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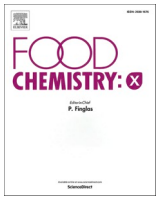

Unveiling the chemical composition of unique flavor profiles in raw Pu-erh tea from different Tea-Producing Mountains

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ABSTRACT

The Chinese proverb "One mountain, one flavor" reflects that raw pu-erh tea (RPT) from different tea-producing mountains (TPMs) possesses distinct flavor profiles. However, limited research has been conducted on the chemical composition underlying distinct flavor profiles. In this study, sensory evaluation and main phytochemical compositions revealed diverse aromas of RPTs from 26 TPMs. A total of 225 volatile compounds were analyzed qualitatively and quantitatively by comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry, including hydrocarbon compounds, ketones, alcohols, and aldehydes. Furthermore, twenty-one common key odor-active compounds, potentially influencing the regional flavor, were identified by gas chromatography-olfactometry and odor activity value. Multivariate statistical analysis showed that geographical factors—longitudes, latitudes, counties, and elevations—account for 37.02 % of the aroma profile variance, implying the possible influence of other potential factors. The study results serve as a reference for a better understanding of the correlation between the RPT flavor characteristics and geographical attributes in TPMs.

1. Introduction

Tea is one of the oldest and most popular beverages in the world, with a history spanning millennia and a global consumer base. The tea plant originated in Yunnan Province, located in southwestern China, a region renowned for its rich tea germplasm resources, particularly the Yunnan large-leaf tea plant (Wang et al., 2022). Pu-erh tea, made from this distinctive variety, is beloved by consumers worldwide for its unique flavor and health benefits (Xia et al., 2019; Zhao, Hu, Sun, Luo, & Zeng, 2023). Based on whether the production process includes pile-fermentation or not, pu-erh tea can be classified into ripe pu-erh tea and raw pu-erh tea (RPT). Specifically, RPT is produced through a process that involves spreading, killing, rolling, sun-drying, steaming, and drying, resulting in an aroma that is both clean, refreshing, strong, and lasting, along with a heavy and mellow flavor accompanied by a sweet aftertaste (Ren et al., 2022; Xu et al., 2021).

The flavor characteristics of RPT are derived from the combination of natural environment and production technology (Xiong et al., 2023). In many developed countries, implementing food-origin certification systems is a common measure to ensure consumer protection (Miyake & Kohsaka, 2023). Pu-erh tea was officially recognized as a Chinese geographical indication tea product in 2008 (GB/T 22111–2008), with 11 cities, 75 counties, and 639 townships designated as its protected areas. Each RPT-producing region develops its distinct flavor profile due to differences in microclimate, soil composition, genetic background of the tea plants, and cultivation methods (Shuai et al., 2022; T. Wang et al., 2018). A Chinese proverb aptly describes this phenomenon: "One mountain, one flavor". In the past, Six Ancient Tea Mountains (Yibang, Yiwu, Youle, Gedeng, Mangzhi, and Manzhuan) were recognized as the production areas of Pu-erh tea (Cao, 2022). However, with the

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increasing cultivation area of tea plants, the current division of Pu-erh tea-producing regions has gradually evolved into four main regions Pu'er, Xishuangbanna, Lincang, and Baoshan (Havemann, 2015). A previous study investigated 20 different areas within these four Pu-erh tea production regions and found that sun-dried green teas from different origins exhibit significant differences in polyphenol and amino acid content, while those from the same or neighboring regions tend to have consistent quality (Ning, Zeng, Zhang, & Wan, 2010). Xiong et al. (2023) distinguished a group of 63 RPTs from Xishuangbanna and Lincang production regions using volatile compounds. Of note is that Pu'er City is the main tea production area, by a subtropical monsoon climate typical of low-latitude plateaus, the region experiences synchronous rainfall and heat, along with favorable light conditions. Wu et al. (2016) observed significant differences in the volatile compounds of RPT between Wuliang Mountain and Jingmai Mountain from Pu'er City, particularly in the terpene alcohols and ketones. Zhang et al. (2020) investigated the volatile compounds of RPT from five different locations in Pu'er City and found that the differences mainly originated from methoxybenzenes, ketones, esters, and alcohols. Previous studies have shown that the flavor and quality of RPT are influenced by the tea production region, particularly the volatile compounds. However, there is limited research on whether different mountains within the same tea production region have distinct "one mountain, one flavor" characteristics. While we can perceive these unique flavors through the senses, our understanding of the underlying metabolic levels is still insufficient.

Aroma is one of the crucial factors determining the quality of tea and significantly influences consumer preferences. There is a wide variety of volatile compounds in tea with diverse properties, present in trace amounts and varying significantly. The mixture of these compounds at different concentrations exerts a combined effect on the olfactory nerves, resulting in the distinctive aroma of tea (Chi-Tang Ho, Zheng, & Li, 2015). Currently, over 700 volatile compounds in tea leaves and infusions have been identified, primarily through the use of gas chromatography–mass spectrometry (GC–MS) (Zhai, Zhang, Granvogl, Ho, & Wan, 2022). With the advancement of analytical chemistry techniques, instruments with stronger separation capabilities and higher resolution, such as comprehensive two-dimensional gas chromatography coupled with quadrupole time-of-flight mass spectrometry (GC × GC–QTOFMS), have been developed and are widely applied to the identification of volatile compounds in tea, leading to the discovery of more trace and ultra-trace volatile compounds (Ouyang et al., 2024). However, only a portion of these volatile compounds contribute to the aromatic profile, resulting in the diversity of aromas among different teas. The odor activity value (OAV) combined with gas chromatography-olfactometry (GC–O) is a commonly used method for identifying key odor-active compounds from the multitude of volatiles that have been characterized (Wen et al., 2023).

In this study, sensory evaluation, instrumental analysis, multivariate statistical analysis, and RDA analysis were conducted on samples of RPT from 26 tea-producing mountains (TPMs) in the tea-production region of Pu'er City, investigating the flavor characteristics of RPTs from different producing areas and the metabolite differences behind "one mountain, one flavor". This study will contribute to a comprehensive understanding of the flavor characteristics of RPT, and provide a theoretical basis for controlling and improving the flavor quality of RPT.

2. Materials and methods

2.1. Reagents and tea samples

Methanol, folin-phenol, indene triketone, anhydrous sodium carbonate (Sinopharm Chemical Reagents, Shanghai, China). *N, N*-dimethylformamide, glacial acetic acid, ethanol, methanol (chromatographic grade, Sinopharm Chemical Reagents, Shanghai, China). Gallic acid, (+)-catechin (C), (−)-epicatechin (EC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin gallate (EGCG), (−)-gallocatechin gallate (GCG), (−)-epigallocatechin (EGC), caffeine, and theobromine (standards, 99 %, Sigma-Aldrich Trading Co., Ltd., Shanghai, China). Aspartic acid (Asp), serine (Ser), glutamic acid (Glu), glycine (Gly), histidine (His), arginine (Arg), threonine (Thr), alanine (Ala), proline (Pro), theanine (Thea), cysteine (Cys), tyrosine (Tyr), valine (Val), methionine (Met), lysine (Lys), isoleucine (Ile), leucine (Leu), phenylalanine (Phe) (standards, 95 %, Waters Technologies Co., Ltd., Shanghai, China). N-alkanes (C7-C28) and n-hexane (J&K Scientific Co., Ltd., Beijing, China). Ethyl decanoate (99.99 %, Anpel-Trace Co., Ltd., Shanghai, China). Purified water (China Resources (Holdings) Co., Ltd., Shenzhen, China).

A total of 26 RPT samples were provided by the Yunnan Pu'er Tea Factory Co., Ltd. in Pu'er City, Yunnan Province, China. The fresh leaf of these samples came from 26 TPMs in Pu'er City respectively and were subsequently made into RPT with the same processing parameters. The TPMs were selected for their geographic representativeness and popularity. Their names and exact geographic locations are shown in Fig. 1.

2.2. Sensory evaluation

The sensory evaluation was performed by six well-trained panelists (three men and three women, aged 23 to 49 years, from Hunan Agricultural University). The evaluation results of the RPTs were assessed according to the Chinese standards "Methodology of sensory evaluation of tea" (GB/T 23776–2018) and "Tea vocabulary for sensory evaluation" (GB/T 14487–2017). A 3.0 g tea sample was infused with 150 mL of boiling water and brewed for 7 min in the tea cup. After the tea infusion was poured out, panelists evaluated the appearance, infusion color, aroma, taste, and infused leaves, and then recorded the comments. Each sample was evaluated three times by each panelist on different days.

