, include smoothness, sweetness, characteristic aroma, astringency, bitterness and thickness. A panel of eight expert panelists, comprising four females and four males, was recruited for the evaluation. Ethical permission was not required to conduct the sensory evaluation. All panelists were professionals trained according to the National Professional Standards for Tea Sensory Evaluation (Profession Code: 6–02–06-11, China). The members of the sensory evaluation panel volunteered and were informed of the research's purpose. Additionally, all volunteers signed an agreement to safeguard the rights and privacy of participants prior to the sensory evaluation.

In brief, 5 g of sample were placed in teapots, followed by the addition of 50 mL of boiling water. The tea leaves were brewed for 1 min, after which the tea infusion was discarded. Subsequently, another 50 mL of boiling water was added, and brewing continued for an

Fig. 1. The processing procedure of samples of tea.

additional minute. The resulting tea infusion was then drained into tea cups for sensory evaluation. The tea tasters were instructed to smell the aroma of the brewed tea leaves and taste the tea infusion after gargling with warm water (Wang & Li, 2024). The expert panel conducted a blind evaluation of the four samples based on the aforementioned six sensory attributes. Each attribute was scored on a scale ranging from 0.0 (non-existent) to 10.0 (extremely strong). Subsequently, the average scores for each sensory attribute were calculated and utilized to construct the radar chart.

2.4. Physicochemical determination

Tea moisture was measured according to the Chinese National standard methods of GB/T8304–2013). The detailed description was followed: placed the weighing dish and cover in a drying oven at 103 ± 2 °C and heated for 1 h. Add 1 g of dry tea (accurate to 0.001 g) in a baking tray of known quality and place it in a drying oven at 120 °C. Keep the lid diagonally open to one side for 2 min, calculated when it rose to 120 °C within 2 min, heated for 1 h, cover the lid and take it out, then cooled to room temperature in a desiccator. The determination of total flavonoids was based on the colorimetric method, and the standard curve was made using rutin standard substances (Zhao et al., 2024). The content of tea polyphenols was determined by the Folin-phenol colorimetric method in the Chinese National Standard (GB/T 8313–2018), and the free amino acids content was quantified in accordance with the Ninhydrin method in the Chinese National Standard (GB/T 8314–2013), respectively. The determination of total soluble sugars was conducted using the anthraquinone sulfate colorimetric method as previously described (Chen et al., 2024). Caffeine content was measured using high-performance liquid chromatography (HPLC; Agilent Co. Ltd., USA) and described by Aaqil's study (Aaqil et al., 2024). All contents of biochemical components (caffeine, tea polyphenols, flavonoids, free amino acids, and total soluble sugars) were expressed as percentages on a dry weight basis (% dry weight).

2.5. Analysis of non-volatile compounds by widely targeted metabolomic

The tea samples were vacuum freeze-dried using a freeze dryer (Scientz-100F, Ningbo Scientz Biotechnology Co., Ltd., China) and ground into powder. Exactly 50 mg of the powder was weighed and transferred into a 2 mL centrifuge tube. Then, 1.2 mL of pre-cooled 70% methanol aqueous solution was added, and the mixture was extracted at 4 °C for 3 h with vortexing every 30 min. Then, the supernatant after centrifuge (12,000 *g*, 3 min, 4 °C) through a microporous membrane (0.22 μm) and transfer into the detection bottle for UPLC-MS/MS analysis.

Liquid chromatography was conducted using a Vanquish UHPLC system (Thermo Fisher Scientific, USA) coupled with an ACQUITY UPLC HSS T3 column (2.1 × 100 mm, 1.8 μm; Waters, Milford, MA, USA), which was maintained at 40 °C throughout the analysis. The mobile phase was delivered at a flow rate of 0.3 mL/min, and the injection volume was 2 μL. For positive-ion (ESI+) mode, the mobile phases consisted of 0.1% formic acid in water (A2) and 0.1% formic acid in acetonitrile (B2). The following gradient program was applied: 0–1 min, 10% B2; 1–5 min, linear increase to 98% B2; 5–6.5 min, held at 98% B2; 6.5–6.6 min, return to 10% B2; 6.6–8 min, re-equilibration at 10% B2. For negative-ion mode (ESI-), separation was achieved using 5 mM ammonium formate in water (A3) and acetonitrile (B3), following the same gradient profile as described above. Mass spectrometric detection was performed using a Q Exactive Focus mass spectrometer (Thermo Fisher Scientific, USA) equipped with an electrospray ionization (ESI) source. The instrument was operated under the following conditions: sheath gas pressure, 40 arbitrary units; auxiliary gas flow, 10 arbitrary units; spray voltage, +3.50 kV for ESI(+) and − 2.50 kV for ESI(−); capillary temperature, 325 °C. Full scan MS spectra were acquired across an m/z range of 100–1000 at a resolving power of 60,000 (FWHM). Data-dependent MS/MS analysis was performed with up to 4 scans per cycle at a resolution of 15,000 (FWHM). The normalized collision energy (NCE) was 30%, and dynamic exclusion was enabled with automatic settings. Raw mass spectrometry data were processed using XCMS (v3.12.0) in R for peak detection, retention time alignment, and feature extraction, yielding a feature table for relative quantification (Want et al., 2013). Metabolite annotation was performed by matching MS/MS spectra against the mzCloud database and the combined spectral libraries of HMDB, KEGG, and LipidMaps (accessed via ChemSpider), supplemented with a custom library from Panomix Biomedical Tech Co., Ltd. (Suzhou, China). To ensure high-confidence annotations and data quality, only features with a fragmentation score ≥ 50 and a relative standard deviation (RSD) ≤ 30% in quality control (QC) samples were retained for subsequent analysis. This integrated approach, leveraging high-resolution MS/MS data and multi-database matching, was employed to ensure the reliable identification of metabolites and to confirm the novelty of their dynamic changes during the FDT refiring process.

2.6. Analysis of volatile compounds by GC × GC TOF MS

A total of 500 mg of tea powder was placed into a 20 mL bottle. A mixture containing 5 mL of saturated sodium chloride solution, 10 μL of 1 mg/L n-hexyl-d13 alcohol solution, and 10 μL of n-alkane (internal standard) was added to each sample. The sample was then incubated at 80 °C for 10 min (Lee et al., 2024; Niu et al., 2025). Then, extraction by the headspace solid-phase micro extraction (HS-SPME) device with a DVB/CAR/PDMS fiber (50/30 μm × 1 cm) obtained from Supelco (Bellefonte, USA) after aged at 270 °C for 10 min (Qi et al., 2020; Yang et al., 2020). Subsequently, inserted the fibers into the gas chromatograph injector immediately and thermally desorbed at 250 °C for 5 min.

Volatile metabolomic analysis were conducted utilizing a LECO Pegasus® 4D instrument (LECO, St. Joseph, MI, USA), which integrates an Agilent 8890 A GC (Agilent Technologies, Palo Alto, CA, USA) system equipped with a split/splitless injector, a dual-stage cryogenic modulator, and time-of-flight mass spectrometer (TOFMS) detector (LECO). A DB-Heavy Wax capillary column (30 m × 250 μm I.D., 0.5 μm film thickness) (Agilent, USA) served as the first-dimension column (^{1}D), while an Rxi-5Sil MS column (2.0 m × 150 μm I.D., 0.15 μm film thickness) (Restek, USA) functioned as the second-dimension column (^{2}D), both of which were employed to conduct GC × GC in this study with a flow rate of carried gas (high-purity helium over 99.999%) at 1.0 mL/min. The temperature program for the oven was as follows: maintained the temperature at 50 °C for 2 min, subsequently increased to 230 °C (5 °C/min) and held for 5 min. The secondary oven temperature was operated at 5 °C higher than the primary oven temperature. The modulator temperature was consistently maintained 15 °C above the temperature of the second column. The modulator operated with a modulation period of 6.0 s. The GC injector temperature was set to 250 °C. The mass spectrometer operates in the electron ionization (EI) mode of 70 eV, with a mass scan range of m/z 35–550 m/z. The temperatures of both the transmission line and the TOF mass spectrometry ion source were set at 250 °C. The raw data were processed with Chroma TOF software for peak picking, deconvolution, and alignment, which provided a data matrix containing peak areas, retention times (1st and 2nd dimension), and mass spectra for all detected features. Tentative identification of compounds was achieved by comparing the deconvoluted mass spectra against the NIST 2020 mass spectral library, with a similarity threshold typically set above 70–80% for confident annotation. Additionally, the retention indices (RI) of volatile compounds were calculated by comparing their retention times to those of a homologous series of n-alkanes (C7–C30). The relative quantification of the identified volatile compounds was performed using the internal standard method. The peak area of each compound was normalized to the peak area of the added internal standard (d13-hexanol) to calculate its relative concentration, thereby correcting for potential instrumental and sample preparation variances. This non-targeted flavoromics approach enables a comprehensive comparison of flavor compound profiles and their abundance trends across different refiring treatment groups (Wu et al., 2019).

