


# Aromatic markers and differences between green and oolong teas: insights from 31 representative cultivars

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## Abstract

Oolong and green teas are two types of tea with distinct aroma characteristics primarily shaped by their processing methods; yet, the influence and contribution of cultivar differences remain insufficiently understood. This research applied headspace solid-phase microextraction combined with gas chromatography–mass spectrometry (HS-SPME-GC-MS) to identify 239 volatile compounds in fresh leaves of 15 green tea (GT) and 16 oolong tea (OT) cultivars. These cultivars, originally bred in various provinces, were cultivated under uniform conditions in a tea germplasm resource garden in Fujian Province. Principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) showed a clear separation between GT and OT. Using the criteria of variable importance in projection (VIP) > 1 and  $p < 0.05$ , 112 volatile compounds with significant differences were identified. Among them, *E,Z*-2,6-dimethylocta-2,4,6-triene and geranyl isovalerate were detected at significantly higher levels in OT, with the highest concentrations observed in 'Mingke 1'. Relative odor activity value (rOAV) analysis combined with random forest analysis revealed that key compounds in GT included nonanal, *Z*-jasmone, 2,6-dimethyl-1,7-octadiene-3,6-diol, linalool, nerol, and 1-decanol. This study analyzed the volatile component variation between GT and OT cultivars, providing a reference for breeding tea cultivars with distinct processing suitability.

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## Introduction

Tea aroma is regarded as a key indicator for evaluating tea quality, with the composition and concentration of aroma compounds varying significantly based on tea cultivars, geographical origin, and processing techniques<sup>[1–3]</sup>. However, tea quality also depends on the raw material, as fresh leaves are rich in various chemical components, including tea polyphenols, theanine, alkaloids, and aromatic substances. Although aromatic compounds represent a relatively minor proportion of fresh leaves, they are highly diverse.

Green tea cultivars are widely grown throughout China, with major production concentrated in Zhejiang and Anhui provinces. Representative cultivars include 'Longjing 43', 'Jiaming 1', and 'Shuchazao'<sup>[4,5]</sup>. Green tea is predominantly characterized by faint scents, sweet, and chestnut-like notes. On the other hand, cultivars for manufacturing oolong tea are cultivated in regions such as southern and northern Fujian, Guangdong, and Taiwan, represented by cultivars like 'Tieguanyin', 'Jinmudan', 'Zhilanxiang', and 'Jinxuan'<sup>[6,7]</sup>. It exhibits more complex aroma characteristics, renowned for its natural floral and fruity notes. The aromatic characteristics of green tea and oolong tea have long been a focal point of extensive research within the field of tea science<sup>[1,8,9]</sup>. Relevant studies have demonstrated that 'Taipinghoukui' green tea is characterized by floral, chestnut-like, and faint scent aromas. The floral aromas are largely attributed to elevated levels of linalool and its oxides, whereas the chestnut-like aroma is primarily intensified through the combined effects of 3-methylbutanal, 2-ethyl-1-hexanol, and indole<sup>[10]</sup>. Wuyi rock tea's aroma is mainly defined by floral,

cinnamon, and roasted notes. Throughout the roasting process, volatile compounds, particularly linalool and  $\beta$ -ionone, play a crucial role in shaping its distinctive fragrance<sup>[11]</sup>. Research into the aroma characteristics of green tea and oolong tea has largely concentrated on dry tea, with an emphasis on how processing techniques influence aromatic composition, and analyzing the aromatic compounds associated with different aroma types<sup>[12–15]</sup>. Nevertheless, there are also a few studies that focus on the aromatic properties of tea plant cultivars. Research indicates that oolong tea made from 'Huangmeigui' and 'Zimudan' has a distinct floral aroma, accompanied by exceptionally strong popcorn, cream, and caramel aromas<sup>[16]</sup>. In addition, green tea made from 'Zhonghuang 1' has a unique aroma of ripe corn, which is completely different from green tea produced from other cultivars<sup>[17]</sup>.

Research on the aroma of fresh leaves suitable for producing specific tea types remains limited and fragmented. To comprehensively compare the volatile compound characteristics of GT (tea cultivars suitable for producing green tea) and OT (tea cultivars suitable for producing oolong tea) fresh leaves, this study employs headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME-GC-MS) to extract and analyze aroma volatile compounds from 31 tea cultivars collected from different regions. SPME is widely recognized for its exceptional sensitivity and selectivity, making it a preferred method for isolating tea aroma compounds<sup>[18–20]</sup>. The findings of this study establish a theoretical framework for understanding cultivar-specific aroma signatures and support the breeding of high-aroma tea varieties.

## Materials and methods

### Tea sample collection

The classification of cultivars as suitable for green tea (GT) or oolong tea (OT) production was based on their processing suitability and prevalent regional usage. GT cultivars such as 'Fuding Dabaicha', 'Shuchazao', and 'Longjing 43' are widely recognized in prior agronomic and breeding studies for their use in green tea manufacturing<sup>[4,5]</sup>. Similarly, cultivars like 'Tieguanyin', 'Huangdan', and 'Rougui' have long been cultivated and processed as oolong tea due to their biochemical profiles and sensory characteristics conducive to oolong tea quality<sup>[6,7]</sup>. The full list of cultivars and their categorization is provided in [Supplementary Table S1](#). The 31 tea cultivars analyzed in this research were collected from the tea germplasm resource garden of Fujian province, China, in May 2024, under uniform conditions. For each cultivar, three to five one-bud and two-leaf (healthy and free of pests and diseases) samples were picked as the experimental materials. The fresh leaves were immediately placed into 25 mL centrifuge tubes and rapidly frozen with liquid nitrogen. After freezing, the tea samples were dried in a freeze dryer (EYELA-FDU-1200, China), ground into fine powder (40-mesh sieve), and then stored at  $-20^{\circ}\text{C}$  for subsequent analysis.

### Collection and analysis of volatile compounds in tea samples

#### SPME extraction of volatile compounds

Volatile compounds were extracted using a solid-phase microextraction (SPME) fiber needle (57328-U, 50/30  $\mu\text{m}$ , DVB/CAR/PDMS), which was preconditioned by thermal desorption at  $230^{\circ}\text{C}$  for 5 min in the GC-MS injection port to eliminate potential contaminants. Then, 0.1g (accurate to 0.001g) of tea powder was weighed and placed into a 20 mL headspace vial. To this, 10  $\mu\text{L}$  of the internal standard, ethyl decanoate (50  $\mu\text{g}/\text{mL}$ ), and 5 mL of boiling distilled water were added. The vial was securely sealed and equilibrated in a  $60^{\circ}\text{C}$  water bath for 5 min, followed by the insertion of the SPME fiber needle for headspace extraction over 60 min. Subsequently, the fiber needle was transferred to the GC-MS injector port at  $250^{\circ}\text{C}$ , where it was extended for 5 min to allow for desorption<sup>[21]</sup>. Perform three biological replicates on each tea sample.

#### GC-MS analysis

The GC analysis was carried out using a Shimadzu GC-MS-QP2020 NX (Shimadzu, Japan) coupled with a DB-5MS capillary column (30 m  $\times$  0.25 mm, 0.25  $\mu\text{m}$ , Agilent, USA). The column temperature program was as follows: Initial temperature  $40^{\circ}\text{C}$  for 3 min, then increased to  $200^{\circ}\text{C}$  for 2 min ( $4^{\circ}\text{C}/\text{min}$ ), and then reached  $270^{\circ}\text{C}$  for 2 min ( $10^{\circ}\text{C}/\text{min}$ ). The injection port temperature was set to  $250^{\circ}\text{C}$  in splitless mode. The carrier was helium gas (purity  $\geq 99.999\%$ , 1.2 mL/min), and a solvent delay of 4 min was applied<sup>[22,23]</sup>.

The MS analysis utilized electron impact ionization (EI), with the ion source temperature configured at  $230^{\circ}\text{C}$ , the quadrupole at  $150^{\circ}\text{C}$ , and the interface temperature set to  $280^{\circ}\text{C}$ . The electron energy was consistently maintained at 70 eV. The analysis was conducted using the selected ion monitoring (SIM) mode to enable accurate qualitative and quantitative ion detection<sup>[24,25]</sup>.