2.3. Color difference detection of the tea infusion and quantification of main phytochemical compositions

The colorimeter (YS6003, 3nh Technology Co., Ltd., Shanghai, China) was used for the color analysis of tea infusion. The extraction method of tea infusion using the method described in the sensory evaluation section. Purified water served as a blank control, and color analysis was conducted on the test infusion after cooling to room temperature. The color evaluation of tea infusion was conducted by the color numeration system of L*a*b* (Pathare, Opara, & Al-Said, 2013). L* value reflects brightness (from 0 in pure black to 100 in pure white), a* value describes the color tendency on the red-green axis (positive values indicate redness, negative values indicate greenness), and b* value describes the color tendency on the yellow-blue axis (positive values indicate yellowness, negative values indicate blueness). To accurately compare the overall color differences among different samples, the total color difference (ΔE) was calculated as follows:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

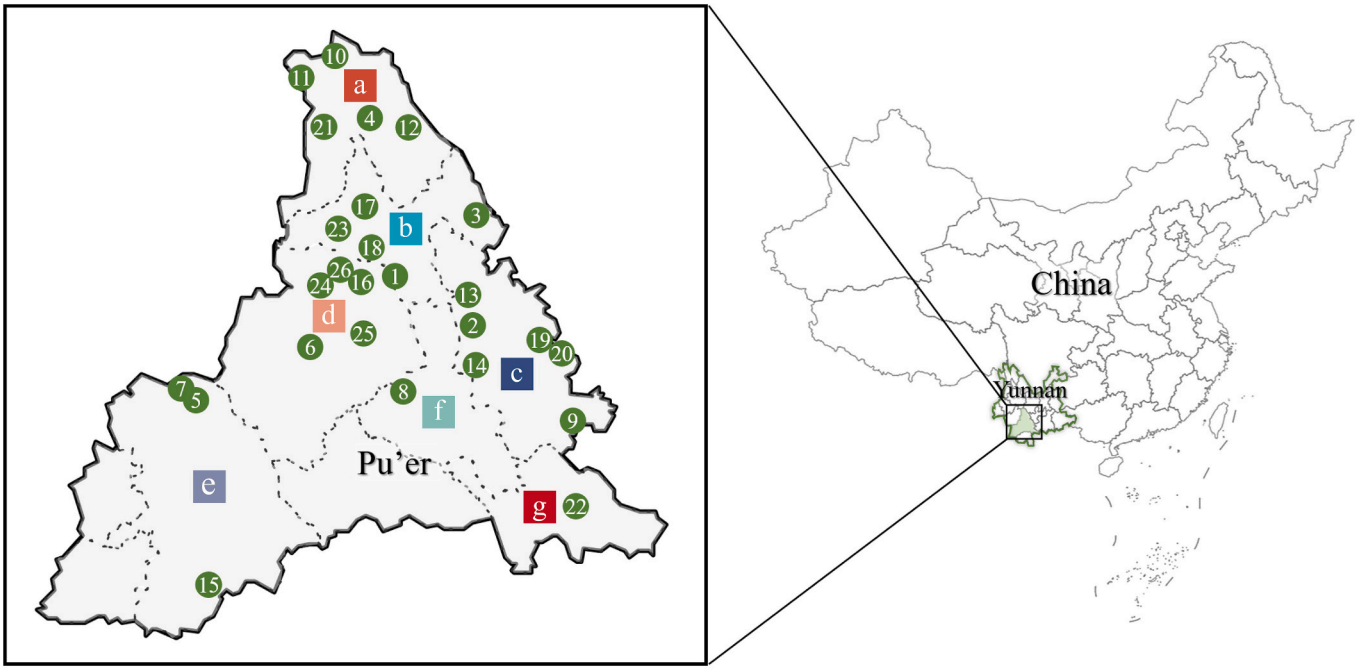
where ΔL^*, Δa^*, and Δb^* are the color parameter differences between the detected sample and the blank control.

The content of water extract and free amino acid was determined as described by GB/T 8305–2013 and GB/T 8314–2013 standards, respectively. Tea polyphenols and soluble sugar were quantified using the folin-phenol colorimetric method and anthrone-sulfuric acid colorimetric assay, respectively (Qiu et al., 2022). The concentration of flavonoids was determined by a colorimetric method (Petry, Ortega, & Silva, 2001). HPLC was employed for the detection of alkaloid, catechin, and amino acid components in tea, as referenced in Y. Li, Li, Gong, and Liu (2011). The analysis was performed three times.

2.4. Volatile compounds detected by HS–SPME/GC × GC–QTOFMS

2.4.1. Volatile compounds extraction by HS–SPME

The volatile compounds in tea samples were extracted by headspace



a JD: Jingdong	b ZY: Zhenyuan	c MJ: Mojiang	d JG: Jinggu	e LC: Lancang	f NE: Ning'er
4 Yubi	1 Tianba	2 Jingxing	6 Tuanjie	5 Bangwei	8 Kunlushan
10 Laocangfude	3 Madeng	14 Tongguan	16 Nanbanhuangcaoba		
11 Manwan	17 Mengda	9 Baliu	24 Yangta	7 Wendong	g JC: Jiangcheng
12 Ailaoxipo	18 Laowushan	19 Xuli	25 Lianhelongtang		
21 Jinding	23 Zhentai	13 Midi	26 Wenshan	15 Jingmai	22 Guoqing
		20 Longba			

Fig. 1. Map of 26 TPMs in the tea production region of Pu'er. The numbers represent the geographic coordinates of the TPMs, while a-g represent the counties where they are located.

solid-phase microextraction (HS–SPME). To obtain the optimal extraction conditions for the samples in the present study, HS–SPME was performed according to previously established methods, with appropriate modifications made as necessary (Chen et al., 2023). First, each sample was initially homogenized and ground to powder. From each tea powder sample, exactly 1.0 g was taken and mixed uniformly to create a quality control (QC) sample. The QC sample was used to examine the stability and repeatability of the applied method. Eventually, the extraction conditions were as follows: A 0.5 g of tea sample powder was weighed and placed inside a 15 mL headspace vial (Agilent, Santa Clara, CA, USA). Subsequently, a magnetic force rotor (6 × 8 mm; Anpel Co., Ltd., Shanghai, China) was added. Introduced into the vial along with 5 mL of boiled water and 10 μL of ethyl decanoate (10 ppm) as internal standard. The headspace vial was tightly sealed using a cap equipped with a silicone headspace septum, then positioned on a magnetic heating stirrer set at 80 °C and 600 r/min. After a 10-min equilibration period, the 85 μm carboxen/polydimethylsiloxane (CAR/PDMS) coating fiber (Sigma-Aldrich Trading Co., Ltd., Shanghai, China) extraction head was pushed out to a position 1 cm from the liquid surface, incubated for 30 min at 200 r/min and 80 °C. At the end of the extraction, the fiber was inserted into the GC × GC-QTOFMS injector for desorption (10 min, 250 °C) and data acquisition. Each sample was repeated three times.

2.4.2. GC × GC–QTOFMS conditions

An agilent GC × GC–QTOFMS (Agilent, Santa Clara, CA, USA) instrument was used to analyze the volatile compounds of RPTs. The one-dimensional GC column was HP-5MS (37.69 m × 250 μm × 0.25 μm; Agilent, USA) and the two-dimensional column was DB-17MS (2.89 m × 180 μm × 0.18 μm; Agilent, USA). High-purity helium (99.99 %) was used as the carrier gas at a flow rate of 1.1 mL/min. The heating program was slightly adjusted based on previous research (Ouyang et al., 2024). After parameter optimization through QC sample testing was completed, the GC × GC conditions were set as follows: The split ratio was 10:1. The injector temperature was set at 250 °C, and the temperature program was as follows: the initial column temperature was 40 °C (held for 1 min), increased to 180 °C at a rate of 4 °C/min, and to 250 °C at 20 °C/min (held for 1 min). The total runtime was 40.5 min.

A solid-state thermal modulator (SSM1820, J &X Technologies, Shanghai, China) was used between the two columns for the heating and cooling stages. The modulator period was 4 s, and the modulation column was HV (C5-C30, 1.3 m × 0.25 mm; J &X Technologies, Shanghai, China). The modulator's inlet and outlet temperatures were synchronized with the GC temperature program.

