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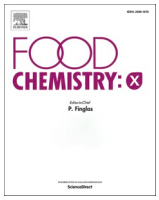

Metabolomics reveals a differential attitude in phytochemical profile of black tea (*Camellia Sinensis* Var. *assamica*) during processing

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ABSTRACT

Black tea's quality and flavor are largely influenced by its processing stages, which affect its volatile and non-volatile phytochemicals. This study aimed to optimized black tea manufacturing by investigating withering time, fermentation time, and temperature's impact on sensory quality. Using a U^*_{15} (15^7) uniform design, optimal conditions were determined: 14 h of withering, 5.6 h of fermentation, and a 34 °C temperature. A verification experiment analyzed the volatile and non-volatile profiles. HPLC, GC–MS, and LC-MS revealed dynamic changes in phytochemicals. Among 157 VOCs and 2642 metabolites, 19 VOCs (VIP > 1.5) were crucial for aroma, while 50 (VIP > 1.5, $p < 0.01$) characteristic metabolites were identified. During processing, fragrant volatile compounds like linalool oxides, geraniol, benzeneacetaldehyde, benzaldehyde, methyl salicylate, and linalyl acetate increased, contributing to rose and honey like aromas. These changes were crucial in developing the characteristic flavor and color of black tea. Twenty-four new compounds formed, while 80 grassy odor volatiles decreased. Non-volatile metabolites changed notably, with decreased catechins and increased gallic acid. Theaflavin compounds rose initially but declined later. This study outlines metabolite changes in *Yunkang* 10 black tea, crucial for flavor enhancement and quality control.

1. Introduction

Tea (*Camellia sinensis* L.), is a famous beverage enjoyed globally for its delightful flavor, aroma, and numerous medical perks (Liu et al., 2019). The physicochemical procedures involved in the manufacturing of black tea have a high influence on its quality. Processing has been shown to gradually alter the permeability of cell membranes, glycosidase activities, and flavor components (Gui et al., 2015), and transform phenolic compounds and catechins. Processing also alters organic acids, sugar (Wu et al., 2019), and other bioactive substances like amino acids (Jabeen et al., 2019). In tea, non-volatile components contribute to medical perks, color, and taste whereas volatile compounds contribute to aroma and flavor (Rawat et al., 2007). The black tea's biochemical potential is mostly determined by plucking, which produces aroma precursors, and withering, which causes membranes to become permeable. Oxidation in tea leaves during withering and fermentation gives processed black tea its red-brown color, sweet aroma, and flavor.

Statistical tools like uniform designs (UD) were used to examine how experimental factors affect responses. A Small sample size allows uniform design (UD) to handle the most levels with the fewest experimental

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runs and is especially suitable for multi-level and multi-factor evaluations (Song et al., 2012). The trials' parameters are determined using the number theoretic method. This means that the selected trials are a good approximation of all operational parameters (Liang et al., 2001). The efficacy of this methodology has been extensively documented across various fields.

High-performance liquid chromatography combined with time of flight mass spectrometry and hybrid quadrupole orbitrap mass spectrometry had been used for mass spectrometry/liquid chromatography-mass spectrometry along with the application of multivariate statistical analysis to differentiate year and seasonal variation, place of production, and processing technology (Tang et al., 2021). High-performance liquid chromatography was used by (X. Fang et al), to trace phenolic acids, purine alkaloids, and flavanols subgroup (catechins). Additionally, a two-dimensional (GC × GC–MS) gas chromatography–mass spectrometry GC–MS was used to detect volatile compounds, pesticides, and herbicides in tea leaves after liquid-liquid microextraction or headspace solid-phase microextraction (Farajzadeh et al., 2020). Numerous non-targeted studies have used techniques like gas chromatography–mass spectrometry (Joshi & Gulati, 2015), nuclear magnetic resonance (Lee et al., 2015), ultra-high-performance liquid chromatography (Dai et al., 2017), and liquid chromatography-mass spectrometry (Xu et al., 2015) to profile tea metabolites. Recently, metabolic profiling has been utilized to investigate the metabolic pathways in various tea cultivars to assess the flavor quality in various subtypes of white tea (Yang et al., 2018). A non-targeted (LC-MS) metabolomics investigation on non-volatile components during fermentation in black tea found 61 non-volatile metabolites (Tan et al., 2016). A comprehensive, stage-by-stage metabolomic analysis of both volatile and non-volatile components was carried out, which goes beyond previous work by integrating advanced statistical tools, optimizing production parameters, and providing a detailed mapping of metabolite changes across the entire manufacturing process. The findings are pivotal for enhancing flavor quality and guiding quality control in black tea production, representing a significant advancement in tea metabolomics. Previous studies limited its focus to isolated stages such as fermentation (Tan et al., 2016), or specific subtypes of tea (Yang et al., 2018). Moreover, none of study has been conducted a thorough multivariate statistical analysis integrating both volatile and non-volatile compounds across different processing stages. This study emphasizes the interaction between specific processing parameters (Withering time, fermentation time and temperature) and their impact on both volatile and non-volatile phytochemicals. In this study, employing the common black tea cultivar *Yunkang* 10 as a model, we conducted extensive GC–MS, LC-MS metabolomics, and HPLC analyses at various black tea processing stages, including plucking, withering, rolling, fermentation, and drying. This approach aims to elucidate the transformation of metabolites occurring at each stage of black tea production.

The optimization of black tea manufacturing was done using a DPS Professional (Version DPSv9.50) data processing system. The U^*_{15} (15^7) mixed level uniform design was selected, and based on experimental design, various tests were performed. The experiments revealed that within the experimental range, the optimal conditions for black tea production were found to be a withering time of 14 h, a fermentation time of 5.6 h, and a fermentation temperature of 34 °C, which is consistent with the model's predicted value (90.02 %). The black tea was manufactured under the result of uniform design, and three samples from each processing stage—fresh, withered, rolled, fermented, and dried—were collected for HPLC analysis of caffeine, four theaflavins, gallic acid, catechins, and seven major quality compounds whereas five samples for LC-MS and GC–MS analysis. LCMS (UHPLC–Q-Exactive HF/MS) and GCMS (HS-SPME-GC–MS) were designed to determine VOCs based on VIP > 1.5 investigate their generation mechanism, and disclose the differences in non-volatile metabolites (VIP > 1.5, $p < 0.01$) at each processing stage of black tea manufacturing.

2. Materials and methods

2.1. Sampling and processing of Black Tea

Fifty kilograms of "*Yunkang* 10" *(Camellia sinensis* L.) fresh tea leaves were harvested in August 2023 from Pu'er Zuxiang Gaoshan tea garden, Yunnan Province, China. The leaves were withered at 25.0–30.0 °C and 50 % relative humidity for 2.5-h intervals with periodic turning. After 1.5 h of rolling by a machine (Fuyang Machinery 6CR-10, China), fermentation at 85 % relative humidity were carried out. Drying occurred at 110 °C for 30 min, followed by 85 °C for 210 min. Thirty kilograms of tea leaves were divided into fifteen portions for experimentation with a uniform design U^*_{15} (15^7) with varying withering time, fermentation time, and temperature. Following optimization, twenty kilograms were processed with specific parameters: 14 h of withering, and 5.6 h of fermentation at 34 °C. The optimized sample was frozen in liquid nitrogen, dried in a vacuum freeze drier at −55 °C for three days, finely powdered, and stored at −20 °C for analysis.

2.2. Uniform experimental design for factor-level selection/optimization of sample

In this paper the optimization of black tea manufacturing was carried out using a uniform design. Three highly influential factors (X1, X2, and X3) were chosen: X1 represents the withering time (hour); X2 the fermentation time (hour); and X3 the temperature during fermentation (°C). As stated in (Table SM 1), each factor has fifteen levels.

A corresponding uniform designed table U^*_{15} (15^7) was developed to design test parameters combinations and the experimental plan was designed based on the factor levels in the (Table SM 1). (Table SM 2) exhibited the combination of the obtained test parameters. The black tea was processed based on the preparation parameters specified in the (Table SM 2). Subsequent performance tests, including sensory analysis, were conducted, followed by other testing.

2.3. Sensory evaluation

All sensory evaluations were conducted following guidelines of CNIS to ensure participant comfort, voluntary participation, and confidentiality of responses. Prior to participating, all panelists provided informed consent after receiving a detailed explanation of the study objectives, procedures, and their rights as participants. Participants were assured of confidentiality, and all data were collected anonymously. Institutional Review Board and Ethics Committee of Yunnan Agricultural University approved this study, and all procedures complied with ethical standards and regulations. The sensory assessment involved evaluating five factors: (a) color of liquor, (b) taste, (c) aroma, (d) infused leaves, and (e) appearance. Fifteen experts from the College of Tea Science at Yunnan Agricultural University, China, were evaluated black tea quality as per tea vocabulary (GB/T14487-2017) for sensory evaluation, and according to national standard of China (GB/T 23776-2018). For the determination of appearance, 100–150 g of tea samples was prepared. Then, 3 g of tea leaves were steeped in 150 mL of boiled water for 5 min. The taste, color, aroma, and infused leaves were assessed sequentially after infusion. A porcelain bowls of white color was used to serve the infusion of tea, with each tea sample assigned a random blind code. The infusion of tea was evaluated by the experts by tasting and smelling, with a 30-s pause among each sample. Through blind evaluation all individual samples was assessed three times.

2.4. Determinations of primary quality components in black tea

The water extract and moisture content were assessed following the guidelines of National Standard of China GB/T (8305-2013) and (GB/T 8304-2013) respectively. The determination of amino acids was carried out with boiling water extraction for 30 min at 570 nm using a UV–vis

spectrophotometer with L-glutamic acid and Ninhydrin assay as the standard following CNIS GB/T 8314-2013. Using a ultraviolet-visible spectrophotometer UV-8000S (Jingmi Instrument, China) with gallic acid as a standard at 765 nm wavelength, as per CNIS GB/T (8313-2018) the content of tea polyphenol content was determined. A D- (+)-glucose as a standard with anthrone reagent was used in UV–vis spectrophotometer to measure total soluble sugars at 620 nm. Pre-existing methodologies of (Ma et al., 2021) were employed to quantify the levels of theaflavins, thearubigins, and theabrownins.