### Qualitative, relative quantitative, and rOAV analysis of volatile compounds

#### Qualitative

The mass spectrometry peaks from the raw data were matched against the NIST 20 database, screening for compounds with a match score exceeding 75. Redundant peaks, background noise, and unidentified low-intensity signals were removed. Only compounds with well-defined peak shapes and reliable retention times

were included in the final dataset. The retention indices (RI) of the target compounds were calculated by referencing the retention times of n-alkanes ( $\text{C}_8\text{--C}_{40}$ ) measured under identical mass spectrometry conditions. The calculated RI values were then validated by comparing them with previously reported data in the literature or accessible through established online resources (<https://webbook.nist.gov/chemistry/>).

#### Relative quantification

The relative concentration of the compound ( $\mu\text{g}/\text{L}$ ) was calculated using the formula: [Concentration of the internal standard (50  $\mu\text{g}/\text{mL}$ )  $\times$  Volume of the internal standard added (0.01 mL)/Volume of water (5 mL)]  $\times$  1,000  $\times$  Peak area of the target compound/Peak area of the internal standard<sup>[26]</sup>.

#### rOAV (relative Odor Activity Value) analysis

The odor activity value is a widely applied metric for evaluating the impact of volatile compounds on the perceived aroma characteristics. The odor threshold is defined as the minimum level of a compound at which its smell becomes detectable in water<sup>[27,28]</sup>.

$$\text{rOAV calculation formula: } \text{rOAV} = \frac{C_i}{\text{OT}_i}$$

where  $C_i$  represents the amount of the compound present in the sample ( $\mu\text{g}/\text{L}$ ).  $\text{OT}_i$  denotes the compound's odor threshold in water ( $\mu\text{g}/\text{L}$ )<sup>[29]</sup>.

### Construction of a random forest model

Using the differential volatile compounds identified across 31 tea cultivars, a random forest model was developed with the R package random forest (version 4.2.0, with the varSelF 0.7.8). Prior to running the model validation, the quantitative data of the volatile compounds were centered and standardized. The model's accuracy was subsequently assessed using the receiver operating characteristic (ROC) curve<sup>[30]</sup>. To assess the performance of the selected features in an independent validation cohort, an ROC curve was generated with the R package pROC (version 3.5.1, with pROC 1.16.2)<sup>[31]</sup>.

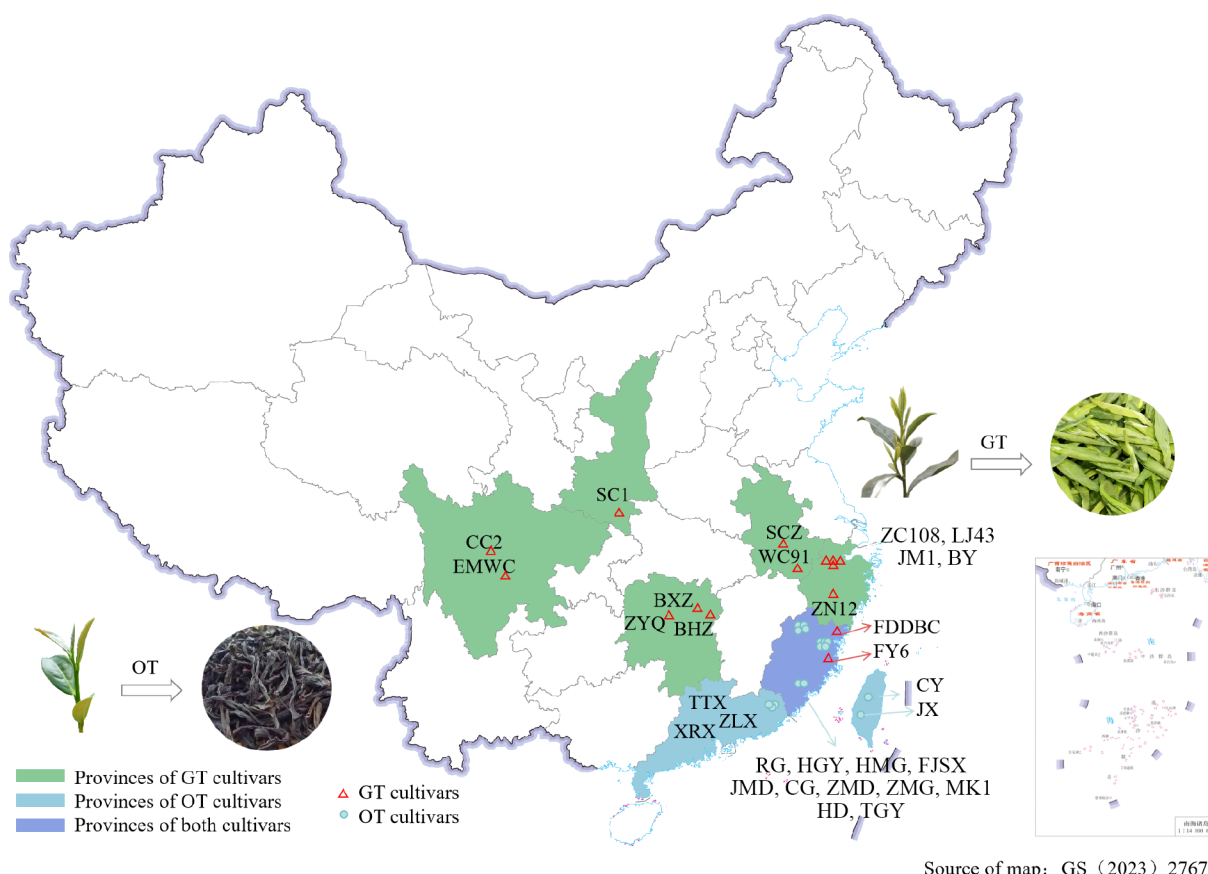
### Statistical analysis

In the study, volatile compounds were collected and analyzed from tea samples, with three biological replicates for each sample. After comparing the raw data with the database, organize it into clean data using Microsoft Office Excel 2012. Duncan's multiple range tests and independent sample t-tests were carried out with IBM SPSS Statistics version 26<sup>[32]</sup>. Multivariate analysis (Principal Component Analysis, PCA, and Orthogonal Partial Least Squares Discriminant Analysis, OPLS-DA) was performed using the Metware Cloud platform (<https://cloud.metware.cn>). Differential volatile compounds were screened according to a  $p$ -value  $< 0.05$  and a variable importance in projection (VIP) value  $\geq 1$ . Draw a visual heatmap using TBtools (version 2.112) software and construct a column chart using Origin 2021 (version 9.8.5).

## Results and discussion

### Original habitat and distribution of the tea plant cultivars used in the experiment

Tea plants from different origins often exhibit distinct quality characteristics. The primary production regions for OT are concentrated in Fujian, Guangdong, and Taiwan, where the tea produced is typically characterized by a high-aroma quality. GT is widely cultivated across several provinces in China, including Anhui, Zhejiang, Sichuan, Hunan, and Shaanxi, with green tea from Zhejiang and Anhui being particularly renowned. Previous studies have reported that green tea and oolong tea exhibit distinct aroma profiles due to differences in processing technologies. In this study, we observed



**Fig. 1** Geographical distribution of 31 tea cultivars. \* Map lines delineate study areas and do not necessarily depict accepted national boundaries.

that GT contained higher levels of volatile compounds compared to OT, with significant differences in the composition of these compounds (Supplementary Fig. S1). On this basis, this study selected 15 tea plant cultivars suitable for making GT and 16 cultivars suitable for making OT, and systematically explored the differences in volatile components in their fresh leaves (Fig. 1).

