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Insights into the flavor profiles of different grades of Huangpu black tea using sensory histology techniques and metabolomics

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ABSTRACT

Significant differences exist in aroma and taste of different grades of large-leaf black tea. In this study, sensory histology combined with metabolomics were used to investigate the sensory characteristics and phytochemical profiles of different grades of Huangpu black tea (HPBT). Sensory evaluation showed that high grade HPBT had high intensity of pekoe, fresh aroma and umami, with aroma and taste scores declining with decreasing grades. 173 non-volatiles were identified, of which 23 marker metabolites could be used as discrimination of different grades HPBT taste. In addition, 154 volatile compounds were identified in the different grades of HPBT, with 15 compounds as key odorants for distinguishing the aroma of different grades of HPBT. Furthermore, correlation analysis revealed that linalool, geraniol and nonanal contributed to the aroma quality score of HPBT. This study will provide a more comprehensive understanding for processing, quality evaluation and grade evaluation system of large-leaf black tea.

1. Introduction

Black tea is one of the world's most popular beverages, with the highest production and consumption among the six varieties types of tea, making up approximately 75% of the world's tea consumption (Chen et al., 2022; Zhang et al., 2019; Zhang et al., 2020). Currently, the main origins of black tea include China, India, Kenya, and Ceylon (Guo et al., 2018). With the differences in growing environment, tea varieties, leaf sizes and processing techniques, black tea can be divided into small and medium-leaf black tea, large-leaf black tea and red crushed tea (Liu et al., 2023). Typically, the processing stages of black tea involve withering, rolling, fermentation, and drying (Su et al., 2022). Based on the tenderness, aroma, taste, and appearance of tea products, black tea can be categorized into various grades (Peng et al., 2023). It was considered that the quality evaluation and grade classification of tea is important for the development of tea industry and consumer purchasing (Zou et al., 2023). Tea grading is related to economic value of tea and directly affects flavor quality and drinking experience (Han et al., 2022; Zou et al., 2023). Currently, the research on black tea grade classification has attracted much attention and made much progress, especially the identification and analysis of the characteristic flavor indicators, such as Keemun black tea, Jiuquhongmei tea, Ning black tea and Hunan black tea (Guo et al., 2018; Liu, Teng, et al., 2023; Lu et al., 2021; Peng et al., 2023; Zou et al., 2023). However, compared with the small and medium leaf black teas, there have been fewer studies conducted on the differences in aroma and taste among the grades of large leaf black tea, especially in revealing the composition of aroma and taste compounds and their dynamics of different grades of teas, as well as how these differences affect flavor quality of tea.

The grade of tea depends largely on the quality of the tea, of which taste and aroma are particularly important factors (Zhai et al., 2022). The taste of black tea is closely related to specific non-volatile compounds of tea, mainly polyphenols, alkaloids and amino acids (Guo et al., 2018; Zhou et al., 2022). The aroma components of black tea are

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composed of volatile compounds from fresh leaves and precursor substances produced during processing (Chen et al., 2022). The large-leaf varieties, especially the Yunnan large-leaf variety(*Camellia sinensis* var. *assamica*), are known for their unique biological characteristics and rich contents, and are regarded as high-quality materials for the producing high-grade black tea (Peng et al., 2023). It has been reported that large-leaf varieties have higher levels of tea polyphenols and caffeine compared to the small and medium-leaf varieties, resulting in black tea with strong flavor (Peng, He, et al., 2023). In addition, the large-leaf black tea contains richer volatile species and higher content of linalool, which is the main aroma presenting substance (Cao et al., 2020). The quality characteristics of tea are influenced by much factors, including tea tree varieties, climatic conditions, the raw materials tenderness and processing technology (Ge et al., 2024; Kang et al., 2019). Therefore, compared with the small and medium-leaf black tea, the special internal composition of the large-leaf fresh leaves imply that their different grades may be unique in terms of aroma and taste as well as its transformation pattern.

Huangpu black tea (HPBT) is produced in Huangpu, Guangzhou, China. It is processed from Yunnan large-leaf variety and has unique floral and fruity aroma and strong taste. However, there are few reports on the flavor profile of different grades of HPBT. In this study, we used sensory histology techniques and metabolomics to conduct a comprehensive and systematic analysis of the aroma and taste components of different grades HPBT, so as to reveal the key flavor components of HPBT and their relationships with tea quality grades. This study will provide a theoretical basis for the processing, quality evaluation and grade determination system of large-leaf black tea.

2. Materials and methods

2.1. Materials and chemicals

Gallic acid (GA), theobromine, theophylline, caffeine, (−)-epigallocatechin gallate (EGCG), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), (−)-epicatechin (EC), (+)-catechin (DL-C), (+)-gallocatechin-3-O-gallate (GCG), glutamic acid, theaflavin (TF1), theaflavin-3-*O*-gallate (TF2a), theaflavin-3′-*O*-gallate (TF2b), theaflavin-3,3′-*O*-gallate (TF3) standards was obtained from Sigma-Aldrich Co., Ltd. (St. Louis, Missouri, USA). Formic acid and acetonitrile for LC-MS grade were purchased from Merck (Darmstadt, Germany) and Thermo (Fisher Scientific, USA), respectively. Folin phenol, ninhydrin, anthrone, and acetic acid were provided by China National Pharmaceutical Corporation (Shanghai, China).