2.7. Statistical analysis

All tea samples were analyzed with three times, and results were expressed as average of three times. The non-volatile and volatile components were analyzed separately on the SIMCA-P (v13.0) and the ropls package of R, include principal component analysis (PCA) and Orthonormal partial least-squares discriminant analysis (PLS—DA) (Trygg & Wold, 2002), and heat map analysis. K-means clustering analysis and volcano plots were performed on the BioDeep Platform (https://www.biodeep.cn). Statistical significance was indicated by a p value of ≤0.05 and a variable importance projection (VIP) value of ≥1. Identified differential metabolites were annotated by KEGG compound database (https://www.kegg.jp/kegg/compound/). The analysis of relative odor activity values was conducted with reference to literature (Li et al., 2023).

3. Results and discussion

3.1. Sensory evaluation

Sensory characteristics-color, aroma, flavor, and appearance are critical indicators of tea quality and directly influence consumer acceptance. Traditional sensory evaluation assessed fermented FDT subjected to different refiring temperatures (Fig. 2A). As shown in Fig. 1, infusion color deepened progressively with increasing temperature, shifting from light yellow (T80) to dark yellow (T100), potentially due to catechin oxidation (Liu et al., 2020). Sensory evaluation scores (Fig. 2B) revealed significant differences in flavor profiles among the temperature groups. Notably, the characteristic aroma intensity was highest for T90 (9.0) and lowest for T100 (7.0). Furthermore, T100 exhibited the lowest perceived astringency, suggesting that higher refiring temperatures reduce the stability of astringent compounds, such as tea polyphenols and flavonoids (Zhang et al., 2025). Conversely, T100 demonstrated the highest sweetness (8.5), followed by T90 (7.5). This enhanced sweetness may arise from high-temperature degradation of tea glycosides into soluble sugars (Ntezimana et al., 2021; Wang & Li, 2024), compounded by T100's minimal astringency, which likely reduces interference with sweetness perception. While T90 presented a rich, refreshing taste with pronounced bitterness and astringency, T80 sensory indicators differed minimally from the group of control, indicating limited impact of 80 °C refiring on FDT flavor. Overall, T100 exhibited the most favorable flavor profile, characterized by high mellow taste and refreshing sweetness scores (averages 8.15 and 8.50, respectively). However, its comparatively lower characteristic aroma intensity precludes identification, based solely on sensory data, of an optimal refiring temperature that universally enhances the overall quality of FDT.

3.2. Dynamic changes in non-volatile metabolites in FDT during refiring

The non-volatile metabolites in tea leaves are the primary contributors to its flavor, and the content of certain key metabolites can directly reflect the quality of tea. To elucidate the key non-volatile metabolites responsible for the improved flavor of FDT through refiring process, this study employed UPLC-MS/MS to analyze four sample groups. Using Proteowizard (v3.0.8789) software, a total of 9948 and 116,308 spectral features were extracted under positive and negative ion modes, respectively. The corresponding representative base peak chromatograms are provided in Fig. S1. A total of 945 non-volatile metabolites were included for statistical analysis. The 945 metabolites identified were classified based on functional groups, including 133 flavonoids, 72 prenol lipids, 71 organooxygen compounds, 48 terpenoids, 48 fatty acyls, 48 benzene and substituted derivatives, 46 carboxylic acids, 45 phenols, 29 alkaloids, and 33 other metabolites (Fig. 3A). Principal component analysis (PCA) of these metabolites provided a comprehensive understanding of the impact of different firing temperatures on the metabolic changes in the FDT refiring process. As shown in Fig. 3B, the first two principal components explained approximately 55% of the total variance (PC1 = 38.6%, PC2 = 16.4%, respectively). The first principal component analysis revealed that the PC1 scores of the T80, T90, and Control groups significantly differed from those of the T100 group, indicating that high-temperature refiring significantly altered the metabolite profiles of the tea. In the second principal component

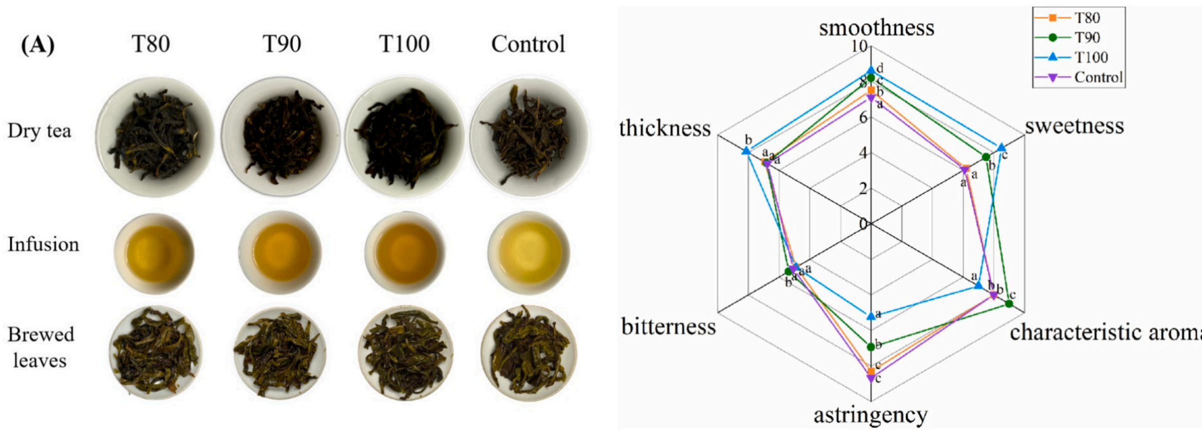

Fig. 2. The sensory evaluation of FDT by different refiring temperature. (A) Images of dry tea and infusion; (B) The sensory radar chart. Data with different superscript letters represent the significant difference between groups (*P* < 0 05).

