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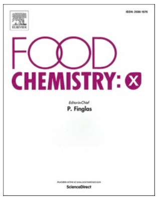

Discrepancy on the flavor compound affect the quality of Taiping Houkui tea from different production regions

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ABSTRACT

Taiping Houkui (TPHK) is prevalent green tea in China, its flavor quality is significantly influenced by different production regions. However, the key flavor compounds responsible for these discrepancies remain unclearly. Here, TPHK samples were produced from fresh leaves of 'Shidacha 2' cultivar planted in 14 distinct production regions. In 14 TPHK samples, a total of 33 non-volatile compounds were identified and quantified. Partial least-squares discriminant analysis (PLS-DA) reveal that theanine and glutamate were the main umami compounds, caffeine imparted with bitterness, which collectively contributed to the variation in the taste flavor of TPHK across different production regions. Furthermore, the profiles of 51 volatile compounds were determined, integrated PLS-DA with odor activity values of volatiles indicated that linalool (165.7–888.5) and geraniol (11.9–141.4) affecting the floral aroma of TPHK among different production regions. Our findings revealed the critical compounds that contributed to the effect of production regions on flavor quality of TPHK.

1. Introduction

Green tea is a non-fermented agricultural product with numerous bioactive compounds, which has been consumed most extensively in China (Bag, Mondal, Majumder, & Banik, 2022; Ridha, Alasady, Azooz, & Mortada, 2024). In addition, catechins can be divided into simple and ester catechins, where ester catechins mainly present astringent and bitter flavors, whereas simple catechins are only associated with bitterness (Ye, Yan, Cui, et al., 2018). Volatiles are crucial component of the flavor of green tea and contribute to its intricate composition (Yin et al., 2022). Previous studies have identified alcohols, aldehydes, ketones, and esters as the primary volatile compounds in green tea (Fang et al., 2022; Ma et al., 2019). The main constituents of chestnut aroma in green tea are 2-methylbutanol, 2,4-dimethylstyrene, D-limonene, phenylethanol, and jasmone, whereas geraniol, nonanal, linalool, and their oxides are the main constituents of floral aroma. (Han et al., 2016; Qu et al., 2023). The release of volatiles in the leaves during processing resulting in dominant clear, floral and chestnut aroma characteristics (Zhu et al., 2018). Despite extensive research dedicated to investigating the effect of variety and processing technology on tea quality, the impact of the production region remains poorly understood.

The environmental conditions surrounding tea cultivation areas, including the soil, light, temperature, and altitude, can create unique microclimates. Research on Wuyi rock tea revealed significant differences in the chemical composition of tea in different production regions, with tea produced in core regions exhibiting higher the caffeine and catechin contents than that produced in non-core regions. This may be due to differences in soil fertility between different production regions (Zhou, Xue, Zhuo, et al., 2019). Another study showed that Wuyi Rock tea has a unique "local flavor", and this flavor is due to production regional variations (Liu, Zhuo, Shi, et al., 2022). Further, differences among production regions in the intensity and light exposure timing contribute significantly to the disparities in the content of secondary metabolites. Studies have shown that red or low-intensity white light increase the content of catechins (Lu et al., 2014), and that shading significantly promotes the accumulation of theanine. (Chen et al., 2017). Additionally, tea quality varies with the altitudes at which it is produced. Terrains located at higher altitudes generally have more suitable

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light and temperature for the growth of tea plants, which results in the production of tea plants of superior quality and are wider acceptance (Wang, Gan, Sun, & Chen, 2022). Along with superior quality additional commercial value is produced. Consequently, core production regions command higher prices than non-core regions (Hong & He, 2020). Thus, a single factor cannot determine tea quality. In addition to tea plant varieties and processing techniques, production regions are also important in the formation of tea flavor and quality.

Taiping Houkui tea (TPHK), one of the ten national geographical indication products (Qi et al., 2020), is recognized for its orchid-like aroma. It is mainly produced in the Huangshan District (Huangshan City, Anhui Province), using new shoots of the "Shidacha" landraces as raw materials (Zhou et al., 2022). Production generally involves the picking of the shoots, withering, fixation, shaping, and drying. The superior geographical environment, unique tea plant varieties and processing technologies result in a TPHK with two leaves and one bud, a flat and straight appearance, and an orchid fragrance (Feng, Li, Li, Wan, & Yang, 2020). TPHK is loved by consumers because of its sweet, smooth taste, and excellent quality. However, the quality of TPHK varies greatly among different production regions owing to factors such as the growth environment, processing technology, and cultivars (Liao et al., 2020). Differences in quality are closely linked to differences in the composition of tea leaves. Therefore, the key components responsible for these differences require further investigation.

In this study, raw materials consisting of fresh leaves from one bud and two leave samples, obtained from plants of identical tree age and landraces that were sourced from fourteen different production regions, were a uniform methodology to produce TPHK. The primary non-volatile and volatile metabolites, were identified through sensory evaluation, and correlation analysis, among other methods, to explore flavor quality differences arising from the different production regions of the TPHK samples. This study provides a theoretical basis for the planting, quality evaluation and comprehensive utilization of TPHK.

2. Materials and methods

2.1. Tea sample

In April 2021, one bud and two leave samples were meticulously handpicked from fourteen distinct regions of the eight-year-old 'Shidacha No.2' clone tea plant cultivar within the TPHK production region of Huangshan District (Huangshan City, Anhui Province). All tea plants were fertilized and watered with same standard. The geographical distribution of the sampling sites is shown in Fig. 1 and Table S1. After picking, a part of the fresh leaves was frozen in liquid nitrogen and stored at -80 °C. The remaining leaves were processed into TPHK through the picking of shoots, withering, fixation shaping and drying.

