Food Chemistry: X 26 (2025) 102253

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x

Decoding the specific minty-like aroma of 'Rucheng baimaocha' (*camellia pubescens*) black tea

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ARTICLE INFO

Keywords:
Rucheng Baimaocha
Black tea
Minty-like aroma
Aroma recombination and omission

ABSTRACT

Rucheng Baimaocha (*Camellia pubescens*) black tea (RCBT) exhibits floral and sweet attributes with a unique minty-like aroma, distinguishing it from traditional and other innovative black teas. However, its key odorants remain unknown. In this study, gas chromatography-olfactometry (GC-O) combined with aroma extraction dilution analysis detected 26 aroma-active compounds (ACCs), of which 20 ACCs were quantified as odorants of RCBT infusion. In addition, aroma recombination models well simulated the overall aroma characteristics of RCBT infusion, which proved the accuracy of identification and quantification. Omission experiments showed that 12 key odorants played crucial roles in aroma formation of RCBT. Among these, four odorants (methyl salicylate, (*E*, *Z*)-2,6-nonadienal, methyl geranate, and (*E*)-2-nonenal), were of great importances for unique minty-like aroma, with significantly higher concentrations compared to other black tea varieties. This study offered a foundational theoretical framework for the processing and quality control of RCBT.

1. Introduction

As the world's second most favored drink globally after water, tea is extensively enjoyed by consumers worldwide for its various health advantages and distinct flavor (Wan et al., 2024; Zhang et al., 2019). Aroma is an essential part of tea's flavor quality, directly affecting the consumer's drinking experience and market acceptance (Su et al., 2022; Wang et al., 2024). However, the aroma of tea is subject to numerous influencing factors. Different tea plant varieties have distinctive aroma attributes and characteristics due to differences in their genetic backgrounds and growing environments (Kang et al., 2019; Zheng, Gan, et al., 2023). For instance, the Fangping green tea originated from the new variety 'Zhonghuang 1' possesses a unique aroma reminiscent of cooked corn, making it stand out from other types of green tea (Liao et al., 2020). The Dianhong tea, made from the Yunnan large-leaf variety, is renowned for its distinctive caramel-like aroma (Ma et al., 2022). Keemun black tea processed from 'Zhuyin' variety exhibits floral, fruity and honey-like aromas, earning it a place among the world's top three premier high-fragrance quality black tea (Wang et al., 2024). As consumer growing demand for high-quality tea and the diversification of market needs, specialty teas of exceptional quality have gained widespread favor among consumers (Ouyang, Jiang, Chen, et al., 2024; Su et al., 2022). 'Rucheng baimaocha' (*Camellia pubescens*), grown in Hunan's Rucheng County, stands out as a special ingredient for crafting top-quality white tea and black tea thanks to its rich silver trichomes and inherent floral scent (Chen et al., 2023; Huang et al., 2019). The processed black tea is covered with golden hairs and has a rich floral and honey fragrance (Huang et al., 2016; Ouyang et al., 2024; Zhong et al., 2023). Interestingly, in repeated sensory evaluations, we found that the aroma characteristics and types of black tea of 'Rucheng baimaocha' variety are quite different greatly from traditional and other innovative black teas, featuring floral and sweet fragrance with a unique minty-like

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https://doi.org/10.1016/j.fochx.2025.102253
Received 1 December 2024; Received in revised form 24 January 2025; Accepted 2 February 2025
Available online 3 February 2025

aroma.