2.5. HPLC analysis of gallic acid, caffeine and catechins

Tea powder (200 mg) was extracted with 5 mL of 70 % methanol solution for 15 h at 4 °C. After centrifugation, the resulting 1 mL supernatant was filtered and stored at −20 °C for HPLC analysis. Gallic acid, catechins, and caffeine were quantified using an Agilent 1200 series HPLC system with a TC-C18 chromatogram column. Solvent A was 0.1 % formic acid methanol solution and solvent B was 0.1 % formic acid aqueous solution. The gradient profile included increases and decreases in solvent A concentration, with a flow rate of 1.00 mL/min and detection at 278 nm. Analytical curves with R2 > 0.990 were used for quantification.

2.6. HPLC analysis of four theaflavins compounds

Tea powder (200 mg) was extracted using 5 mL of 70 % methanol solution at 70 °C for 10 min. A 0.45 μm nylon membrane filtered 5 μL of the extract for analysis on an Agilent 1100VL system. Theaflavin compounds were quantified using an Agilent column (250 × 4.6 mm, 5 μm) with solvents A (water solution with EDTA-2Na, acetic acid, and acetonitrile) and B (water solution with EDTA-2Na, acetic acid, and methanol). The gradient program for solvent A varied from 100 % to 70 % over 0–30 min. Separation occurred at 278 nm, 35 °C, and 0.7 mL/min flow rate. Quantitative content was calculated using analytical curves (R2 > 0.990).

2.7. Assessing volatile metabolite by HSPME-GC–MS

In a 2 mL tube, 50 mg sample was mixed with 500 μL methanol-water solution (4:1), then 200 μL chloroform was added. After centrifugation, the supernatant was dried with nitrogen. Following oximation and derivatization, samples were analyzed by GC–MS. An 8890B gas chromatograph with a 5977B mass selective detector and DB-5MS capillary column was used. GC conditions: 60–310 °C at 8 °C/min with 1 mL/min helium flow. MS conditions: 50–500 *m/z* range, 150 °C quadrupole temperature, and 230 °C ion source temperature. Data preprocessing with MassHunter workstation involved removing false positive peaks and internal standards. Metabolite identification utilized NIST, Fiehn, and MS-DIAL databases.

2.8. Assessing non-volatile metabolites by LC-MS/MS (UHPLC–Q-Exactive HF/X) analysis

A 400 μL solution of methanol:water (4:1, *v*/v) with L-2-chlorophenylalanine (0.02 mg/mL) as internal standard was used for solid sample extraction of 50 mg. After settling at −10 °C, the sample underwent crushing, ultrasound treatment, and protein precipitation. LC-MS/MS analysis was performed using a Thermo Fisher Scientific system (UHPLC-Q Exactive HF/X) with an HSS T3 column. Solvent gradient changed from 0 % to 100 % B over 0.1–13 min, with a 2 μL sample injection and 0.4 mL/min flow rate at 40 °C. Data was acquired in positive/negative ion modes using Data Dependent Acquisition (DDA) mode. Raw LC/MS data was processed with QI Progenesis software, removing false positives and identifying metabolites using databases like Metlin and HMDB.

2.9. Statistical analysis

Experimental data were analyzed using DPS Professional (Version DPSv9.50) to optimize black tea formulation. GC/MS and LC/MS analyses were replicated five times, while tea macro compounds and HPLC analysis were replicated three times, with results presented as mean ± standard deviation (SD). SPSS 20.0 for Windows was used for one-way ANOVA and Duncan's multiple range comparison tests, with significance set at $p < 0.05$. Metabolomics data underwent multivariate statistical analysis including principal component analysis (PCA) and partial least squares discriminant analysis (PLSDA) using the R package ropls (Version 1.6.2). Data were visualized as heat maps via hierarchical clustering analysis (HCA) using Scipy (Python 1.0.0) to examine metabolite distribution patterns across tea samples. Significant metabolites were identified based on variable importance in project (VIP) values and student's *t*-test (*p*-value), with $p < 0.01$ and VIP > 1.5 considered significant. Identified metabolites underwent metabolic pathway and enrichment analysis using KEGG to map their involvement in biochemical pathways.

3. Results and discussions

3.1. Uniform design and experimental results

After the use of uniform design in the current study U^*_{15} (15^7), the black tea was manufactured through different methods including different withering times, fermentation times, and fermentation temperature. Following the processing of black tea using various techniques, a sensory assessment of five factors, including (a) appearance (b), aroma (c), liquor color (d), taste and (e) infused tea leaves, were assessed by tea professionals and the result for each production method (Table 1). The factors' ranges were as follows: X_1 (14-22h), X_2 (2−10h), and X_3 (26–34 °C).

To determine the optimized condition of parameters, stepwise polynomial regression analysis was conducted, examining the impact of withering time (X1), fermentation time (X2), and fermentation temperature (X3) on the sensory attributes of black tea (Y). DPS software exhibits the polynomial regression evaluation between the performance score "Y" and various factors and the regressed equations are mentioned below:

$$\begin{aligned} Y = {} & 83.5421744 + 0.8672351103X_1 + 1.3744538494X_2 \\ & -0.4087980366X_3\ 0.16110716588X_2X_2 \\ & + 0.022866518190X_3X_3 + 0.06808768923X_1X_2 \\ & -0.04911968380X_1X_3 - 0.014688133782X_2X_3 \end{aligned} \quad (1)$$

Table 1
Uniform design experimental plan U^*_{15} (15^7) with results.

Test/ Process	Factors			Y Sensory Score
	X1 Withering Time (hrs)	X2 Fermentation Time (hrs)	X3 Fermentation Temperature (°C)	
1	1 (14)	5 (10)	13 (30)	85.6
2	2 (16)	10 (10)	10 (34)	87.46
3	3 (18)	15 (10)	7 (28)	86.26
4	4 (20)	4 (08)	4 (32)	88.47
5	5 (22)	9 (08)	1 (26)	88.8
6	6 (14)	14 (08)	14 (32)	88.3
7	7 (16)	3 (06)	11 (26)	89.88
8	8 (18)	8 (06)	8 (30)	88.16
9	9 (20)	13 (06)	5 (34)	86.26
10	10 (22)	2 (04)	2 (28)	86.3
11	11 (14)	7 (04)	15 (34)	89.76
12	12 (16)	12 (04)	12 (28)	86.64
13	13 (18)	1 (02)	9 (32)	85.17
14	14 (20)	6 (02)	6 (26)	83.56
15	15 (22)	11 (02)	3 (30)	83.3

From the analysis of quadratic polynomial regression, the correlation indices including the correlation coefficient (R) of the equation, the overall F-statistic (F) value, the level of significance (*p*-value), the residual standard deviation (S), the adjusted correlation coefficient (Ra), and the Durbin-Watson statistic (d), were determined as follows: $R = 0.9335$, $F = 5.0854$, $p = 0.0314$ ($p < 0.05$), $S = 1.1053$, $Ra = 0.8367$, and $d = 2.6777$, respectively. If the ($p < 0.05$), Ra is closer to 1, and the Durbin-Watson statistic value is close to 2, it signifies statistical significance in the regression analysis and from the above statistical parameters it can be seen that this regression equation is well fitted. According to (Xu & Yun, 2003) if the R value is closer to 1, then stronger the correlation in the predicted and obtained value. In this study, the correlation coefficient $R = 0.9335$ indicates strong agreement between predicted and experimental values. A comparison among the observed value, fitted value and fitting error, and the correlation related to each factor is shown in (Table 2a), whereas (Table 2b) represents the relevant statistical results of quadratic polynomial stepwise regression analysis.

The T-test value assesses the credibility of statistical results, with a larger t-value indicating higher credibility. Based on the t-test values, the order of influence of different factors and their interactions on evenness is as follows: X2X2 > X1X3 > X1X2 > X1 > X2 > X3X3 > X2*X3 > X3. The regressed equations derived from the uniform design results are applicable only within the specified ranges of the investigated parameters. The analysis results indicated a good fit for the equation. Subsequently, for solving the regression equation a DPS software was used (1), following the method previously described by (Zhang & Zheng, 2012). Using Eq. (1), the optimal parameters of the uniform design for producing optimized black tea were determined, as illustrated in (Table 3).

The optimized black tea processing involved withering for 14 h, and fermentation for 5.6 h at 34 °C. Following this, the sample underwent comprehensive analysis, including metabolomics, determination of tea quality components, and determination of catechins, gallic acid, caffeine and theaflavin compounds.

Table 2
(a) Comparison among the observed value, fitted value and fitting error (b) Results of quadratic polynomial stepwise regression.

(a)

Experiment No:	Observed value	Fitted value	Fitting error
1	85.6	86.1288	−0.5288
2	87.46	86.7653	0.6947
3	86.26	86.654	−0.394
4	88.47	87.6027	0.8673
5	88.8	88.967	−0.167
6	88.3	88.5621	−0.2621
7	89.88	88.5051	1.3749
8	88.16	88.1002	0.0598
9	86.26	87.6411	−1.3811
10	86.3	86.1114	0.1886
11	89.76	89.5725	0.1875
12	86.64	87.526	−0.886
13	85.17	84.8088	0.3612
14	83.56	84.2379	−0.6779
15	83.3	82.7373	0.5627

(b)

Factors	Partial Correlation	T-test	*p-value*
r(y,X1)	0.26	0.6594	0.5307
r(y,X2)	0.2164	0.543	0.604
r(y,X3)	−0.0489	0.12	0.9079
r(y,X2*X2)	−0.7778	3.0314	0.0191
r(y,X3*X3)	0.1712	0.4256	0.6832
r(y,X1*X2)	0.4207	1.1359	0.2934
r(y,X1*X3)	−0.4343	1.1811	0.2761
r(y,X2*X3)	−0.1221	0.3014	0.7719

Table 3
A uniform design optimal parameters.