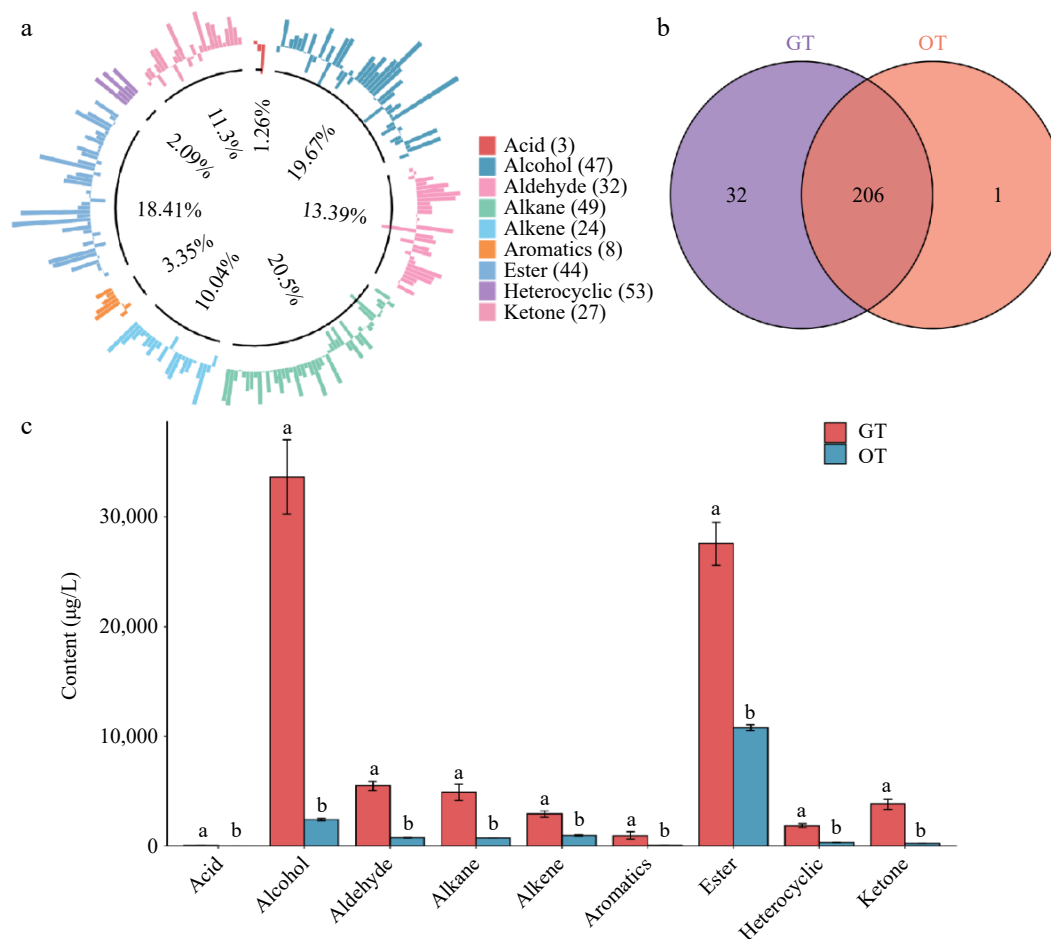
### Identification and comparison of volatile components in GT and OT

An overall count of 239 volatile compounds was successfully identified across 31 tea plant cultivars. These compounds were categorized into nine distinct groups (Fig. 2a), consisting of 49 alkanes, 47 alcohols, 44 esters, 27 ketones, 24 alkenes, eight aromatic hydrocarbons, five heterocyclic compounds, and three acids. Among these, alkanes, alcohols, and esters together comprise over half of the volatile compounds, contributing 20.5%, 19.67%, and 18.41%, respectively, highlighting their dominant presence among the volatile constituents in fresh leaves. Notably, Alkanes account for a high proportion of volatile compounds, but they mainly present a waxy and oily odor, with limited influence on the overall fragrance of green and oolong teas. In contrast, alcohols and esters play a dominant role in shaping the floral and fruity aromas in green and oolong teas [33,34]. Based on processing suitability, the 31 tea plant cultivars were classified into two groups: those suitable for green tea processing (GT) and those suitable for oolong tea processing (OT). From the analysis of the Venn diagram (Fig. 2b), it is found that both GT and OT shared 206 volatile compounds. The GT contains 32 volatile compounds with specificity, including 2,6-dimethyl-1,7-octadiene-3,6-diol, ethyl hexanoate, and 2-octanone, among others. However, *E,Z*-2,6-dimethylocta-2,4,6-triene was only found within OT, which may be an important reason for its floral and balsamic aromas. Further investigation identified notable differences in the total

amounts of volatile compounds among different categories between GT and OT (Fig. 2c). The overall level of alcohol compounds in GT (33,662.78  $\mu\text{g/L}$ ) is markedly greater compared to that in OT (2,399.53  $\mu\text{g/L}$ ), with the main differences observed in the contents of linalool, Z-linalool oxide (furanoid), and phenylethanol. These glycosidically bound volatile compounds (GBVs), considered precursors of tea aroma, are highly enriched in green tea, but do not contribute to aroma production during oolong tea processing [35–38]. The observed differences highlight the importance of volatile alcohols in shaping the sweet and floral aromas of GT. Moreover, variations exist in the overall concentration of ester compounds between the fresh leaves of GT (27,575.02  $\mu\text{g/L}$ ) and OT (10,784.90  $\mu\text{g/L}$ ). Research indicates that esters, which contribute floral and fruity notes, are more identified in OT [39]. Among the esters in GT, methyl salicylate (17,022.62  $\mu\text{g/L}$ ) exhibited the highest relative concentration, followed by geranyl isovalerate (2,777.80  $\mu\text{g/L}$ ). In contrast, the trend was reversed in OT, where geranyl isovalerate (6,117.11  $\mu\text{g/L}$ ) was the dominant ester, and methyl salicylate (2,071.62  $\mu\text{g/L}$ ) ranked second. The results highlight distinct differences in the major esters that characterize GT and OT. Methyl salicylate is identified as the predominant ester in GT, whereas geranyl isovalerate is the principal ester in OT.

### Multivariate analysis and differential analysis of volatile compounds in GT and OT

Principal component analysis (PCA) revealed (Fig. 3a) that PC1 explained 20.39% of the total variation, while PC2 contributed 7.13%, showing a distinct differentiation between the GT and OT groups. One outlier was identified in the OT group that did not significantly affect the overall distribution. Based on the PCA results, orthogonal partial least squares discriminant analysis (OPLS-DA) was performed to determine the classification and differences



**Fig. 2** GC-MS analysis of volatile compounds in GT and OT. (a) The proportion of volatile compounds in the total components. (b) Venn diagram of the volatile compounds of GT and OT. (c) Total amount of different volatile compound categories in GT and OT (different letters indicate significant differences,  $p < 0.05$ , GT:  $n = 15$ ; OT:  $n = 16$ ).

within the data (Fig. 3b). The OPLS-DA analysis clearly demonstrated a pronounced segregation between GT and OT, with inter-group differences contributing 18.3% and intra-group differences contributing 19.5%. These findings aligned closely with those of PCA. The model evaluation metrics, including  $Q^2 = 0.985$ ,  $R^2Y = 0.995$ , and  $R^2X = 0.47$  (Fig. 3c), indicated a reliable model. Following 200 permutation tests (Fig. 3d), the results confirmed that the model was not overfitted and its validation was reliable. Therefore, the findings effectively highlight the substantial differences in volatile components of fresh leaves between GT and OT.