Black tea contains a rich variety of volatile substances, with over 600 types identified through detection and analysis. These substances are mainly derived from the inherent volatile compounds in the fresh leaves, as well as those newly formed during the processing stages (Chen et al., 2022). The diverse in types and quantities of these aromatic substances endows black tea with a broad range of unique fragrance types and characteristics (Kang et al., 2019; Zheng, Hu, et al., 2023). Currently, some progress has been made in the research on volatile compounds and odorants of 'Rucheng baimaocha' black tea (RCBT). Huang et al. (2019) found that the congou black tea made from 'Rucheng baimaocha' variety had a strong and long-lasting floral aroma, which mainly consisted of 15 volatile constituents including geraniol, methyl salicylate, linalool, nerolidol, dimethyl sulfide, and cis-jasmone. Compared to the 'Zhuyeqi' variety, studies on the flavor quality of 'Rucheng baimaocha' No. 1 and No. 2 revealed that the total volatile components, as well as the relative content of fruity and floral aroma compounds, were much greater than those found in the control black tea (Zhong et al., 2023). Additionally, in previous research, we used relative odor activity value (ROAV) alongside gas chromatography-olfactometry (GC-O) to explore the key aroma components of 'Baihaocha' black tea from Guangxi, Guangdong, and Hunan (Ouyang et al., 2024). We found that 23 compounds can serve as indicators for distinguishing black tea produced in the three different regions, and geraniol, linalool oxide IV, and (*Z*)-jasmone were associated with floral attribute; 1-methyl-4-(1-methylethenyl) benzene, neral, 2,6-dimethyl-5-hepten-2-one, citral linked to fruity odor; 1-nonanol, (*E*)-2-nonenal, 2,6-dimethyl-5-hepten-2-one and 1-heptanol related to fresh odor; and 6-methyl-5-hepten-2-one, (*E*, *E*)-2,4-heptadienal and (*Z*)-4-heptenal connected to roasted property (Ouyang et al., 2024). However, current studies on the aroma of RCBT have not conducted precise quantitative experiments, nor have they applied recombination or omission techniques to verify the results. It was evident that the research on the molecular basis of the RCBT aroma remains incomplete. In recent years, sensomics has emerged as a powerful approach for identifying key odorants in foods by integrating olfaction, odor thresholds, aroma recombination and omission (Wei et al., 2024; Zhai et al., 2022). Liao et al. (2020) found that dimethyl sulfide plays a notable role in the aroma of green tea with cooked corn-like. Yu et al. (2023) discovered that geraniol, (*E*, *E*)-2,4-heptadienal and (*E*)-β-ionone had significant effects on floral flavor contribution by comparing common and premium Lu'an Guapian green tea. To date, there has been little research on the unique minty-like aroma of RCBT, and it remains unclear whether a single compound or a combination of multiple odorants is responsible for the characteristic minty-like aroma.

Hence, the objective of this study was to systematically reveal the RCBT key aroma-contributing compounds. The following experiments were conducted: (1) identification of aroma active compounds(AACs) using GC-O coupled with aroma extraction dilution analysis(AEDA); (2) quantification of the screened AACs by internal standard method and calculation of odor activity value(OAV); (3) Determination of key AACs through recombinant and omission test for RCBT; (4) Compared with other black tea varieties to further verify the key odorants responsible for the minty-like aroma in RCBT.

2. Materials and methods

2.1. Chemicals

Ethyl decanoate and n-alkanes (C7-C25) were purchased from Sigma-Aldrich (Shanghai, China). Aroma standards were commercially available and used for volatile identification: hexanal, (*E*)-2-Hexenal, (*Z*)-4-heptenal, heptanal, benzaldehyde, 6-methyl-5-hepten-2-one, butanoic acid, hexyl ester, nonanal, (*E*)-2-Hexen-1-ol, β-Myrcene, 2-heptanol, benzyl alcohol, (*E*, *Z*)- 2,6-nonadienal, terpinen-4-ol, citral, (*Z*)-3-hexen-1-ol, methyl geranate, acetophenone, α-ionone, 3-octanone, 2-n-butyl furan (Aladdin, Shanghai, China); benzoic acid, 1-heptanol, methyl ester, linalool, phenylethyl alcohol, (*E*)-2-nonenal, acetic acid, phenylmethyl ester, nerol, geraniol (J&K Scientific, Beijing, China); 1-octen-3-ol and methyl salicylate (Sigma-Aldrich, Shanghai, China); neral (Kaiwei chemical Co., Ltd., Shanghai, China); 2,4-nonadienal and trans-β-ionone (Macklin Biochem, Shanghai, China); β-damascenone (Leyan, Shanghai, China).

2.2. Tea samples

We gathered 19 RCBT tea samples from the enterprises in Rucheng County. Based on the experimental requirements, 13 typical RCBT samples with a distinct minty-like aroma were selected. Additionally, to compare with other types of black tea, Keemun black tea (KM), Yingde black tea (YH), and Dianhong tea (DH) were purchased from their respective production areas. All tea samples were prepared according to the traditional process of black tea.