2.2. Tea samples

The samples of different grade HPBT were provided by the Huangpu Innovation Research Institute of Hunan Agricultural University (Guangzhou, China). These materials consisted of three different grades of HPBT samples, including special grade (HPBT1), first grade (HPBT2) and second grade (HPBT3). The HPBT samples were made using traditional local processing techniques to ensure more consistent quality. All samples were processed in March 2023 and each grade sample was stored at −20 °C for subsequent analysis.

2.3. Flavor evaluation of different graded HPBT samples

2.3.1. Traditional sensory evaluation

The sensory assessment of HPBT was performed by a panel of seven well-trained assessors, comprising three males and four females, whose ages ranged from 25 to 30 years. It was worth mentioning that all the sensory evaluation trials were carried out with the formal approval and ethical permission by the Ethics Committee of Hunan Agricultural University (approval number, 2023–138). Prior to the start, all participants were informed about the specifics of trial and volunteered to join. The traditional sensory evaluation was conducted according to the Chinese standard method (GB/T 23776–2018). In brief, 3 g of black tea was steeped in 150 mL of boiling water for a duration of 5 min. Subsequently, the tea leaves were strained and the panelists evaluated the quality and give a score accordingly.

2.3.2. Quantitative descriptive analysis (QDA) of the aroma

Based on the traditional sensory evaluation, we evaluated the aroma profiles of the three grades of HPBT. All assessors have previously undergone specific training to identify, describe and quantify different aroma characteristics. Subsequently, we selected six aroma quality descriptors that best represented the common aroma qualities, including fruity, floral, sweet, roasted, fresh, and pekoe. Finally, a 5-point rating scale(0 denoted non-existent, 3 represented medium intensity, and 5 signified very strong) was employed to evaluate the aroma attributes of each sample according to our previous research (Chen et al., 2023; Ouyang et al., 2024). The sensory attribute intensity was based on the average ratings given by the seven panelists.

2.3.3. Electronic tongue evaluation

The taste intensity of tea was quantified by an ET (Insent, Atsugi-Shi, Japan) equipped with four sensors. The pre-treatment of the tea infusion was consistent with the traditional sensory, and the tea infusion was cooled to room temperature for taste intensity determination. The four electronic sensors are capable of sensing different taste in the tea The four electronic sensors are capable of sensing different taste in the tea infusion, including AAE (umami), COO (bitterness), AE1 (astringency), CAO (sourness), aftertaste-astringency(aftertaste-A), aftertaste-bitterness(aftertaste-B), and richness. Each measurement cycle for a given sample involves sequential steps of cleaning of the anionic, cationic solutions, reference solution cleaning, sample measurement, aftertaste measurement, and thorough cleaning. The potential difference converted by the electronic tongue sensor is processed through a pattern recognition system to analyze the taste attributes and intensity of each tea sample. Each sample was measured four times, and the results from the last three times replicates were chosen for statistical analysis.

2.4. The determination of main chemical composition

The aqueous extracts and total polyphenol of black tea were determined according to previous published method (Huang et al., 2024). Free amino acid (FAA) was measured according to national standards (GB/T 8314–2013). The catechins, gallic acid(GA), and alkaloids were detected using high performance liquid chromatography (Shimadzu, Japan) according to our previously methods (Lu et al., 2021). The theaflavin fractions were determined based on the national standards (GB/T 30483–2013).

2.5. Untargeted non-volatile compound analysis

The sample extraction method was modified according to our previous method (Li et al., 2021). Briefly, 0.5000 g of tea sample was weighed into 25 mL of 70% (*v*/v) methanol-water and extracted by ultrasonication for 30 min, with shaking every 10 min. Then the mixture was centrifuged at 12000*g* for 10 min at 10 °C. The supernatant was aspirated and filtered for subsequent analysis. In addition, pooled quality control (QC) samples were used to assess the stability and reproducibility of metabolomics.

The non-volatile determination of black tea was performed by ultra-high-performance liquid chromatography-Orbitrap-Q Exactive-Orbitrap mass spectrometry (UHPLC-Q Exactive-Orbitrap-MS, Thermo Fisher Scientific, USA). Black tea extract was separated by chromatography using a hypersil gold C18 column (2.1 mm × 100 mm, 1.9 μm; Thermo Fisher Scientific) at 35 °C with a flow rate of 300 μL/min. The mobile phase was composed of 0.1% water-formic acid (A) and 0.1% acetonitrile-formic acid (B). The elution gradients were as follows:

0–1.5 min: 0% B; 1.5–1.6 min: 0–5% B; 1.6–18 min: 5–18% B; 18–34 min: 18–95% B; 34–38.4 min: 95% B; 38.4–38.5 min: 95–0% B; and 38.5–42 min: 0–0% B. The injection volume was 2 μL. The mass spectra of tea samples were collected at voltages of 3.5 kV and 2.5 kV for positive and negative ion modes, respectively, with full scans mass from 100 to 1500 *m/z*. The mass spectrometry conditions were as follows: ion transfer tube temperature, 325 °C; nebulizer, 300 °C; scanning period, 3 s. Raw data from UHPLC-Q Exactive-Orbitrap-MS were subjected to peak extraction and peak matching using compound discoverer 3.2 software (Thermo Fisher Scientific, USA) to obtain retention times and peak intensities. Metabolites were identified based on precise molecular, ms2 fragmentation information, PubChem and relevant references.