The mass spectrometer parameters were as follows: 70 eV electron ionization energy and mass scan range from 45 to 500 *m/z*, 50 Hz/s acquisition rate, solvent delay time of 3.0 min, quadrupole temperature of 150 °C, and ion source temperature of 200 °C.

2.4.3. Qualitative and quantitative analysis of volatile compounds

The data obtained from GC was analyzed using the Canvas software (version 1.0.0.25117, J & X Technologies, Shanghai, China). The minimum peak detection signal-to-noise ratio was set to 10. The information

of each spectrum peak was compared with the main library (*mainlib*), the replicates library (*replib*), and the retention indexes library (*nist_ri*) of the NIST20 standard library. Normal alkanes C7-C19 (Sigma Aldrich Corporation, Saint Louis, MO, USA) served as external references to calculate the retention index (RI) of each compound. The calculation method of RI referred to Ouyang et al. (2024). Volatile compounds were screened with a penalty intensity of "very strong", retaining volatile compounds with a forward match >700, reverse match >800, and RI deviation <15.

Quantification was based on the internal standard solution. The concentrations of the volatile compounds were calculated in ng/g by comparing their peak areas with the peak area of ethyl decanoate.

$$c = (A/A_s \times c_s \times v_s)/m$$

where c is the concentration of volatile compounds, ng/g; A and A_s are the peak area of volatiles and the internal standard, respectively; c_s is the concentration of the internal standard, mg/μL; v_s is the volume of the internal standard, μL; m is the quantity of the tea sample, g.

2.5. GC–O and OAV analysis

An olfactory detection port (Sniffer 9100; Brechbühler, Schlieren, Switzerland) was used to analyze the evaluation of the aroma compounds of RPT. The column temperature program for GC–O analysis is consistent to that of GC × GC–TOFMS analysis, and the aroma extract was split between the TOFMS and Olfactory Detection Port with a 1:1 proportion. High-purity helium (99.99 %) served as the carrier gas for GC–O. The temperature of the GC–O injector was set to 230 °C, and the transfer line was set to 260 °C.

The GC–O analysis of odor-active compounds in the QC samples of RPTs was performed by three trained panelists (one male and two females). All panelists were tea experts with at least 5 years of sensory evaluation experience, and were well-trained over 90 h to become familiar with diverse odor descriptions using varying standards with different concentrations. During the olfactory assessment, panelists recorded the odor descriptors, retention time, and intensity values. A scale ranging from 0 to 4 was used to evaluate the aroma intensity: 0 (none), 1 (weak), 2 (moderate), 3 (strong), 4 (extremely strong). The odor descriptions that attributes detected by at least two panelists at the same retention time were retained. The aroma intensity value was calculated as the average of the corresponding panelists. Compound identification of aroma was confirmed by MS, RI, and other methods.

The impact of a volatile compound on the overall aroma was measured using odor activity value (OAV). The OTs were obtained from the handbook of flavor ingredients, reported literature, or relevant websites.

$$OAV = c/OT$$

where *OAV* is the odor activity value of the volatile compound; c is the concentration of the volatile compound, ng/g; *OT* is the odor threshold of the volatile compound in water, μg/kg.

2.6. Statistical analysis

All analyses were repeated at least 3 times and results were expressed as mean ± standard deviation by Microsoft Office Excel 2016. SPSS (version 26.0, International Business Machines Corporation, USA) performed a one-way analysis of variance, and $p < 0.05$ was considered statistically significant. The heat map was performed using the Omic-Studio tools at https://www.omicstudio.cn/tool (Lyu et al., 2023). R (version 4.2.2) was used for principal component analysis (PCA), hierarchical cluster analysis (HCA) (Euclidean distance), consensus clustering analysis (K-means, Euclidean distance), and Pearson's correlation analysis, Redundancy analysis (RDA). The veen plot was performed in Origin (version 2022, OriginLab, USA). The bar chart was performed using GraphPad Prism (version 8.0.0 for Windows, GraphPad Software, USA).

3. Results and discussion

3.1. Sensory analysis and Main phytochemical compositions analysis

The sensory analysis of RPTs from different TPMs was conducted for appearance, infusion color, aroma, taste, and infused leaves (Table S1). A high level of similarity in appearance among all RPTs is shown in Fig. S1, which suggests the good tenderness of the raw materials. Five infusion hues were recorded, including golden yellow, light orange yellow, orange yellow, deep orange yellow, and yellowish orange. Then, a colorimeter was used to make measurements on each sample (Table S2), and the color sensation was shown in Fig. 2A. The total color difference ΔE* values showed varieties among different hues. As the color deepens, the a* value remains relatively stable, whereas the range of b* and ΔE* values distribution gradually increases, representing a change in the direction in hue from golden yellow to yellow orange. The color of the tea infusions usually correlates with the oxidation degrees of the tea polyphenols after processing (Feng et al., 2019). The average content of tea polyphenols tends to increase as the color lightens (Fig. 2B, Table S3), and a similar trend is observed in total catechin components (Table S4). However, flavonoid and caffeine content differences were not significant among the tea infusions. Therefore, the hue difference may be attributed to the contents of the oxidation products of tea polyphenols.

For the taste evaluation, RPTs were primarily characterized as heavy and thick, while some exhibited a mellow quality. Varying degrees of astringency were observed in the tea infusions, which was mainly due to the high content (82.08 %) of ester catechins, including EGCG, ECG, and GCG (Table S5). Moreover, the content of amino acids in tea contributes significantly to its taste. The analysis revealed that umami amino acids accounted for the largest proportion (54.65 %) of taste-active amino acids in RPTs, followed by bitter (35.64 %) and sweet (8.58 %) amino acids, which is similar to findings from other studies (Zhou et al., 2020). However, the content of these compositions in 26 RPTs generally showed no significant distribution pattern (Fig. 2C).

Of the aroma type, a majority of RPTs featured floral and fruity notes. However, there were significant variations in their intensity and persistence. Samples 1 and 15 were particularly prominent, characterized by pronounced and enduring floral-fruit aromas, further enriched with sweet characteristics. Some of the samples presented a clean and pure aroma, which can be attributed to their basic aroma of RPTs, and exude no strange odors. Of particular note, several samples displayed special aromas, such as betel nut aroma (sample 5) and pekoe flavor (sample 24), which may be attributed to the differences in the accumulation of precursor compounds in fresh tea leaves. The diversity of the aromatic profiles of RPTs may contribute significantly to the formation of "one mountain, one flavor".

3.2. Identification and quantification of the volatile compounds in PRTs from different TPMs by GC × GC–TOFMS

To further identify the volatile compounds contributing to the aroma of RPTs from different TPMs, HS–SPME–GC × GC–QTOFMS analysis was conducted. Confirmation of identification was completed by comparing the linear retention index (RI) with N-alkane standard solution (C7-C19) (Table S6). The total ion chromatography (TIC) plots of N-alkane and QC sample are shown in Fig. S2-S3, while the surface plots of samples are shown in Fig. S4. A total of 225 volatile compounds were identified from 26 samples, with the detailed results presented in Table S7. It can be classified into 8 categories based on chemical structure (Fig. 3A), including 81 hydrocarbon compounds (36 %), 40 ketones (17.78 %), 30 alcohols (13.33 %), 30 aldehydes (13.33 %), 22 heteroxy compounds (9.78 %), 14 esters (6.22 %), 5 nitrogen compounds (2.22 %), and 3