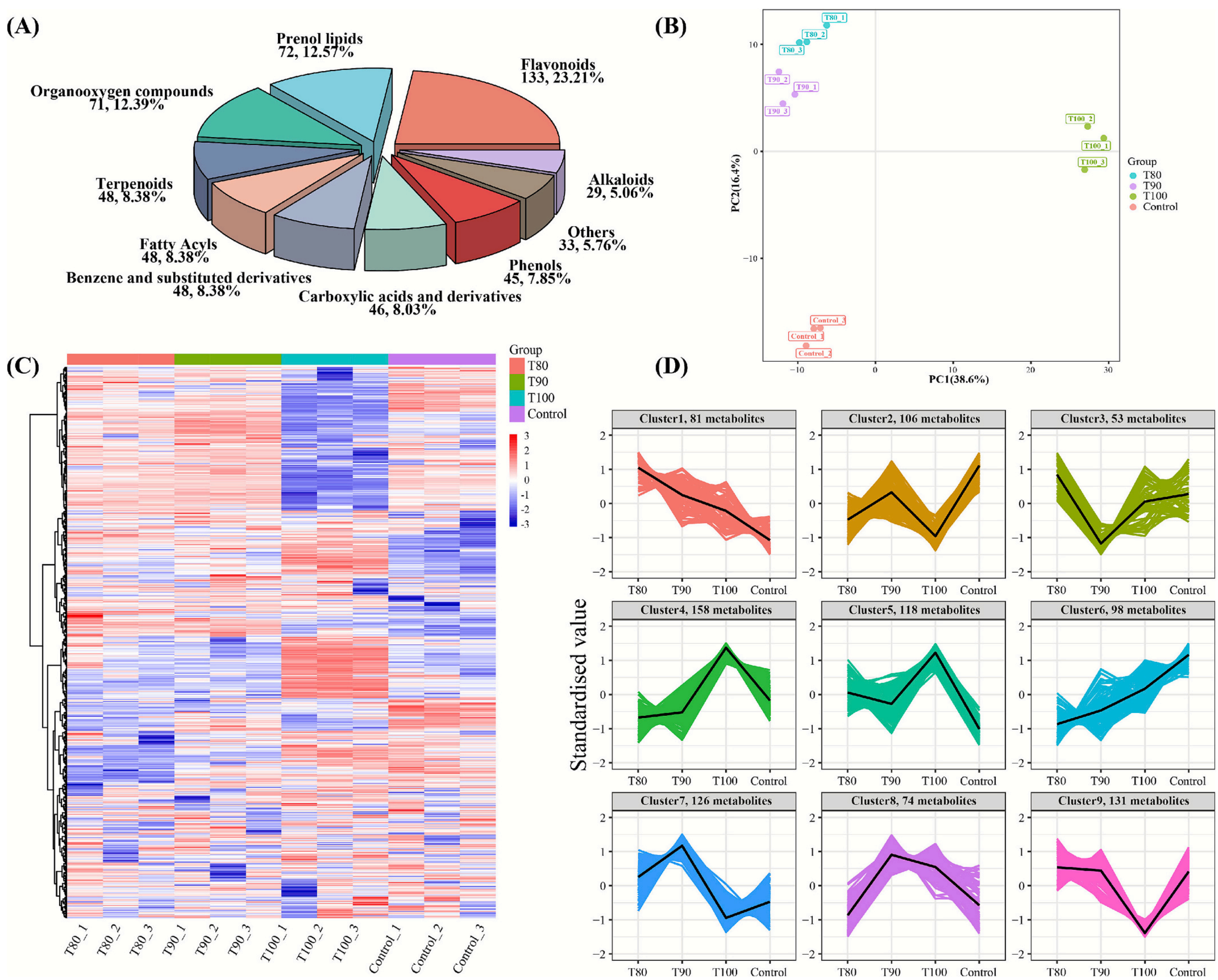

Fig. 3. Multivariate statistical analysis for all detected non-volatile metabolites of the four tea samples by different refiring temperatures in FDT. (A) Pie chart of different types; (B) PCA score scatter plot analysis; (C) Heat map analysis and (D) The relative contents of metabolites were processed by unit scaling variance, followed by K-means cluster analysis. In these results, the X-axis represents the different samples, and the Y-axis represents the relative standardized content of the metabolites.

analysis, the PC2 scores indicated significant differences between the T80, T90, and T100 groups and the Control group, suggesting that the refiring process promoted the generation of specific metabolites. To further investigate the transformation trajectories of non-volatile metabolites during the refiring process, orthogonal partial least squares discriminant analysis (OPLS-DA) was applied to examine the differences in metabolites between the four sample groups (Fig. S2). The results showed clear differentiation between the groups, consistent with the PCA findings. After 200 permutation tests, the R^2 and Q^2 values of the model were 0.99 and 0.73, respectively, confirming the reliability of the model and ruling out any over fitting (Fig. S3).

A heatmap was used to visualize the clustering of metabolite levels across the T80, T90, T100, and Control groups (Fig. 3C). Clustering analysis of all metabolites and samples revealed significant inter-group differences, with clear distinctions in metabolite content, particularly between the T100 group and the other groups. Additionally, K-means clustering (Fig. 3D) was applied to categorize the identified secondary metabolites into nine clusters to examine the variation in metabolite

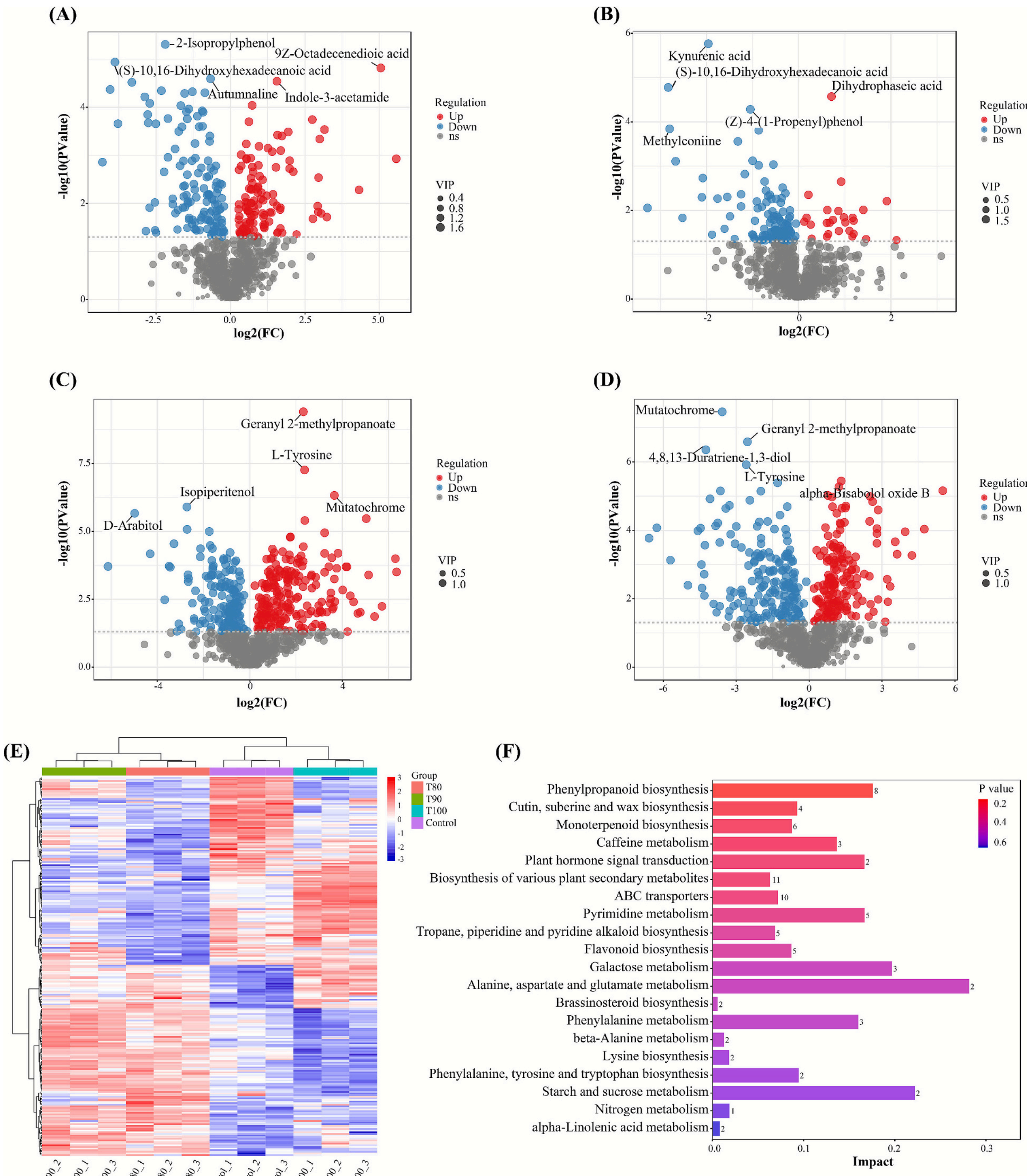

Fig. 4. Differences analysis for the non-volatile metabolite compositions of group in FDT. (A-D) Volcano plot analysis of Control_vs_T80, T80_vs_T90, T90_vs_T100 and T100_vs_Control; (E) Heat map analy sis; (F) Bar chart of the KEGG impact factors for different metabolic pathway. The vertical and horizontal axis represent the metabolic pathways and influencing factors, respectively. The numerical values in this figure indicate the quantities of metabolites involved in the corresponding pathways.