2.6. Volatile compound analysis

The volatile compounds were extracted using headspace solid-phase microextraction (HS-SPME). Briefly, 0.5000 g of each black tea sample powder was weighed accurately into a 20 ml headspace bottle. The headspace vials were filled with 5 mL of boiling water and 10ul of ethyl decanoate (8.63 mg/L) as an internal standard by CTC autosampler, and equilibrated at 600 r/min and 60 °C for 10 min. After that, divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS; 50/30 μm) fiber head (Sigma-Aldrich Trading Co., Ltd., Shanghai) was inserted into a headspace and incubated for 30 min under the same conditions. Then, the fiber was desorbed at 250 °C for 10 min at the GC inlet for subsequent analysis.

Volatile compounds were identified by comprehensive two-dimensional gas chromatography-quadrupole-time-of-flight mass spectrometry (GC × GC-Q-TOF-MS, Agilent, USA). HP-5MS and DB-17MS from Agilent Technologies were utilized as 1D and 2D chromatographic columns, respectively. The solid-state modulator SSM1800 (J&X Technology Co., Ltd., China) was heated and cooled between the two columns, modulating once every 4 s. The temperature of GC inlet was kept constant at 250 °C, while the split ratio was set to 10:1. The gas chromatography column was heated as follows: initially at 40 °C for 1 min, then to 180 °C at 4 °C/min, then to 250 °C at 20 °C/min, and finally held for 1 min. Ionization for mass spectrometry was carried out by electron bombardment of the ion source with an electron energy of 70 eV, 200 °C for the ion source and 150 °C for the quadrupole. Only compounds with forward match >700, reverse match >800, and retention index (RI) deviation <15 were retained (Chen et al., 2023; Ouyang et al., 2024). RIs were determined for each compound using the n-alkane line ranging from C7 to C25. Ethyl decanoate was used for the relative quantification of volatile components, and the odor activity values (OAV) indicate the contribution of individual volatile compounds to the aroma according to our previous method (Chen et al., 2023; Wen et al., 2023).

2.7. Statistical analysis

All analyses were performed with a minimum of three biological replicates and expressed as the mean ± standard deviation (SD). The analysis of differences was conducted using SPSS22 software (IBM, Armonk, USA) through Duncan's multiple tests of one-way ANOVA, with statistical significance set at $p < 0.05$. The software SIMCAP+ 12.0 (Umetrics, Sweden) was utilized for conducting principal component analysis (PCA), partial least squares-discriminant analysis (PLS-DA), hierarchical cluster analysis (HCA), and Partial Least Squares (PLS). Heatmaps were plotted using TBtools (https://github.com/CJ-Che n/TBtools).

3. Results and discussion

3.1. Flavor characteristic analysis of different grades of HPBT

In order to explore the flavor differences between the different grades of HPBT, we performed traditional sensory evaluation of the three grades of HPBT, and the results were presented in Fig. 1A and Table S1. Generally, the tea trichomes are considered an important indicator of the tenderness of tea. Expectedly, the tea trichomes in HPBT decrease significantly with decreasing grades. All three grades of HPBT showed red and bright characteristics in the tea infusion. Additionally, it was important to highlight that the aroma and taste scores of HPBT decreased as its rank decreases. Among them, the sweet odor was the basic aroma of HPBT, and the higher grades of HPBT had higher umami and pekoe attributes. Furthermore, all HPBT had strong taste properties, which may be related to the higher content of polyphenols in the large-leafed varieties. Overall, there were significant flavor differences between the different grades of HPBT.

QDA is a widely used evaluation method for flavor analysis. It is capable of providing detailed flavor descriptions and intensities, allowing for the distinction between various tea samples (Ouyang et al., 2024; Zheng et al., 2023). It was evident that the three grades of HPBT have significant differences among their aroma attributes in Fig. 1B. Briefly, HPBT1 has distinct pekoe and fresh attributes, which was related to its tenderness and lighter fermentation process. HPBT2 has the highest floral and fruity attributes, and HPBT3 has the highest roasted. In addition, all three grades of HPBT exhibited high sweet property. Overall, the results of QDA were generally consistent with the trends of traditional sensory evaluation.

The electronic tongue technology, which mainly mimics the mammals taste system to classify the taste of substances being tested, is now widely used in food quality assessment studies (He et al., 2009; Peng, Li, et al., 2023). In order to objectively quantify the taste of different grade black tea samples, ET was used to analyze the taste profile of the tea infusion, and the results were shown in Fig. 1C. It was evident that the umami intensity of the tea samples decreased with decreasing HPBT rank, which was consistent with the traditional sensory evaluation. The astringency intensity of HPBT2 was the highest compared to HPBT1 and HPBT3. Notably, while the intensity of sourness detected by ET increases across different grades HPBT, the levels of sourness of three grades HPBT and astringency identified in HPBT1 and HPBT3 fall below the taste threshold detectable by ET. It suggested that human taste buds may be more sensitive than the ET device in certain aspects of taste perception.

3.2. Analysis of non-volatile compounds of different grades of HPBT

3.2.1. Analysis of main chemical composition of different grades of HPBT

The tea grades are influenced by a number of factors, and taste is one indicator of tea quality. In this study, we quantified the water extract, alkaloids, total polyphenols, catechin, FAA, GA, and theaflavin fractions of black tea, and the results were presented in table S2. The level of water extract reflects the amount of soluble substances in the tea and is strongly linked to the intensity of the tea taste (Huang et al., 2024). Polyphenols are the main secondary metabolites of tea and contribute significantly to the flavor and health benefits of tea. Catechins affect the formation of the bitter and astringency of tea (Zhang et al., 2020; Zhou et al., 2022). In both fresh leaves and unfermented tea, catechins are the major tea polyphenols, and their levels notably decrease during fermentation (Guo et al., 2018). In our study, the water extract of HPBT2 was significantly higher than that of HPBT1 and HPBT3. In additional, the content of the galloylated catechin EGCG and GCG decreased with decreasing tenderness, in agreement with previous studies, which may be related to the specific synthesis and accumulation mechanism of phenolics in tea plants (Guo et al., 2018; Jiang et al., 2013). Furthermore, theaflavin, produced through the oxidative dimerization of catechins, is regarded as a significant indicator of black tea quality (Guo et al., 2018). It was evident that HPBT1 had the highest content of TF3, while TF1 and TF2b had the lowest.