2.2. Chemicals

The (−)-catechin (C), (−)-epicatechin (EC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), (−)-gallocatechin gallate (GCG), (−)-epigallocatechin gallate (EGCG) and caffeine (all >99% pure, as determined through HPLC), as well as methanol, acetic acid, and acetonitrile (all HPLC grade) were purchased from Aladdin (Shanghai, China). The sodium carbonate, forinol reagent, gallic acid, potassium dihydrogen phosphate, sodium dihydrogen phosphate dihydrate, ninhydrin, stannous chloride, theanine standard, caffeine standard, *n*-alkane mixed label (C7-C40) ethyl decanoate, standards (Z)-3-hexen-1-ol, β-myrcene, β-ocimene (mixture of isomers), linalool, phenylethyl alcohol, linalool oxide II (pyran), methyl salicylate, neral, geraniol and cis-3-hexenyl hexanoate were purchased from Sigma-Aldrich (Shanghai, China).

2.3. Sensory evaluation

Sensory evaluation of the tea samples was conducted by three experts with national tea evaluator qualifications in the Tea Evaluation Laboratory of the Anhui Agricultural University. Since there is no ethics committee or formal documentation, we have taken some measures to protect the people participating in this study: all samples are food grade and harmless to humans; all participants can withdraw from this study at any time without reason; all participants gave oral or written consent to participate in this study. The experts independently evaluated the tea samples using a sensory evaluation method outlined by Zhou et al., assessing five factors: appearance, tea infusion, aroma, taste, and leaf characteristics (Zhou et al., 2022). The evaluation results were recorded using a percentage system, in which the weights corresponding to these factors were 25, 10, 25, 30 and 10%, respectively.

2.4. Analysis of non-volatile metabolites

Tea polyphenol content was determined using the Folin-Ciocalteu method, and a standard curve was plotted using gallic acid as the

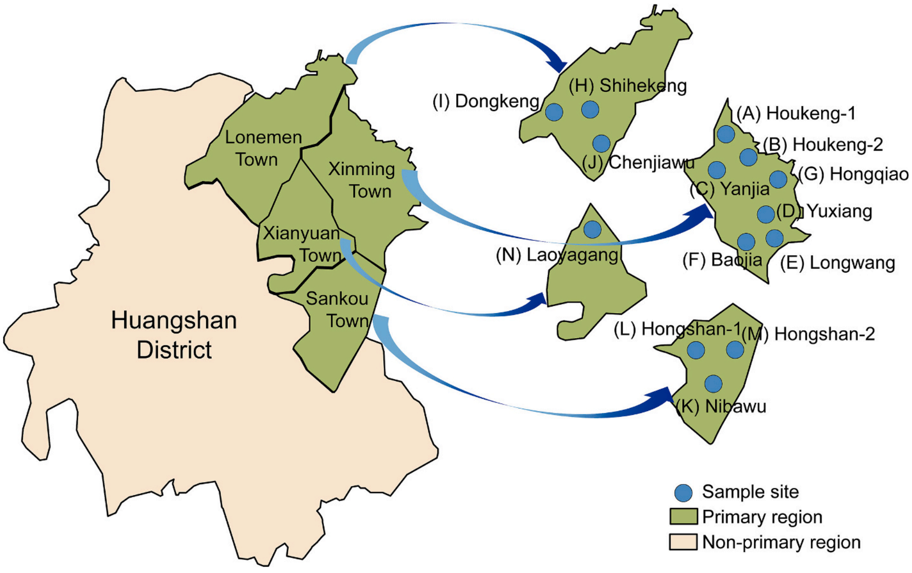

Fig. 1. Geographical distribution of the locations fourteen TPHK samples were collected from the Huangshan district of China (A-N).

standard reference. The content was calculated by determining the absorbance of the sample as previously described (Qiao et al., 2023). The resulting standard curve is expressed through the equation: $Y = 0.0084\times + 0.0156$, $R^2 = 0.9936$, where Y represents the absorbance at 765 nm and X represents the content in μg/mL. Catechins and caffeine contents were determined using a Waters Model 2695 HPLC system equipped with a C18 column (Phenomenex, 4.6 × 250 mm, 5 μm). Catechin and caffeine standards were used to produce standard curves for each quantification, according to Zhao et al. (Zhao et al., 2020). The free amino acid fraction was determined using an amino acid analyzer according to the method described in previous study (Qiao et al., 2023). All components were tested in triplicate.

2.5. Extraction of volatiles by HS-SPME-GC/MS

Ground dry tea samples weighting 2.0 g of were placed in 20 mL headspace vials. Aliquots containing 5 μL of ethyl decanoate (200 ng/mL) were subsequently added as the internal standard and the vials were sealed immediately. A 50/30 μm DVB/CAR/PDMS extraction fiber needle was subsequently inserted, and the sample was allowed to adsorb in a 60 °C water bath for 50 min, before being detected in the inlet port of an Agilent 7890B gas chromatograph, equipped with an Agilent 5977B mass spectrometer detector.

GC–MS analysis conditions were set as follows: an HP-5MS (30 mm × 0.25 mm × 0.25 μm) column was employed, using He (purity >99.999%) as the carried gas with a flow rate of 1.0 mL/min. The inlet, detector, and column temperatures were 250, 280 and 50 °C, respectively. Sample injection was performed in no splitting mode. The temperature program comprised the following stages: initially, the temperature was maintained 50 °C for 3 min; then raised at 3 °C/min until 90 °C and maintained for 2 min; then raised at 4 °C/min until 180 °C and maintained for 2 min; and finally raised at 20 °C/min until 250 °C and maintained for 3 min. MS ion source and quadrupole temperatures were 230 and 150 °C, respectively. The selected ionization method was electron ionization. Electron energy was 70 eV, ion scan range was 30–400 *m/z*, and solvent delay was 0 min. The SPME fiber was inserted into the injection port and desorbed for 5 min for detection.