content between the samples. Among these, 199 non-volatile metabolites (clusters 1 and 5) had lower levels in the Control group compared to the experimental groups, while 206 metabolites (clusters 2 and 6) showed higher levels in the experimental groups, indicating that the refiring process influenced the transformation and production of non-volatile metabolites. This mainly involved a reduction in phenolic compounds, glycosides, and some amino acids, such as (−)-epigallocatechin 3-(3-methyl-gallate), epigallocatechin gallate, chavicol, bergaptol, delphinidin 3,5-diglucoside, tricin 7-glucoside, and L-isoleucine. The decrease in phenolic compounds led to a reduction in astringency and an increase in the richness of the tea infusion, while glycosides hydrolyzed under high temperature into soluble sugars, imparting a fresh, sweet taste to the tea (Yang et al., 2024), these findings were consistent with the sensory analysis results (Fig. 2B). In clusters 4 and 5, a total of 276 metabolites exhibited significantly higher levels in the T100 group compared to the other groups. These metabolites were primarily acids, lipids, and pigments, such as theaflavins, indicating that the high-temperature re-firing process is accompanied by oxidation.

3.3. Analysis of differential non-volatile metabolites and metabolic pathways

Differences in metabolites can directly affect the similarities and differences in taste characteristics (Zhang et al., 2022). To elucidate the impact of refiring temperature on the non-volatile metabolites in FDT, we investigated key differential metabolites between the samples. Specifically, 234 differential metabolites were selected from the 946 non-volatile metabolites based on the criteria of $P \leq 0.05$ and VIP ≥ 1 (Table S1). Volcano plots were used to depict the upregulated and downregulated metabolites (Fig. 4A-D). A total of 246 differential metabolites (115 up, 131 down) were identified between the Control and T80 groups. Among these, 2-isopropylphenol, (*S*)-10,16-dihydroxyhexadecanoic acid, and autumnaline were the most significantly downregulated metabolites. (*S*)-10,16-Dihydroxyhexadecanoic acid can undergo the Maillard reaction at high temperatures, generating caramel-colored substances. These substances, along with theaflavins, thearubigins, and other coloring substances, contribute to the final tea color and enhance the glossiness of the tea leaves. Autumnaline, an alkaloid with various bioactivities, including antitumor and cardiovascular protective effects, is also a precursor of colchicine. Colchicine used as a specific drug for acute gout currently, has shown some promise in cancer treatment, and its synthesis primarily relies on plant extraction, with biosynthesis being a hot research topic (Gao et al., 2024; Nett et al., 2020). Therefore, refiring process at 80 °C plays a significant role in the transformation of active compounds in tea, particularly the conversion of colchicine. A total of 149 differential metabolites (26 up, 123 down) were identified between the T80 and T90 groups, with (*S*)-10,16-dihydroxyhexadecanoic acid again being one of the most significantly downregulated metabolites, indicating that the Maillard reaction is a key pathway for the transformation of non-volatile metabolites during the refiring process in FDT. Between the T90 and T100 groups, 352 differential metabolites (202 up, 150 down) were found, with geranyl 2-methylpropanoate and L-tyrosine significantly upregulated. Geranyl 2-methylpropanoate, a terpene ester compound, exhibits floral and fruity aromas, which are characteristic flavor notes in Yashixiang of FDT. In contrast, between the T100 and Control groups, 360 differential metabolites (173 up, 187 down) were identified, where both geranyl 2-methylpropanoate and L-tyrosine were significantly downregulated. This suggests that excessively high refiring temperatures (100 °C) may have a negative impact on the aroma of the tea (Huang et al., 2025). Therefore, the range of refiring temperature has a crucial impact on the aroma of FDT and deserves further study.

To reveal the overall trend of metabolites changes and inter-group differences in FDT under refiring with different temperature conditions, a heatmap clustering analysis was conducted on the differential metabolites from the four sample groups, as shown in Fig. 4E. The T80 and T90 groups clustered together, indicating similar metabolite compositions within the temperature from 80 to 90 °C. In contrast, the T100 group exhibited distinct differences from the Control group, highlighting the significant impact of high-temperature (100 °C) refiring on the expression of tea metabolites. This change is attributed to the inhibition or activation of enzyme-catalyzed reactions induced by high temperatures, which further affect the synthesis and degradation of metabolites in tea (Zhang et al., 2024). Additionally, certain metabolites were significantly downregulated in the Control group but upregulated in the T80 group, suggesting that they are temperature-sensitive metabolites, which warrants further quantitative analysis and investigation of their regulatory mechanisms. KEGG pathway enrichment analysis was performed on all differential non-volatile metabolites (www.metaboanalyst.ca). As shown in Fig. 4F, 20 major metabolic pathways were identified across the T80, T90, T100, and Control groups, with the top six pathways being: phenylpropanoid biosynthesis; cutin, suberine, and wax biosynthesis; monoterpenoid biosynthesis; caffeine metabolism; plant hormone signal transduction; and biosynthesis of various plant secondary metabolites. These pathways may play an important role in plants' response to abiotic stress. Phenylpropanoid biosynthesis involves the conversion of phenylalanine into various phenylpropanoids, such as flavonoids, lignins, and coumarins. Studies had shown that phenylalanine metabolism was a key pathway in Dancong tea (Sun et al., 2023), and further research is needed to investigate the potential connection between these two pathways. Metabolites such as sinapyl alcohol, coniferaldehyde, 5-hydroxyferulic acid, 4-vinylphenol, isoeugenol, 5-hydroxyconiferaldehyde, chavicol, and (Z)-4-(1-propenyl) were derived from phenylpropanoid biosynthesis. The dynamic changes of (*S*)-10,16-dihydroxyhexadecanoic acid, a key intermediate in the cutin, suberin, and wax biosynthesis pathway, provide a compelling link between lipid oxidation and the Maillard reaction during tea refiring. Its highest content in the T90 group (Fig. S4), followed by a significant downregulation in higher temperature groups, suggests a temperature-dependent interplay between lipid-derived pathways and glycation chemistry. From a biochemical perspective, this fatty acid derivative can undergo oxidative degradation under thermal stress, generating a suite of reactive carbonyl species, such as aldehydes and ketones. These lipid-derived carbonyls can then actively participate in the Maillard reaction as alternative reactants, a process often referred to as "lipid-Maillard interaction".