Alkaloids are important quality components of tea, contributing to bitter and a variety of health benefits, including caffeine, theobromine and theophylline. Among them, caffeine is the main alkaloid and

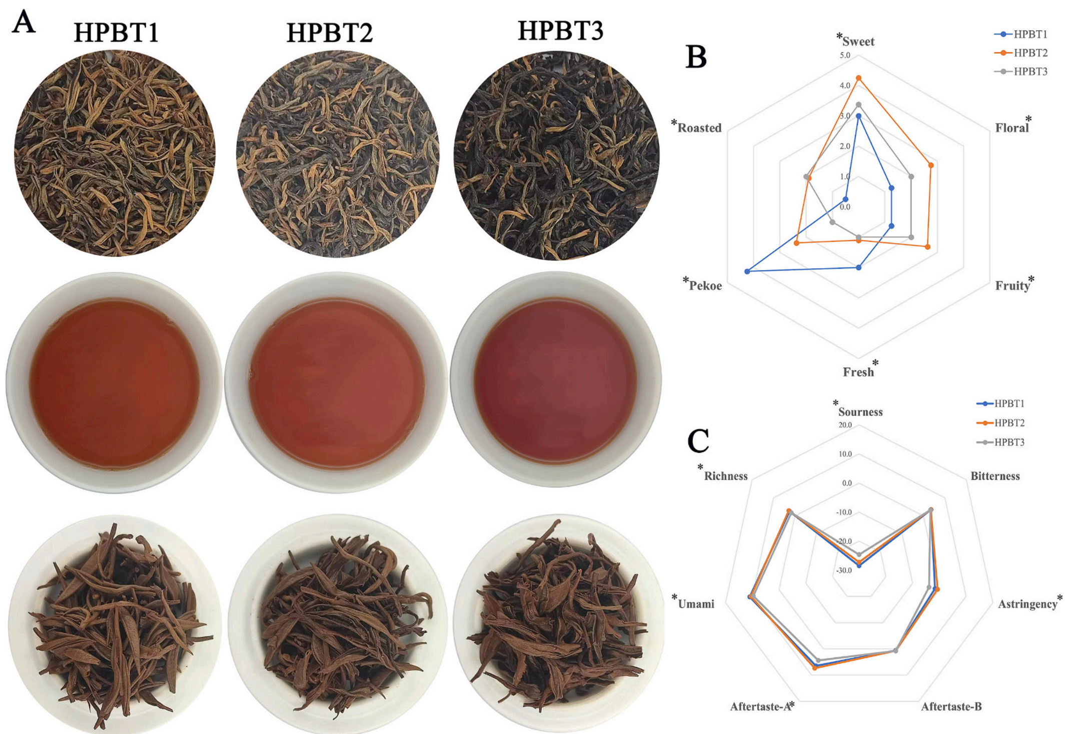

Fig. 1. The appearance and infusion of different grades of HPBT(A); Quantitative descriptive analysis of aroma (B); Electronic tongue analysis of HPBT(C). (HPBT1: special grade Huangpu black tea, HPBT2: first grade Huangpu black tea; HPBT3: second grade Huangpu black tea).

contributes significantly to the bitter of tea (Zhang et al., 2020). Caffeine can also combine with theaflavins to enhance the umami of tea. In our study, the caffeine content of HPBT2 was significantly higher than that of HPBT1 and HPBT3, while the theobromine content decreased with the decreasing grade. FAA are crucial in forming the umami and aroma of tea and generally more abundant in higher grade tea. The amount of FAA decreased with decreasing rank, which was consistent with previous studies (Guo et al., 2021). The GA contributes to the astringency of tea, typically not found in fresh leaves but slightly increases after the fermentation of galloylated catechins (Guo et al., 2018). In our study, the GA content of HPBT1 and HPBT2 was significantly higher than HPBT3.

3.2.2. Analysis of non-volatile metabolites of different grades of HPBT

Although major chemical components can account for the differences in various grades of HPBT, a considerable portion of compounds showed no significant correlation with the tea grades. It is essential to identify additional chemical distinctions among various grades of HPBT through high-precision untargeted metabolomics. In this experiment, the non-volatile compounds of different grades of HPBT were performed by UHPLC-Q Exactive-Orbitrap-MS, and representative total ion chromatograms were shown in Fig. S1. After analysis, 3710 metabolite ions features were detected across different grades of HPBT. Finally, 173 compounds were obtained based on retention time, accurate mass, tea metabolomics databases and relevant literature and the results were shown in table S3, including 12 catechins, 26 amino acids, 31 flavonoids and flavone glycosides, 22 phenolic acids, 17 nucleosides, 24 organic acids, 4 alkaloids,19 lipids and 18 others.