The volatile-free matrix, identical to commercially available sample without volatile compounds, was prepared in a slightly modified way from the previous method (An et al., 2022; Du et al., 2013). Briefly, equal amounts of ground and mixed tea samples were placed in distillation flasks and repeatedly rotated with water at 40–50 °C to eliminate aromatic components. During the evaporation process, the powder was regularly inspected until all volatile components were undetected. Finally, the samples were freeze-dried to facilitate future analysis.

2.3. Sensory evaluations

Twelve experienced members (25–30 years old, four men and eight women) from Hunan Agricultural University conducted a sensory evaluation of black tea aroma in a specific room. Prior to the formal experiments, each assessor underwent at least 90 h of specialized training to ensure they could precisely identify and measure the various aroma types and characteristics. It was crucial to mention that all participants were fully informed before the experiment and had the option to discontinue at any time. Subsequently, sensory evaluation was carried out following the Chinese national standard (GB/T 23776–2018). First, 3 g of tea samples were measured and infused in 150 mL of boiling water for a period of 5 min. Subsequently, the tea residue was removed through filtration and subjected to evaluation. The intensity of the aroma attributes for both the original and reconstituted model tea infusions was evaluated using a 5-point intensity scale (0 represented as perceptible, 3 as medium and 5 is very strong) according to our previous method (Ouyang, Jiang, Chen, et al., 2024). The scores for the attributes in the quantitative descriptive analysis (QDA) were derived by calculating the mean ratings supplied by a panel of 12 experts.

2.4. Extraction of volatile compounds

The volatiles of RCBT were extracted by headspace solid-phase microextraction (HS-SPME) based on our previously refined methods (Ouyang, Jiang, Xu, et al., 2024). For each sample, a precise 0.500 g tea sample powder was measured and transferred into 20 mL headspace vials. After that, the headspace vial was positioned in the CTC's sample tray, and the CTC introduced 10ul of ethyl decanoate (8.63 mg/L) and 5 ml of boiling water into it. Sequentially, the headspace vial was maintained at 60 °C and 600 r/min for 10 min to equilibrate the sample. A fiber head made of 50/30 μm divinylbenzene/carboxy/polydimethylsiloxane was inserted into the headspace and subjected to extraction under identical circumstances for a duration of 30 min. Lastly, the fiber underwent desorption at the GC inlet for 10 min at 250 °C.

2.5. GC × GC- O-Q-TOFMS analysis

The volatile compounds were detected by comprehensive two-dimensional gas chromatography quadrupole time-of-flight mass spectrometry (GC × GC-Q-TOF-MS, Agilent technologies, USA) coupled to the olfactory detection port. The 1D and 2D columns were from Agilent, HP-5MS and DB-17MS, respectively, and were both located in the column oven. The heating steps for the 1D and 2D columns were the same, detailed as follows: an initially temperature of 40 °C held for 1 min, followed by a gradual rise of 4 °C per minute until reaching 180 °C, then a rapid increase of 20 °C per minute to 250 °C, with a final hold at temperature for 1 min. The SSM1800 modulator aided column heating/cooling with a 4-s cycle. The mass spectrometer ran in electron ionization mode at 200 °C(source) and 150 °C(quadrupole), utilizing 70 eV electrons to scan 45–500 amu. The retention index (RI) was derived using the n-alkane series as a basis for calculation. Based on our previous criteria, compounds were kept if the forward match exceeded 700, the reverse match was over 800, and the RI deviation was below 15 (Ouyang, Jiang, Chen, et al., 2024).

In the 2D mode, the 1D column sample mixes with helium, splits into two equal parts. One part to ODP, and the other to mass detector. In this study, three experienced panelists conducted the GC-O detection process. Prior to the official GC-O, all panelists conducted extensive sniffing tests to identify and describe the aroma characteristics of the different standards. For AEDA analysis, the enriched volatile compounds were subjected to stepwise dilution test by altering the split ratio (1:2, 1:4, 1:8, 1:16, …) of the injection port, and then the diluted sample was analyzed by GC-O. The sample was continuously diluted from a high concentration until it was no longer detectable by smell, and the maximum dilution perceivable corresponds to the odorant's flavor dilution (FD) factor.