3.4. Dynamic changes in volatile metabolites in FDT during refiring

Aroma is a key quality indicator of tea, and the aromatic compounds in tea primarily originate from its volatile metabolites, as well as the conversion of non-volatile precursor substances such as fatty acids, carotenoids, amino acids, and soluble sugars (Wang, Bi, et al., 2023; Yin et al., 2022). To further investigate the impact of the refiring process on the metabolites of FDT, volatile metabolites were analyzed using HS-SPME-GC × GC-TOF-MS. The GC × GC technique employs two chromatographic columns, significantly enhancing separation capability and sensitivity for complex mixtures that are challenging to resolve with traditional GC. Accordingly, the two-dimensional total ion current plots for all FDT samples refired at different temperatures are presented in Fig. S5. After raw data processing and the removal of contaminant signals, over 4100 peak features were tentatively annotated from the raw data using the NIST library (Fig. S6). As shown in the PCA score plot (Fig. 5A), the scores of the first principal component (PC1) for the T80 and T90 samples exhibited significant differences compared to those of the Control and T100 samples. This finding suggests that the temperature during the refiring process substantially altered the volatile metabolites of FDT. Analysis of the second principal component (PC2) further revealed a distinct separation between the T100 and Control groups. This clear inter-group difference indicates that the high refiring temperature induced a marked compositional shift in the volatile

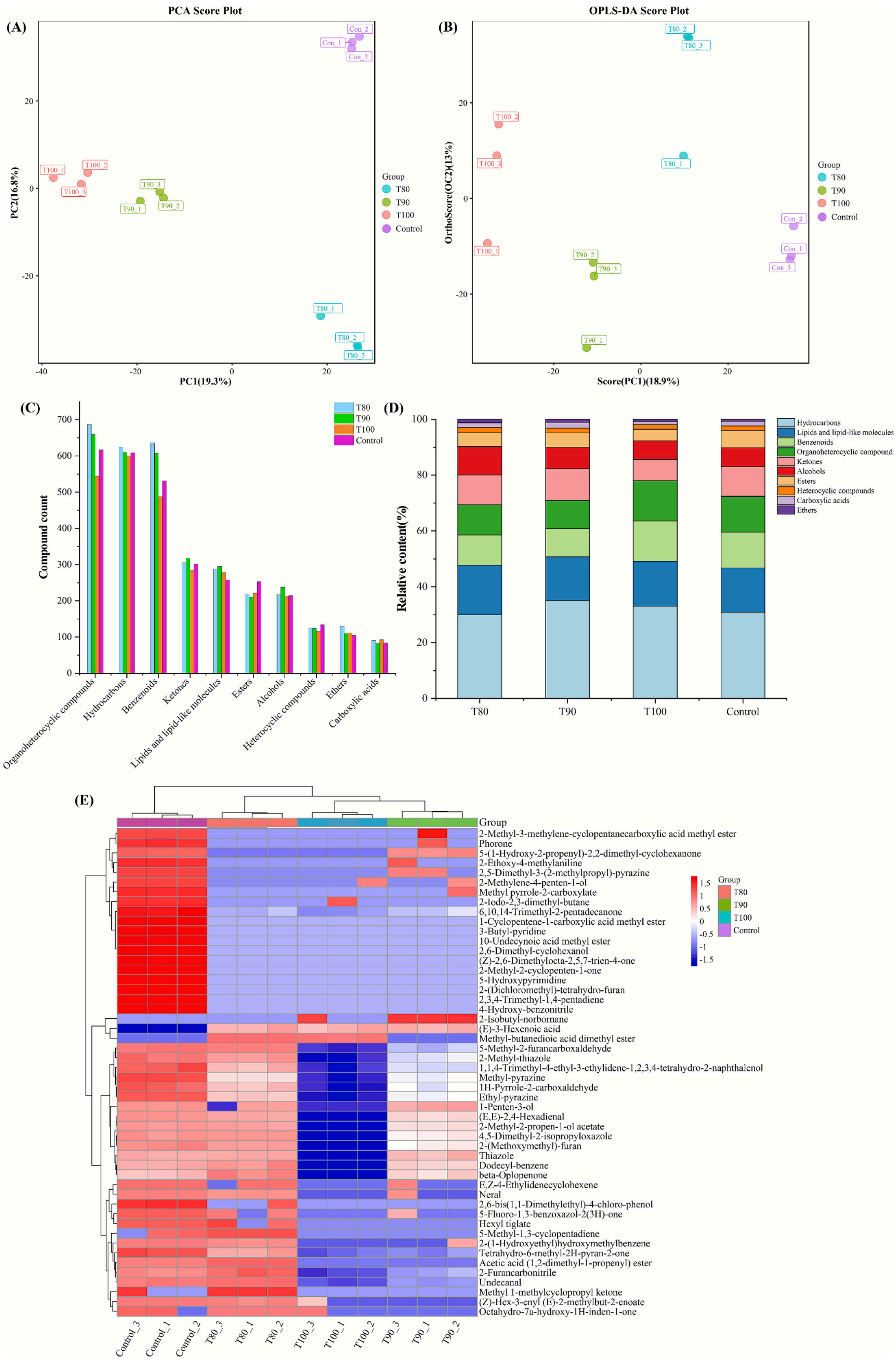

Fig. 5. Multivariate statistical analysis for all detected volatile compounds of the four tea samples by different refiring temperatures, T80 (samples treated at 80 °C), T90 (treated at 90 °C), T100 (treated at 100 °C), and Control (untreated samples). (A) PCA score scatter plot analysis; (B) OPLS-DA score scatter plot analysis; (C) Bar chart of compounds counts of different classes and (D) compounds relative contents (%) of the Top10 each classes. (E) Heat map analysis of the levels of Top 50 differential volatile compounds in FDT.

metabolites. To track the transformation of volatile metabolites during refiring, OPLS-DA was utilized to examine differences among the four groups (Fig. 5B). The results demonstrated significant distinctions between the groups. Following 100 permutation tests, the R^2 and Q^2 values for the metabolites were 1.0 and 0.26, respectively, thereby confirming the reliability of the model with no overfitting (Fig. S7).

To investigate whether the changes in tea aroma during the refiring process were related to the composition of the volatile compounds, we analyzed the types of volatile metabolites involved (Fig. 5C). The top three compound categories with the highest diversity were organoheterocyclic compounds, hydrocarbons, and benzenoids, with the highest number of species observed at 80 °C, which were 687, 624, and 636, respectively. As the temperature increased, the number of compound types decreased to 659 (organoheterocyclic compounds), 610 (hydrocarbons), and 608 (benzenoids) at 90 °C, and further to 545, 600, and 488 in the T100 group. The Control group contained 617, 609, and 531 species of these respective compounds. Ketones, lipids and lipid-like molecules, and alcohols exhibited higher diversity at 90 °C compared to other groups. Compounds such as β-ionone, α-ionone, cis-jasmone, and 3-octen-2-one in the ketone group, as well as linalool, geraniol, and hotrienol in the alcohol group, were considered characteristic markers of the aroma of FDT (Qin et al., 2023). Among the lipids, γ-caprolactone was identified as a key component contributing to the honey-like flavor of FDT (Chen et al., 2022). Therefore, based on the diversity of volatile metabolites, 80 °C and 90 °C resulted in the richest variety of volatile compounds. While the overall diversity of volatile metabolites decreased with temperature, a quantitative analysis of the major compound classes revealed a more complex pattern. To further compare the compositional characteristics among temperature treatments, we assessed the relative content of the top 10 compound categories (Fig. 5D). The top ten volatile components across all groups were hydrocarbons, lipids and lipid-like molecules, benzenoids, organoheterocyclic compounds, ketones, esters, alcohols, carboxylic acids, heterocyclic compounds, and ethers. Under refiring conditions of 80–90 °C, the concentrations of hydrocarbons and ketones increased, peaking at 90 °C. However, this peak was followed by a decline at higher temperatures, indicating that the effect of heating on these compounds is dependent on temperature. Overall, moderate refiring at 90 °C optimally preserved or even enhanced the levels of critical aroma compounds like ketones and hydrocarbons, whereas higher temperatures led to a widespread loss of both volatile diversity and content, the analysis indicated that the refiring temperature should not exceed 90 °C.