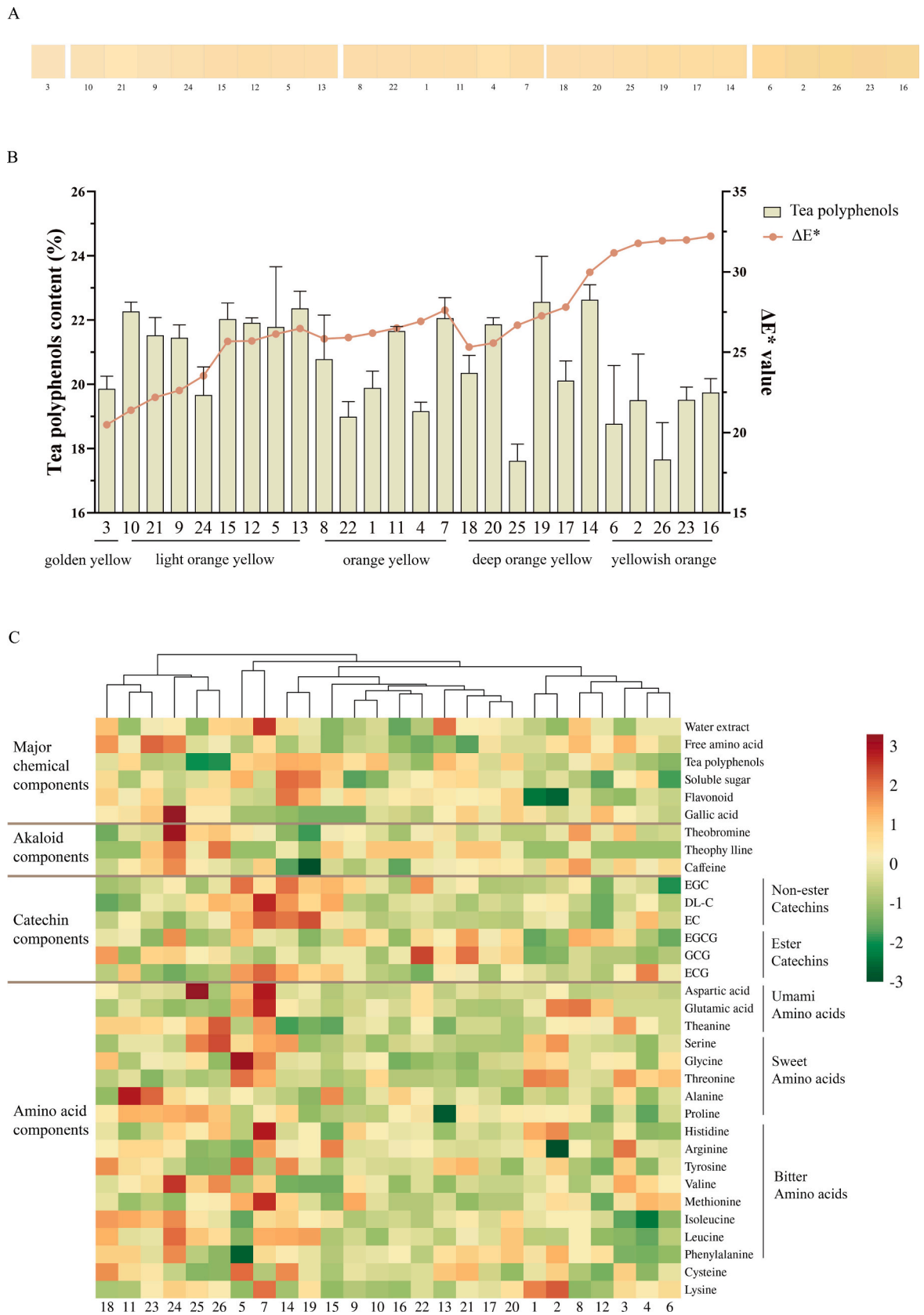


Fig. 2. Color difference and main phytochemical compositions of the tea infusion in RPTs from 26 TPMs. (A) The color sensation of the tea infusions by the color numeration system of L*a*b*. (B) Variation trend of tea polyphenols content and ΔE* value in different color tea infusions. (C) Heat map of the content of main phytochemical compositions.

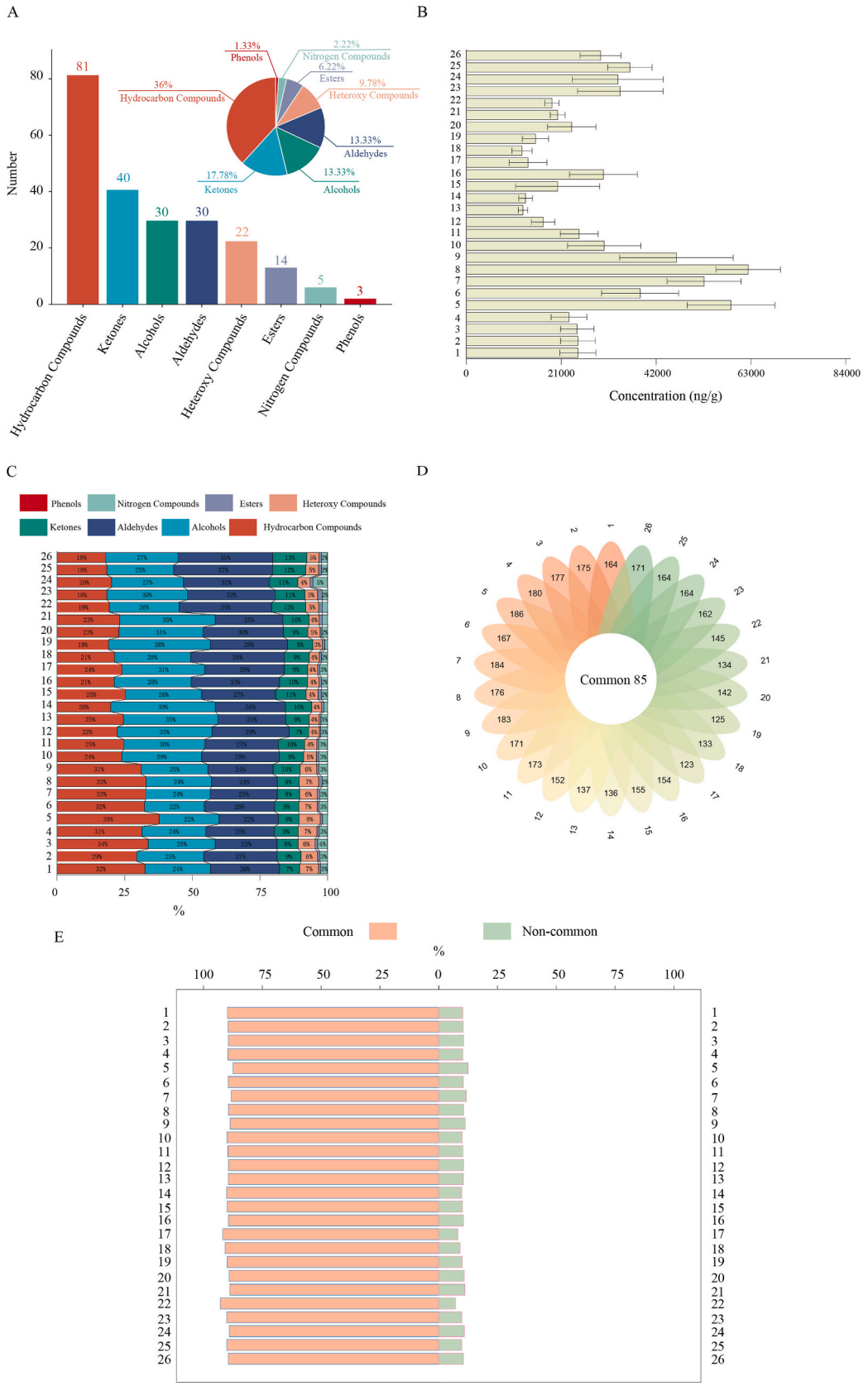


Fig. 3. The volatile compounds in RPTs from 26 TPMs. (A) The number and percentage of total volatile compounds. (B) The total content of volatile compounds. (C) Proportion of volatile compounds. (D) The number of volatile compounds. (E) Comparison of relative abundances of common and non-common volatile compounds.

phenols (1.33 %). Hydrocarbon compounds, ketones, alcohols, and aldehydes together constitute over 80 % of the total aroma compounds, which is consistent with previous findings (Guo et al., 2023).

There is a significant difference in the total concentration of volatile compounds among different RPTs (Fig. 3B). It is worth noting that tea samples with high content of volatile compounds may not necessarily have strong and lasting characteristics in aroma sensory evaluation, nor can they represent a specific aroma type (Feng et al., 2019). Alcohols (21 %–38 %), aldehydes (22 %–37 %), hydrocarbon compounds (15 %–34 %), and ketones (10 %–16 %) represented the principal volatile components of RPTs, accounting for nearly 90 % of the total concentration (Fig. 3C). Alcohols and ketones have strong positive effects on the floral and fruity aroma of tea samples, and some alcohols are accompanied by woody aroma (e.g., linalool, linalool oxides, and cedrol). Among ketones, α-ionone and β-ionone contribute to the sweet, floral, and woody aromas in RPT, and damascenone imparts intense sweet and honey-like characteristics (He et al., 2016). In addition, alkanes and alkenes in hydrocarbon compounds also contribute to the woody and fruity aroma. These typical volatiles have good harmonization with the aroma of RPT. Notably, the samples in this study generally had a high content of aldehydes, but it was not consistent with previous studies that reported relatively low aldehydes content (W. Zhang et al., 2019). The variation in raw material, geography, manufacturing process, and aroma detection method could contribute to the differences. Aldehyde aroma compounds mainly impart fresh and grassy notes, such as benzaldehyde, (E)-2-octenal, and nonanal, which contribute to the green-grassy aroma.