2.6. Quantification analysis of volatile compounds

The quantification of volatiles was performed using a combination of internal and external standards. The external standard method was used for aroma compounds with commercially available standards (Table S1). Briefly, aroma standards were prepared as a mixed solution of five different concentration gradients using ethanol as the solvent. Then, 10ul of ethyl decanoate, 10ul of mixed standard and 5 ml of boiling water were added to a 0.5 g 'volatile-free' matrix. The extraction was performed according to section 2.4, followed by GC × GC-Q-TOFMS analysis. The content of the analyzed compound was calculated according to the corresponding standard curve. Compounds without standard substances were relatively quantified using ethyl decanoate.

2.7. OAV calculation

Referring to evaluation methods, the content of each AAC was calculated by brewing 3 g tea in 150 ml of water. OAVs were calculated as the ratio of AAC concentration to their respective odor threshold (OT) in water. A value of OAV above 1 notably contributes to the overall flavor (Zheng, Gan, et al., 2023). The OTs were extracted from relevant literature and websites.

2.8. Aroma recombination and omission

For aroma reconstitution experiments, volatiles pinpointed through GC-O analysis and OAV were mixed at realistic quantitative concentration in 'volatile-free' matrix. In mission tests, recombinant models were used to deletion of one or a class of odorants from the triangular tests, thus further narrowing down the range of characteristic aroma substances. The initial and the reconstituted tea infusions were assessed by experienced panelists, with each trial being conducted three times. The levels of significance for the triangulation experiment were set as: a difference was deemed significant ($p<0.05$) with eight correct responses, highly significant ($p < 0.01$) with 9, and very highly significant ($p < 0.001$) with 10 correct responses from panelists (Wei et al., 2024).

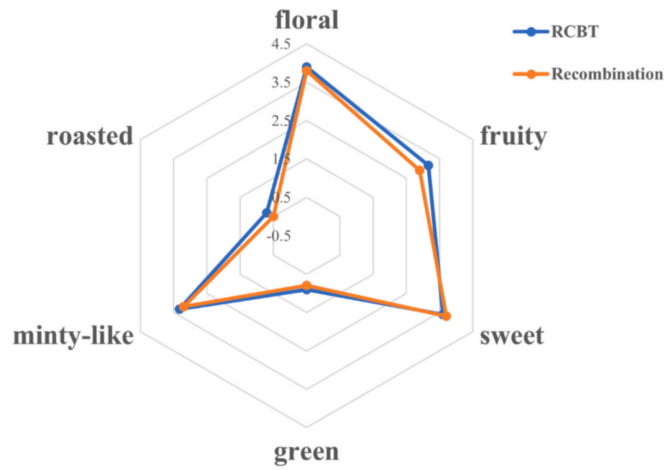

Fig. 1. The aroma profile of 'Rucheng baimaocha' black tea (RCBT) and the fully aroma recombinant.

Table 1
Aroma active compounds of 'Rucheng baimaocha' black tea (RCBT).

No.	RI [a]	Aroma active compounds	Odor descriptiona [b]	FD [c]	Identification method [d]
1	801	Hexanal	green apple, cut grass	16	RI/MS/O/STD
2	900	(*Z*)-4-Heptenal	baked potatoes, biscuit	128	RI/MS/O/STD
3	980	1-Octen-3-ol	mushroom-like	64	RI/MS/O/STD
4	986	6-Methyl-5-hepten-2-one	mushroom, pepper	128	RI/MS/O/STD
5	1045	Benzeneacetaldehyde	floral, honey	16	RI/MS/O/STD
6	1074	Linalool oxide I	sweet	2	RI/MS/O/ STD
7	1086	Linalool oxide II	roasted, sweet	4	RI/MS/O/STD
8	1094	Benzoic acid, methyl ester	fruity	32	RI/MS/O/STD
9	1099	Linalool	citrus-like, floral	512	RI/MS/O/STD
10	1104	Nonanal	floral	128	RI/MS/O/STD
11	1116	Phenylethyl Alcohol	floral, sweet, honey	32	RI/MS/O/STD
12	1155	(*E*, *Z*)-2,6-Nonadienal	fresh, cucumber	128	RI/MS/O/STD
13	1162	(*E*)-2-Nonenal	fresh, cucumber-like	32	RI/MS/O/STD
14	1164	Acetic acid, phenylmethyl ester	fruity, fresh	32	RI/MS/O/STD
15	1177	Terpinen-4-ol	fresh, woody	128	RI/MS/O/STD
16	1192	Butanoic acid, hexyl ester	fruity, fresh	32	RI/MS/O/STD
17	1192	Methyl salicylate	fresh, mint	64	RI/MS/O/STD
18	1214	2,4-Nonadienal	deep fried, fat	64	RI/MS/O/STD
19	1240	Neral	citrus, lemon	64	RI/MS/O/STD
20	1255	Geraniol	floral	512	RI/MS/O/STD
21	1273	Citral	fruity, like lemon	256	RI/MS/O/STD
22	1322	Methyl geranate	fruit, fresh	64	RI/MS/O/STD
23	1386	*β*-Damascenone	sweet, honey	512	RI/MS/O/STD
24	1395	(*Z*)-Jasmone	floral, like jasmine	16	RI/MS/O/STD
25	1426	α-Ionone	floral	16	RI/MS/O/STD
26	1486	Trans-*β*-Ionone	floral, sweet	64	RI/MS/O/STD