3.5. Multivariate analysis to explore differential volatile metabolites

The aroma of the final tea product is primarily formed during the processing stages. Extensive research has been conducted on the influence of different processing techniques on the aroma of oolong tea (Lan et al., 2022; P.-P. Liu et al., 2018; Wang, Li, Wang, et al., 2022). To investigate the variations in volatile compounds of FDT subjected to different refiring temperatures, we conducted OPLS-DA analysis and *t*-tests, employing a significance threshold of VIP ≥ 1 and $P \leq 0.05$. A total of 663 characteristic differential volatile compounds were identified (Table S1), including 96 organoheterocyclic compounds, 81 benzenoids, 74 hydrocarbons, 63 esters, 52 ketones, 49 lipids and lipid-like molecules, 33 alcohols, 27 aldehydes, and others. To further investigate the impact of refiring temperature on volatile compounds, clustering heatmap analysis was conducted on the top 50 differential metabolites (Fig. 5E). A clear distinction in the flavor compounds between the experimental and Control groups was observed ($P < 0.05$). Specifically, the refiring process led to a significant reduction in the volatile compounds in FDT. We further analyzed the types of aromas associated with the significantly reduced differential volatile compounds and found that these compounds primarily exhibited green aromas, such as 2-methyl-3-methylene-cyclopentanecarboxylic acid methyl ester, 2,5-dimethyl-3-(2-methylpropyl)-pyrazine, and 2-methylene-4-penten-1-ol, as well as herbal aromas, which included phorone and 5-(1-hydroxy-2-propenyl)-2,2-dimethyl-cyclohexanone. Critically, these diminished aroma attributes do not constitute characteristic markers of FDT. Additionally, the refiring process mitigates the negative aroma properties of certain compounds. For instance, 2-ethoxy-4-methylaniline and 3-butylpyridine release a strong and unpleasant fishy odor(Liu et al., 2024), while 2-iodo-2,3-dimethyl-butane presents a typical musty smell. Furthermore, 2-(dichloromethyl)-tetrahydro-furan contributes a significant chemical solvent odor.

The significant reduction in nitrogen-containing heterocyclic compounds, notably 2,5-dimethyl-3-(2-methylpropyl)-pyrazine and 3-butylpyridine ($P < 0.05$), can be mechanistically explained by the competitive binding and trapping actions facilitated by tea polyphenols during refiring. As elucidated in the study by Dong et al. (2023), phenolic acids such as caffeic acid (CA) and gallic acid (GA) can effectively trap reactive α-dicarbonyl compounds (e.g., methylglyoxal, MGO), which are critical intermediates for the formation of pyrazines and pyridines via the Strecker degradation pathway (Dong et al., 2023). In our FDT system, the heat-labile catechins and other phenolic compounds are likely degraded or transformed under thermal stress, and in this process, they may act as carbonyl trappers. By covalently binding to MGO and other dicarbonyls, these polyphenolic derivatives directly compete with amino acids (e.g., valine, leucine) for the reactive intermediates, thereby shunting the reaction pathway away from the formation of nitrogen-containing volatiles and toward the formation of phenolic-MGO adducts. This mechanism not only explains the decrease in specific pyrazines but also provides a biochemical rationale for the observed reduction in overall Maillard-type aroma intensity at certain refiring temperatures (Lan et al., 2022). It is noteworthy that aldehydes were not included among the top 10 volatile compounds in all the detected groups. However, aldehydes accounted for 28.13% of the differences in volatile compounds across the groups. The observed differences are hypothesized to be due to the oxidation of aromatic alcohols during the re-ignition process, leading to the formation of aldehydes (Zhai et al., 2022). (E)-3-Hexenoic acid, an unsaturated fatty acid in plants (such as the cis-isomer), may undergo thermal-induced isomerization at high temperatures, converting to the trans-isomer. This process involves changes in the position of the double bond and the isomeric configuration, typically occurring as a non-enzymatic reaction driven by heat, which accounts for the decrease in this compound during re-ignition. Temperature affects the release of volatile compounds both directly (accelerating physical volatilization) and indirectly (increasing biosynthesis). Understanding this relationship is crucial for predicting the impact of climate change on plant chemical composition, plant-environment interactions (such as pest and disease resistance, and pollinator attraction), and the role of plant-derived volatile organic compounds in atmospheric chemistry.

3.6. Correlation network diagram and key aroma-active volatiles analysis

The aroma composition of food is complex and diverse, with the same type of aroma being produced by different combinations of volatile compounds. In this study, the volatile compounds obtained were compared with the FlavorDB database to identify the sensory flavor characteristics of FDT. A related network diagram illustrating volatile compounds and aroma profiles was created to provide a comprehensive overview of the flavor-contributing substances in FDT (Fig. 6). The figure indicates that FDT was characterized by rich fruity, fresh, sweet, and floral aromas, consistent with findings from previous studies (Qin et al., 2023). A total of 53 volatile compounds were identified to support the fruity aroma, including 3,7-dimethyl-1,6-octadien-3-olformate, 3,7,11,15-tetramethyl-2-hexadecen-1-ol acetate, 2-butoxyethyl acetate, 1-hexanol, 2,6,6-trimethyl-1-cyclohexene-1-acetaldehyde, and 1-penten-3-ol. The fresh aroma was mainly provided by compounds such as (E,E)-2,4-hexadienal, (E)-2-octenal, 3,6-dihydro-4-methyl-2-(2-methyl-1-propenyl)-2H-pyran, and 3-hexen-1-ol benzoate. Volatiles

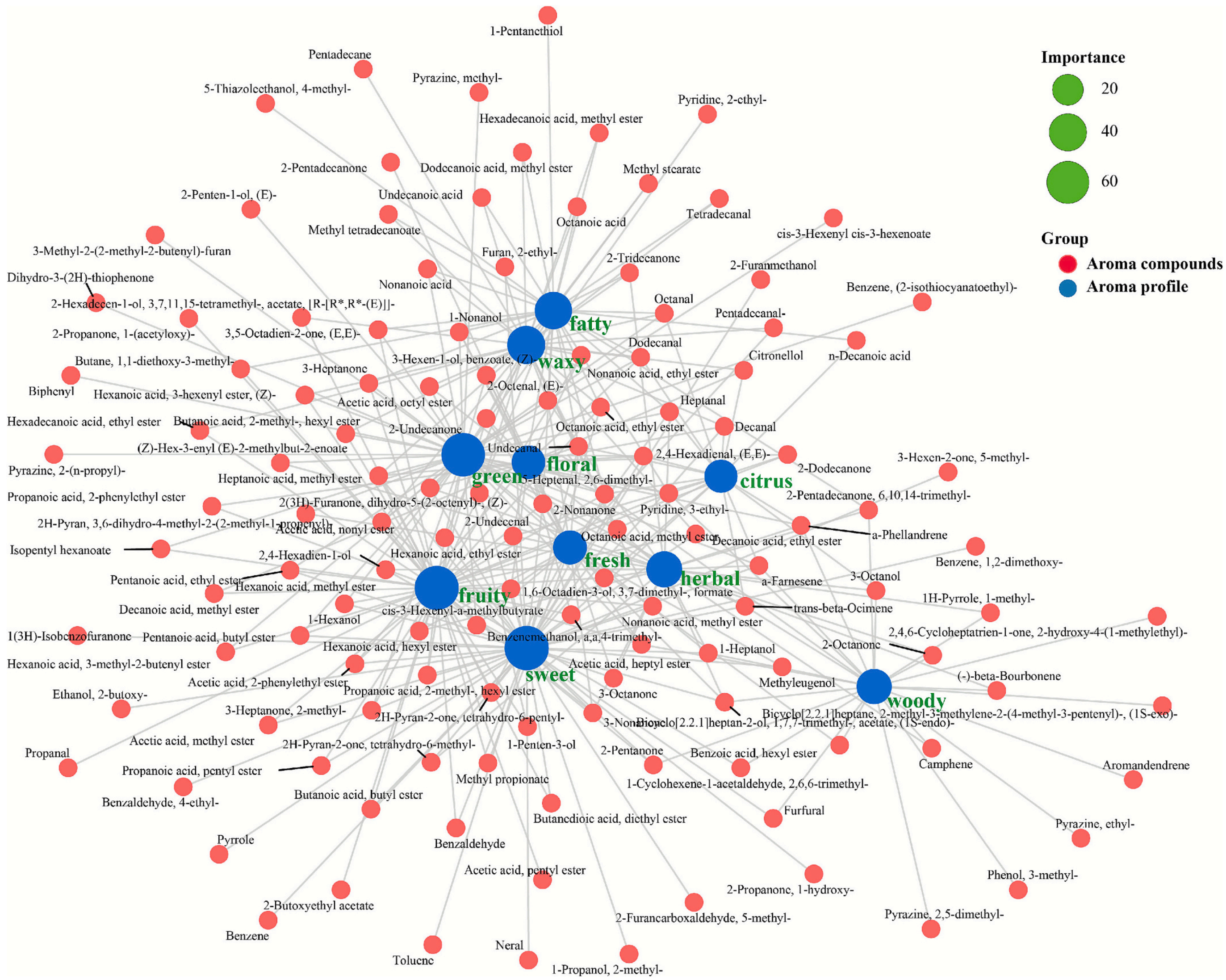

Fig. 6. Sensory flavor characteristics and correlation network diagram for the flavor compounds. The blue circles indicate sensory characteristics, and the red circles indicate flavor compounds. The larger the blue circle, the more relative compounds it indicates. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

compounds such as 1(3H)-isobenzofuranone, (*Z*)-dihydro-5-(2-octenyl)-2(3H)-furanone, and 2-ethyl-furan contributed significantly to the sweet aroma.