2.6. Qualitative and quantitative determination of volatiles

Both a qualitative and a quantitative method were used in the determination of volatiles. In the qualitative method, the MS ion map from the mass spectrometry was compared with maps published in the NIST library, using a matching index exceeding 80% as the substance identification standard. Subsequently, the retention index (RI) was calculated from the retention time of 1 μL of an n-alkane (C7-C40) mixture and that of the substance to be measured. The calculated RI was then compared with the reported RI of the substance for qualification. The following eq. (1) was used for the calculation of the RI.

$$RI = 100 \times \left(\frac{log_{10}X_i - log_{10}C_n}{log_{10}C_{n+1} - log_{10}C_n} + n \right) \tag{1}$$

where X_i is the retention time (min) of compound X, C_n is the retention time of the n-alkane to the left of the peak of compound X, n is the number of carbon atoms in the n-alkane, and C_{n+1} is the retention time of the n-alkane to the right of the peak of compound X.

The volatiles were quantified using the internal standard method. The relative content of volatiles (ng/kg) was determined by multiplying the area under the peak of the volatiles by 1000 and dividing the result by the product of the area under the peak of the internal standard and the dry weight of the sample. For the quantification of volatiles using standards, a standard curve was drawn based on the area under the peak of the volatiles and the corresponding standard content (Table S2).

2.7. Odor activity values (OAVs)

The OAVs were calculated by dividing the concentration of volatiles by the odor threshold in water. Volatiles with OAVs ≥1 were considered as significant component contributing to aroma in the samples.

2.8. Statistical analysis

Data means and standard deviations were calculated using EXCEL 2019. One-way analysis of variance with Duncan's multiple comparison test was performed using SPSS Statistics 20. Partial least-squares discriminant analysis (PLS-DA) of biochemical compounds was performed using SIMCA 14.1. Graphing and heatmap analyses were performed using the OriginPro 2021 and TBtools, respectively. (Chen et al., 2020).

3. Results

3.1. Sensory evaluation of TPHK across different production regions

To investigate the effect of the production regions on the quality of TPHK, samples from 14 different production regions were subjected to sensory evaluation (Fig. 2, Table 1). As expressed through the coefficient of variation (CV) of the sensory scores shown in Fig. S1, taste and aroma were varied between TPHK sample from 14 different regions. For the taste evaluation, the CV was 3.2%, with the L region presenting umami, mellow and fragrant flavors, with the highest score (96 points), while the F and K regions received low scores (88.5 points) with slightly mellow and thick flavor. For the aromatic evaluation, the CV was 2.7%; the L region also obtained the highest score (96 points) and an exhibited intensive floral scent, while an impure baking-like aroma was detected in samples from the I, J, and K regions, which presented the lowest score (88 points). Other tea samples exhibited sweet and pure aroma characteristics. This suggests that aroma and taste are the dominant factors influencing tea quality. The H and K regions had the highest scores (92 points) for appearance. After brewing for 5 min, the tea infusion was light yellow and green, with TPHK from the E, M and N regions receiving the highest scores (94 points). For leaves, the G region had the lowest score (88 points). Combining all scores, the top four TPHK were from regions L (96 points), N (92 points), and A and M (91.75 points, tied in third). Sensory evaluation suggested that the qualities of TPHK vary among different production regions.

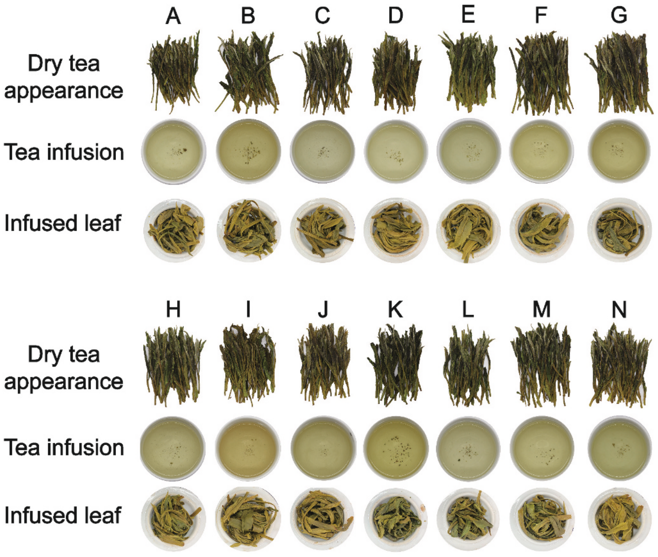

Fig. 2. Appearance of dry tea, tea infusion and infused leaf of TPHK from fourteen different production regions.

Table 1
Sensory evaluation in TPHK tea samples.