Variations were observed in the number of volatile compounds detected in 26 RPTs (123–186), with 85 common compounds (Fig. 3D). However, common compounds constituted just 45–75 % of the total, suggesting substantial variation in the quantities of non-common compounds across RPTs from different TPMs. Interestingly, the common compounds in each tea sample exhibited a relatively stable and high percentage content (Fig. 3E). Despite variations in the composition and

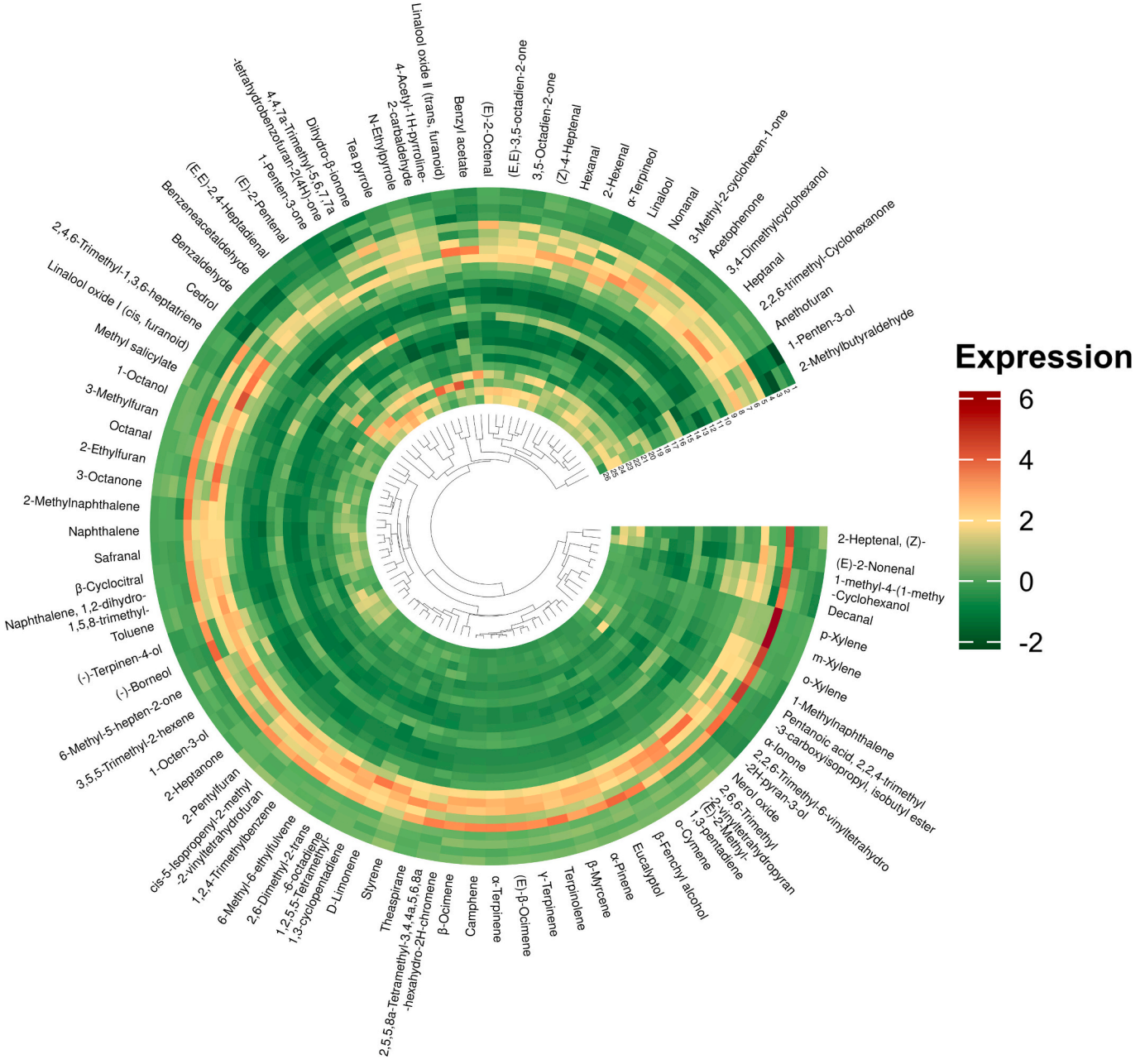


Fig. 4. Heat map of the content of common volatile compounds in RPTs from different TPMs.

content of volatile compounds, the relative abundance of common compounds across each RPT was consistently high (>85 %). These results suggest their importance in the formation of aroma characteristics. The heat map (Fig. 4) presented in detail the concentration distribution of 85 common compounds in each sample. It was mainly composed of hydrocarbon compounds, aldehydes, and alcohols. The contents of linalool, D-limonene, α-terpineol, benzaldehyde, nonanal, hexanal, 2,2,6-trimethyl-cyclohexanone, o-cymene, 2-methylbutyraldehyde, and 2-pentylfuran were relatively high (Table S8). These compounds are characterized by a floral and fruit fragrance, as well as a fresh and cool aroma (Du et al., 2014), which may be related to the features of RPTs in aroma sensory evaluation. Notably, the concentration changes of these compounds had a high degree of fit with the corresponding trend of total volatile compound content in the mountain, indicating the significant importance of these substances in the formation of the unique aroma of RPTs.

3.3. GC–O and OAV analysis of the volatile compounds

In GC–O analysis, 29 odorants were identified in RPTs (Table 1). The aroma intensity of linalool with sweet floral, grassy, and vanilla flavors was the highest (3.5), followed by β-cyclocitral (lemon peel, green leaves, cool, penetrating, 3.3), 6-methyl-5-hepten-2-one (metallic, shiitake, rubber, enoki mushroom, 3.2), geraniol (lemony, passion fruit, herbaceous aroma, strong floral, 3.2), β-ionone (violet, floral, 3.2) and damascenone (green fruit, molasses, strong honey, strong sweet, 3.0). Most of them are associated with floral, grassy, and citrusy fragrances. However, some volatile compounds with high content did not show significant aroma intensity nor were detected in olfactory records. It may be attributed to a high odor threshold or similar peak times, making it difficult to distinguish by smell. The odor-active compounds can be categorized into four classes by odor description (Table 1): (A) Sweet Aromatic Type, represented by compounds such as linalool, geraniol, β-ionone, damascenone, and methyl salicylate, which exhibit sweet floral, fruity, or honey-like; (B) Fresh Aromatic Type, including

Table 1
Odor-active compounds of RPTs from different TPMs.