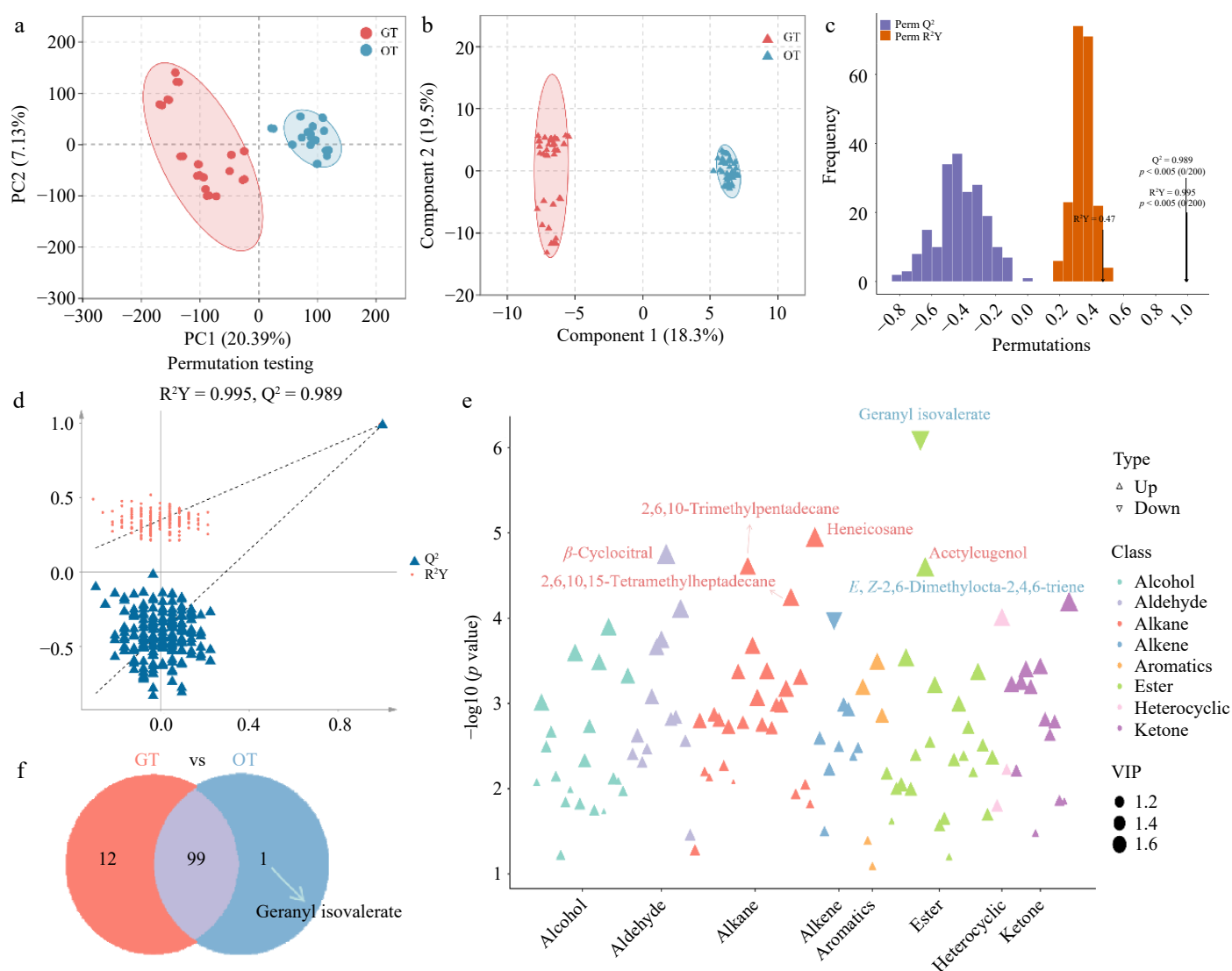
To investigate the variation in volatile compounds in the fresh leaves of GT and OT, 112 differential compounds were identified using the criteria of  $p < 0.05$  and  $VIP > 1$  (Fig. 3f). Among these, 99 compounds were detected in both groups, 12 compounds were unique to GT, and only one compound was unique to OT. The differential compounds were predominantly concentrated in the categories of alkanes, esters, and alcohols, indicates that esters and alcohols are the primary contributors to the aroma of fresh leaves in GT and OT, which is consistent with previous findings (Fig. 3e). Among the 112 differential volatile compounds, 110 exhibited significantly higher abundance in GT, including heneicosane,  $\beta$ -cyclocitral, 2,6,10-trimethylpentadecane, acetyleugenol, 2,6,10,15-tetramethylheptadecane, phytone, and tridecanal, among others. Notably, the  $VIP$  value of  $\beta$ -cyclocitral exceeded 1.6, with its odor characteristics described as sweet, faint scent, and floral<sup>[36,40]</sup>, highlighting its substantial impact on the overall fragrance of GT. Phytone and tridecanal primarily contribute woody and waxy aromas to tea, while

acetyleugenol contributes sweet and fruity aromas. Comparatively, acetyleugenol plays a more prominent role in shaping the aroma of fresh leaves. In contrast, *E,Z*-2,6-dimethylocta-2,4,6-triene and geranyl isovalerate were found at higher levels in OT, both of which contribute to its characteristic floral aroma. Meanwhile, the former was only detected in OT, indicating its unique contribution to OT's aroma. Overall, these findings support the distinctive role of *E,Z*-2,6-dimethylocta-2,4,6-triene within OT's aroma, which we will focus on in subsequent analyses.

### rOAV analysis of differential volatile compounds

rOAV represents the significance of individual aroma compounds within the overall fragrance and acts as a standard for identifying key aromatic components in tea<sup>[41]</sup>. Research indicates that an rOAV value  $\geq 1$  suggests that the aroma compound is crucial in defining the overall aroma, whereas an rOAV value  $\geq 10$  signifies an extremely significant contribution to the overall aroma<sup>[42–44]</sup>. We calculated the odor thresholds of 67 compounds and analyzed their rOAV (Table 1; Supplementary Table S2). Among them, 23 volatile compounds in GT had  $rOAV > 1$ , while only eight compounds in OT exhibited  $rOAV > 1$ . These compounds include aldehydes, esters, alcohols, ketones, and alkenes. It is noteworthy that the only alkene, *E,Z*-2,6-dimethylocta-2,4,6-triene, is a unique differential volatile compound in OT ( $rOAV > 10$ ), with an rOAV exceeding 100 in MK1. It possesses floral and balsamic notes and may be a key compound in high-aroma OT. Nonanal, hexyl formate, geranyl butyrate, methyl salicylate, and geranyl isovalerate all exhibited rOAVs greater than





**Fig. 3** Multivariate analysis of volatile compounds and screening of differential volatile compounds. (a) Score plots for the PCA analysis of the volatile compounds. (b) Score plots for the OPLS-DA analysis of the volatile compounds. (c), (d) OPLS-DA model validation and 200 permutation tests. (e) Scatter plot of differential volatile compounds. (VIP > 1,  $p < 0.05$ ). (f) The Venn diagram of differential volatile metabolites between GT and OT.

one in both OT and GT, and these compounds are predominantly linked to floral, fruity, and sweet aromas. At the same time, the rOAV of Z-3-hexenyl butyrate exceeds 10, while the rOAV of linalool is greater than 100. Both compounds are associated with floral and fruity aromas, and their rOAVs in GT are more than 10 times higher than those in OT. These findings demonstrated their significant contributions to the aroma of both tea types. Moreover, the rOAV values of volatile compounds, including 2,6-dimethyl-1,7-octadiene-3,6-diol, Z-jasmone, heptanal, 3-heptanone, 2-nonanone, 1-decanol, 1-nonanol, and nerol in GT, were all greater than one. These compounds are mainly associated with faint scents and sweet and floral and fruity aromas. Earlier research has identified nonanal and Z-jasmone as key volatile compounds influencing the floral aroma of green tea [33,45]. Therefore, the above-mentioned volatile compounds are likely critical in defining the aromatic profile of GT.

### Identification of key volatile compounds using a random forest model

To further analyze the key volatile compounds between GT and OT, we constructed a random forest model utilizing 112 differential volatile compounds (Fig. 4a), and evaluated the model using ROC

curve analysis. The ROC curve's area (AUC) functions as a critical measure for determining the accuracy and reliability of the classification model, with an AUC value closer to one indicating higher accuracy. When  $0.5 < \text{AUC} < 1$ , the model is considered optimal. The AUC value of this classifier model is one, manifesting the model's effectiveness (Fig. 4b) [46,47]. Based on the constructed model, the mean decrease in accuracy for each compound was calculated, and a higher value reflected a greater importance of the volatile compound [48]. According to this value, the top 10 differential volatile compounds were identified as nerol, geranyl isovalerate, 2,6-dimethyl-1,7-octadiene-3,6-diol, geranyl butyrate, 2,6,10-trimethyltridecane, hexadecane, 2,4-di-tert-butylphenol, phthalaldehyde, 3-ethyl-3-methylheptane, and 1-decanol. Building on previous analysis, the impact of alkanes on the overall aroma is very low, so we focus on alcohol and ester compounds. Among them, the average reduction accuracy of nerol is the highest (> 30), and its prominent floral and faint scent characteristics help strengthen overall aroma of the tea [49]. Geranyl isovalerate and geranyl butyrate have faint scent, sweet, and floral notes [50]. 2,6-Dimethyl-1,7-octadiene-3,6-diol has a faint scent and citrus aroma, and is a unique aromatic compound in GT. ROC curve analysis was performed on the selected 10