Region	Appearance Score	Tea infusion score	Aroma score	Aroma evaluation	Taste score	Taste evaluation	Leaf score	Total score
A	87	92.5	93.5	sweet	92	slightly mellow and thick	92	91.75
B	85	90	89	pure	91	slightly mellow and thick, slightly astringent	92	89
C	88	92.5	92	sweet, pure	92	slightly mellow and thick	90	90.85
D	90	90	89	pure	89	slightly mellow and thick, slightly astringent	91	89.55
E	90	94	94	sweet, floral	95	mellow and thick	92	93.1
F	89	92.5	91	sweet	88.5	slightly mellow and thick	91	89.9
G	90	89	91.5	sweet	86	slightly mellow and thick, slightly astringent	88	88.875
H	92	91.5	90	pure	92	mellow and thick, slightly astringent	89	91.15
I	85	92.5	88	high-fired	89	high heat fired, slightly mellow and thick	91	88.3
J	89	92.5	88	high-fired	93	slightly mellow and thick	89	90.3
K	92	92	88	high-fired	88.5	slightly mellow and thick	90	89.75
L	89	93	96	flowery	96	umami, mellow and fragrant	92	93.55
M	89	94	90	pure	95	mellow and slightly thick	91	91.75
N	87	94	93	sweet	95	mellow and slightly thick	91	92

3.2. Comparative analysis of non-volatile compounds

The taste of tea infusions is determined by the types and contents of non-volatile compounds in the dry tea (Shan et al., 2024). Considering the observed discrepancy in taste, the samples were subjected to HPLC analysis to measure the contents of 33 main non-volatile compounds, including tea polyphenols, free amino acids, caffeine and 6 monomers of catechin (EGC, C, EC, EGCG, GCG, and ECG). Detailed information of these contents is shown in Table S3 and Fig. S2. Total gallate catechins (TGC) encompass the contents of EGCG, ECG, and GCG, whereas EGC, C,

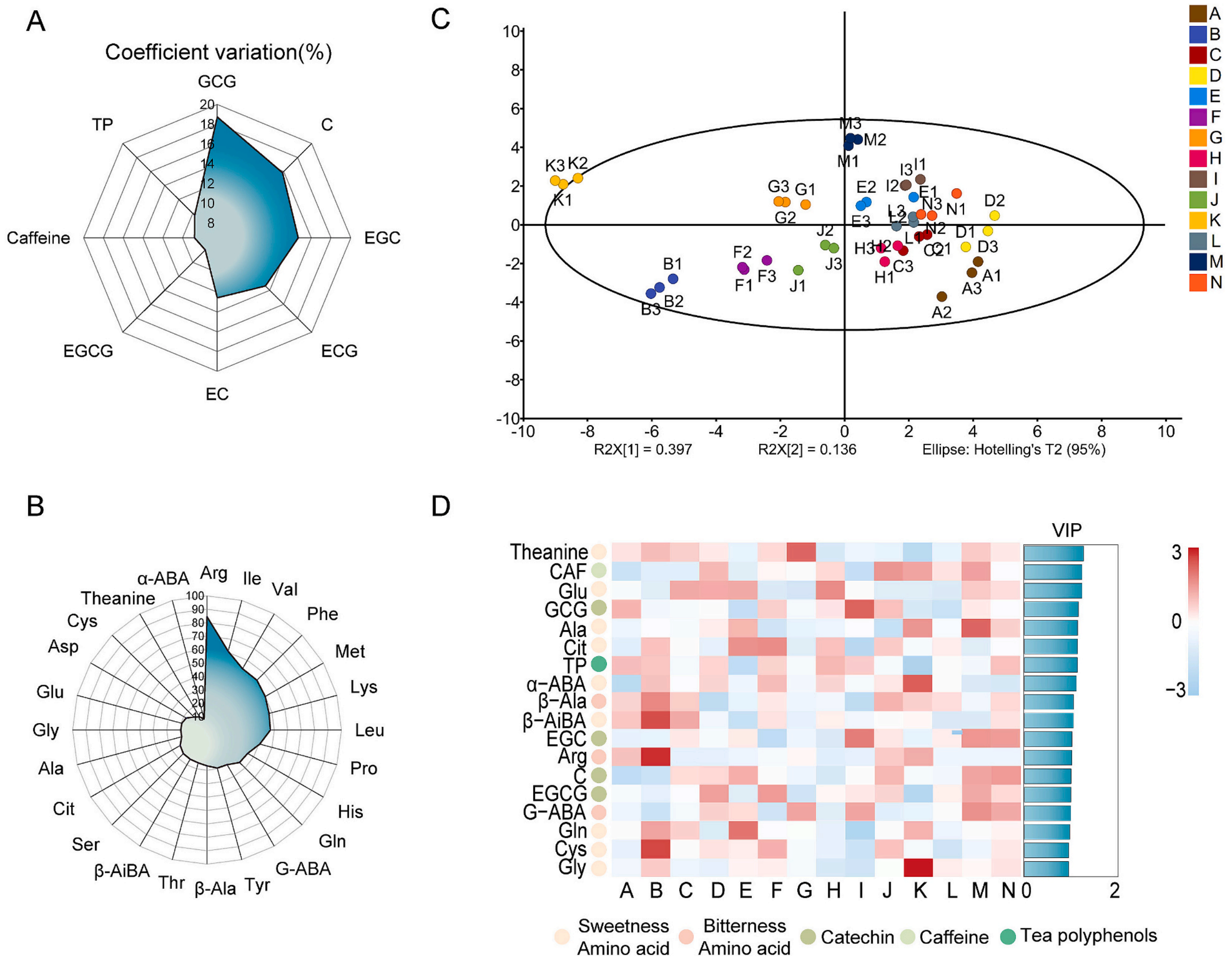

Fig. 3. Effect of the production region on the contents of non-volatile metabolites of TPHK. (A). Coefficient variation of TP, catechins and caffeine and (B). free amino acids in tea samples from fourteen different regions. (C) Score plot of the PLS-DA for non-volatile metabolites of tea samples from fourteen different regions, (D). Heatmap of the content of substances with VIP ≥ 1(blue) calculated through PLS-DA. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and EC constitute total non-gallate catechins. Of these, EGCG accounts for approximately 79% of TGC, which is the major contributor to catechins. Notably, the content of one catechin monomer, GCG, varied significantly from 1.25 to 2.59 mg/g, exhibiting the highest CV (19.5%) compared to other non-volatile compounds (Fig. 3A). Tea polyphenols (TP), which contribute significantly to the taste and colour of tea infusions, exhibited the lowest CV (4.93%), indicating that TP did not fluctuate among the TPHK samples. These results suggest that the GCG content of TPHK varies greatly across the different production regions.