Volatile compounds only exhibit their theoretical aroma when they reach a certain concentration. Therefore, the unique aroma of tea is influenced not only by the concentration of volatile metabolites but also by their odor thresholds. The Relative Odor Activity Value (ROAV) serves as a quantitative measure to assess the individual contribution of various volatile metabolites to the overall aroma of tea. Typically, volatile metabolites with an ROAV ≥ 1 are considered key aroma components of tea samples. The higher the ROAV value, the greater the aroma impact. Additionally, studies have shown that metabolites with $0.1 \leq$ ROAV <1 have a significant modulatory effect on the aroma of tea, while those with $0.01 <$ ROAV <0.1 are considered potential aroma components (Li et al., 2023). This study conducted ROAV analysis on the volatile compounds in FDT to determine their contribution to the overall aroma characteristics of the tea. During the refiring process, a total of 16 volatile compounds with ROAV >0.1 were selected, and 9 key aroma compounds with ROAV ≥ 1 were identified (Table 1), and their mass spectra were provided in Fig. S8. Among them, 2-methyl-butanal and (E)-2-nonenal exhibited significantly higher ROAV values than other volatile compounds, followed by 2-pentyl-furan, 2,3-butanedione, heptanal, 2-undecanone, 1-octen-3-one, (E)-2-octenal, and 3-methyl-butanoic acid. 2-methyl-butanal exhibited cocoa, coffee, nut, or fruity aromas, while (E)-2-nonenal contributed fatty and fresh notes, playing a significant role in the aroma of all samples. With the progress of refiring to 100 °C, the contribution of 2-methyl-butanal decreased sharply. This aldehyde is a classic Strecker degradation product of amino acids like L-isoleucine. Its dynamic change reflects the consumption of these precursor amino acids (as shown in Section 3.2) and the progression of thermal reactions, which subsequently promote the formation of other aroma compounds, such as 2,3-butanedione, whose ROAV value increased notably at higher refiring temperatures. Notably, 2,3-butanedione, a compound commonly found in foods, is most abundant in dairy products and plays a key role in butter flavor. In this study, the content of 2,3-butanedione increased significantly during refiring, suggesting that the unique creamy flavor of oolong tea may be linked to the substantial production of 2,3-butanedione during the process. The ROAV value of (E)-2-nonenal in the samples after re-firing at 90–100 °C increased significantly, indicating that the process enhanced the fatty flavor of oolong tea. It is important to note that the ROAV model assumes additivity of odors and does not account for potential synergistic or masking interactions among aroma compounds. To further confirm the sensory impact of these compounds, future aroma reconstitution and

Table 1
The volatile compounds with ROAV >0.1 in the FDT during refiring.

Name	Class	CAS	Odor Character	T80	T90	100	Control
2-Methyl-butanal	Aldehydes	96–17-3	Cocoa, Almond	85.46	85.02	33.33	90.12
(E)-2-Nonenal	Aldehydes	18,829–56-6	Fatty, Cucumber	66.67	82.80	89.89	66.67
2-Pentyl-furan	Organoheterocyclic compounds	3777-69-3	Green Beans, Vegetable	8.41	15.94	26.67	10.55
2,3-Butanedione	Ketones	431–03-8	pleasant, buttery	5.96	7.93	26.18	9.20
Heptanal	Aldehydes	111–71-7	Citrus, Fatty, Rancid	10.10	6.49	8.61	8.85
2-Undecanone	Ketones	112–12-9	Orange, Fresh, Green	7.74	5.85	5.86	8.41
1-Octen-3-one	Ketones	4312–99-6	Mushroom-Like	6.25	10.39	17.85	7.06
(E)-2-Octenal	Aldehydes	2548-87-0	Nuts, Green, Fatty	7.40	4.75	9.80	6.64
3-Methyl-butanoic acid	Benzenoids	503–74-2	Rancid Cheese, Sweaty, Putrid	0.99	2.49	4.37	1.95
(E,Z)-2,6-Nonadienal	Aldehydes	557–48-2	Cucumber, Green	0.72	0.22	0.67	0.97
2-Methyl-propanal	Aldehydes	78–84-2	pungent	0.29	0.09	0.30	0.25
2-Ethyl-1-Hexanol	Alcohols	104–76-7	Rose, Green	0.28	0.39	0.32	0.22
Methyl isovalerate	Esters	556–24-1	Apple	0.02	0.11	0.11	0.21
Acetic acid	Carboxylic Acids	64–19-7	Pungent, vinegar	0.25	0.23	0.42	0.20
Pentanal	Aldehydes	110–62-3	sickening, rancid, decayed	0.05	0.10	0.25	0.08
2-Heptanone	Ketones	110–43-0	sweet, mushroom	0.13	0.12	0.24	0.04

omission experiments would be highly valuable, for instance, 2,3-butanedione to the creamy note and (E)-2-nonenal to the fatty character could be definitively confirmed and quantified.

In addition to sensory correlation, the molecular mechanisms by which these key compounds (e.g., 2-methylbutanal, 1-octen-3-one) elicit their specific aroma perceptions still remain to be fully understood. Future studies employing molecular docking could further explore the interactions between these ligands and human olfactory receptors, thereby providing atom-level insights into the structure-activity relationships that underlie FDT's complex aroma. (E,Z)-2,6-nonadienal, 2-methyl-propanal, 2-ethyl-1-hexanol, methyl isovalerate, acetic acid, pentanal, and 2-heptanone, as potential compounds in the samples, also