**Table 1.** Differential volatile compounds between GT and OT.

Category	RT	RI	Compounds	Content (µg/L)		Threshold (µg/L)	Odor characteristics
				GT	OT		
Aldehyde							
1	6.390	796	Hexanal	116.98 ± 13.97 a	25.14 ± 0.44 b	4.5 <sup>A</sup>	Grassy, green, leafy, vinegar
2	10.210	902	Heptanal	262.74 ± 11.05 a	25.92 ± 1.28 b	2.8 <sup>C</sup>	Faint scent, herbal, citrusy
3	12.449	960	Benzaldehyde	1,008.64 ± 144.59 a	67.81 ± 10.30 b	350 <sup>C</sup>	Sweet, bitter, almond, cherry
4	12.562	962	1,2-Benzenedicarboxaldehyde	711.78 ± 35.67 a	2.60 ± 0.21 b	1,000 <sup>F</sup>	Mild, aromatic
5	14.105	1,002	Octanal	88.24 ± 1.90 a	16.17 ± 1.09 b	7 <sup>A</sup>	Citrusy, fruity, waxy
6	17.921	1,103	Nonanal	578.00 ± 7.10 a	40.67 ± 1.30 b	1 <sup>F</sup>	Citrus, orange, peel
7	21.577	1,204	Decanal	114.07 ± 10.61 a	24.33 ± 0.87 b	2.6 <sup>A</sup>	Citrusy, waxy
8	21.987	1216	β-Cyclocitral	160.36 ± 2.49 a	15.46 ± 1.23 b	3 <sup>A</sup>	Lemon, citrus, floral
9	23.887	1,272	4-Propyl-benzaldehyde	268.71 ± 3.44 a	63.65 ± 0.72 b	10 <sup>F</sup>	Floral, almond
10	28.276	1,407	Dodecanal	98.92 ± 17.18 a	7.27 ± 0.39 b	14 <sup>F</sup>	Citrusy, waxy, fruity, fatty
11	28.277	1,408	Tridecanal	91.29 ± 1.28 a	2.81 ± 0.37 b	70 <sup>F</sup>	Citrus, faint scent, soapy
12	36.369	1,686	3-Methyl-butanal	27.11 ± 4.59 a	0.56 ± 0.01 b	0.1 <sup>B</sup>	Pungent, fruity, nutty
13	41.828	1,900	Pentadecanal	123.51 ± 6.32 a	17.79 ± 0.65 b	430 <sup>F</sup>	Waxy, fatty, floral
Ester							
14	9.086	871	Hexyl formate	19.91 ± 1.18 a	2.35 ± 0.23 b	0.1 <sup>F</sup>	Fruity
15	13.394	984	Vinyl hexanoate	39.90 ± 1.49 a	1.01 ± 0.30 b	50 <sup>F</sup>	Faint scent, fruity
16	13.690	992	Geranyl butyrate	318.80 ± 12.74 a	13.80 ± 0.36 b	0.1 <sup>F</sup>	Sweet, fruity, floral, orange
17	13.883	997	Ethyl hexanoate	26.88 ± 3.34 a	0 b	5 <sup>A</sup>	Fruity, sweet, faint scent
18	17.581	1,094	Benzyl acetate	15.66 ± 1.04 a	0 b	364 <sup>G</sup>	Sweet, floral, fruity, balsamic
19	20.838	1,184	Z-3-Hexenyl butyrate	295.40 ± 6.60 a	35.37 ± 2.14 b	0.1 <sup>F</sup>	Faint scent, fruity, green, sweet
20	21.082	1,190	Methyl salicylate	17022.62 ± 975.42 a	2071.62 ± 116.92 b	40 <sup>A</sup>	Caramel, minty, herbal
21	23.102	1,249	Geranyl isovalerate	2,777.80 ± 77.04 b	6,117.11 ± 153.55 a	100 <sup>F</sup>	Floral, fruity, herbal, citrus
22	26.122	1,339	Acetylcugenol	29.22 ± 1.75 a	5.02 ± 0.83 b	n.f.	Spicy, sweet, fruity, woody
23	26.440	1,349	Cinnamyl isovalerate	44.19 ± 6.57 a	1.35 ± 0.08 b	n.f.	Sweet, spicy, fruity
24	27.031	1,368	Hexyl hexanoate	28.89 ± 1.07 a	1.52 ± 0.09 b	6,400 <sup>A</sup>	Fruity, sweet, creamy
25	27.322	1,377	4Z-4-Hexenyl butyrate	304.75 ± 14.37 a	1.47 ± 0.09 b	n.f.	Fruity, sweet, faint scent, apple
26	27.337	1,378	Neryl hexanoate	232.94 ± 2.00 a	17.08 ± 1.46 b	781 <sup>C</sup>	Fruity, green, pear, pineapple
27	27.590	1,385	E-2-Hexenyl hexanoate	16.36 ± 2.75 a	0.91 ± 0.10 b	n.f.	Fruity, sweet
28	33.074	1,568	Nerol benzoate	152.34 ± 2.19 a	51.13 ± 2.35 b	500 <sup>C</sup>	Faint scent, floral
29	33.531	1,584	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	37.89 ± 1.07 a	0 b	n.f.	Mild, sweet, fatty
30	33.737	1,591	Ethyl laurate	35.88 ± 5.61 a	3.56 ± 0.56 b	n.f.	Sweet, fruity, fatty
31	34.710	1,626	Decyl decanoate	131.49 ± 21.24 a	20.23 ± 0.88 b	n.f.	Mild, fatty, oily
32	39.104	1,791	Ethyl tetradecanoate	46.03 ± 3.72 a	17.34 ± 0.55 b	4,000 <sup>F</sup>	Sweet, fatty, fruity
33	42.374	1,922	Methyl palmitate	70.55 ± 1.01 a	8.60 ± 0.65 b	2,000 <sup>E</sup>	Waxy, sweet
34	44.065	1,990	Ethyl palmitate	555.70 ± 1.19 a	171.98 ± 5.31 b	2,000 <sup>E</sup>	Mild, fatty
35	46.868	2,061	Phytyl acetate	270.72 ± 24.58 a	11.79 ± 2.61 b	2 <sup>C</sup>	Floral, green, faint scent
36	47.879	2,085	Ethyl linolenate	214.69 ± 16.09 a	63.76 ± 0.48 b	450 <sup>G</sup>	Oily, fatty, nutty, green
Alkane							
37	14.034	1,001	3,6-Dimethyldecane	14.24 ± 0.45 a	0 b	n.f.	Mild, fatty, hydrocarbon
38	14.678	1,017	Undecane	40.26 ± 1.81 a	0 b	10,000 <sup>E</sup>	Odorless, oily, waxy
39	16.129	1,056	5-(2-Methylpropyl)-nonane	137.29 ± 23.92 a	19.82 ± 0.10 b	n.f.	Mild, fatty, hydrocarbon
40	16.131	1,057	3-Ethyl-3-methyl-heptane	64.16 ± 3.05 a	19.70 ± 1.46 b	n.f.	Mild, fatty, hydrocarbon
41	16.199	1,058	2,3,5,8-Tetramethyl-decane	176.24 ± 10.97 a	8.25 ± 0.86 b	n.f.	Odorless, oily
42	16.335	1,061	3,7-Dimethyl-decane	66.14 ± 1.73 a	0 b	n.f.	Odorless, oily
43	21.429	1,200	Dodecane	376.64 ± 23.67 a	49.92 ± 3.52 b	1000 <sup>E</sup>	Mild, hydrocarbon, petroleum
44	21.733	1,209	3,4-Dimethyl-undecane	31.33 ± 2.28 a	2.29 ± 0.07 b	n.f.	Odorless
45	23.539	1,262	3-Ethyl-3-methyl-decane	52.59 ± 3.52 a	10.34 ± 0.95 b	n.f.	Mild, hydrocarbon
46	23.989	1,275	4,6-Dimethyl-dodecane	582.01 ± 80.64 a	86.94 ± 0.96 b	n.f.	Odorless, oily
47	24.257	1,283	5-Butyl-nonane	95.06 ± 17.35 a	0.49 ± 0.10 b	n.f.	Odorless
48	26.890	1,371	2,6,10-Trimethylpentadecane	69.46 ± 1.95 a	5.41 ± 0.32 b	n.f.	Oily,waxy
49	28.047	1,399	Tetradecane	339.47 ± 33.31 a	48.08 ± 0.89 b	1,000 <sup>E</sup>	Mild, waxy, odorless, oily
50	28.398	1,411	3,8-Dimethyl-decane	44.83 ± 0.80 a	2.19 ± 0.11 b	n.f.	Hydrocarbon
51	29.075	1,433	2,6,11-Trimethyl-dodecane	65.22 ± 8.29 a	7.33 ± 0.41 b	n.f.	Odorless, oily
52	29.898	1,460	2,6,10-Trimethyl-tridecane	191.03 ± 22.37 a	30.74 ± 1.70 b	n.f.	Odorless, oily
53	30.719	1,487	5-Methyl-5-propylnonane	143.54 ± 21.80 a	37.71 ± 0.56 b	n.f.	Odorless
54	31.749	1,522	2,6,10-Trimethyl-tetradecane	26.96 ± 2.17 a	5.95 ± 0.29 b	n.f.	Odorless
55	33.947	1,598	Hexadecane	438.21 ± 32.59 a	11.97 ± 0.91 b	13,000,000 <sup>E</sup>	Odorless, oily
56	33.971	1,599	3-Methyl-5-propylnonane	50.05 ± 3.83 a	1.19 ± 0.14 b	n.f.	Odorless, oily, waxy
57	35.296	1,647	2,6,10,15-Tetramethyl-heptadecane	117.06 ± 9.36 a	16.75 ± 0.63 b	n.f.	Oily, waxy, petroleum
58	36.697	1,698	8-Methyl-heptadecane	139.98 ± 5.29 a	17.17 ± 0.25 b	n.f.	Mild, hydrocarbon