To better understand the metabolic patterns of the three different grades of HPBT, multivariate statistical analyses were used to explore differences in metabolites. As shown in Fig. 2A, the PCA analysis revealed that the first two principal components accounted for 57.8% and 34.6% of the variance, respectively, effectively distinguishing the three different grades of HPBT along PC1. Next, we used the supervised PLS-DA to investigate metabolites that distinguish the three grades of HPBT ($R2X = 0.987$, $R2Y = 0.999$, and $Q2 = 0.996$), and cross-validation analyses showed the PLS-DA model was reliable ($R2 = 0.29$, $Q2 = -0.28$) in Fig. 2B-C. These results indicated that the metabolic profiles of the three grades of HPBT were significantly different. Finally, 23 differential metabolites with variable importance projection (VIP) scores of ≥ 1 and $p < 0.05$ were identified, including 5 catechins, 3 amino acids, 6 flavonoids and flavone glycosides, 5 phenolic acids, 2 alkaloids, 1 organic acids and 1 other in three grades of HPBT (Fig. 2D-E).

It has been reported that black teas retain a certain amount of polyphenols (especially galloylated catechins) at the end of fermentation, which is beneficial to the concentration and intensity of the tea infusion (Lu et al., 2021). In this study, the catechin results for the metabolome were consistent with the trend of the liquid chromatography. It was reported that Leucine was positively correlated with umami (Yang et al., 2018). The Leucine level of HPBT1 and HPBT2 were significantly higher than that of HPBT3, suggesting that it may contribute to the formation of umami (Yang et al., 2018). Theanine is the most abundant amino acid in tea, accounting for about 50% of the FAA, and contributes significantly to the umami of tea. On the other hand, it can also neutralise the bitterness and astringency and reduce the overstimulation caused by the high concentration of caffeine in the tea (Zhu et al., 2021). It was observed that theanine and FAA had similar distributions in different grades of HPBT. Flavonoids and glycosides have a wide range of health benefits and exert significant influence on the astringency of tea due to their very low threshold (Perez-Vizcaino & Duarte, 2010; Scharbert et al., 2004). Previous studies have shown that most myricetin-*O*-glucoside, quercetin-*O*-glucoside and kaempferol-*O*-glucoside were higher in Shoumei and Baimudan than in silver needles (Yang et al., 2018). In our study, the levels of kaempferol 3-*O*-glucoside, myricitrin, quercetin-3β-D-glucoside, cynaroside, and rutin were significantly higher in HPBT2 and HPBT3 than in HPBT1, which may be related to the higher astringency of HPBT2 and HPBT3. Phenolic acids are a cluster of aromatic compounds containing carboxyl and hydroxyl groups, exerting a significant impact on the astringency and sourness of tea (Ye et al., 2022). It was evident that the content of quinic acid and