[a] RI, retention index; [b] Odor description of aroma-active compounds was obtained at the olfactory port; [c] FD (flavor dilution) factor was measured by AEDA. [d] MS, mass spectra; O, olfactometry; STD, authentic reference compounds.

2.9. Statistical analysis

The data were replicated threefold, averages were subsequently computed. Radar plots were prepared using Excel software.

3. Results and discussion

3.1. Aroma profile and volatile compound composition of RCBT

To understand the aroma profile of RCBT, sensory evaluation of RCBT was performed through the QDA. We selected 6 aroma properties as representatives: floral, sweet, fruity, minty-like, roasted, and green. The result showed that the floral and sweet aroma of RCBT were the most intense attributes, followed by fruity aroma (Fig. 1). In addition, the minty-like characteristic of the RCBT was very distinct and special compared to other black teas, which may be related to the uniqueness of its variety. Overall, the sensory evaluation of RCBT aligned well with the overall trend observed in our previous results (Ouyang et al., 2024).

The aroma of tea is related to the presence of volatiles, RCBT volatile compounds were extracted and detected by SPME combined GC × GC-Q-TOFMS. A total of 194 common volatile compounds were identified in 13 minty-like scent RCBT samples, including 37 alcohols, 30 aldehydes, 29 esters, 36 alkenes, 20 ketones, 9 alkanes, 10 aromatic compounds, 12 oxygen heterocyclic compounds, 3 acids, and 8 other compounds (Table S2). Among them, alcohols and aldehydes were the most dominant in minty-like scent RCBT according to their concentration, which was similar to the results of previous studies (Ouyang, Jiang, Chen, et al., 2024). Previous studies have shown that most of the alcohols and aldehydes were associated with the floral, fruity, sweet and fresh aroma of black tea (Zhai et al., 2022). Therefore, it was essential to further analyze the aroma-active compounds in order to identify the key odorants of minty-like RCBT.

3.2. Identification of the AACs in RCBT

The olfactory technique has emerged as an effective tool for screening AACs and is now widely adopted in the analysis of food flavors (Ouyang, Jiang, Chen, et al., 2024; Zhai et al., 2022). Additionally, the combination of GC-O and AEDA can determine the aroma FD factors of each flavor compound and rank their importance, thereby assessing their degree of contribution to the overall scent (Feng et al., 2015; Zhai et al., 2022). The AACs of RCBT were analyzed using GC × GC-O-Q-TOFMS, resulting in the identification of 26 compounds with FD factors varying between 2 and 512 (Table 1), including 9 aldehydes, 7 alcohols, 5 esters, and 5 ketones, which was in line with our previous trend of distribution of RCBT in terms of the main volatile compound species (Ouyang et al., 2024). To pinpoint the aroma active compounds of RCBT, we conducted a comparison of their retention index and odor descriptions against authentic reference chemicals.