(to be continued)

Table 1. (continued)

Category	RT	RI	Compounds	Content (µg/L)		Threshold (µg/L)	Odor characteristics
				GT	OT		
59	36.828	1,703	Phytane	47.43 ± 1.29 a	4.03 ± 0.10 b	n.f.	Odorless, oily
60	37.776	1,740	3,8-Dimethyl-undecane	25.78 ± 1.40 a	3.67 ± 0.31 b	n.f.	Mild, fatty, hydrocarbon
61	39.330	1,799	Octadecane	70.36 ± 0.45 a	1.91 ± 0.19 b	20 <sup>E</sup>	Mild, waxy, petroleum
62	48.494	2,100	Heneicosane	393.56 ± 19.59 a	30.00 ± 1.74 b	n.f.	Waxy, oily, greasy
Ketone							
63	5.896	782	3-Hexanone	74.30 ± 0.79 a	0 b	1,000 <sup>C</sup>	Herbaceous, fruity
64	6.224	791	2-Hexanone	9.51 ± 0.19 a	0.91 ± 0.05 b	24 <sup>F</sup>	Mild, fruity, solvent
65	9.614	885	3-Heptanone	66.35 ± 1.62 a	6.82 ± 0.39 b	1 <sup>F</sup>	Fruity, banana, spicy, solvent
66	12.202	953	6-Methyl-2-heptanone	188.52 ± 5.75 a	24.91 ± 0.90 b	8.1 <sup>F</sup>	Fruity, banana, spicy, solvent
67	13.521	987	2-Octanone	786.02 ± 85.85 a	0 b	350 <sup>F</sup>	Fruity, banana, spicy, buttery
68	16.043	1053	2-Tetradecanone	24.05 ± 0.84 a	0.65 ± 0.07 b	10 <sup>F</sup>	Mild, fatty, fruity
69	16.212	1,058	2-Dodecanone	74.54 ± 4.57 a	1.34 ± 0.15 b	42 <sup>F</sup>	Fatty, waxy
70	16.467	1,064	Acetophenone	854.90 ± 65.93 a	29.31 ± 0.90 b	170 <sup>D</sup>	Sweet, almond, fruity, floral
71	17.414	1,089	2-Nonanone	259.56 ± 51.80 a	24.21 ± 1.03 b	5 <sup>F</sup>	Herbaceous, spicy
72	27.717	1,389	Z-Jasmone	221.25 ± 5.09 a	11.07 ± 0.30 b	7 <sup>A</sup>	Sweet, floral, herbaceous, fruity
73	29.430	1,445	6,10-Dimethyl-5,9-undecadien-2-one	111.62 ± 1.68 a	20.60 ± 1.49 b	3,000 <sup>D</sup>	Fruity, sweet
74	31.486	1,513	2-(2-Propenyl)-1,3-cyclohexanedione	107.72 ± 0.60 a	22.54 ± 1.40 b	n.f.	Mild, herbal, spicy
75	40.304	1,838	Phytone	48.91 ± 1.10 a	7.71 ± 0.22 b	n.f.	Woody, herbaceous, earthy
Alcohol							
76	8.878	865	6-Methyl-1-heptanol	75.94 ± 3.40 a	9.50 ± 1.00 b	n.f.	Faint scent, fatty,herbal
77	9.018	869	1-Hexanol	140.47 ± 9.33 a	3.95 ± 0.54 b	500 <sup>A</sup>	Fruity, alcoholic, sweet, green
78	10.243	903	4-Methyl-2-hexanol	17.39 ± 0.96 a	0.84 ± 0.06 b	n.f.	Faint scent, alcoholic, mild, floral
79	14.172	1,004	2,6-Dimethyl-1,7-octadiene-3,6-diol	432.70 ± 8.06 a	0 b	10 <sup>F</sup>	Faint scent, herbal, citrus, lemon
80	14.538	1,014	2-Butyl-1-octanol	38.72 ± 3.21 a	4.42 ± 0.05 b	n.f.	Mild, fatty, hydrocarbon
81	14.782	1,020	4-Pentenol	10.83 ± 0.63 a	1.81 ± 0.17 b	1 <sup>F</sup>	Faint scent, herbal, fruity
82	16.674	1,035	1-Decanol	542.52 ± 25.03 a	40.97 ± 1.24 b	23 <sup>C</sup>	Mild fatty, waxy, floral, orange
83	17.274	1,086	Z-Linalool oxide (furanoid)	830.56 ± 104.82 a	77.69 ± 2.41 b	320 <sup>C</sup>	Earthy, floral, sweet, herbal
84	17.699	1,097	Linalool	8,805.09 ± 298.70 a	1,424.74 ± 32.19 b	0.6 <sup>A</sup>	Sweet floral, citrus
85	19.675	1,151	Z-3-Nonenol	25.93 ± 1.18 a	0.85 ± 0.03 b	n.f.	Faint scent, herbal, green
86	20.369	1,171	1-Nonanol	379.02 ± 23.01 a	26.90 ± 0.51 b	5.3 <sup>C</sup>	Faint scent, floral,dusty
87	20.456	1,173	E-Linalool oxide (pyranoid)	104.72 ± 6.25 a	25.07 ± 1.61 b	3,000 <sup>G</sup>	Sweet, floral, woody, herbaceous
88	21.910	1,214	7-Methyl-3-methylene-6-octenol	59.19 ± 5.26 a	0 b	n.f.	Faint scent, herbal,green
89	22.344	1,227	3Z-3,7-Dimethyl-3,6-octadienol	114.09 ± 0.66 a	1.78 ± 0.41 b	n.f.	Sweet, floral, rose, citrusy
90	22.879	1,242	2-(4-Methylidenecyclohexyl) prop-2-enol	34.60 ± 1.38 a	4.53 ± 0.31 b	n.f.	Mild, herbal, floral
91	23.084	1,248	Nerol	18,033.70 ± 2247.35 a	296.16 ± 38.27 b	290 <sup>A</sup>	Floral, fruity, lemon, faint scent
92	28.280	1,407	E-2-Decenol	42.70 ± 1.80 a	2.40 ± 0.14 b	n.f.	Faint scent, fatty, waxy, herbal
93	29.294	1,440	Dihydro-β-ionol	30.35 ± 1.97 a	3.36 ± 0.22 b	n.f.	Floral, woody
94	32.648	1,453	2-Isopropyl-5-methyl-1-hexanol	13.52 ± 1.23 a	2.16 ± 0.35 b	50 <sup>F</sup>	Faint scent, floral,herbal
95	42.868	1,491	Phytol	41.35 ± 0.71 a	3.33 ± 0.55 b	640 <sup>E</sup>	Herbaceous, woody, faint scent
Alkene							
96	5.610	774	1,3,5-Cycloheptatriene	11.15 ± 0.58 a	1.50 ± 0.11 b	n.f.	Mild, solvent
97	8.894	865	2,6-Dimethyl-2-heptene	40.50 ± 0.82 a	14.93 ± 0.37 b	n.f.	Mild, hydrocarbon
98	15.760	1,046	3-Carene	45.07 ± 3.96 a	0 b	770 <sup>F</sup>	Faint scent, pine, citrusy, sweet
99	18.843	1,128	E,Z-2,6-Dimethylocta-2,4,6-triene	0 b	10.29 ± 0.06 a	0.03 <sup>F</sup>	Floral, herbal, balsamic
100	18.848	1,129	2,6-Dimethyl-2,4,6-octatriene	104.78 ± 12.48 a	15.90 ± 0.29 b	n.f.	Strong, herbal, green, faint scent
101	29.622	1,451	E-β-Farnesene	118.87 ± 14.63 a	15.47 ± 1.12 b	87 <sup>B</sup>	Sweet, floral, fruity, woody
102	31.511	1,514	Copaene	56.27 ± 5.40 a	19.76 ± 0.44 b	6 <sup>C</sup>	Spicy, honey, faint scent, piney, woody, citrusy
103	31.604	1,517	(+)-Delta-cadinene	74.74 ± 2.43 a	16.49 ± 1.44 b	1.5 <sup>C</sup>	Herbal, woody
104	31.689	1,520	E-Calamenene	37.46 ± 0.36 a	6.81 ± 0.28 b	n.f.	Spicy, woody
Aromatics							
105	8.481	854	Ethylbenzene	27.87 ± 2.53 a	0 b	170 <sup>C</sup>	Aromati
106	9.718	888	Styrene	237.98 ± 22.96 a	9.99 ± 0.70 b	730 <sup>D</sup>	Balsamic, gasoline, sweet,
107	13.245	980	α-Methylstyrene	93.35 ± 1.74 a	17.97 ± 6.59 b	n.f.	Aromatic, sweet