No.	CAS	Odorants	OT (ug/kg)[a]	OAV[b]	Odor descriptors	Aroma intensity	Identification	Classification
1	96-17-3	2-Methylbutyraldehyde	1.3	417.56	mild smoky, green, almond	2.8	MS, O	B
2	1629-58-9	1-Penten-3-one	1	496.21	green, chestnut	1.7	MS, O	B
3	108-88-3	Toluene	6	59.68	enoki mushroom	1.6	MS, RI, O	C
4	66-25-1	Hexanal	4.5	232.75	green grass, fresh	1.5	MS, RI, O	B
5	6728-31-0	(Z)-4-Heptenal	0.06	3099.88	baked potatoes, biscuit, fat, chestnut	2.3	MS, RI, O	D
6	80-56-8	α-Pinene	41	2.11	toasted, resin, turpentine	1.6	MS, RI, O	D
7	3391-86-4	1-Octen-3-ol	1.5	159.75	earthy, mushroom, enoki mushroom-like	2.7	MS, RI, O	C
8	110-93-0	6-Methyl-5-hepten-2-one	100	4.65	metallic, shiitake, rubber, enoki mushroom	3.2	MS, RI, O	C
9	470-82-6	Eucalyptol	1.1	28.1	cool, eucalyptol, floral	1.3	MS, RI, O	A
10	122-78-1	Benzeneacetaldehyde	4	81.92	pleasant earthy, berry	0.8	MS, RI, O	C
11	586-62-9	Terpinolene	200	2.07	woody, pine	0.8	MS, RI, O	C
12	34995-77-2	Linalool oxide II(trans, furanoid)	320	1.08	citrus, leaf	1.5	MS, RI, O	B
13	78-70-6	Linalool	6	541.5	weak herbal green, floral, sweet flower, vanilla	3.5	MS, RI, O	A
14	16647-04-4	6-Methyl-3,5-heptadien-2-one	375–380	<1	soapy, smooth, sweet fruity, cool	1.3	MS, RI, O	A
15	18368-95-1	p-1,3,8-menthatriene	15	<1	green fruit, cedarwood	0.8	MS, RI, O	C
16	18829-56-6	(E)-2-Nonenal	–	–	cucumber, diluted floral	2	MS, RI, O	B
17	693-54-9	2-Decanone	3	10.21	plastic, kraft paper, slightly toasted	0.8	MS, RI, O	D
18	119-36-8	Methyl salicylate	40	2.13	mushroom aroma, almond	2.4	MS, RI, O	C
19	432-25-7	β-Cyclocitral	5	64.18	lemon peel, green leaves, cool, penetrating	3.3	MS, RI, O	B
20	106-24-1	Geraniol	7.5	3.43	lemony, passion fruit, herbaceous aroma, strong floral	3.2	MS, RI, O	A
21	21964-48-7	1,12-Tridecadiene	–	–	unripe fruit, paper aroma, green	1.7	MS, RI, O	B
22	112-12-9	2-Undecanone	5.5	<1	woody aroma, penetrating, mushroom aroma, aroma after aging	1.6	MS, RI, O	C
23	91-57-6	2-Methylnaphthalene	10	3.46	coconut, wood, aroma after aging	0.8	MS, RI, O	C
24	13341-72-5	Isomintlactone	–	–	lime blossom, sour, fruity, fresh mint	1.6	MS, RI, O	A
25	31501-11-8	(Z)-3-Hexenyl hexanoate	–	–	mild sweet, molasses	1.8	MS, RI, O	A
26	23726-93-4	Damascenone	0.002	8432.98	green fruit, molasses, strong honey, strong sweet	3	MS, RI, O	A
27	127-41-3	α-Ionone	10.6	4.83	sandalwood, floral	0.8	MS, RI, O	A
28	14901-07-6	β-Ionone	8.4	21.81	violet, floral	3.2	MS, RI, O	A
29	15356-74-8	4,4,7a-Trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	0.17678	210	coumarin, musk	1.2	MS, RI, O	C

[a] OTs: Odor thresholds in water. "-" signifies that the OT value was not found in the search.
[b] OAV: Odor Activity Value.

β-cyclocitral, 2-methylbutyraldehyde, and (E)-2-nonenal, which are associated with green or grassy notes; (C) Aged Aromatic Type, including 6-methyl-5-hepten-2-one, 1-octen-3-ol, and methyl salicylate, which exhibit woody, aged, or mushroom-like aromas; (D) Roasted Aromatic Type, including (Z)-4-Heptenal, α-Pinene, and 2-Decanone. Class A exhibited the highest total aroma intensity (19.7), followed by Class C (15.9), Class B (14.5), and Class D (4.7). We speculate that 35.95 % of the sweet aromatic type constitutes the foundation of flowery and fruity aroma, 26.46 % of the fresh aromatic type is instrumental in the clean and pure characteristics, and 29.01 % of the aged aromatic type contributes positively to the overall aroma tone of RPTs.

Compounds with OAV values greater than 1 are considered to have an appreciable impact on the aroma profile of RPTs (Table 1). High OAVs (159.75–8432.98) were exhibited, with compounds such as linalool, damascenone, 2-methylbutyraldehyde, 1-octen-3-ol, and (Z)-4-heptenal, which correspond to high olfactory intensity. Damascenone and (Z)-4-heptenal greatly contribute to the aroma because of their extremely low odor thresholds. Damascenone is synthesized through two primary pathways: oxidative cleavage of carotenoid xanthophylls and acid-catalyzed hydrolysis of plant secondary metabolites or glycosides (Tian et al., 2024). Recognized as a main flavor component in the red berry and apple, it is also believed to significantly contribute to the fruity note of RPT. (Z)-4-heptenal is a potential marker for RPT, which is significantly influenced by wet-hot conditions and plays a crucial role in shaping the unique flavor of RPT (Xu et al., 2021). Additionally, except β-ionone, the remaining compounds were consistently detected in all tea samples, suggesting their significance as common key odorants among RPTs.

A total of 21 key odor-active compounds were identified by combining the GC–O and OAV analysis, including 2-methylbutyraldehyde, 1-penten-3-one, toluene, hexanal, (Z)-4-heptenal, α-pinene, 1-octen-3-ol, 6-methyl-5-hepten-2-one, eucalyptol, benzeneacetaldehyde, terpinolene, linalool oxide II, linalool, 2-decanone, methyl salicylate, β-cyclocitral, geraniol, 2-methylnaphthalene, damascenone, α-ionone, and 4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one.

3.4. Multivariate statistical analysis of RPTs from different TPMs

To explore potential influencing factors that contribute to flavor diversity in different TPMs, we used multivariate statistical analysis to classify and investigate the volatile compounds. Principal component analysis (PCA) is an unsupervised statistical dimensionality reduction technique. It can transform multiple correlated variables into a few uncorrelated variables through matrix transformation to reflect the potential relation of samples (Ivosev, Burton, & Bonner, 2008). Considering the significant impact of both concentrations and the ratios of aroma compounds on the overall tea flavor profile, PCA was used to analyze the concentrations and relative percentages of 225 volatile compounds in RPTs from different TPMs. For the concentrations, PC1 contributed 67.7 % and PC2 contributed 7.9 %, cumulatively explaining 75.6 % of the total variance. As shown in Fig. 5 Ab, most samples were concentrated in the first and fourth quadrants; however, some samples exhibited unique outlier characteristics. These distribution patterns are not only caused by differences in total volatile compound concentrations among samples but may also be related to compounds unique to certain samples. For the ratios, PC1 contributed 43.3 % and PC2 contributed 16.8 %, collectively accounting for 60.1 % of the total variance. It is worth noting that the samples in the first quadrant were primarily composed of aldehydes, which constituted the highest proportion of their chemical composition. In contrast, samples in the second quadrant were mainly characterized by hydrocarbon compounds, while those in the fourth quadrant were primarily associated with alcohols. Nevertheless, these initial results are not what we expected, and the underlying relationships still require further exploration.

Therefore, we revisited the PCA results based on the aroma types (Fig. 5 Aa) of the samples which are from the results of sensory evaluation, as well as their longitude (Fig. 5 Ba), latitude (Fig. 5 Ca), and elevation (Fig. 5 Da) of the origin of raw materials. Furthermore, their significantly different volatile compounds (Table S9) were screened out based on the fold change (| log2(Fold Change) | > 0.5) and *t*-tests ($p < 0.05$).

3.4.1. Aroma types

Based on sensory evaluation results (Table S1), RPTs exhibit diverse aromatic profiles, they can be mainly classified into two categories: one highlights sweet, floral, and fruity aromas (floral and fruit type), and the other tends to have clean and pure characteristics (pure type). Based on the concentrations (Fig. 5 Ab) or the ratios (Fig. 5 Ac) of volatile compounds, samples of the two aroma types were not completely separated on the PCA score plot. A possible reason is subtle variations in a few odor-active compounds, which prevents us from distinguishing them in the broader profile (Yu et al., 2023). Interestingly, a more distinct separation trend was observed in Fig. 5 Ac compared to Fig. 5 Ab, suggesting that the compositional ratios of volatile compounds may be the key to the formation of their aroma characteristics.