Amino acids impart the sweetness and bitterness to tea infusions and are generally considered important contributors to their flavor (Scharbert & Hofmann, 2005). In this study, we detected 25 free amino acids associated with sweetness and bitterness. The amino acid content was the highest in samples from region B (44.58 mg/g) and the lowest in region I (33.17 mg/g), with theanine being the most important contributor and accounting for approximately 55% of the total amino acids. Additionally, arginine (Arg) exhibited the highest CV (68.94%) with contents ranging from 0.15 to 0.99 mg/g among different production regions. In contrast, alanine (α-ABA) showed the lowest CV (8.28%), reflecting its comparable levels among production regions, with a range from 0.051 to 0.071 mg/g (Fig. 3B). This suggests that the amino acid content of TPHK varies greatly across the different production regions.

To further identify the crucial non-volatile compounds that contributed to taste of TPHK, PLS-DA was conducted on the contents of 33 non-volatile metabolites. The high model parameters obtained with this procedure ($R^2X = 0.987$, $R^2Y = 0.897$, and $Q^2 = 0.581$) indicate high confidence and predictive ability without overfitting phenomena, as illustrated in Fig. S3 through 200 repetitions of calculations of the validated model. As the result showed that regions B and K differed the most from the other regions, whereas the rest of regions showed closer similarity to each other (Fig. 3C). Subsequently, we calculated the variable importance projection (VIP) values of 33 non-volatiles based on the result of PLS-DA. After filtering the compound with VIP <1, a total of 18 non-volatiles metabolite with VIP ≥ 1 was identified as principal compounds, which were considered important contributors to the classification (Qiu et al., 2023). This included 4 catechins, 12 amino acid, tea polyphenols and caffeine (Fig. 3D). Theanine, caffeine (CAF), and glutamate (Glu) exhibited the highest VIP values, which indicates that these compounds may be mainly responsible for the differences in taste between TPHK samples of different production regions.

3.3. *Identification and profiling of volatiles*

Distinct aromas are released by various tea leaves from different regions and play a crucial role in evaluating the flavor quality of TPHK (Qin et al., 2024). HS-SPME-GC–MS was used for the detection of volatiles in TPHK, identifying a total of 51 volatiles (Table S4 and Fig. S4). All volatiles were classified into eight groups: 12 alcohols, 11 esters, 6 aldehydes, 6 alkenes, 6 terpenes, 4 ketones, 3 aromatics, and 3 others (Fig. 4A). Volatiles were dominated by alcohols and esters, accounting for 65.9% of the total volatiles (Table S5 and Fig. S5). Total volatile content ranged from 2.72 to 7.55 μg/kg. TPHK from region D had the highest total volatile content surpassing samples from other regions by significant margin, except for the sample from region M, while region F had the lowest content. The alcohol content ranging from 1.1 to 3.4 μg/kg between samples, with the maximum amount detected in sample from region D, which was significantly greater than that of other samples, while the minimum corresponded to the sample from region B. Ester contents were in the range of 0.4 to 2.4 μg/kg, with the sample from region M exhibiting significantly higher contents than other regions, whereas the lowest contents were detected in region F. Among