(to be continued)

Table 1. (continued)

Category	RT	RI	Compounds	Content (μg/L)		Threshold (μg/L)	Odor characteristics
				GT	OT		
108	14.981	989	<i>p</i> -Cymene	54.96 ± 0.79 a	3.96 ± 1.16 b	11.4 <sup>A</sup>	Woody, citrus, aromatic, sweet
109	24.602	1,293	1-Methylnaphthalene	111.86 ± 3.83 a	17.52 ± 1.09 b	14 <sup>D</sup>	Aromatic, coal-tar, oily, solvent
Heterocyclic compound							
110	28.991	1,430	Coumarin	95.51 ± 3.87 a	7.25 ± 1.09 b	11 <sup>A</sup>	Sweet, vanilla, hay ,almond
111	31.174	1,502	2,4-Di-tert-butylphenol	652.49 ± 20.81 a	59.30 ± 2.73 b	500 <sup>C</sup>	Phenol, sweet, rubbery, plastic
112	40.098	1,830	Caffeine	352.30 ± 26.56 a	110.84 ± 5.90 b	n.f.	Mild bitter, herbal

'n.f.', odor thresholds of related compounds not found in the literature. All the odor thresholds and odor description were obtained from: A<sup>[26]</sup>; B<sup>[56]</sup>; C ([www.thegoodscentscompany.com/search2.html](http://www.thegoodscentscompany.com/search2.html)); D<sup>[16]</sup>; E<sup>[57]</sup>; F<sup>[58]</sup>; G<sup>[59]</sup>. 'a, b', significant differences in the relative content of differential volatile compounds between GT and OT. (*p* < 0.05, GT: *n* = 15, OT: *n* = 16).

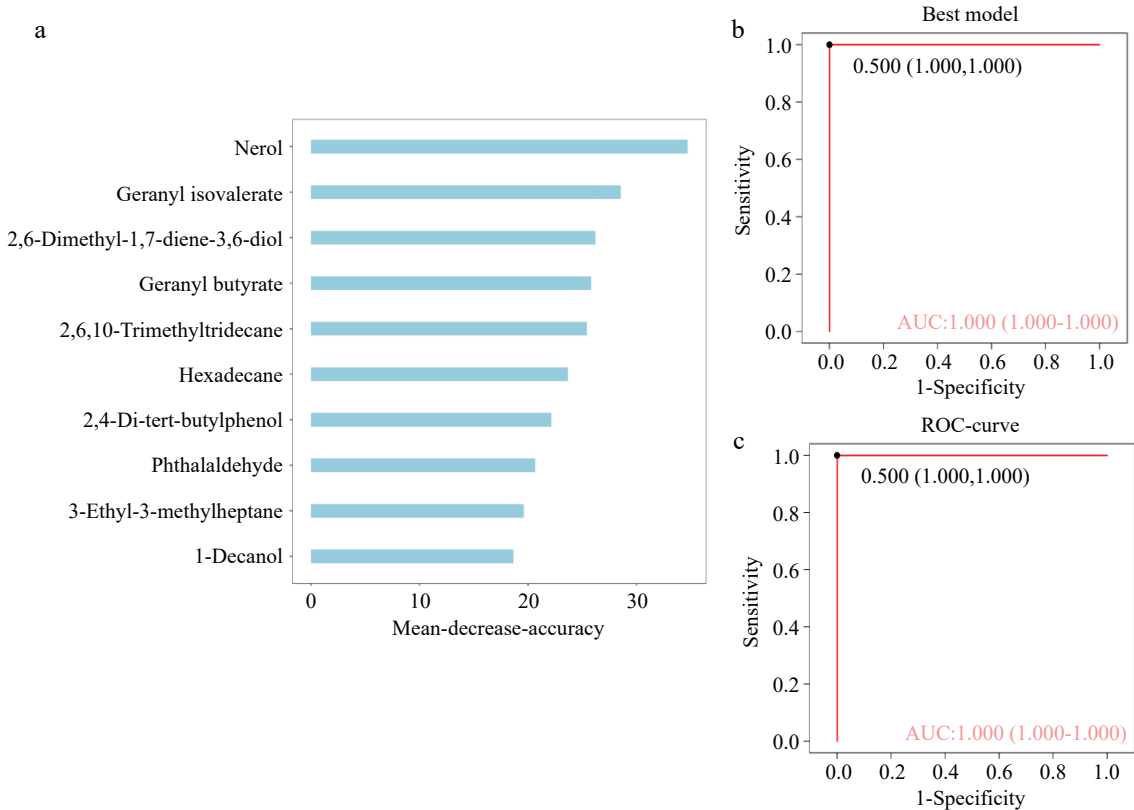


Fig. 4 Random forest analysis and ROC curve of differential volatile compounds between GT and OT. (a) Ranking of mean decrease accuracy, the higher the accuracy, the greater the importance of the metabolite, and the top 10 compounds were selected for display. (b) The ROC curve was used to assess the performance of the random forest classifier. (c) ROC curve assessment of the top 10 key compounds.

volatile compounds (Fig. 4c), yielding an AUC value of 1, demonstrating the reliability and accuracy of these compound combinations in distinguishing the aroma of GT and OT.