There were 24 and 22 differential volatile compounds identified in terms of concentration and ratio level respectively, mainly including hydrocarbon compounds, ketones, and alcohols. Higher levels of aldehydes and ketones with grassy, green, and woody scents may contribute to the formation of pure aroma, including pentanal, biphenyl, (E)-2-pentanal, 1-penten-3-one, germanylacetone, β-homocyclital, isomenone, isophorone, and 4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one. In addition, high proportions of alcohols and hydrocarbon compounds such as mesityl oxide, hotrienol, (E)-2-methyl-1,3-pentadiene were found in floral and fruit aroma samples. Notably, hotrienol possesses distinct floral-fruit attributes, with a low aroma threshold and relatively high boiling point, which plays an important role in the formation of tea aroma (Kang, Zhu, Zheng, Liang, & Lin, 2018). Mesityl oxide, with its honey-like odor, may also be a key contributor to the floral-fruit character of RPT.

3.4.2. Geographic locations

By analyzing the PCA results across dimensions of longitude, latitude, and county, (Fig. 5Ba, 5Ca, 5 Da), we noticed that the PCA results from the former two have a high overlap in confidence intervals, but exhibit different distribution patterns. (Fig. 5 Bb, 5Bc, 5Cb, 5Cc). It may suggest that the influence of geographical factors on tea aroma differs along both longitudinal and latitudinal directions. The separation degree of TPMs categories divided by county appeared more distinct in the PCA plot compared to the two natural geographical attributes of longitude and latitude. (Fig. 5 Db, 5Dc). We speculate that this phenomenon could be attributed not only to the integrated effects of longitude and latitude but also possibly to differences in cultivation and management standards for tea trees within individual counties. Moreover, the differences in volatile compound accumulation among samples from different TPMs may also contribute to these disparities (Wu et al., 2016).

The 192 differential compounds were present in RPTs from the three county groups, with particularly high concentrations and ratios in LC county. The concentration and proportion of compounds with woody and herbaceous fragrances were relatively high, such as (E)-β-ocimene, α-phellandrene, β-ocimene, camphene, 4-isopropenyltoluene, terpinolene, γ-terpinene, α-terpinene, α-pinene, β-myrcene. In addition, the distinctly sweet and fruity compounds, such as 2-undecanone, ethyl hexanoate, nerol oxide, 2-decanone, and decanal, were also concentrated in this region, which may contribute to the floral and fruit aroma of RPTs. Overall, differential compounds characterized by green, woody, citrus, floral, and fruity notes were enriched in LC county, most of which also possessed higher concentrations in the southern part of Pu'er City. Thus, the aroma profile of LC county RPTs is likely shaped by the latitude.

For JG county, there was a remarkable enrichment of compounds

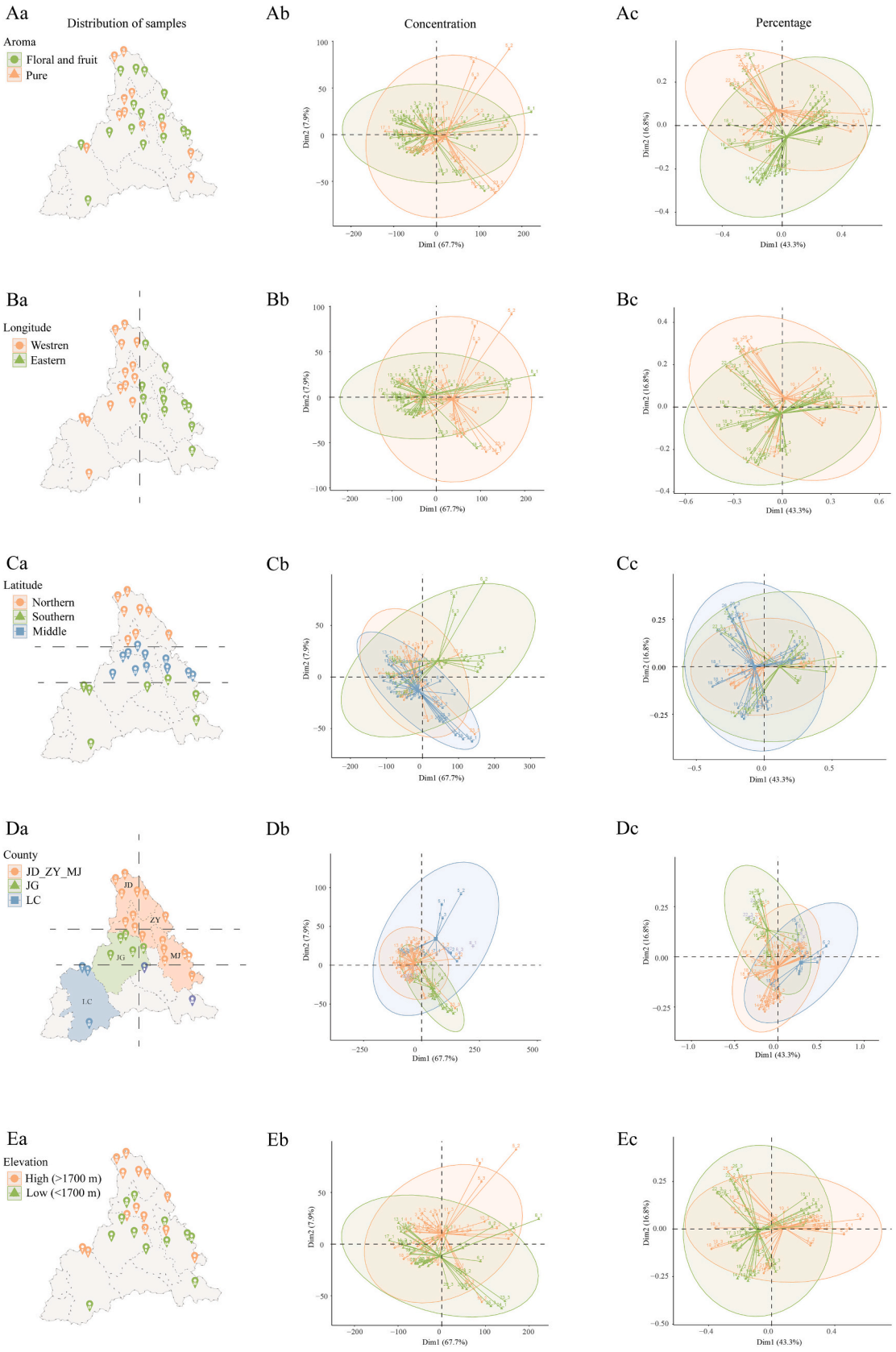


Fig. 5. Principal Component Analysis (PCA) of RPTs from 26 TPMs. A-E represent different classification criteria, while a-c represent PCA classification results, concentration results and percentage results, respectively (ellipses indicate 95 % confidence intervals).

exhibiting green, almond, and fatty aromas, including (E, E)-2,4-heptadienal, (E, E)-3,5-octadien-2-one, isovaleraldehyde, 2-hexenal, 1-penten-3-one, 3-ethyl-2-methyl-1,3-hexadiene, pentanal, and (E)-2-pentenal. Meanwhile, benzeneacetaldehyde and 2,6,6-trimethyl-2-cyclohexene-1,4-dione contributed significantly to the floral and fruit attributes distinctive to JG samples. Benzeneacetaldehyde is one of the important aromatic substances that affect the essential tea aromas, with a fragrance similar to hyacinth (Jiang, Boorboori, Xu, & Lin, 2021). Notably, isophorone (cedarwood, spice, tobacco), 2,4-dimethylbenzaldehyde (mild, sweet, bitter-almond), β-ionone (floral, sweet, violet) accumulated in the western part of Pu'er City, as well as in LC county and JG county. It may suggest that longitude change exerts some effect on the distribution of such compounds.

For JD_MJ_ZY county, 7 contributing compounds had the highest content compared to the other two regions, including hotrienol, anthracene, undecanal, 3-methylpentane, dodecanal, 1-penten-3-ol, and cedrol. These compounds have significant sweet and fatty aroma characteristics. Among them, undecanal (rose, woody, and orange peel aroma) was also a characteristic differential compound in the northern part of Pu'er City. It has been detected in various teas and crops and is recognized as a pivotal contributor to their aromatic profiles (Xuexue et al., 2022; Yu, Liu, Zhang, Luo, & Zeng, 2021).

3.4.3. Elevations

High-mountain tea gardens generally have relatively low temperatures, high humidity, and abundant clouds and fog, which are conducive to the synthesis and accumulation of nitrogen compounds and aromatic substances in tea trees (Wang et al., 2022). The elevation of Yunnan's TPMs from Pu'er City is generally high ranging from 1090 m to 2350 m, however, they can still be affected by the relative differences in elevation (Xiong et al., 2023). Two categories were divided based on the average elevation of TPMs: high elevation and low elevation, with 1700 m as the dividing line (Fig. 5 Ea). There was a minor disparity in the concentrations and percentages of volatile compounds across different elevations, presenting a subtle trend toward separation (Fig. 5 Eb, 5Ec).