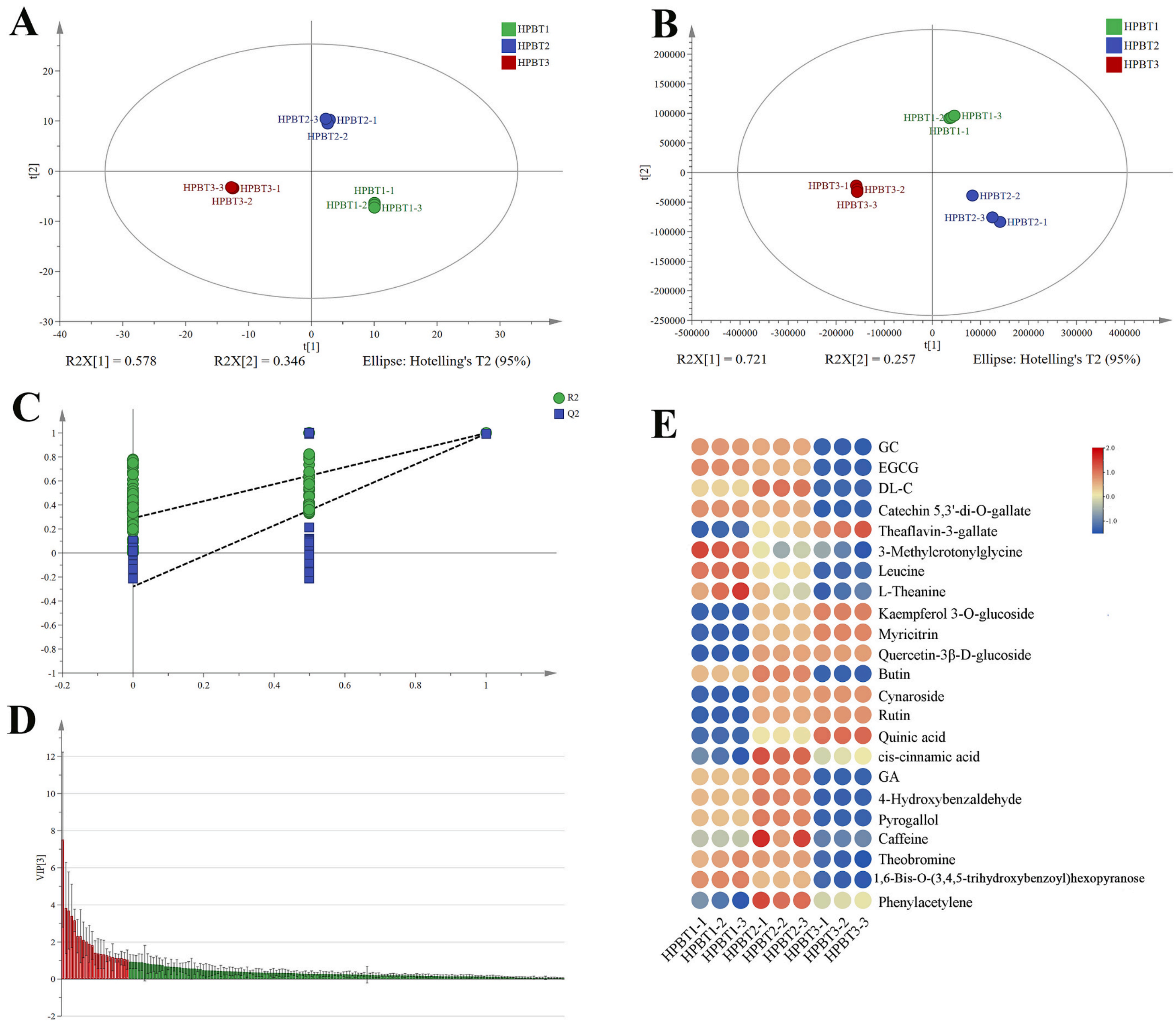

Fig. 2. Multivariate statistical analysis of non-volatile compounds. PCA(A); PLS-DA(B); Cross-validation results(C); VIP plot(D); Heatmap of key non-volatile metabolites of different grades HPBT(E).

cis-cinnamic acid were higher in HPBT2 and HPBT3 than in HPBT1. Interestingly, quinic acid has also been identified as a key marker compound to distinguish different grades of Keemun black tea (Guo et al., 2018). In contrast, the GA level in HPBT1 and HPBT2 was significantly higher than that in HPBT3. GA is deemed an essential umami-enhancing compound in green tea, and sensory evaluation have shown that it can significantly increase the umami intensity of sodium L-glutamate (Kaneko et al., 2006).

3.3. Analysis of volatile compounds of different grades of HPBT

3.3.1. Overall analysis of volatiles composition in HPBT

In order to have a comprehensive understanding of the volatile compounds of different grades of HPBT, GC × GC-Q-TOFMS analysis was performed and representative total ion chromatograms were shown in Fig. S1. 154 volatile compounds were identified in three grades of black tea samples, including 27 aldehydes, 25 alkenes, 20 alcohols, 20 esters, 18 alkanes, 16 ketones, 12 aromatic hydrocarbons, 9 oxygen heterocyclic compounds, 2 sulfides, 2 pyrrole derivatives, 3 other compounds (Table S4). The profiles of the volatile compounds of HPBT were shown in Fig. S2. It was evident that alcohols, aldehydes and esters were the main volatile compounds of HPBT, which was consistent with the results of previous black tea studies (Kang et al., 2019; Ouyang et al., 2024). In particular, similar to Dianhong tea, the proportion of alcohols in HPBT was the highest, which may be related to the genetic basis of large-leaf black tea and the growing environment (Peng et al., 2021). It can also be observed that the volatile content of different grade HPBT also varies considerably, with alcohols decreasing with decreasing grade and aldehydes increasing with decreasing grade. The average content of 10 volatile compounds exceeded 100 ng/g, including linalool, methyl salicylate, benzeneacetaldehyde, α-terpineol, trans-β-ionone, D-limonene, hexanal, benzaldehyde, linalool oxide I and linalool oxide II. These high levels of volatile components mainly exhibited floral, sweet and fresh attributes, which were consistent with the trend of sensory evaluation of HPBT. Among them, it was noteworthy that the linalool content of the different grades of HPBT exceeds 45% and decreased with decreasing grade. It was reported that three aroma types of black tea: the first being floral with linalool and linalool oxide as the dominant components, the second being intermediate with linalool and geraniol, and the third being rosy with geraniol (Su et al., 2022). Clearly, HPBT belongs to the first category according to the volatile content, which was also consistent with the characteristics of large-leafed varieties. Methyl salicylate with fresh and minty odors had the second highest content of the three grades of HPBT and was considered key odorants of Ceylon black tea (Kang et al., 2019). In addition, the levels of benzeneacetaldehyde with floral and sweet attributes and benzaldehyde with nutty odor, both of which were identified as key aroma active compounds of Keemun, Assam and Ceylon black tea, increased with decreasing grades (Kang et al., 2019). In conclusion, there were significant differences in the volatile components of different grades of HPBT.

In order to understand the similarities and differences in the different grades of HPBT, we performed multivariate statistical analyses on 154 volatile compounds. Firstly, two unsupervised methods (PCA and HCA) were used according to the content of the identified volatile compounds and the results were presented in Fig. 3A-B. The PCA results (PC1 = 52.7%; PC2 = 33.3%) indicated a close distribution between HPBT2 and HPBT3, with a distinct separation of HPBT2/HPBT3 and HPBT1 along PC1. The clustering results of HCA were consistent with the trend of PCA. The Venn plot showed that 139 volatile compounds were common to all three grades of HPBT, in addition to 4 and 3 compounds specific to HPBT1 and HPBT2, respectively (Fig. 3C). Furthermore, the PLS-DA model can effectively separate the three different grades of black tea, which good predictive capability (R2Y = 0.994, Q2 = 0.986) without signs of overfitting (Fig. 3D-E). 31 compounds were identified as key differential compounds for the three grades of HPBT based on VIP > 1.0 and $p < 0.05$ (Fig. 3F). These differential compounds mainly include alcohols, aldehydes and esters, which were consistent with the main aroma compounds of black tea reported previously (Xiao et al., 2017; Zhai et al., 2022).