Factors	X1 Withering Time	X2 Fermentation Time	X3 Fermentation Temperature
Best Combination	14 h	5.6831 h	34 °C
Predictive value of sensory analysis	90.024		

3.2. Characteristics of metabolites at different processing stages

3.2.1. Assessing determination of volatile compounds by HS-SPME-GC–MS (Chemometrics Analysis) during processing of black tea

Investigating the unique flavor of *Yunkang* 10 black tea and its correlation with processing, we conducted GCMS-SPME extraction to isolate volatile compounds and a total of 157 volatile components, including 7 aldehydes, 3 organosulfur compounds, 2 phenols, 8 alcohols, 3 amines, 27 Terpenoids, 13 esters, 9 hydrocarbons, 5 ketones, 24 heterocyclic compounds, 2 ethers and 52 others, were identified in *Yunkang 10* black tea. The comparative contents within each group were assessed across various processing steps, as shown in (Fig. 1). Notable variations were observed in the quantities of all the groupings across different stages.

The TIC plots (Fig. 2a) showed the differences in volatile compounds among the five processing stages (FTL, WL, RL, FL, and DL) and revealed that the five stages, differed from each other, particularly for 19 volatile compounds, including 2-methyl butanal, Hexanal, Benzeneacetaldehyde/Phenyl Benzeneacetaldehyde, Linalool, Linalyl acetate, 3-Carene, (+)-4-Carene, Linalool oxide I, Linalool oxide II, Geraniol, Hexanoic acid,3-hexenyl ester, Indole, Butanoic acid, Trans-Linalool oxide(furanoid), 3- hexenyl ester, Benzaldehyde, Methyl salicylate, Benzyl alcohol, (2*R*)-2-hydroxy-3-methylbutanenitrile, and Isonitrosoacetylacetone. The major volatile compounds found in FTL, were 2 methyl butanal (6.0 %), Hexanal (b) (5.68 %), Linalool.

(5.31 %), 3-Carene (24.53 %), (+)-4-Carene (24.41 %), Linalool oxides (Linalool oxide I, Linalool oxide II, and Trans-Linalool oxide) (3.26 %), Geraniol (6.47 %), Hexanoic acid, 3-hexenyl ester (4.96 %), Indole (3.24 %), Butanoic acid, 3-hexenyl ester (2.65 %), Benzaldehyde (0.49 %), Methyl salicylate (5.43 %), Benzyl alcohol (2.02 %), and (2*R*)-2-hydroxy-3-methylbutanenitrile (5.52 %) (Fig. 2b). Yet, in dried leaves, 2 methyl butanal, Hexanal, 3-Carene, (+)-4-Carene, Indole, isonitroacetylacetone, (2*R*)-2-hydroxy-3-methylbutanenitrile, Butanoic acid, III hexenyl ester, and Hexanoic acid, III hexenyl ester completely vanished, whereas the concentration of linalool oxides containing (Linalool oxide I/II and Trans-Linalool oxide) (13.42 %), geraniol (13.27 %), Benzeneacetaldehyde (28.42 %), benzyl alcohol (7.97 %), Benzaldehyde (5.88 %), Methyl salicylate (15.98 %, and linalyl acetate (6.97 %) kept increasing throughout processing (Fig. 2b).

The multivariate statistical analysis, PLSDA, revealed significant fluctuations in volatile compounds throughout black tea processing. The PLS-DA model cross-validation (Fig. 3a, and b) demonstrated a high level of accuracy in distinguishing volatile components throughout the black tea processing. The Q^2 value above 0.95 indicates a significant impact of black tea processing on the volatile compounds. Moreover, we observed Q^2 and R^2Y values of 0.992 and 0.999, respectively. Additionally, the intercept of Q^2 and the Y-axis was below 0, indicating that without over fitting the model possessed effective predictive ability. In differentiation among processing stages, not all volatile components played a crucial role. Following PLS-DA analysis, compounds having a VIP > 1.5 were identified as a key differential volatile and were screened for further investigation. Subsequently, 19 VOCs showed a VIP value over 1.5 (Fig. 3c).

Within the set of 157 detected volatile compounds, twenty-four compounds were found to be newly produced. In contrast, 80 volatile compounds responsible for the grassy odor in black tea, declined to

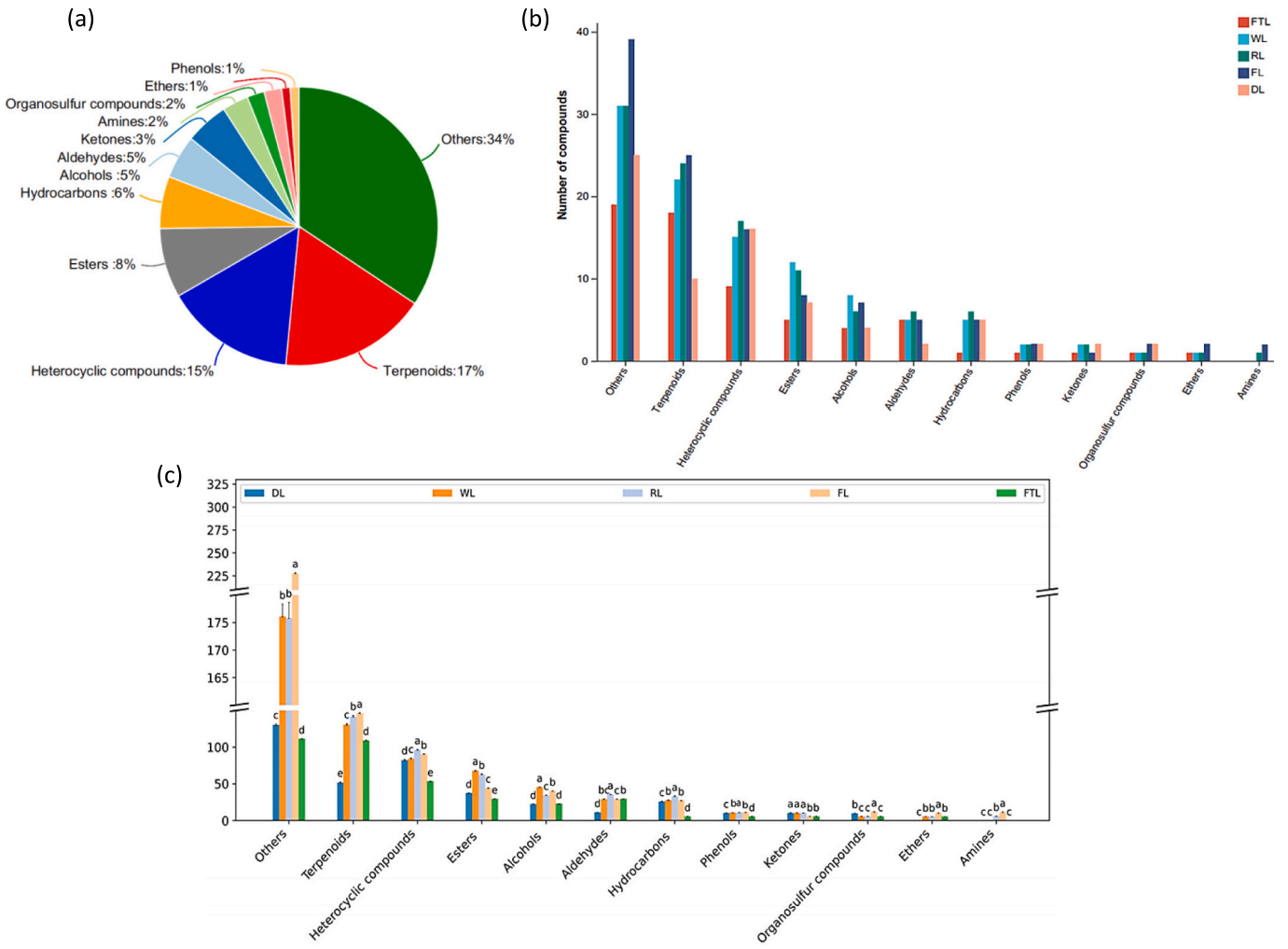

Fig. 1. The proportion of identified volatiles of various categories. (a) The ratios of the 157 volatile components during processing of black tea. (b) The quantity of various volatile metabolites assessed at each stage of processing. (c) Volatile compounds classification. Significant variations at the $p < 0.05$ level are shown by lowercase letters within the same volatile categories.

negligible during manufacturing and the rest of 53 VOCs displayed fluctuating levels during the manufacturing stages.