In this study, 9 aroma-active aldehydes were found in RCBT. Among them, citral (256; fruity, like lemon), (*Z*)-4-heptenal (128; roasted), nonanal (128; floral), (*E*, *Z*)-2,6-nonadienal (128; fresh, cucumber-like) had the highest FD factors, followed by 2,4-nonadienal (64; cereal and fat), neral (64; fruity, lemon), (*E*)-2-nonenal(32; fresh, cucumber-like), hexanal(16; green and cut grass), benzeneacetaldehyde (16; floral, rose-like). Previous research has indicated that most of the aldehydes found in tea have predominantly citrus and fresh aroma characteristics, with some also contributing flavors such as honey, fat and metal (Flaig et al., 2020; Ma et al., 2022; Zhai et al., 2022). Aldehydes in tea are primarily produced through two processes: lipid oxidation catalyzed by lipoxygenase and Strecker degradation (Ho et al., 2015). A significant portion of aldehydes, such as (*E*, *Z*)-2,6-nonadienal, 2,4-nonadienal, (*E*)-2-nonenal and hexanal, are produced through the pathway of lipoxygenase-catalyzed lipid oxidation (Ho et al., 2015; Ma et al., 2022; Zhai et al., 2022).

Alcohols with floral and sweet attributes are the third major group of volatiles in tea, existing both in free form and as glycosides (Zhai et al., 2022). In this research, 7 aroma active compounds were detected, including geraniol, linalool, linalool oxide I, linalool oxide II, phenylethyl alcohol and terpinen-4-ol. Linalool (512; floral) and geraniol (512; floral, lemon peel) stood out with highest FD factors, indicating their crucial role in shaping the scent profile of RCBT. Both linalool and geraniol are two significant terpene alcohols sharing the common precursors in tea, linalool is mainly associated with the fermentation and drying phases of tea processing, while geraniol is more closely linked to the rolling and fermentation stages of tea (Liu et al., 2024; Wang et al., 2020; Zhai et al., 2022). However, linalool oxide I (2; floral) and linalool oxide II (4, baked, sweet) showed lower FD values. These four volatiles served as significant AACs in black teas, and it was noteworthy that they all originate mainly from glycosidic hydrolysis in fresh leaves (Xiao et al., 2017; Zhai et al., 2022). Furthermore, terpinen-4-ol (128; herb, must) and 1-octen-3-ol (64; mushroom-like) were also recognized as contributing to the green attribute.

Esters are common volatiles in tea and are mainly associated with fruity, floral and sweet scent (Zhai et al., 2022). Five ester aroma active compounds were found, with methyl salicylate (64; fresh, mint, wintergreen-like) and methyl geranate (64; fresh, fruit) exhibiting the highest FD factors. Our previous studies have also shown that methyl salicylate had the largest relative abundance in RCBT and was the most identified aroma active compound in RCBT (Ouyang, Jiang, Chen, et al., 2024). In addition, it was observed that the methyl geranate content in RCBT was notably higher compared to that in congou black tea made from Jianghua Kucha and Sangzhi Daye. Moreover, methyl geranate was undetectable in 'Zhuyeqi' variety black tea, suggesting that the methyl geranate may play an significant role in the distinct minty-like scent of RCBT (Huang et al., 2019; Zhong et al., 2023).

Moreover, five ketones were detected and *β*-damascenone (512; sweet, honey) possessed the highest FD factor. *β*-Damascenone makes significant contributions to the aroma of black tea owing to its very low

Table 2
Quantification and odor activity value (OAV) calculation of 'Rucheng baimaocha' black tea (RCBT) aroma active compounds.

Aroma active compounds	The sensory evalution concentration (μg/L,3 g of tea in 150mLof water)	OT / (μg/ L)	OAV
(*E*, *Z*)-2,6-Nonadienal	28.75	0.0045	6388.64
trans-*β*-Ionone	42.23	0.007	6032.65
β-Damascenone	2.92	0.002	1459.67
(*E*)-2-Nonenal	15.49	0.08	193.58
Benzeneacetaldehyde	733.42	4	183.35
Linalool	978.65	6	163.11
Hexanal	549.19	4.5	122.04
Geraniol	496.71	7.5	66.23
2,4-Nonadienal	3.31	0.05	66.10
1-Octen-3-ol	62.67	1	62.67
(*Z*)-4-Heptenal	3.38	0.06	56.35
Citral	31.26	1	31.26
Nonanal	30.72	1	30.72
Terpinen-4-ol	165.59	6.4	25.87
(*Z*)-Jasmone	27.59	1.9	14.52
Methyl salicylate	578.03	40	14.45
α-Ionone	4.62	0.4	11.55
Methyl geranate	121.63	21.44	5.67
Acetic acid, phenylmethyl ester	41.72	30	1.39
Phenylethyl alcohol	988.52	1000	1.02
Neral	45.34	100	<1
6-Methyl-5-hepten-2-one	8.49	100	<1
Benzoic acid, methyl ester	1.49	73	<1
Butanoic acid, hexyl ester	1.77	250	<1

Note: OTs: Odor thresholds in water. These values were sourced from published literature or relevant websites.