The differential compounds identified were mainly hydrocarbon compounds (55.17 %), such as camphene hydrate, γ-terpinene, cis-1-methyl-4-(1-methylethenyl)-cyclohexanol, β-ocimene, terpinolene, (E)-β-ocimene, and α-phellandrene. The contents of these compounds were higher in high-elevation RPTs than in low-elevation, and they were identified as the main contributors to the grass and woody aromas in tea. At the same time, compounds with floral and orange aromas such as 2-undecanone (orange, pineapple, rose), decanal, and undecanal (floral, citrus, fat, oil, sweet) were also enriched in high elevation, coordinating the floral and fruit aroma of RPTs. The ratio of benzyl alcohol was higher in high elevation, making an important contribution to the aromatic profile of tea with its faint apple-like flavor. It likely originates from phenylalanine metabolism in tea plants and accumulates in fresh leaves due to the influence of high-elevation climates (Jiang et al., 2021).

3.5. RDA analysis

RDA is a multivariate statistical method that combines the features of multiple regression analysis and PCA for exploring the relationship between a multivariate dataset and one or more sets of explanatory variables. Therefore, RDA analysis was used in this study to assess the effects of longitude, latitude, county, and elevation on differences in aroma type (Fig. 6A). The first two axes from RDA accounted for 61.68 % and 15.24 % of the total variance in aroma type differences, respectively. The PERMANOVA test showed that longitude ($R^2 = 0.1268$, $p = 0.001$), latitude ($R^2 = 0.1789$, $p = 0.001$), county ($R^2 = 0.4025$, $p = 0.001$), and elevation ($R^2 = 0.0503$, $p = 0.021$), significantly influenced aroma, with the county having the highest proportion. However, these elements explained only 37.02 % of the variance in volatile components (Fig. 6B), this may be one of the reasons that samples of different aroma types were poorly differentiated in RDA and PCA. Meanwhile, it also reminded us that more factors should be considered.

4. Conclusion

This study investigated the flavor characteristics of RPTs, as well as the relationship between the tea-producing regions and the metabolic profiles. In main phytochemical compositions, tea polyphenols and catechins are likely responsible for the variations in tea infusion color.

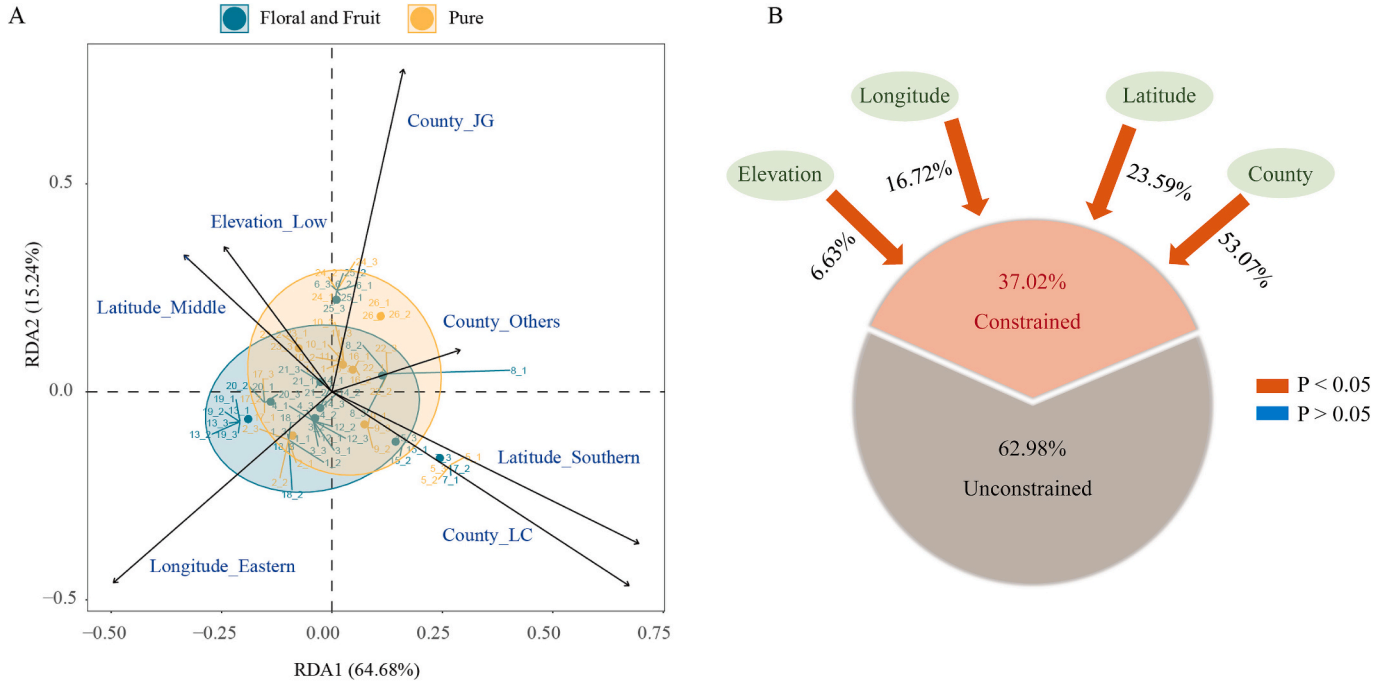


Fig. 6. Redundancy analysis (RDA) of RPTs from 26 TPMs. (A) The effects of longitude, latitude, county, and elevation on differences of aroma type (floral and fruit & pure). (B) Degree of explanation by each factor in RDA.

These components exhibit varying proportions across the 26 RPTs, without a clear distribution pattern in taste. The aroma characteristics exhibited a relatively higher degree of diversity. A total of 225 volatile compounds were identified in RPTs from different TPMs by HS–SPME–GC × GC–QTOFMS analysis, mainly alcohols, aldehydes, hydrocarbon compounds, and ketones, which exhibit floral, fruity, woody, and fresh aroma characteristics. Subsequently, 21 common compounds were identified as key odor-active components with a significant effect on the aroma of all RPTs by GC–O and OAV analysis. Further, multivariate statistical analysis was used to explore the differences in volatile compounds between different aroma types, longitudes, latitudes, counties, and altitudes. A relatively limited degree of explanatory power (37.02 %) had been proved by RDA among these geographical factors.

Results of this work indicated that while the RPTs from different TPMs exhibited a degree of flavor diversity, the extent of variation observed was less pronounced than what the principle of "one mountain, one flavor" might imply. However, the high mountain tea gardens have special microclimate environments, while environmental factors such as temperature, precipitation, and soil types also significantly influence the aroma of tea. Thus, increasing the number of mountain samples collected in the RPT production regions, and comprehensively considering the correlations between multiple factors and "one mountain, one flavor" should be priorities for subsequent research. Concurrently, metabolomic analysis should be employed to investigate the influence of non-volatile compounds on RPT flavor.

Ethical statement

The authors guarantee that the described work has been conducted in compliance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for human experimentation.

The national laws do not require ethical approval for sensory evaluation. There are no human ethics committees or formal documentation procedures available for sensory evaluation.

Participants gave informed consent via the statement "I am aware that my responses are confidential, and I agree to participate in this survey" where an affirmative reply was required to enter the survey. They were able to withdraw from the survey at any time without giving a reason. The tested products were safe for consumption. Before starting the sensory evaluation and analysis, the informed consent was obtained from all participating tea reviewers.

CRediT authorship contribution statement

Xinyi Zhang: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Hongyu Chen:** Writing – review & editing, Visualization, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Yang Liu:** Methodology, Investigation, Data curation. **Xingchang Ou:** Methodology, Investigation, Data curation. **Lun Liu:** Supervision, Resources, Project administration. **Jian Ouyang:** Supervision, Project administration, Methodology, Conceptualization. **Ronggang Jiang:** Methodology, Formal analysis, Conceptualization. **Xiaoqin Yi:** Methodology, Formal analysis, Conceptualization. **Ligui Xiong:** Writing – review & editing, Supervision, Methodology. **Zhonghua Liu:** Supervision, Project administration, Methodology, 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.

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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.2024.102001.

Data availability

Data will be made available on request.

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