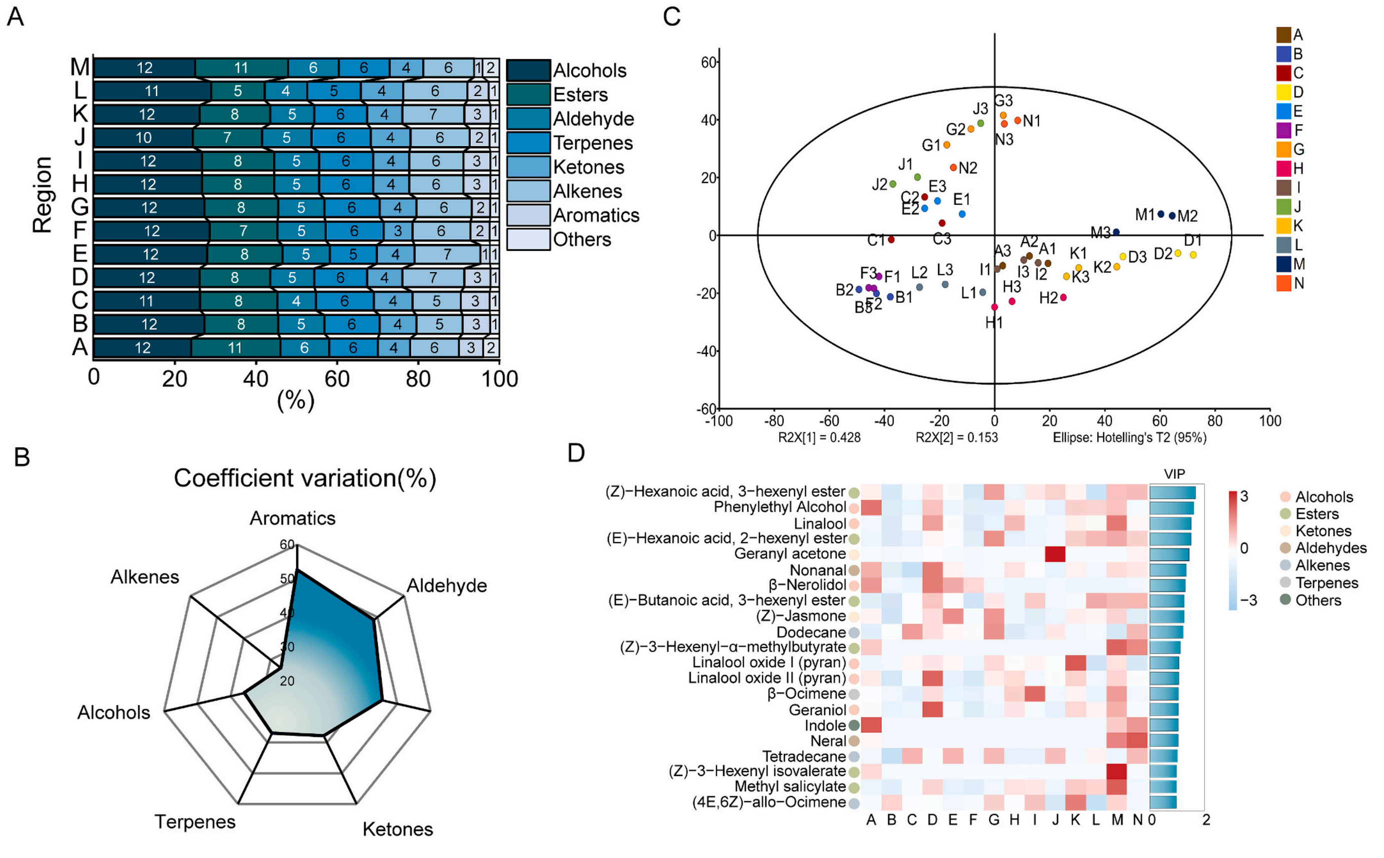

Fig. 4. Volatiles profiles of TPHK from fourteen different production regions (A) Number of volatiles in each category. (B) Coefficient variation of different categories, (C). PLS-DA score plot, (D). Heatmap of the content of volatiles with VIP ≥ 1(blue) calculated through PLS-DA. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

these groups, alkenes showed the lowest CV (26.01%), with contents ranging from 0.34 to 0.67 μg/kg, whereas the contents of aromatics exhibited significant variation from 0.026 to 0.12 μg/kg, with the highest CV (52.52%), as illustrated in Fig. 4B.

To further investigate the crucial volatile influencing TPHK in different production regions, PLS-DA was conducted on the contents of 51 volatiles (Fig. 4C), yielding robust model parameters ($R^2X = 0.987$, $R^2Y = 0.944$, and $Q^2 = 0.8$), that indicate high confidence and predictive ability without overfitting phenomena (illustrated in Fig. S6, with 200 repetitions of calculations of the validated model). As the result showed that the volatile profiles varied significantly among TPHK samples from different regions, as evidenced by the clustering of TPHK samples in Fig, 4C. The PLS-DA model generated VIP values, indicating 21 compounds with VIP ≥ 1 as potential candidate compounds, including 6 alcohols, 6 esters, 2 ketone, 2 aldehydes, 3 alkenes, 1 terpene, and 1 other (Fig. 4D). Except for dodecane, (4E,6Z)-allo-ocimene, and tetradecane, which were not characterized by odor types, the other volatiles provided floral or fruity aromas to TPHK. These volatiles are the main contributors to the production regional differences in the aroma of TPHK.

3.4. *Characterization and evaluation of crucial volatiles*

The contents of main volatiles influencing the aroma of TPHK varied significantly among samples of different production regions, especially samples from regions D and M exhibiting particularly high content. To verify this observation, a few representative volatiles were selected for absolute quantification, with the results presented in Fig. 5 and Fig. S7. The main volatiles compounds were linalool, geraniol, β-myrcene, (Z)-3-hexen-1-ol, methyl salicylate, phenylethyl alcohol, β-ocimene, linalool oxide II, cis-3-hexenyl hexanoate, which gave the tea a floral and fruity aroma. However, the contents of these volatiles differed significantly between samples from different regions. For instance, cis-3-hexenyl hexanoate, which is responsible for the fruity aroma, was not detected in region L and (Z)-3-hexen-1-ol was not detected in region J.

Volatile contributions to TPHK aroma were evaluated using the OAVs method, which is frequently applied to assess the contribution of a single volatile compound to the overall aroma. Absolute quantification revealed that five volatiles presented OAVs ≥1 (Table 2). The OAVs of linalool (165.7–888.5), geraniol (11.9–141.4), β-myrcene (16.6–88.8) and cis-3-hexenyl hexanoate (0–19.7) exceeded 1 in all regions, except for cis-3-hexenyl hexanoate, which was not detected in region L. Linalool and geraniol are common odor-active compounds in tea (Han et al., 2016), mainly exhibiting a citrus-like or rose-like floral fragrance. Additionally, methyl salicylate presented an OAV ≥ 1 in samples from both H and M, as did phenylethyl alcohol in region A and β-ocimene in region I, all displaying floral aromas. Subsequently, we identified three characteristic floral volatiles in TPHK sample with OAVs ≥1 and VIP ≥ 1, namely linalool, geraniol, and cis-3-hexenyl hexanoate. Of these volatiles, linalool and geraniol were consistent with the distinctive compounds in green tea, as previously described (Liu et al., 2023; Wang et al., 2020). Additionally, cis-3-hexenyl hexanoate presented fruity aromas in TPHK, and presented OAVs ≥1 and VIP ≥ 1. The concentrations of linalool and geraniol exhibited significant variation among different production regions, with linalool levels ranging from 96.08 to 515.35 ng/g and geraniol levels from 13.09 to 155.07 ng/g, respectively. This wide range, with several-fold differences between the lowest and highest values, indicates that these volatiles contribute significantly to the production regional variation in the aroma of TPHK.