3.2.2. Key attributes of volatile metabolites during various stages of processing

The grassy odor has been claimed to be produced by aromatic compounds such as Hexanal, 2-methyl butanal, butanoic acid-3-hexenyl ester, indole, hexanoic acid-3-hexenyl ester, 3-Carene, and (+)-4-Carene. The contents of the compounds, mentioned above including Isonitroacetylacetone and (2*R*)-2-hydroxy-3-methylbutanenitrile, were found to be gradually decreased during processing and finally disappeared in dried leaves. Significantly, geraniol, benzyl alcohol, linalool oxides (Linalool oxide I/II and Trans-Linalool oxide), benzeneacetaldehyde, methyl-salicylate, benzaldehyde, and linalyl acetate rose as the main volatile constituents in the final tea product, exhibiting increased content. The content of benzeneacetaldehyde, geraniol, and linalool oxides in dried leaves was considerably higher than that of fresh tea leaves. The aforementioned compounds exhibited a favorable correlation with black tea's floral and sweet taste. These characteristics volatile compounds ($p < 0.05$), and (VIP > 1.5) showed that black tea processing facilitated the accumulation of volatile compounds, notably terpenoids and aldehydes. Additionally, we found that accounting for almost 25 % of all volatile chemicals detected in *Yunkang* 10 black tea, aldehydes were the most prevalent group. During fermentation, alcohols undergo oxidation to form aldehydes, leading to a transformation of the tea aroma to fruity from green grassy, roasted, floral, or sweet and converts phenethyl alcohol and benzyl alcohol into phenylacetaldehyde and Benzaldehyde. Phenyl acetaldehyde/Benzeneacetaldehyde is noted for its floral and honey-like aroma in tea, Methyl salicylate is known to play a crucial role in the overall aroma of tea, lending it a floral and sweet fragrance, benzyl alcohol and linalool lend a sweet flowery flavor to black tea, a rose flower-like fragrance is contributed by geraniol which is characteristic of *Yunkang 10* black tea. In black tea, the linalool content increased in FTL from 5.31 % to 7.97 % in DL. Linalool is recognized as the black tea's characteristic aroma compound, primarily produced during fermentation from the hydrolysis of glucoside. Enzymatic formation of linalool oxides from glycoside precursors contributes to its pleasant, flowery aroma. Usually, phenylethyl-alcohol, linalool and its oxide (furanoid), primarily originate from the hydrolysis of trans-linalool 3,6 oxide-6-O-β-D glucopyranoside, cis-linalool 3,6-oxide-6-O-β-D glucopyranoside, Linalyl β-D glucopyranoside, and phenylethyl β-D glucopyranoside catalyzed by β-glucosidase (Ni et al., 2021). During the drying process, the Maillard reactions in tea leaves contribute to an increase in the levels of linalool and its oxides, thereby enriching the black tea's aroma. The breakdown of carotenoids also leads to the production of aromatic volatiles. Throughout the processing of black tea, Group I volatile compounds, primarily comprising ketones, aldehydes (such as trans-2 hexenal, heptanal, and hexanal), and alcohols with a grassy flavor, undergo a rapid increase during withering and fermentation, followed by a sharp decline during drying. Conversely, Group II VFC,

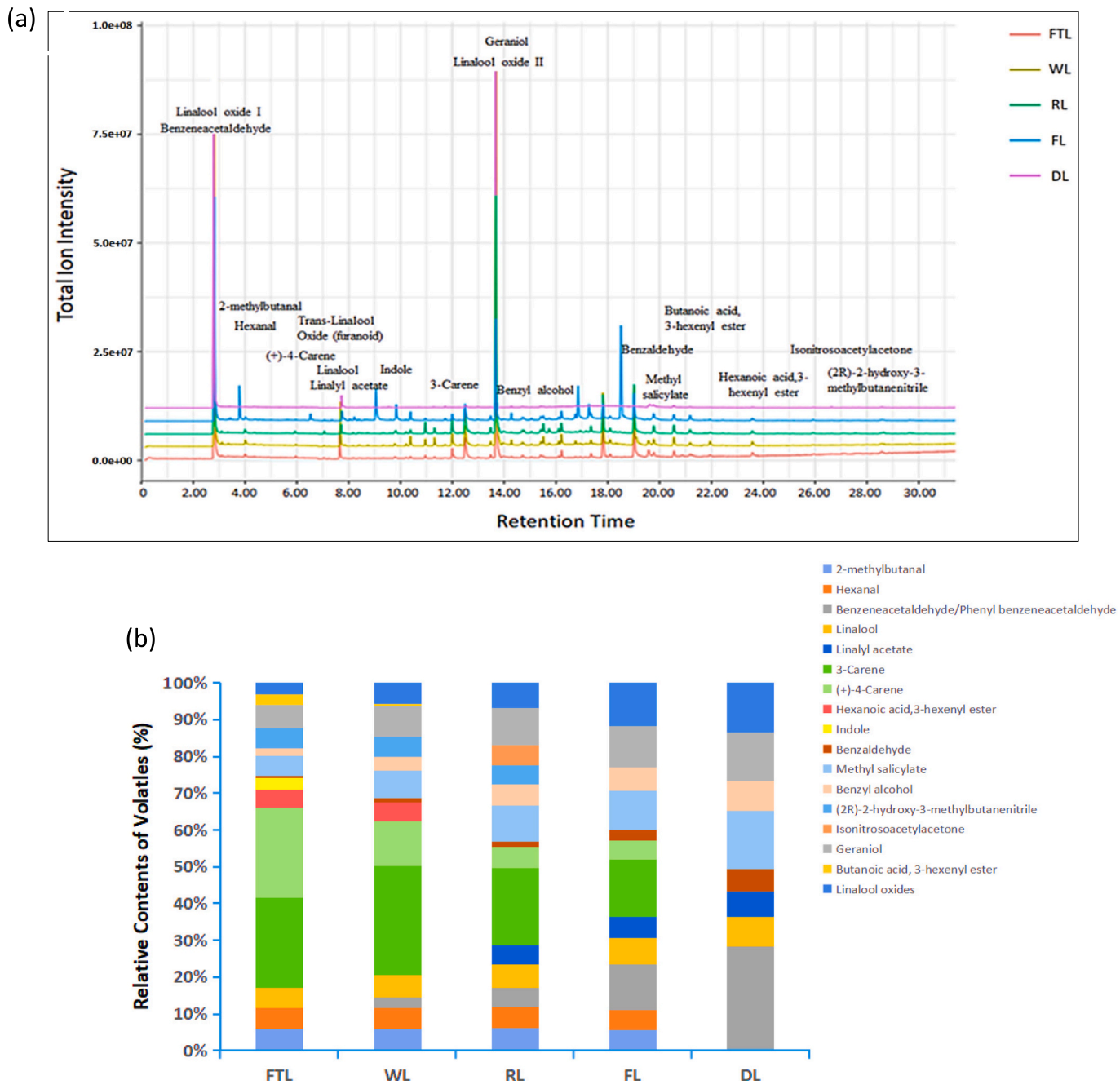

Fig. 2. (a) Total ion chromatogram representing each processing stage of black tea manufacturing including plucked leaves (FTL), withering (WL), rolling (RL), fermentation (FL) and drying (DL). (b) Changes in volatile components of tea leaves during five processing steps including FTL, WL, RL, FL, and DL. The combined contents of linalool oxides I, II, and (furanoid) are referred to as linalool oxides.

dominated by phenols, esters (such as methyl salicylate), and terpenes (like geraniol, linalool, and phenylacetaldehyde), with sweet and flowery flavors, maintains elevated levels during the DL (Fig. 2b), leading to a substantial rise in the dry tea's flavor.

3.3. Non-volatile metabolite profiling by LC-MS (UHPLC-Q Exactive HF/X) and multivariate statistical analyses

By employing Progenesis QI software, 2642 metabolites were identified through qualitative matching analysis in positive and negative ion modes. Among these, 1890 metabolites (1032 in positive mode and 858 in negative mode) met fragmentation score criteria ≥50 and RSD criteria ≤30 for statistical analysis. Base peak chromatograms of FTL, WL, RL, FL, and DL showed robust signal detection and significant peak capacity across all samples. Multivariate data analysis was conducted on non-volatile metabolites to examine variations across black tea processing stages. During analysis a stable performance of the experimental platform was observed by using the quality control sample (QC) as shown in the PCA plot (Fig. SM 1), showed tight clustering, and ensured the reliability of the test data. This clustering also demonstrates the good stability and reproducibility of the non-targeted approach using the LC/MS (UHPLC-Q-Exactive HF/MS) methodology. The PCA score plot demonstrates distinct group patterns for the five processing stages, indicating significant alterations in non-volatile compounds throughout black tea processing revealing clear sample separation in both positive and negative ion modes (Fig. 4a, and b). PC1 and PC2 explained 54.10 %

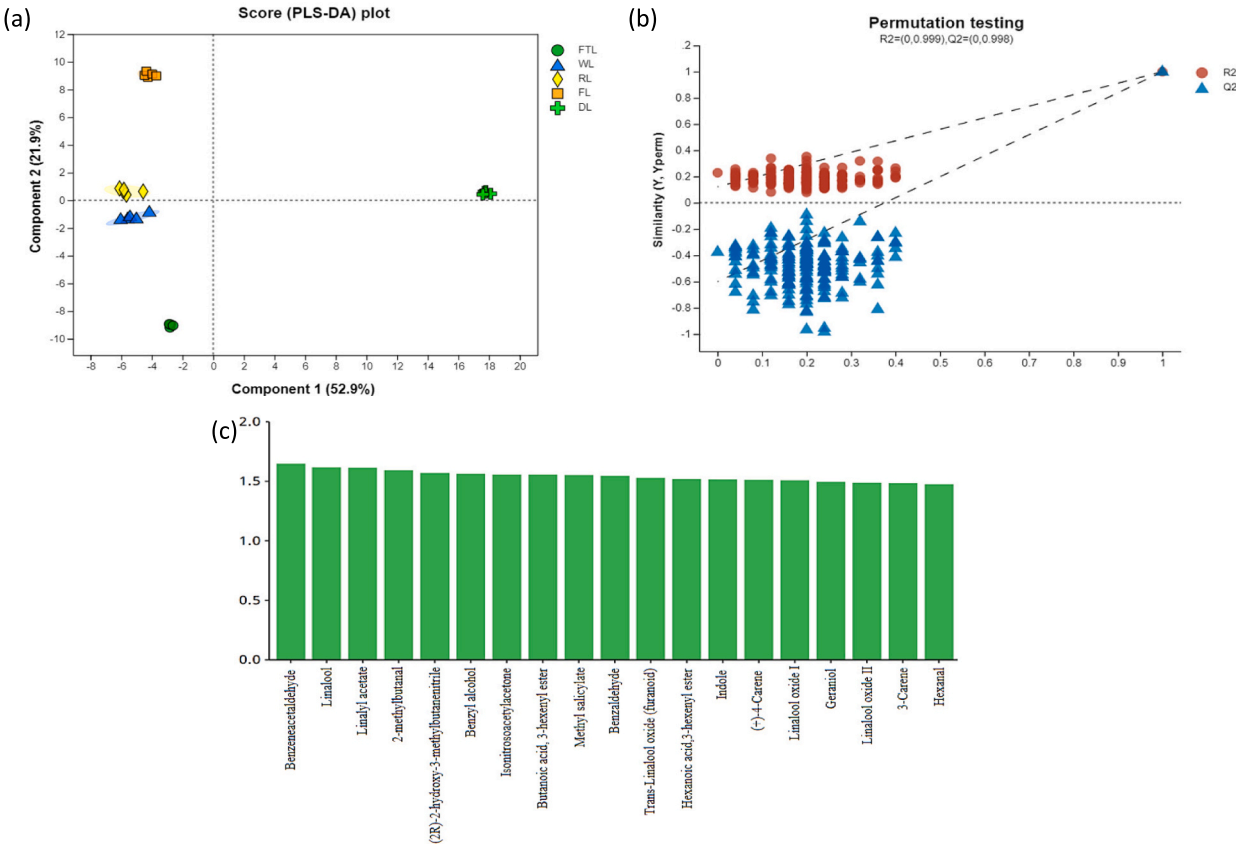

Fig. 3. (a) The volatile components PLS-DA scores plot (b) The volatile components permutation test plots (c) The VIP values of the 19 volatile components found in black tea.