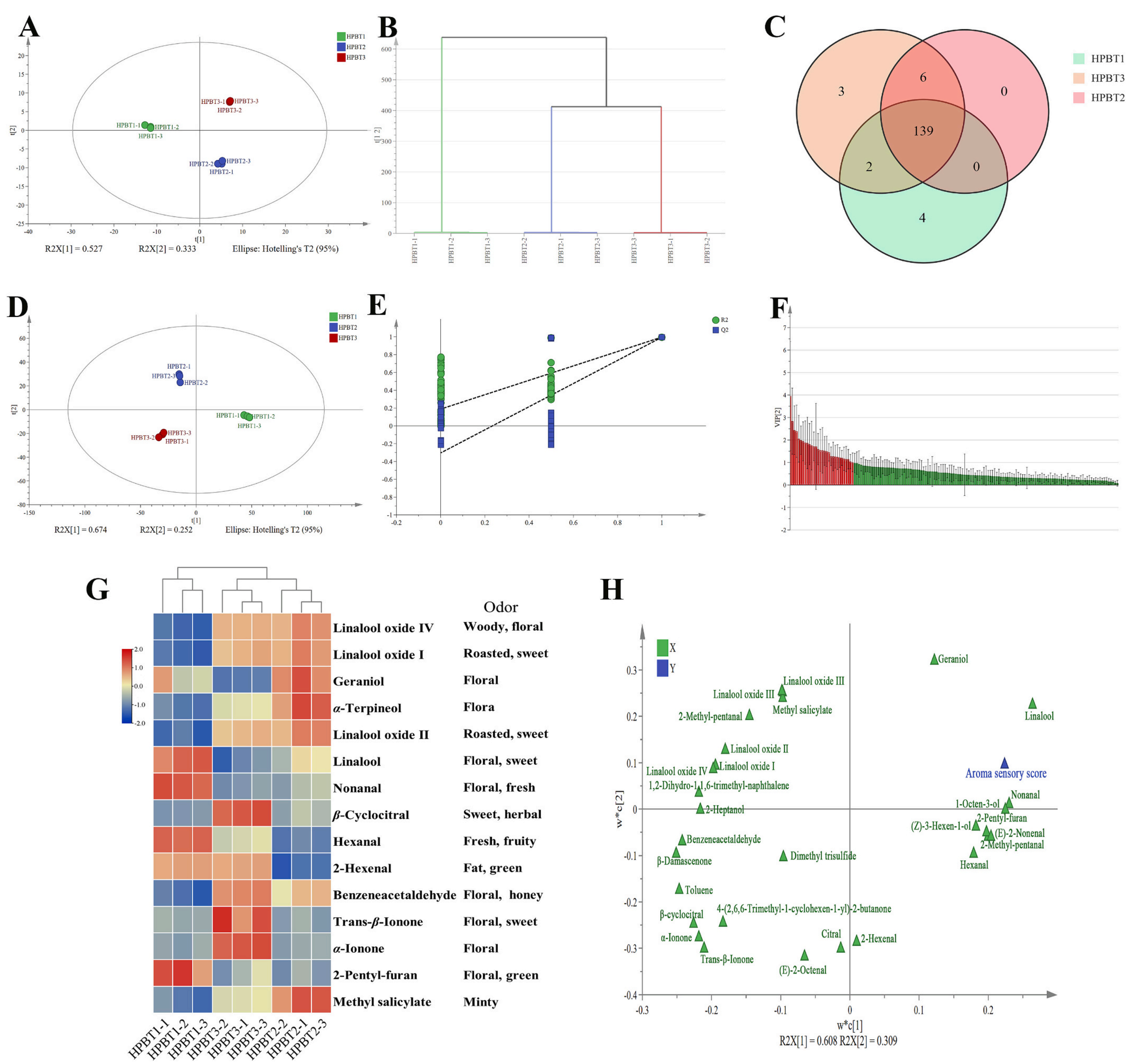

Fig. 3. Multivariate statistical analysis of volatile compounds. PCA(A); HCA(B); Venn plot (C); PLS-DA(D); Cross-validation results(E); VIP plot(F); Heatmap of key odorants for different grades HPBT(G); PLS plots for aroma active compounds and aroma sensory score(H).

3.3.2. Screening of key aroma active compounds

It is widely known that the contribution of volatiles to the overall aroma of tea is not only closely related to their concentration, but also depends on their aroma threshold (Zheng et al., 2022). In food flavor research, OAV is considered a key indicator for assessing aroma contribution, and compounds with an OAV >1 are generally considered to be important aroma active components (Kang et al., 2019). In this experiment, we identified 29 volatile compounds with an OAV exceeding 1(Table. S5). Among them, 6 aroma compounds had OAV values >50 across all three categories of HPBT, including trans-*β*-ionone, *β*-damascenone, linalool, (*E*)-2-nonenal, *α*-ionone and benzeneacetaldehyde. These high levels of aroma active compounds mainly exhibited floral, fruity, sweet and fresh attributes, which were in line with the trend of the sensory evaluation of HPBT, indicating their significant contribution to the fundamental aroma profile of HPBT. In addition, we further explored the key aroma compounds that distinguish the three grades of HPBT. 15 compounds were considered key aroma compounds of three grades (OAV > 1, $p < 0.05$) combined with PLS-DA, including 6 alcohols and 5 aldehydes, and the results were shown in Fig. 3G and table. S6. Previous studies have shown that alcohols and aldehydes were the primary aroma compounds of black tea, aligning with our study (Ouyang et al., 2024).