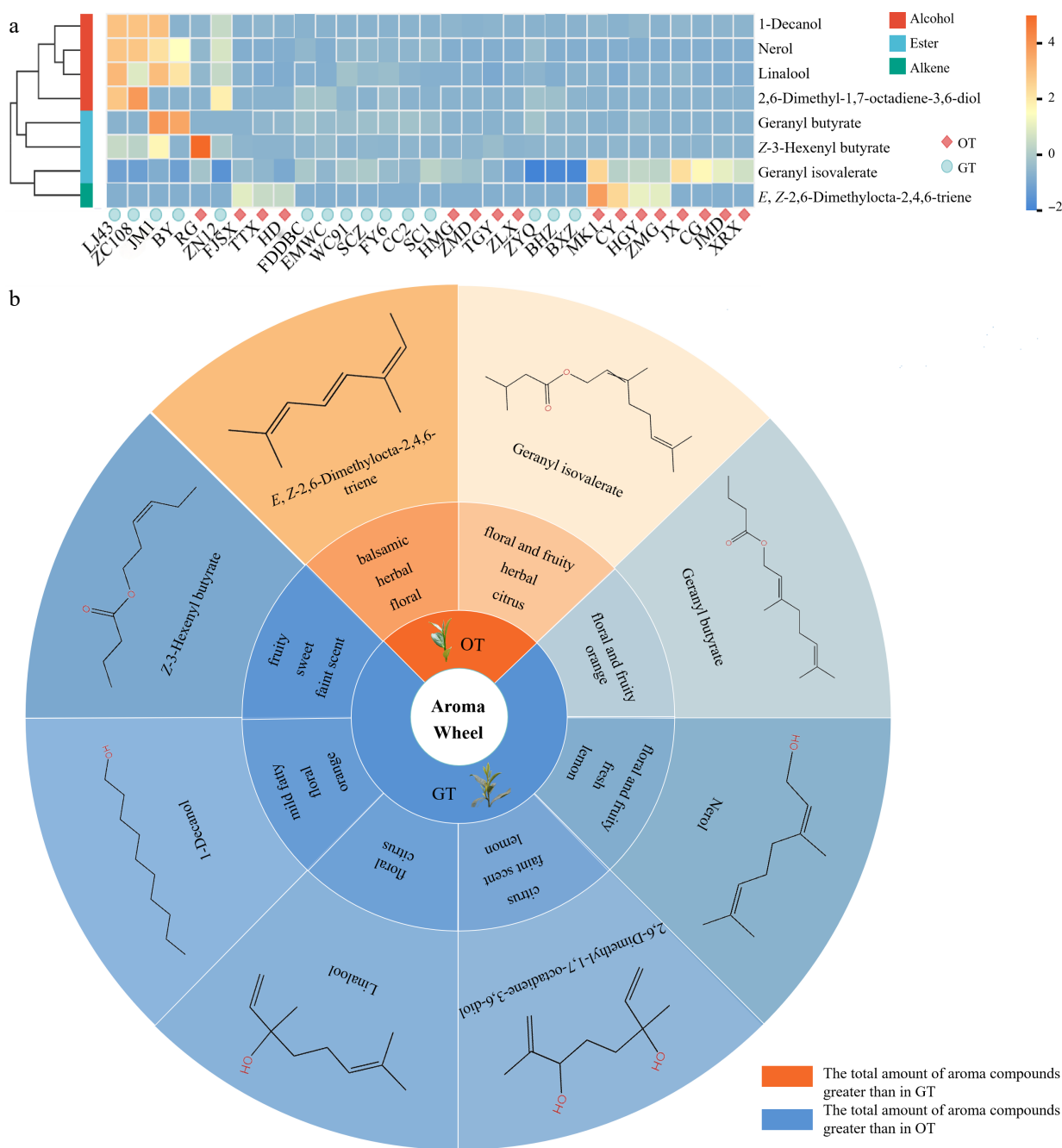
Comparative analysis of volatile compound variations among cultivars

Eight major volatile compounds with high mean decrease accuracy and *rOAV* > 1 were selected. To observe the variation trends of these compounds between GT and OT, their relative content at the sample level was normalized, followed by heatmap and clustering analysis (Fig. 5a). The results demonstrated that *E,Z*-2,6-dimethylocta-2,4,6-triene, within the OT group, showed the highest relative content in MK1 and CY cultivars. In the GT group, 2,6-dimethyl-1,7-octadiene-3,6-diol, with distinctive aroma characteristics, had the highest content in ZC108, followed by LJ43 and ZN12. At the same time, the results revealed that the dominant alcohols in GT are associated with floral and faint scents, which aligns with existing

research showing that linalool enhances the floral aroma of green tea<sup>[8,51]</sup>. Geranyl butyrate was found in higher concentrations in JM1 and BY cultivars, while other esters contributing to floral and fruity aromas were mainly concentrated in OT, including RG, MK1, CY, and JX. In terms of sensory evaluation, RG is not only known for its unique cinnamon flavor but also for its floral and fragrant aroma<sup>[52]</sup>. At the same time, MK1 also exhibits obvious floral and fruity characteristics<sup>[53]</sup>, which is a manifestation of high-quality oolong tea. These findings indicate that compounds with floral and fruity aromas play a significant role in shaping the overall aroma of OT. To analyze the impact of these eight volatile compounds on the overall aroma profile of tea, they were divided into GT and OT groups and visualized in a flavor wheel based on their specific aroma characteristics (Fig. 5b). Among them, *E,Z*-2,6-dimethyl-2,4,6-octatriene, which has floral and balsamic notes, and geranyl isovalerate, with floral and fruity aromas, play a major role in



## Aromatic differences between green and oolong teas



**Fig. 5** Heatmap of volatile compounds across different varieties and flavor wheel of aromatic compounds. (a) Heatmap analysis of key volatile compounds selected across different cultivars. (b) Flavor wheel of eight key volatile compounds in GT and OT (roAV > 1).

determining the flavor quality of OT. The remaining six volatile compounds, including linalool, Z-3-hexenyl butyrate, and 2,6-dimethyl-1,7-octadiene-3,6-diol, help form floral and faint scents of GT. This visual analysis allows for a clear comparison of the aroma differences between GT and OT. In summary, the aroma characteristics of the GT group are primarily composed of alcohols with faint and floral scents, while esters with floral and fruity notes are the primary compounds responsible for shaping the aroma of the OT group.

Although the current analysis was conducted on fresh tea leaves, many of the identified key compounds, such as linalool, nerol, and *E, Z*-2,6-dimethyl-2,4,6-octatriene, are known to persist through tea processing or transform into other aroma-active derivatives<sup>[54]</sup>. Previous studies have reported that linalool and its oxides contribute significantly to the floral aroma of both green and

oolong teas after processing<sup>[6,10,11,51]</sup>. Notably, nerol has been reported to significantly increase during the 'yaoqing' (tossing) stage of oolong tea processing. *E, Z*-2,6-dimethyl-2,4,6-octatriene has been shown to accumulate during the 'zuoqing' (green-making) process, contributing to the formation of oolong-specific aroma traits<sup>[55]</sup>. Therefore, the presence and abundance of these compounds in fresh leaves may serve as effective indicators for the aroma potential of different cultivars, contributing to the final flavor quality of the dry tea.

## Conclusions

In this study, volatile compounds in fresh leaves from 15 cultivars suitable for GT and 16 cultivars suitable for OT were analyzed using HS-SPME-GC-MS. A comprehensive analysis identified 239 distinct

volatile compounds. Multivariate analysis further revealed notable distinctions in the aroma profiles between GT and OT, with alcohols and esters being the main classes of differential compounds. Alcohol compounds with floral and faint scent notes are predominantly found in GT, including linalool, nerol, 1-decanol, and 2,6-dimethyl-1,7-octadiene-3,6-diol. Additionally, key aroma compounds unique to GT also included 1-nonanal and Z-jasmone. In contrast, ester-based volatile compounds with floral and fruity aromas were primarily found in OT, with geranyl isovalerate being the predominant compound. The aroma compound unique to OT was *E,Z*-2,6-dimethylocta-2,4,6-triene. Future investigations should aim to elucidate the specific mechanisms underlying these key volatile compounds and their metabolic regulatory networks, thereby advancing our understanding of aroma formation in tea and facilitating the optimization of tea cultivation and processing practices.

## Ethical statement

The appropriate protocols for protecting the rights and privacy of all participants were utilized during the execution of this research.

## Author contributions

The authors confirm contribution to the paper as follows: conceptualization: Lei X, Yan J, Wang P; methodology: Lei X, Yan J, Xiao Y, Lei W, Fan W, Zhang Y, Li H, Wang P; investigation: Lei X, Xiao Y, Lei W, Fan W; data curation, formal analysis, software: Lei X; experimental designing: Wang P; writing – original draft: Lei X, Yan J; writing – review & editing: Yu Y, Wang P; funding acquisition, project administration: Xu Q, Ye N, Yu Y, Wang P. All authors reviewed the results and approved the final version of the manuscript.

## Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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