and 21.80 % of total the variance in positive ion mode (Fig. 4c), and 60.5 % and 21.5 % in negative ion mode (Fig. 4f). Metabolite profiles accurately classified samples from each stage, highlighting the substantial impact of processing on tea metabolome. A clear separation was observed between stages, particularly evident between FL and DL, indicating significant metabolite changes during the final stage. A supervised analytic technique called PLS-DA was used to observe the variations between the processing stages (Fig. 4d, and g). In agreement with the PCA finding, the PLS-DA score plot showed clear variations among the non-volatile components at various processing stages. The permutation plots generated from PLS-DA demonstrated the model's strong predictive ability without over fitting (Fig. 4e and h). Following 200 permutations, the intercept values of R2 were 0.999 for ESI+ and 1.0 for ESI−, with corresponding Q^2 values of 0.992 and 0.996, respectively. These results indicate the model's good credibility and interpretability. R^2 measures the goodness of fit, while Q^2 determines the predictive ability of the model in the confidence test. The 200-times confidence test shown in (Fig. 4e, and h), suggests that the multivariate statistical analysis's findings are reliable.

Around 50 differential characteristic metabolites were point out after conducting multivariate statistical analysis, with VIP > 1.5 and *p*-value <0.01, including 18 flavonoids/flavonoids glycosides, 6 organoheterocyclic compounds, 4 alcohol and polyols, 3 amino acids and its derivatives, 2 organic acids, 2 benzoic acids and derivatives, 1 carbonyl compounds, 2 phenylpropanoids, 1 terpenoid/terpene glycosides, 1 alkaloids, 1-carobohydtrate/sugar acid derivative, and 9 others.

Hierarchical clustering based on heat map visualization was used to investigate the differences in the contents of 50 metabolites during processing (Fig. 5). Obvious changes can be seen for those 50 biomarker metabolites in which the blue color represents lower levels of metabolites, while the red color represents higher levels.

The major pattern observed for alterations in metabolite was 1) An increased level was observed of Amino Acids and derivatives during processing (Theanine, L-Theanine, and Temocaprilat) 2) A decreased level was observed in flavonoids and flavonoid glycosides including (hyperoside, Catechin 7-O-gallate, 6"-Caffeoylhyperin, Rutin, Epicatechin, Theaflavate A, Kaempferol 3-rhamnosyl-(1- > 3) (4‴-acetylrhamnosyl)(1- > 6)-glucoside, Carthamidin 6,7-diglucoside, (2R) Naringenin 8-C alpha-L-rhamnopyranosyl-(1- > 2)-beta-D-glucopyranoside, Quercetin 3-[rhamnosyl-(1- > 2)-rhamnosyl-(1- > 6)-glucoside], Isoneotheaflavin, (−)-Epicatechin Gallate, (−)-Epiafzelechin 3-gallate, Quercetin) whereas (−)-Epigallocatechin gallate, Schaftoside, (+/−)-Catechin, and 6"-O-Acetylglycitin showed a rising trend during processing. 3) Alcohol and Polyols showed an increasing trend (5-p-coumaroylquinic acid, Theogallin, Quinic Acid, and 4-p-Coumaroylquinic acid). 4) Among the organoheterocyclic compounds Indoleacrylic acid, Azinphosmethyl oxon, and 5'-Hydroxypiroxicam showed a decreasing trend whereas Paramethadione, Indoline, and Ethyl maltol rose with processing. 5) Among the organic acids Vanillic acid 4-O-glucuronide showed an increasing trend while Isocitrate decreased as processing progressed. 6) Benzoic Acids and derivatives showed the content of 2,6-Digalloylglucose was found to be increased as processing progressed whereas Methyl Gallate decreased. 7) Carbonyl compounds (Keto-1,4-benzoquinone) were found to be increased as processing progressed. 8) Phenylpropanoids (Ellagic Acid and Sanguiin H4) showed an increasing trend. 9) Alkaloids (23-Acetoxysoladulcidine) were increased as processing progressed and showed maximum values at the drying stage. 10) Carbohydrates/Sugar Acid derivatives (Gluconic acids) initially decreased then increased and finally decreased at the drying stage. 11) Others metabolites showed a wavy fluctuations during processing. The heat map analysis shows the accumulation of amino acids, alcohols and polyols, Carbonyl compounds, Phenylpropanoids,

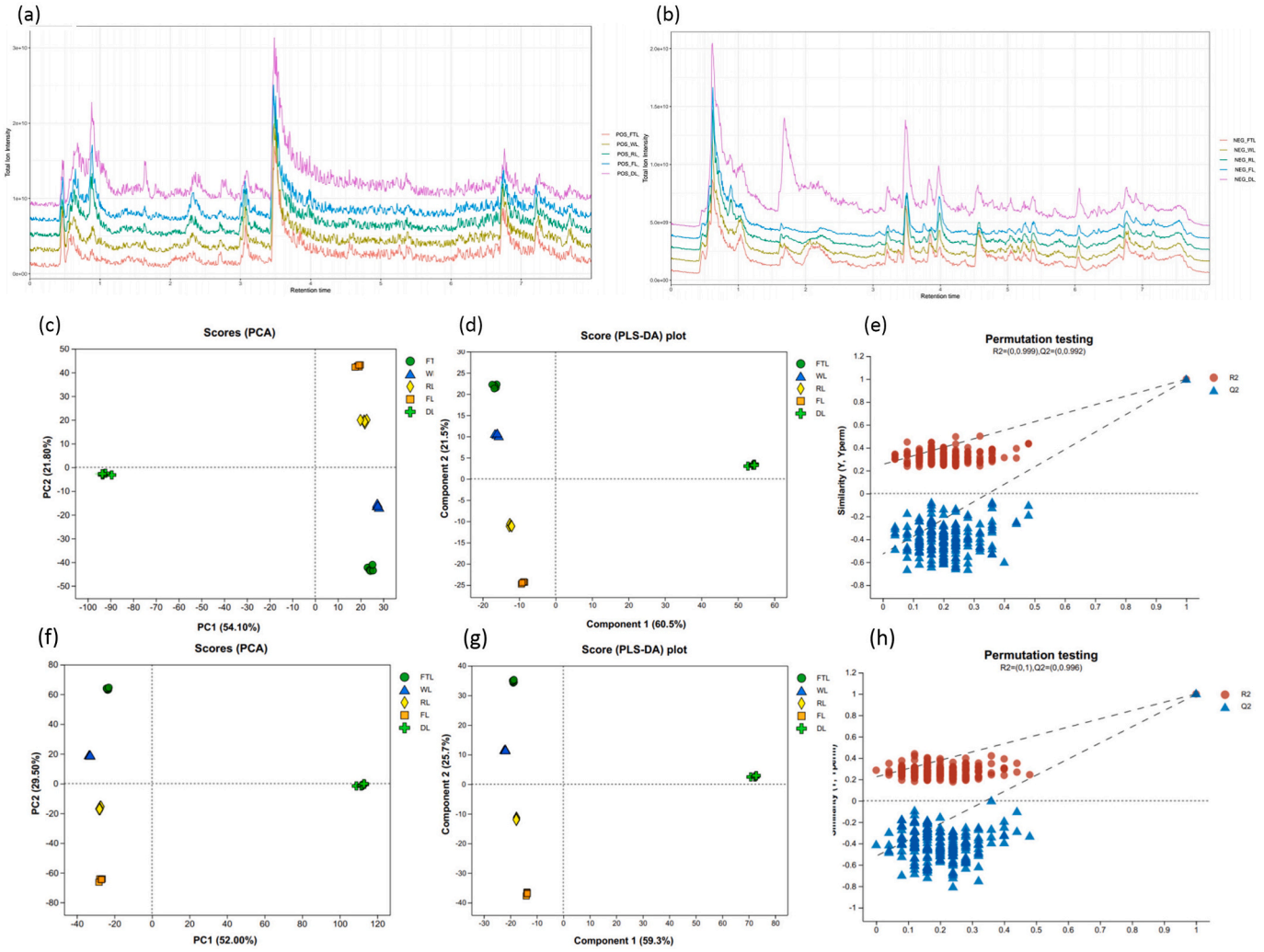

Fig. 4. Results of multivariate statistical analysis for metabolites differences at each processing stage. (a) Base peak chromatograms of black tea at each processing stage were obtained using LC-MS in positive mode of ionization. (b) A black tea's base peak chromatograms at each processing stage were obtained using LC-MS in negative ionization mode. (c) The score of PCA in positive mode of ionization. (d) The PLS-DA score in positive mode of ionization. (e) The PLS-DA model validation in positive ion mode. (f) The score of PCA in negative mode of ionization. (g) The score of PLS-DA in negative mode of ionization. (h) The PLS-DA model validation in negative mode of ionization.