OT (Ma et al., 2022). Additionally,α-ionone(16; floral), *β*-ionone(64; floral and sweet)and 6-methyl-5-hepten-2-one(128; mushroom, metal) were common volatile substances generated from carotenoids and have been found as key odorants in black tea (Ho et al., 2015; Xiao et al., 2017).

3.3. Quantification of aroma active compounds

Tea comprises numerous matrix components, including polyphenols and alkaloid, which readily undergo physical or chemical interactions with volatile substances, thereby affecting the emission of odorants from the tea (Weng, 2020). The evaluation of the AEDA method reflects the importance of odor active substances in volatiles. However, AEDA focuses solely on olfactory perception and neglecting the matrix's impact on odorants release (Zhai et al., 2023). To analyze the influence of individual components on the overall flavor, the actual concentrations of 24 AACs with aroma FD factors ≥8 in the RCBT were calculated using external standard methods (Table 2). The results showed that phenylethyl alcohol (988.52 μg/L) was the most concentrated compound among the aroma active compounds followed by linalool (978.65 μg/L), benzeneacetaldehyde (733.42 μg/L), methyl salicylate (578.03 μg/L), hexanal (549.19 μg /L), geraniol (496.71 μg/L), and all of them were above 400 μg/L in RCBT. It was stated that there are three forms of black tea based on the content of volatiles, the first contains high content of linalool and linalool oxide, the second is an intermediate type dominated by linalool and geraniol, and the third is high in geraniol (Ouyang, Jiang, Xu, et al., 2024; Su et al., 2022). In this research, the levels of linalool and geraniol in RCBT were both high, categorizing it as an intermediate type. The trend was similar to that of 'Renhua Baimaocha' black tea, which may be attributed to their similar growing environments (Ouyang et al., 2024).

The effect of individual volatile on the overall flavor is influenced by both their concentrations and their odor thresholds within the specific matrix (Wang et al., 2022). Therefore, OAV was utilized to evaluate the significances of each AAC in shaping the sample flavor (Wang et al., 2022; Wei et al., 2024). As Table 2 shown, it was seen that 20 AACs in the tea infusion had OAVs exceeding 1, suggesting they were key aroma contributors. Of these, (*E*, *Z*)-2,6-nonadienal (OAV:6386.67), trans-*β*-ionone (OAV: 6032.65), *β*-damascenone (OAV: 1459.67), (*E*)-2-nonenal (OAV: 193.58), benzeneacetaldehyde (OAV: 183.35), linalool (OAV: 163.11), hexanal (122.04) exhibited high OAVs, indicating that these substances were main sources of the RCBT aroma. Among them, carotenoid derivatives (trans-*β*-ionone and *β*-dammarone) and terpenoids (linalool and geraniol) add to the floral and sweet aroma of RCBT. Phenylacetaldehyde is converted from *L*-phenylalanine and has floral and honey properties, it is also a major scent component in the world's four well-known black teas and Xinyang black tea (Kang et al., 2019; Yao et al., 2022). Furthermore, (*E*, *Z*)-2,6-nonadienal and (*E*)-2-nonenal produced by lipid oxidation showed predominantly fresh and cucumber-like properties, which could be connected to the minty-like aroma of RCBT.

3.4. Aroma recombination and omission tests

The simulation of recombination test is an effective method for verifying the precision of identification and measurement (Wang et al., 2020). In this research, we added 20 AACs with OAV ≥ 1 to the deodorized matrix based on their actual concentrations in RCBT. Twelve panelists evaluated the aroma attributes of both the original and reconstituted tea infusion, with findings presented in Fig. 1. It was clear that there was no notable disparity in properties within the RCBT reconstituted and original tea infusions, demonstrating an accurate replication of the aroma characteristics. The above findings indicated that we have successfully pinpointed and measured 20 crucial AACs.