For the HPBT1 sample, five key odorants had the highest levels compared to the other two grades, including linalool, nonanal, 2-pentyl-furan, hexanal, and (*E*)-2-hexenal, which mainly contributed to the floral, fruity, and fresh aroma. Among these compounds, linalool has floral and sweet properties. Previous studies have shown that the content of linalyl primeverosides and total primeverosides decreased with increasing leaf maturity (Wu, 2021). It was also reported that the linalool content of Yingde black tea is the highest in special and first grade tea and decreases significantly in second and third grade tea, which was considered to be the most critical characteristic odorants of Yingde black tea (Fan, 2021). Nonanal contributes to the floral and fresh flavor. In our study, the linalool and nonanal content decreased with decreasing HPBT tenderness grade, which was in general agreement with the trend of previous studies. In addition, (*E*)-2-hexenal and hexanal derived from fatty acids and 2-pentyl-furan with floral and green odors were identified as key odorants of black tea, which may contribute to the formation of fresh attribute in HPBT (Su et al., 2022; Xiao et al., 2017).

For the HPBT2 sample, six contributors were identified as key odorants, including methyl salicylate, geraniol, *α*-terpineol, linalool oxide I, linalool oxide II and linalool oxide IV, which mainly exhibited floral, fruity, sweet and roasted aroma. It was reported that methyl salicylate has the highest content in first grade Jiuqu hongmei tea, which was consistent with our trend (Guo et al., 2021). Geraniol is one of the most common alcohols found in tea and is considered key odorants of the four most famous black teas worldwide (Kang et al., 2019; Zhai et al., 2022). In our study, HPBT2 possessed the highest geraniol content, which may be attributed to the fact that the first leaves of the new shoots contain the highest content of geranyl primeverosides (Wu, 2021). *α*-Terpineol and linalool oxide IV contribute floral attribute (Zhai et al., 2022). In addition, the contents of linalool oxide I and linalool oxide II in HPBT2 and HPBT3 were close to and significantly higher than that of HPBT1, which may contribute to the formation of roasted odor (Kang et al., 2019).

For the HPBT3 sample, four contributing compounds were identified as discriminatory odorants, including benzeneacetaldehyde, *α*-ionone, trans-*β*-ionone and *β*-cyclocitral. Among them, benzeneacetaldehyde is derived from the degradation of phenylalanine, which contributes to the honey aroma of Hunan black tea and Xinyang black tea. (Yao et al., 2022; Yin et al., 2022). Trans-*β*-ionone and α-ionone both have floral and sweet attributes and were considered to be key aroma components of black tea (Kang et al., 2019; Ma et al., 2022). *β*-Cyclocitral with sweet and herbal attributes was considered key aroma substance for ripened Pu-erh tea (Pang et al., 2019). It has been reported that its relative content increases with decreasing grade in new Jiuquhongmei black tea, which was consistent with the trend of our study (Guo et al., 2021).

To further investigate the contribution of aroma active compounds (OAV > 1) to the aroma quality of HPBT, we performed PLS analyses of odorants and aroma sensory score. It is generally accepted that when two variables are located close to each other, it indicates a positive correlation between them in a PLS loading plot (Liu, Teng, et al., 2023). As shown in Fig. 3H, it was evident that linalool, geraniol and nonanal were positively correlated with the aroma sensory score. These compounds present pleasant odors, mainly contributing floral and sweet attributes, which were favourable for the aroma quality of HPBT. Previous aroma recombination and omission test showed that linalool and geraniol contribute significantly to the overall aroma profile of high-grade Dianhong tea with floral, sweet and caramel-like attributes (Ma et al., 2022). It was also observed that the above compounds have high content in high grade HPBT, which further indicated that they are important aroma components of HPBT. In addition, (*E*)-2-octenal, 2-heptanol, and *β*-cyclocitral were negatively correlated with the aroma sensory score. These compounds mainly presented green, fat and herbal odors, which had a negative effect on the aroma quality of HPBT. Furthermore, 2-heptanol and *β*-cyclocitral increased with decreasing grade.

4. Conclusion

In this study, sensory histology combined with metabolomics techniques were used to investigate the sensory characteristics and phytochemical profiles of different grades of HPBT. Sensory evaluation showed that high grade HPBT had high intensity of pekoe, fresh aroma and umami, with aroma and taste scores decreasing as the grade decreased. 173 non-volatiles were identified by UHPLC-Q Exactive-Orbitrap-MS, of which 23 marker metabolites could be used as discrimination of different grades of HPBT taste, mainly including catechins, amino acids, phenolic acids, flavonoids and flavonoid glycosides. In addition, GC × GC-Q-TOMS identified 154 volatile compounds in the three grades of HPBT, with 31 volatile compounds as potential key aroma components. Combined with the OAV analysis, 15 compounds were considered key odorants for distinguishing the aroma of different grades of HPBT, mainly producing floral, fruity, sweet and fresh aromas. Furthermore, correlation analysis revealed that linalool, geraniol and nonanal contributed to the aroma quality score of HPBT. In conclusion, this study will provide a theoretical basis for the quality evaluation and grade determination of HPBT.

CRediT authorship contribution statement

Ronggang Jiang: Methodology, Investigation, Formal analysis. **Hao Xu:** Software, Data curation. **Shuai Wen:** Software, Methodology. **Changwei Liu:** Formal analysis, Data curation. **Yang Liu:** Formal analysis, Data curation. **Hongyu Chen:** Methodology, Investigation. **Yuke Zhai:** Software, Methodology. **He Xie:** Software. **Jinhua Chen:** Methodology. **Shi Li:** Methodology. **Kunbo Wang:** Funding acquisition. **Zhonghua Liu:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Jian-an Huang:** Writing – review & editing, Supervision, Project administration, Funding acquisition, 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

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101600.

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