Terpenoids/Terpene Glycosides, and Alkaloids, whereas, among the flavonoids and flavonoid glycosides, the accumulation was found in (−)-Epigallocatechin gallate, Schaftoside, (+/−)-Catechin, and 6"-O-Acetylglycitin, in organoheterocyclic compounds Paramethadione, Indoline, and Ethyl maltol, in organic acids Vanillic acid 4-O-glucuronide, in Benzoic Acids and derivatives 2,6-Digalloylglucose, and among others metabolites (Quinone A and Pheophorbide a).

3.3.1. *Major changes in differential non-volatile metabolites*

3.3.1.1. Amino acids and derivatives. During processing of black tea, significant ($p < 0.01$) variations in amino acids and derivatives were found. L-theanine is one of the key amino acidsthat contribute flavor to black tea (Yu & Yang, 2019) and was found to be increased at the drying stage. Theanine, known for enriching the sensory characteristics, and black tea's flavor with its distinctive increased sweetness, and umami taste was observed to be elevated during the drying stage. L-Theanine was found to be relatively stable at a withering stage which was in agreement with (Fang et al., 2023) who also declared that some amino acids were found to be increased after withering. Theanine and L-theanine were low at initial stage (FTL) of processing but rose significantly in fermented leaves, and then declined at the final stage (DL). Temocaprilat levels were observed to be elevated in fresh tea leaves, decreased during the fermentation (FL), and peaked again at the final stage of drying (DL). Through a Maillard reaction, L-theanine also plays a role in generating roasted and caramelized aromas, such as pyrazine (Zhou et al., 2019) which may explain why at the drying stage its content is comparatively lower than that of the fermentation stage.

3.3.1.2. Organic acids. The main organic acids found were vanillic acid 4-O-glucuronide and isocitrate which were found to be decreased at the fermentation stage which is declared by (Xiao et al., 2022) that some of the organic acids significantly decreased ($p < 0.05$) during fermentation. Isocitrate was found to be highest in the withered leaves (WL) and negligible in the dried leaves. In contrast vanillic acid 4-O-glucuronide showed a negligible amount of increase at the drying stage which contributes a sweet and aromatic flavor and enhanced sensory profile by imparting subtle vanilla-like notes which can complement the natural flavor of black tea. One of the crucial factors for the smoothness, mellowness, and decreased sourness and bitterness in black tea may be the variation in these metabolites.

3.3.1.3. Flavonoids/flavonoids glycosides. Heat map analysis and one-way ANOVA exhibited a significant variation ($p < 0.01$) among 19

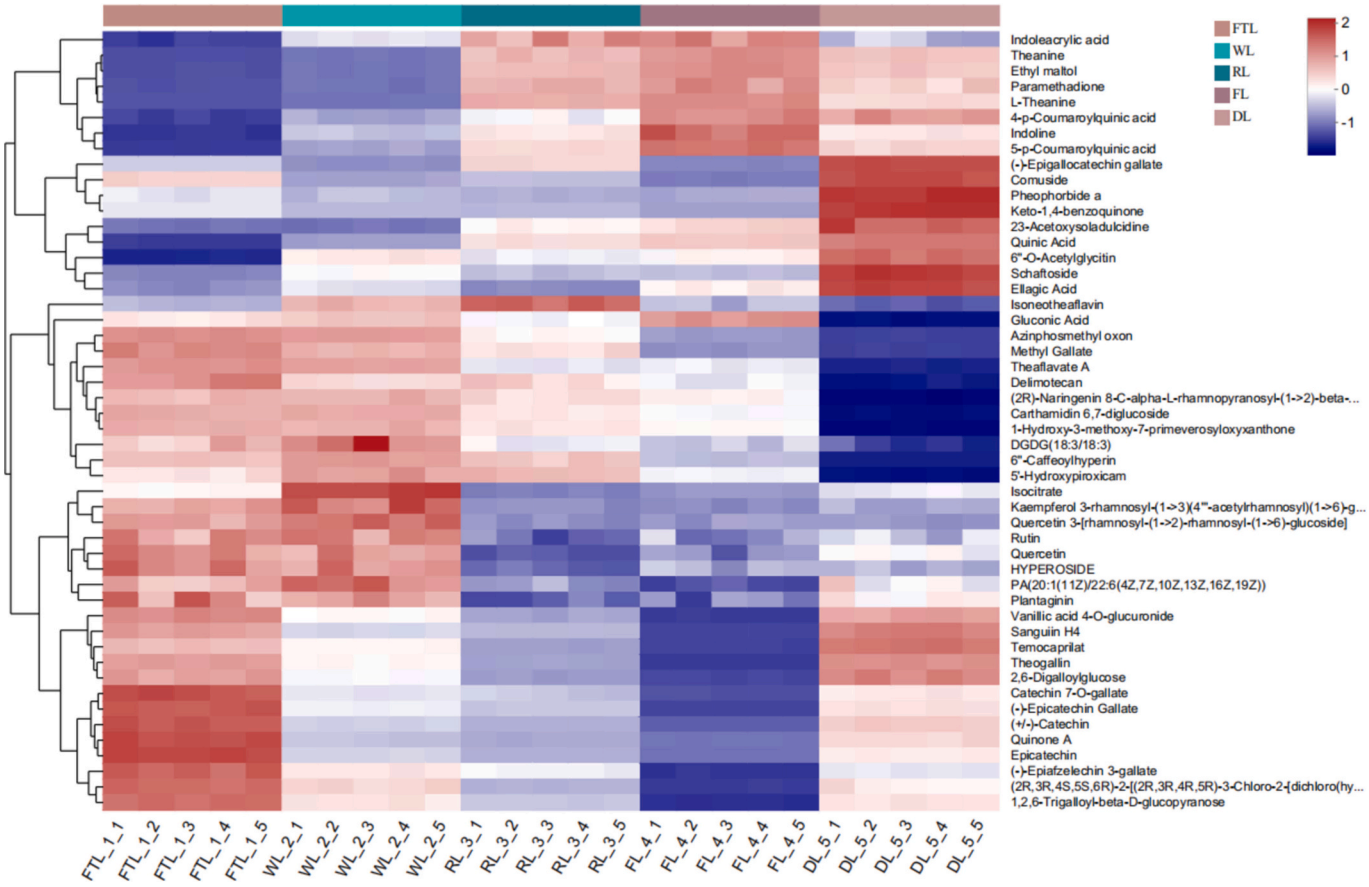

Fig. 5. A hierarchical clustering heat map for 50 different Non-volatile metabolites, meeting the criteria of $p < 0.01$ and VIP > 1.5, demonstrating the unique influence of processing on characteristic metabolites.

flavonoids and their glycosides during black tea manufacturing. Four differential flavonoids and their glycosides; (−)-Epigallocatechin gallate, Schaftoside, 6''-O-Acetylglycitin, and comuside were significantly ($p < 0.05$) increased during processing and found maximum at the drying stage whereas the rest of fifteen metabolites including hyperoside, Catechin 7-O-gallate, 6''-Caffeoylhyperin, Rutin, Epicatechin, (−)-Epigallocatechin gallate, Theaflavate A, Kaempferol 3 rhamnosyl-(1- > 3) (4‴-acetylrhamnosyl)(1- > 6)-glucoside, Schaftoside, Carthamidin 6,7-diglucoside, (2*R*)-Naringenin-8-C-alpha-L-rhamnopyranosyl-(1 > 2)-beta-D-glucopyranoside, Quercetin 3-[rhamnosyl-(1- > 2)-rhamnosyl-(1- > 6)-glucoside], Isoneotheaflavin, (−)-Epicatechin Gallate, (−)-Epiafzelechin-3-gallate, (+/−)-Catechin, 6''-O-Acetylglycitin, and Quercetin showed a significant variation particularly, at the withering stage and were found to be decreased significantly ($p < 0.05$) during processing and showed minimum values at drying stage. This significant decrease in most of the catechins compounds is most probably due to Catechins transformation into theaflavins by polyreaction and condensation during withering. Previous literature extensively documented the significant impact of flavones and flavonols on the bitterness and astringency of tea infusions. Therefore, the conversion or degradation of flavones and flavonols could potentially influence the distinctive taste profile of dark tea. During black tea processing, flavonoids and flavonoid glycosides undergo enzymatic oxidation by enzymes polyphenol peroxidase and oxidase, which tend to decrease in their concentration. The oxidation leads to theaflavins and thearubigins formation, which impart the aroma, flavor, and characteristic color to black tea. This transformation is crucial for developing the unique sensory properties and overall quality of black tea. Catechins are susceptible to high temperature, making them prone to isomerization and degradation during both the heating and storage. Moreover, during rolling and fermentation, tea catechins decrease due to decomposition caused by autoxidation by enzymes polyphenol peroxidase and oxidase. During rolling, the stress on leaves causes vacuolar membranes to rupture, potentially allowing PPO and PO enzymes to come into contact with catechins, initiating oxidation. This process leads to intermediate orthoquinones formation, which react to produce theaflavins and thearubigins a phenolic derivatives during the rolling and fermentation process of black tea (Obanda et al., 2001). Formation of these compounds leads to the color alteration from green to copper-brown, accompanied by a significant alteration in the aroma of the tea as fermentation progresses. The reduced levels of EGC and EC can be attributed to the polyphenolic compounds oxidation, like catechins, whereby benzotropolone transforms them it into brown colored substance with enriched aroma. Additionally, during processing the decline in catechin compounds correlates with the elevated presence of free amino acids detected in *Yunkang* 10 tea leaves, potentially enhancing their enrich taste and imparting a smooth and mellow flavor.