4. Discussion

The quality of tea cultivated in diverse geographical locations differs owing to changes in factors such as altitude, soil and light. Taste is a key factor in evaluating tea quality. We identified non-volatile metabolites in TPHK tea samples grown in different production regions and found significant differences between them, which were related to the regional environmental change. The production regions selected for this study included the towns of Longmen, Xinming, Xianyuan, and Sankou (Fig. 1). Of these, Xianyuan is located at the highest altitude, reaching 561 m above sea level. The average altitude of both Xinming and Longmen approximately 420 m. In contrast, Sankou is located at 167 m, the lowest altitude (Table. S1). Research indicates that as altitude rises,

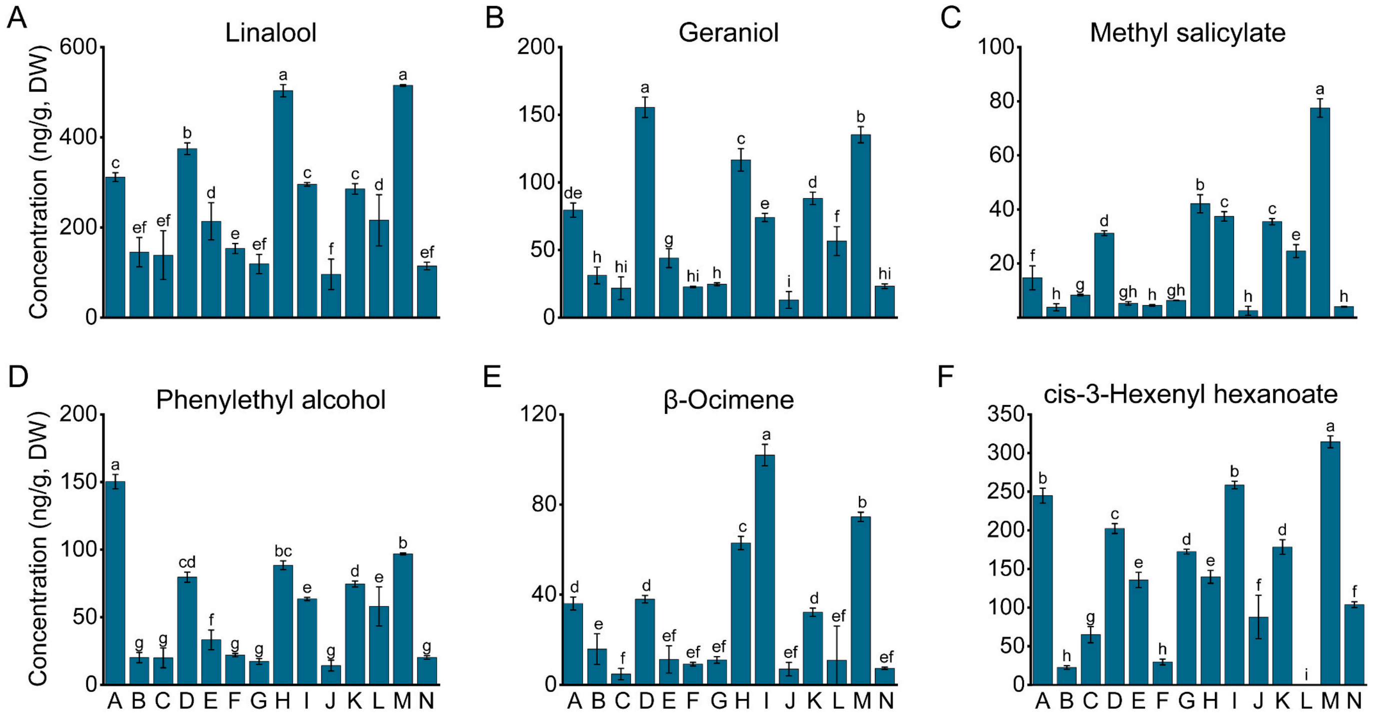

Fig. 5. Content of six representative volatiles in TPHK. Values are presented as the mean ± SD from three biological replicates, and the different letters indicate significant differences at $P < 0.05$ according to Duncan's test.

the amino acid (AA) content increases, and the TP content decreases, leading to a significant decrease in the TP/AA ratio (Han et al., 2017), which is an important factor affecting the taste of green tea. In present study, we detected 25 amino acids (Table S3), including sweetness and bitterness amino acids, and we found that changes in TP and AA contents were irregular. We detected comparable levels of TP across all production regions, while observing significant discrepancies in AA content among the regions (Table. S3). The two regions with the highest total amino acid content were B and G regions, which located in Xinming, which is not the highest altitude among all regions. Sensory evaluation revealed that samples from region L which is located in Sankou with the lowest altitude presented the most favorable taste (Table. 1). This variation may be attributed to the combined effects of light and temperature on tea plants cultivated at varying altitudes, which affect their metabolite levels (Wang et al., 2022). Based on the VIP value identified from 33 metabolites, theanine, caffeine, and glutamate were characterized as the most important non-volatile that influencing the TPHK sample from different regions (Fig. 3), similar result was found in green tea that caffeine and glutamate were served as main bitter and umami compound, respectively (Yu, Yeo, Low, & Zhou, 2014). In consistent with previous studies, theanine and glutamate were also characterized as key compounds influenced the umami flavor discrepancy in Oolong tea from different regions (Wang, Gan, et al., 2022). Collectively, these results suggested the content difference of theanine, caffeine, and glutamate may associate with the taste flavor discrepancy of TPHK.