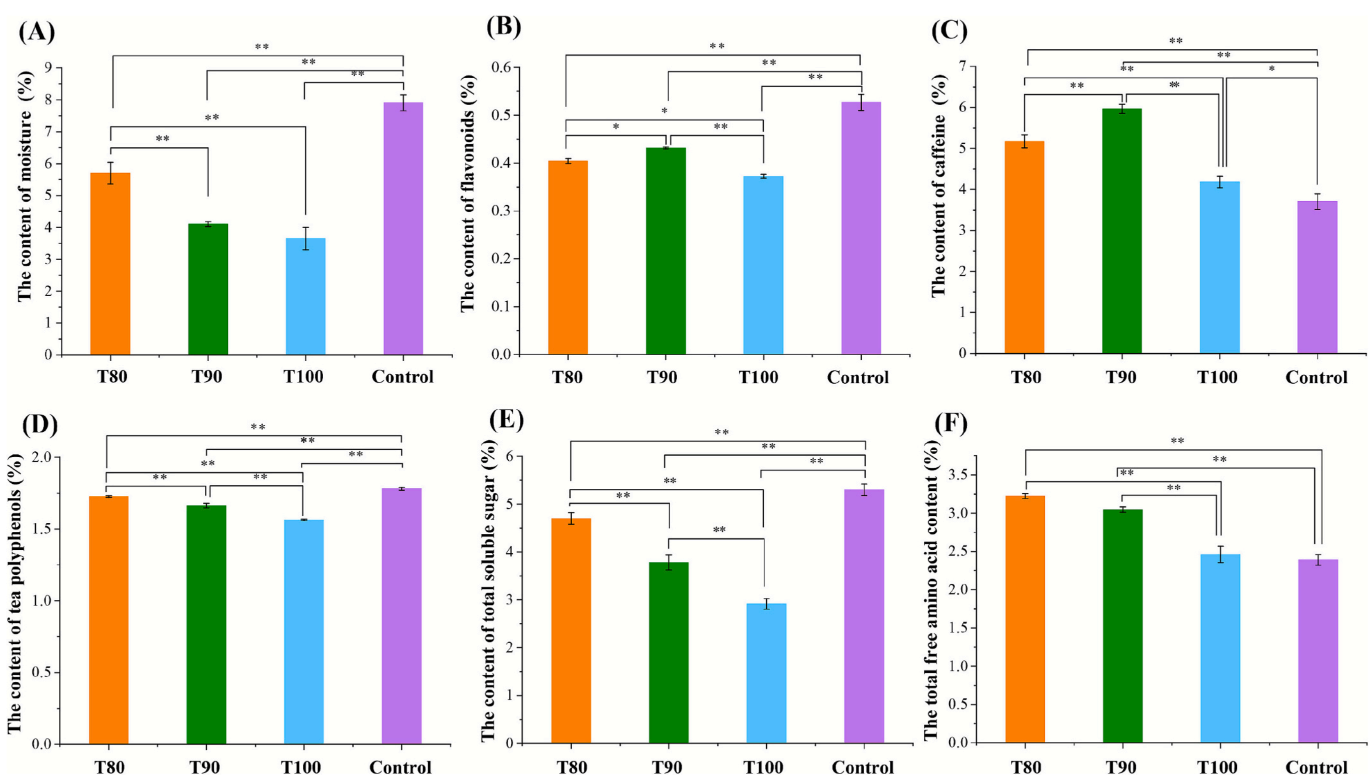

Fig. 7. The content of (A) moisture, (B) flavonoids, (C) caffeine, (D) tea polyphenols (E) total soluble sugar and (F) total free amino acids of the four tea samples by different refiring temperatures in FDT. The different superscripts show significant differences ($P < 0.05$) according to Duncan's test. All contents of biochemical components were expressed as percentages on a dry weight basis (% dry weight).

played a supportive role in the overall aroma of the tea.

3.7. Quantitative analysis of key quality indicators of tea during the refiring process

Moisture content is regarded as one of the most critical parameters in tea grading, as it directly influences the shelf life of tea. Additionally, both the aroma quality and flavor profile of tea are intricately linked to its moisture content (Huang et al., 2021). Oolong tea with moisture content in the range of 3–5% exhibited better quality and storage stability compared to tea with higher moisture content (Chen et al., 2014; Chen & Weng, 2010), reducing the moisture content of tea was also one of the primary objectives of the refiring process. As shown in Fig. 7A, the moisture content of tea significantly decreased ($P < 0.05$) under refiring at different temperatures. As the refiring temperature increased, the moisture content decreased to 5.70%, 4.10%, and 3.65%, respectively. In contrast, the control group exhibited a moisture content of 7.91%, which was much higher than that of the final oolong tea, resulting in poorer storage stability. Therefore, the refiring process is essential before oolong tea is marketed. As secondary metabolites, flavonoid impart characteristic bitterness and astringency to tea even at trace concentrations (Wang, He, et al., 2024). As shown in Fig. 7B, a significant difference in flavonoids content was observed between the group of refiring and control ($P < 0.05$). However, the flavonoids content did not follow a linear relationship with increasing refiring temperature. At 100 °C, the flavonoids content was lowest (0.37 mg/g), while it was highest at 90 °C (0.53 mg/g). Previous studies have demonstrated that the accumulation of caffeine is mainly influenced by metabolic pathways of degradation (Zhao et al., 2020). As one of the most important quality indicators in tea, caffeine plays a decisive role in the taste of tea. The caffeine content in FDT showed a trend of increasing and then decreasing after refiring, with the highest caffeine content (5.97%) observed at 90 °C (Fig. 7C). Tea polyphenols, a general term for phenolic compounds in tea, are key components responsible for the health benefits of tea. The content of tea polyphenols significantly decreased during refiring (Fig. 7D). The Control group had the highest tea polyphenol content (1.78%), which dropped to 1.56% at 100 °C. This decrease is related to non-enzymatic isomerization, oxidation, polymerization, and degradation of tea polyphenols during heat treatment (Fan et al., 2016). Soluble sugars are important components contributing to the sweetness of oolong tea infusions (Liu et al., 2018), Fig. 7E shows that the total of soluble sugar content significantly decreased during refiring ($P < 0.05$). The Control group had the highest soluble sugar content (5.3%), which decreased to 2.92% at 100 °C due to Maillard reactions involving polysaccharides and amino compounds, resulting in the formation of high-molecular-weight melanoidin. Amino acids play an indispensable role in determining the taste and color of tea. The content of total free amino acids showed an increasing trend in refired tea at temperatures below 100 °C (Fig. 7F), rising from 2.39% in the Control group to 3.05% at 90 °C. This increase resulted from thermal degradation of proteins and peptides, generating additional amino acids (Wang, Ren, et al., 2023); then, a decrease was observed with the content dropping to 2.46% at 100 °C. Notably, sugars and amino acids are key substrates in the Maillard reaction, which promotes the synthesis of pyrazines and other heterocyclic compounds, leads to a stronger roasted aroma in tea (Wang, Li, Lin, et al., 2022).

While the controlled experimental design using a single tea batch and a fixed refiring duration successfully isolated the specific effects of temperature on both volatile and non-volatile metabolite reprogramming during refiring, we acknowledge that this approach does not capture batch-to-batch biological variability or the full time–temperature kinetics of the underlying reactions. Importantly, the primary objective of this study was not to provide statistically generalized conclusions across diverse raw materials, but rather to elucidate temperature-driven mechanistic trends governing polyphenol transformation, aroma compound formation, and their collective contribution to sensory modulation under strictly controlled conditions. By minimizing raw material heterogeneity, this design enabled a clearer interpretation of pathway-level changes, including phenylpropanoid metabolism, controlled oxidation, and Maillard-related reactions. Nevertheless, we recognize that biological replication using multiple tea batches, together with kinetic modeling, will be essential in future studies to validate the robustness, scalability, and industrial applicability of these findings and to further optimize refiring strategies for targeted quality and flavor development.

4. Conclusions

This study demonstrates that refiring can significantly improve the sensory quality of FDT by reducing bitterness and enhancing sweetness, smoothness, and aromatic complexity under the investigated conditions. These effects are primarily associated with controlled oxidation and Maillard reactions, which collectively reshape both volatile and non-volatile metabolites during refiring. Moderate refiring at 90 °C was found to effectively balances flavor development and bioactive compound retention, whereas higher refiring at 100 °C tended to intensifies fatty–buttery notes while compromises fruity aromas, underscoring the importance of precise temperature control.

From an analytical perspective, the findings reveal that refiring serves as an important modulatory step in oolong tea processing, enabling targeted regulation of flavor-related metabolites. Such integrative approaches are expected to advance both the scientific understanding and potential industrial application of refiring technology toward achieving more consistent, health-promoting, and consumer–preferred tea products.

CRediT authorship contribution statement

Mo Ding: Writing – review & editing, Writing – original draft, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Shanshan Meng:** Supervision, Software, Methodology, Data curation. **Lei Hu:** Resources, Methodology, Funding acquisition. **Hongyan Wang:** Validation, Supervision, Investigation. **Jianjian Huang:** Validation, Supervision, Data curation. **Fengnian Wu:** Writing – review & editing. **Zhengchao Yu:** Writing – review & editing. **Jean W.H. Yong:** Writing – review & editing, Funding acquisition. **Hui Zhu:** Writing – review & editing, Project administration, Methodology. **Zhong Hu:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization.

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Declaration of competing interest

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

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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.2025.103469.

Data availability

Data will be made available on request.

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