3.3.1.4. Alcohol and polyols. The main non-volatile differential metabolites (alcohol and polyols) found in black tea were 5-p-coumaroylquinic acid, Theogallin, 4-p-coumaroylquinic acid, and Quinic Acid which exhibited a significant ($p < 0.01$) variation during black tea processing. The 4-p-coumaroylquinic acid was found to be minimum in withered leaves and maximum in fermented and dried leaves whereas 5-p-coumaroylquinic acid was found to be minimum at the withering stage and maximum in fermented leaves and then decreased again in dried leaves. Quinic Acid showed a gradual increase from the rolling stage and showed a maximum content in dried leaves. Theogallin was found to be negligible in WL and then showed minimum values at the FL and at the DL found to be increased. Quinic acid (polyol) contributes to the

astringency and sourness of tea and found to be significantly decreased at a withering stage. Theogallin is the main color contributing factor to plain tea (Biswas et al., 1973), and contributes umami flavor to tea. The 4-p-coumaroylquinic acid and 5-p-coumaroylquinic acid exhibited a decreasing trend after fermentation. During withering, tea leaves lose moisture, which may result in a slight concentration of non-volatile compounds like polyols. However, there are no specific transformations or reactions targeted at alcohols and polyols during this stage. During rolling breakdown of cell wall occurs which initiates oxidation alcohol and polyols content are likely minimal and incidental.

3.3.1.5. Alkaloids, Organoheterocyclic compounds, benzoic acid and derivatives, carbonyl compounds, Phenylpropanoids, Carbohydrates/Sugar Acid derivatives, and other trace non-volatile metabolites. The alkaloid found in this study was 23-Acetoxysoladulcidine which had shown a significant variation during processing stages and was found to be negligible at the rolling stage and after rolling gradually increased. The six Organoheterocyclic compounds exhibited significant ($p < 0.05$) changes during the black tea processing, in which Indoleacrylic acid (Indoles and derivatives), Azinphosmethyl oxon, 5'-Hydroxypiroxicam showed a decreasing trend whereas Paramethadione (Oxazolidines), Indoline, and Ethyl maltol (Pyranones and derivatives) has been significantly increased during processing. This decreasing trend is probably caused by oxidation, degradation, and potential formation of new compounds, leading to changes in their concentration and properties whereas the increase in some organoheterocyclic metabolites is due to the formation and release of compounds from precursors, as well as concentration effects during moisture removal. Indoleacrylic acid contributes aroma and flavor to black tea in trace amounts, while azinphosmethyl oxon, 5-hydroxyoyroxicam, paramethadione, indoline, and ethyl maltol are not typically associated with black tea quality as important as catechins, flavonoids, and alkaloids and are found in trace amount. Benzoic acid and derivatives include 2,6-Digalloylglucose which showed a significant variation during processing, at the FL found to be minimal but at the DL showed the maximum values and contributed to the astringency, color, antioxidant properties, and overall sensory characteristics of black tea, enhancing its quality whereas Methyl Gallate which contributes to the antioxidant properties significantly decreased during processing. The carbonyl compound Keto-1,4-benzoquinone showed an increased during processing and was found to be maximum in the dried sample which contributes to black tea quality by enhancing aroma, flavor, color, and chemical stability (Lin & Blank, 2003), declared that carbonyl metabolites are important flavor components and could be produced by the degradation of lipids. Carbohydrates/Sugar acid derivatives include gluconic acid as a differential metabolite in this study which was found to be negligible at the RL and then rose in the FL and at the DL again found to be minimal. Although gluconic acid may be present in trace amounts in black tea due to microbial activity or fermentation during processing. Phenylpropanoids include Ellagic Acid and Sanguiin H4. In this study, the content of phenylpropanoids showed significant variation and were found to be on the peak in the dried sample. Phenylpropanoids in black tea contribute to flavor, aroma, antioxidant properties, color, and chemical stability, enhancing its overall quality. Among other trace non-volatile metabolites Quinone A and Pheophorbide, a showed a significant variation and found to be lower at the withering, rolling, and fermentation stage but increased at the drying stage in which Pheophorbide a showed the maximum value at the drying stage. Quinone A, also known as 5-O-caffeoylquinic acid, likely contributes to the antioxidant properties, color stability, and potential health benefits of black tea. Pheophorbide A, a chlorophyll derivative, may influence color and potentially offer antioxidant properties. All other trace non-volatile metabolites found to be significantly decreased during black tea processing.

3.4. Dynamic variations in macro-compounds during the processing stages

3.4.1. Attitude of macro compounds toward processing

The one-way ANOVA using Duncan's test revealed significant variations in macro compounds during the processing stages. Free amino acids, water extract, tea polyphenols, and total soluble sugar decreased significantly ($p < 0.05$), while theaflavins, thearubigins, and theabrownin showed a significant increase ($p < 0.05$) (Fig. 6). Similarly, gallic acid exhibited a notable increase ($p < 0.05$), total catechins demonstrated a decreasing trend while caffeine content and four theaflavin compounds showed non-significant wavy fluctuations.

In this study, total soluble sugar had shown a significant decrease ($p < 0.05$), with fresh tea leaves and dried leaves showing a notable difference. However, during withering, rolling, and fermentation, non-significant fluctuations were noted. The soluble sugar content in fresh leaves was 6.18 % whereas in dried leaves it was decreased to 3.88 % which is most probably due to the plucked tea leaves losing their capacity for photosynthetic sugar synthesis, causing a significant reduction in glucose and fructose content during withering. Endogenous enzymes break down saccharides into monosaccharides, leading to a decline in disaccharides like sucrose, which becomes negligible during fermentation and drying. The hydrolysis of sucrose results in increases content of fructose and glucose post-withering. Following the rolling process, the presence of monosaccharides such as fructose, glucose, and galactose could enhance the sweet flavor of black tea. It has been confirmed that soluble sugar in black tea decreases due to loss of photosynthetic ability, respiration consuming sugars, enzymatic breakdown of higher molecular weight saccharides, reduction of disaccharides like sucrose, and accumulation of monosaccharide after rolling. Water extract content showed a significant ($p < 0.05$) decrease during processing. Fresh leaves exhibited the highest content at 29.12 %. Fermented and dried tea leaves showed no significant difference, with water extract contents of 22.32 % and 21.68 %, respectively. The decrease in water extract content is attributed to the leaching of soluble compounds during processing, enzymatic oxidation, fermentation, and alterations in the solubility of compounds, leading to reduced extractability in water. In this study, non-significant change in total amino acid content was noted in tea samples during the withering and rolling stages, but at fermentation and drying stage a significant decline was noticed. The free amino acid content exhibited an increasing trend at the withering stage (3.95 %), followed by a gradual decrease, reaching approximately 2.83 % in dried samples. Amino acids play indispensable role in determining quality of tea in terms of taste and color. This rise is attributed to degradation of protein by peptidases and proteases from ruptured cells (Yao et al., 2006) or amino acids derived from sugars. Amino acids have the potential to react with with orthoquinones and results in aroma change to sweet flowery from green grassy, potentially explaining the decrease in free amino acids during later processing stages. Tea polyphenol content significantly decreased at each processing stage of black tea, starting from 31.38 % in fresh leaves and decreasing to 14.83 % after withering, rolling, fermentation, and drying. This decline is linked to chemical changes like polymerization, oxidation, and transformation of polyphenols during tea processing (Fan et al., 2016). Withering induces physicochemical alterations in tea leaves, reducing content of moisture and enhancing permeability of cell membrane, while also contributing to the aroma formation of tea. The softened leaves facilitate rolling, which further disrupts cell structure, accelerating the enzymatic oxidation of polyphenols. The levels of black tea pigments, such as theaflavins, thearubugins, and theabrownins, showed a significant increase during processing. Initially, the theaflavins content in fresh tea leaves was recorded at 0.01 %, increasing to 0.04 % in withered leaves. However, no significant difference was noted during the first two stages of processing. Subsequently, during rolling, fermentation, and drying, a notable increase was observed, with the levels reaching 0.16 %, 0.33 %, and 0.42 %, respectively. The content of thearubugins was 2.94 %,4.04 %, 5.09 %, 6.01 %, and 7.03 % whereas of theabrownins was 1.95 %,