Green tea typically has a clean, refreshing and chestnut-like aroma. TPHK is known for its noble orchid aroma. However, the aroma of TPHK varies greatly among different production regions, with some exhibiting a floral aroma, whereas others have a roasted or distinctive odor resulting from the complex interplay between volatiles. We combined two indicators, OAVs and VIP exceeding 1 (Fig. 4, Table. 2), to screen the crucial floral volatiles influencing the aroma of TPHK in different regions, such as linalool and geraniol, as in previous studies (Zhou, Wang, Lei, & Huang, 2018). Although cis-3-hexenyl hexanoate also presented OAVs and VIP >1, it displayed as a fruity aroma, whereas the main quality characteristic of TPHK should be floral aroma. Terpenes, alcohols, esters, and aldehydes represent the primary volatile types in TPHK (Fig. 4), as the previously described (Zhu et al., 2017). Studies have shown that the main volatiles affecting the aroma of TPHK are twenty volatiles (Zhou et al., 2022). These include methyl epi-jasmonate, linalool, geraniol, phenylethyl alcohol, and (*Z*)-jasmone, consistent with the finding of present study, where linalool, geraniol, and phenylethyl alcohol were also identified as major volatiles (Fig. 5). Differential from the aroma of TPHK from different production regions is influenced by linalool and geraniol, 2-methylpyrazine was identified that conferred Shuixian tea with regional aroma (Yuan et al., 2024). Similarly, methyl salicylate was considered as the key volatile that distinguished 'Baimaocha' tea in Rucheng from other different regions (Ouyang et al., 2024), suggesting that the regional aroma of distinct tea was influenced by different volatiles. During the growth process of tea, the accumulation of volatiles is affected by varying light intensities and wavelengths, and previous studies had shown that red light causes green tea to concentrate more aroma compounds (He et al., 2023). The different altitudes of the production regions of TPHK lead to different light exposure during the growth process. Additionally, the synthesis of these aromatic components is affected by the soil composition of the different production regions, previous study had shown that the soil composition vary in different regions of Huangshan district (Xiang et al., 2024). Magnesium (Mg) has been proved to enhance the metabolism of sugars, nucleic acids, and vitamins while decreasing the biosynthesis of terpenes and amino acids (Zhang et al., 2023). Consequently, differences in soil composition and light-quality may be responsible for the differences in aroma of TPHK samples. However, the regional environment is complex and diverse, and many factors may affect the quality of TPHK from different production regions, whether and how these factors affect the quality of tea needs further verification and analysis.

Table 2
Odor activity values of key volatiles in TPHK tea samples.

Odorants	OT*(μg/kg)	odor type	A	B	C	D	E	F	G	H	I	J	K	L	M	N	Average OAV
Linalool	0.58	Flora	537.3	250.4	238.8	646.4	368.4	264.3	205.3	868.3	510.0	165.7	492.4	372.4	888.5	197.4	429.0
Geraniol	1.1	Flora	72.3	28.4	19.7	141.4	40.0	20.6	22.4	106.1	67.4	11.9	80.2	51.5	123.0	21.1	57.6
cis-3-hexenyl hexanoate	16	Fruity	15.3	1.4	4.1	12.6	8.5	1.9	10.8	8.7	16.2	5.5	11.1	0.0	19.7	6.5	8.7
Methyl salicylate	40	Flora	<1	<1	<1	<1	<1	<1	<1	1.1	<1	<1	<1	<1	1.9	<1	<1
Phenylethyl alcohol	140	Flora	1.1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
β-Ocimene	97	Flora	<1	<1	<1	<1	<1	<1	<1	<1	1.05	<1	<1	<1	<1	<1	<1

* : OT, odor thresholds in water. Odor thresholds in water were obtained from Zhou, Liu et al., 2022; Czerny et al., 2008 and the Leibniz-LSB@TUM Odorant Database (https://www.leibniz-lsb.de/en/databases).

5. Conclusions

In this study, we found that there were differences in the sensory quality of TPHK tea among different production regions, and explored the differences in flavor quality between TPHK samples from different production regions through determination their non-volatile and volatile profiles. A total of 33 non-volatiles are detected in TPHK, with theanine, caffeine and glutamate were characterized as key taste compounds. Subsequently, GC–MS identified a total of 51 volatiles in 14 different TPHK sample, with alcohols and esters showed dominance. Combined with the OAV comparison, linalool and geraniol were identified as the crucial floral aroma compounds. These compounds collectively shaping the varying flavor of TPHK across different production regions.

Ethical approval

National law does not require ethical approval for sensory assessments, so appropriate steps were taken to protect the privacy and rights of participants. Participants in this study agreed verbally or in writing to participate in this study, and could withdraw from the experiment at any time without reason during the study. The samples tested were safe for consumption.

CRediT authorship contribution statement

Songyan Huang: Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation. **Linlin Xu:** Visualization, Data curation. **Mingtao Shu:** Methodology, Investigation, Data curation. **Dahe Qiao:** Software, Methodology, Investigation. **Huilin Wen:** Resources, Methodology. **Hui Xie:** Resources, Data curation. **Hongrong Chen:** Resources, Data curation. **Shengrui Liu:** Supervision. **Deyu Xie:** Supervision. **Chaoling Wei:** Supervision, Funding acquisition. **Junyan Zhu:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no competing interests.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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