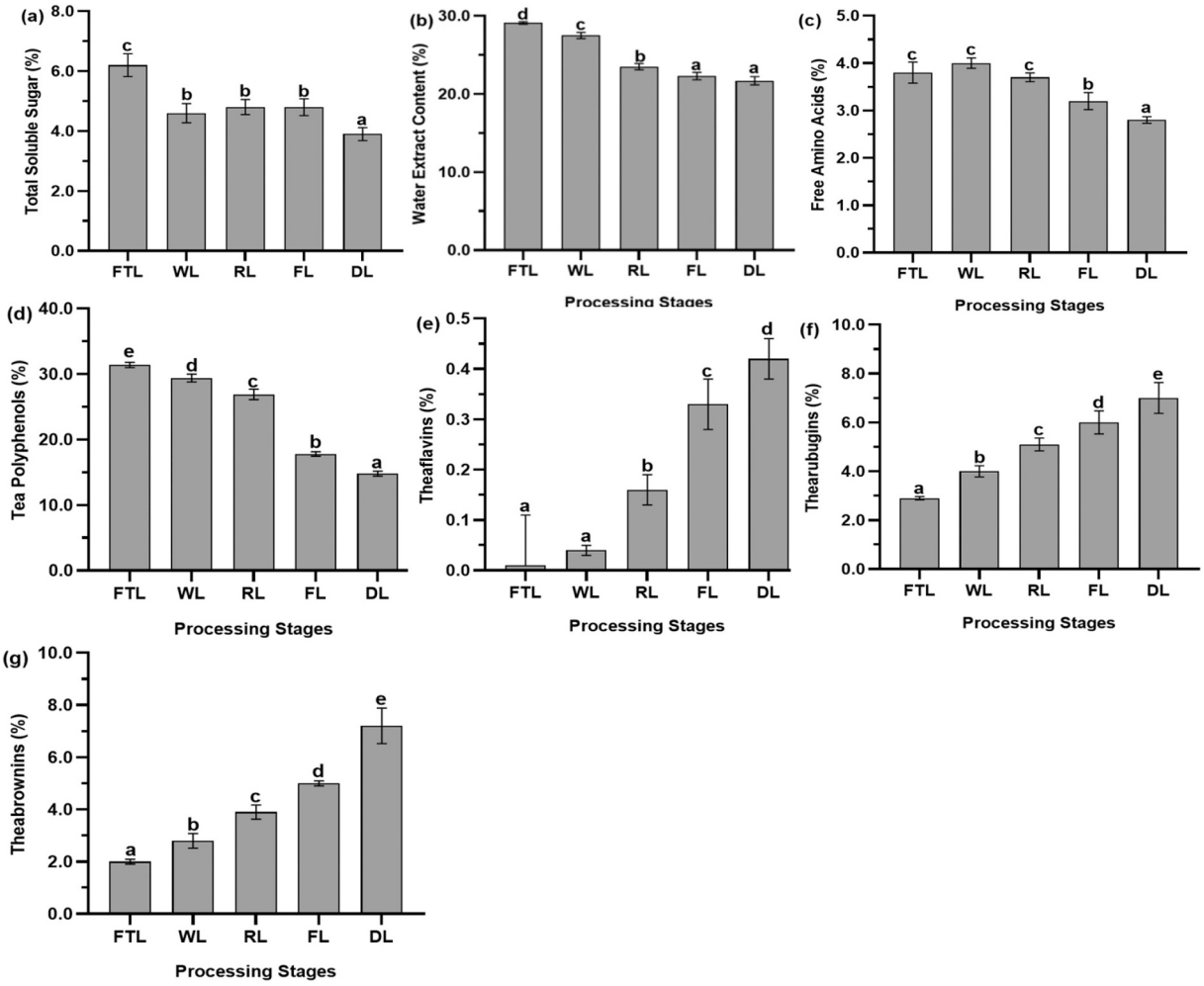

Fig. 6. The relative total content of (a) soluble sugar, (b) water extract, (c) free amino acids, (d) tea polyphenols (e) theaflavins, (f) thearubugins, and (g) theabrownins. The different superscripts show significant differences ($p < 0.05$) according to Duncan's test.

2.78 %, 3.94 %, 5.05, and 7.21 in FTL, WL, RL, FL, and in DL respectively.

In this study, Catechins (C), epicatechin (EC), epigallocatechin (EGC), epicatechingallate (ECG), and Epigallocatechin gallate (EGCG) revealed a significant ($p < 0.05$) decrease (Fig. SM 2), having a relative content in fresh tea leaves approximately 3.42 mg/g, 7.07 mg/g, 15.30 mg/g, 13.78 mg/g, and 26.75 mg/g, respectively. In dried leaves, these values decreased to about 0.98 mg/g, 1.36 mg/g, 1.92 mg/g, 1.05 mg/g, and 1.01 mg/g, respectively. These compounds exhibited a gradual decline throughout the processing stages. Significant variations in gallic acid content during black tea processing: fresh, withered, rolled, fermented, and dried tea leaves of 1.34 mg/g, 1.70 mg/g, 3.59 mg/g, 3.16 mg/g, and 2.27 mg/g, respectively (Table 4). A dramatic increase was observed at the rolling stage, consistent with findings (Miao et al., 2013). Caffeine content showed non-significant variation during processing, with levels in fresh, withered, rolled, fermented, and dried tea leaves recorded as 27.08 mg/g, 32.70 mg/g, 33.17 mg/g, 32.52 mg/g, and 32.07 mg/g respectively. Among the four major theaflavin compounds found in black tea, theaflavin appeared after withering (Table 4), and during the rolling and fermentation stages, the theaflavin levels reached a peak before gradually decreasing. This decline may have been caused by the breakdown of the theaflavins or their transformation into different substances, like thearubigins. The four theaflavins compounds appear after withering and peak during rolling and fermentation before declining at the drying stage. This decline may be due to degradation or conversion into compounds like thearubigins (Tanaka et al., 2002). Theaflavins formed during fermentation are oxidized further by epicatechin quinone, producing thearubigins. At the final stage of processing, TF was the most abundant dimeric theaflavin at 0.95 mg/g, followed by TF-3-G at 0.70 mg/g, TF-3′-G at 0.58 mg/g, and TFDG at 0.57 mg/g. During rolling and fermentation, TF and TF-3′-G decreased significantly from 1.85 and 0.75 to 1.31 and 0.63 mg/g, respectively, while TF-3-G and TFDG contents exhibited negligible change. Hence, the levels of each theaflavin compound are affected by both their quantity of catechin precursors and stereo chemical configuration.

4. Conclusion

The study optimized the manufacturing process of *Yunkang* 10 black tea using the uniform design (UD) method, focusing on withering time, fermentation time, and fermentation temperature. Tests based on the UD experimental design were conducted, resulting in optimal parameters of withering time of 14 h, fermentation time of 5.6 h, and fermentation temperature of 34 °C. Verification experiments were carried out under these conditions, and the optimized sample underwent phytochemical profiling using metabolomics and HPLC analysis. Analysis of 157 VOCs and 2642 metabolites identified 19 key aroma-active compounds (VIP > 1.5) and 50 characteristic metabolites ($p < 0.01$, VIP > 1.5), highlighting the influence of each processing stage on the aromatic attribute and overall quality of *Yunkang* 10 black tea. Flavonoids/flavonoid glycosides were the major subclass in non-volatile

Table 4
Dynamic changes in content of Catechins (catechin, epicatechin, epigallocatechin, epicatechingallate, epigalloctachingallate), gallic acid, caffeine and four theaflavin compounds (theaflavin, theaflavin-3-gallate, theaflavin-3′-gallate, and theaflavin-3,3;-digallate) during processing black tea.

Components	FTL	WL	RL	FL	DL
Catechins (C)	3.42 ± 0.62c	3.08 ± 0.07bc	2.64 ± 0.24^{b}	1.06 ± 0.08^{a}	0.98 ± 0.12^{a}
Epicatechin (EC)	7.07 ± 0.51^{d}	5.61 ± 0.56^{c}	3.26 ± 0.32^{b}	2.04 ± 0.17^{a}	1.36 ± 0.39^{a}
Epigallocatechin (EGC)	15.30 ± 0.44^{d}	13.24 ± 0.61c	8.05 ± 0.53^{b}	3.14 ± 1.84^{a}	1.92 ± 0.99^{a}
Epicatechinsgallate (ECG)	13.78 ± 0.42^{e}	11.96 ± 0.11^{d}	7.04 ± 0.24^{c}	2.07 ± 0.85^{b}	1.05 ± 0.33^{a}
Epigallocatechinsgallate (EGCG)	26.75 ± 0.28^{e}	22.20 ± 0.19^{d}	17.65 ± 0.22^{c}	2.38 ± 0.37^{b}	1.01 ± 0.14^{a}
Gallic Acid (GA)	1.34 ± 0.34^{a}	1.70 ± 0.41^{ab}	3.59 ± 0.43^{c}	3.16 ± 0.05^{c}	2.27 ± 0.41^{b}
Caffeine (CAF)	27.08 ± 0.27^{a}	32.70 ± 0.81^{b}	33.17 ± 1.62^{b}	32.52 ± 0.22^{b}	32.07 ± 0.08^{b}
Theaflavin (TF)	ND	1.17 ± 0.04^{ab}	1.85 ± 0.27^{c}	1.31 ± 0.11^{b}	0.95 ± 0.18^{a}
Theaflavin-3-gallate (TF-3-G)	ND	0.61 ± 0.03^{a}	0.89 ± 0.10^{bc}	0.93 ± 0.15^{c}	0.70 ± 0.18^{ab}
Theaflavin-3′-gallate (TF-3′-G)	ND	ND	0.75 ± 0.04^{c}	0.63 ± 0.13^{ab}	0.58 ± 0.09^{a}
Theaflavin-3,3′-digallate (TFDG)	ND	0.57 ± 0.10^{a}	0.63 ± 0.07^{a}	0.62 ± 0.02^{a}	0.57 ± 0.02^{a}

Note: N.d." indicates "not detected." Each tea sample underwent three replications, and data were presented as mean values ± standard deviation. Different lowercase letters of superscript (a, b, c, d and e, $p < 0.05$) denote levels of compounds with statistically significant differences determined by one-way ANOVA (Duncan's multiple comparison test).

compounds, while Terpenoids dominated volatile compounds. Among the 19 volatile compounds, linalool oxides (linalool oxide I, linalool oxide II, and trans-linalool oxide), geraniol, Benzeneacetaldehyde, benzyl alcohol, benzaldehyde, methyl salicylate, and linalyl acetate were found to be accumulated in the dried sample which contributes the characteristic color and flavor to black tea. Among the non-volatile metabolites, the accumulation of amino acids and their derivatives was observed during processing. This study provides insight into the fluctuations in the tea metabolome during manufacturing of black tea, offering valuable guidance for future research aimed at enhancing the nutritional value and sensory qualities of black tea. However, the biological roles of these metabolites, as well as their potential health benefits, require further validation through targeted biochemical and clinical studies. Future research should focus on confirming the health-related impacts of these compounds, through in vivo and in vitro studies. This research paves the way for deeper exploration into the functional properties of tea metabolites. By elucidating the dynamic changes in volatile and non-volatile profiles during processing, this research contributes to improving functional compounds and flavor in tea. Ultimately, it enhances scientific understanding of how each processing step influences metabolite formation in black tea.

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CRediT authorship contribution statement

Muhammad Aaqil: Designed the study, performed the experiments, contributed to data collection and wrote the manuscript. **Muhammad Kamil:** Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation. **Ayesha Kamal:** Visualization, Validation, Software, Resources, Project administration, Funding acquisition. **Taufiq Nawaz:** Writing – original draft, Investigation, Data curation. **Chunxiu Peng:** Software, Resources, Methodology. **Ibrahim A. Alaraidh:** Visualization, Validation, Software, Methodology. **Saud S. Al-Amri:** Software, Resources, Funding acquisition. **Mohammad K. Okla:** Visualization, Validation, Methodology, Funding acquisition. **Yan Hou:** Writing – original draft, Visualization, Validation, Software, Methodology. **Shah Fahad:** Writing – review & editing, Project administration, Methodology, Data curation. **Jiashun Gong:** Writing – original draft, Supervision, Methodology, Investigation, Data curation, Conceptualization.

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

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