To examine the impact of individual AAC on the overall scent profile of RCBT, aroma omission models were conducted. Referring to the reconstituted model, experienced panelists identified differences in the omission model through triangle tests. As illustrated in Table 3, twelve key odorants were pinpointed as major contributors to aroma formation of RCBT, including hexanal, (*Z*)-4-heptenal, benzeneacetaldehyde, linalool, (*E*, *Z*)-2,6-nonadienal, methyl salicylate, geraniol, citral, methyl geranate, trans-*β*-ionone, *β*-damascenone, (*E*)-2-nonenal. Among them, the omission of hexanal and (*Z*)-4-heptenal significantly reduced the green and roasted properties, respectively ($p<0.05$). Eight panelists noted the absence of citral in RCBT tea infusion, which significantly reduced the fruity aroma, indicating citral's important role in RCBT's fruity characteristics ($p<0.05$). In addition, the omission of five key AACs (benzeneacetaldehyde, linalool, geraniol, trans-*β*-ionone, *β*-damascenone) significantly reduced the floral and sweet aroma attributes of RCBT tea infusion. When (*E*, *Z*)-2,6-nonadienal, methyl salicylate, methyl geranate and (*E*)-2-nonenal were omitted, respectively, significant reductions in minty-like aroma were all observed compared to the fully recombinant model. In particular, the omission of (*E*, *Z*)-2,6-nonadienal correctly identified significant changes in the overall scent of the tea infusion by 10 tea panelists. Furthermore, the impact of individual minty-like aroma compounds on the intensity of minty-like aroma was further evaluated using omission trials. As shown in Table 4, the omission of (*E*, *Z*)-2,6-nonadienal resulted in a significant decrease in the minty-like aroma (−26.25 %), followed by methyl salicylate (−18.13

Table 3
The results of the triangle test for key aroma active compounds in 'Rucheng baimaocha' black tea (RCBT).

Key aroma active compounds	Right of selection [a]	Significance	Description of odor difference
Hexanal	8	*	Less green
(Z)-4-Heptenal	8	*	Less roasted
1-Octen-3-ol	5		
Benzeneacetaldehyde	9	**	Less floral and sweet
Linalool	8	*	Less floral and sweet
Nonanal	5		
Phenylethyl Alcohol	7		
(E, Z)-2,6-Nonadienal	10	***	Less mint-like
Methyl salicylate	8	*	Less mint-like
2,4-Nonadienal	6		
Geraniol	8	*	Less floral
Citral	8	*	Less fruity
trans-Geranic acid methyl ester	9	**	Less mint-like
(Z)-Jasmone	3		
trans-β-Ionone	8	*	Less floral and sweet
α-Ionone	4		
β-Damascenone	8	*	Less sweet
Terpinen-4-ol	4		
Acetic acid, phenylmethyl ester	5		
(E)-2-Nonenal	9	**	Less mint-like

[a] Number of correct judgments from 12 experts. "*" representing significant ($p < 0.05$); "**" representing significant ($p \leq 0.01$); "***" representing significant ($p \leq 0.001$).

Table 4
Results of Omitting minty-like Odorants in 'Rucheng baimaocha' black tea (RCBT) recombination.

Group	Omitted odorants	Intensity of minty-like	Variance (%)
Re-RCBT		3.2	
I	(*E*, *Z*)-2,6-Nonadienal, methyl salicylate, methyl geranate, (*E*)-2-nonenal	2.0	37.50
II	(E, Z)-2,6-Nonadienal	2.36	26.25
III	Methyl salicylate	2.62	18.13
IV	Methyl geranate	2.88	10.00
V	(*E*)-2-Nonenal	2.80	12.50

Table 5
Concentration and odor activity value (OAV) of different black tea volatiles.

Compounds	OAV				OTs (μg/L)	The concentration of sensory evalution (ug/L,3 g of tea with 150mLof water)			
	RCBT	KM	YH	DH		RCBT	KM	YH	DH
(*E*, *Z*)-2,6-nonadienal	6386.67 ± 1369.85	401.40 ± 59.19	–	–	0.0045	28.75 ± 6.08	1.81 ± 0.22	–	–
Methyl salicylate	14.45 ± 2.07	6.64 ± 0.32	3.19 ± 0.12	1.65 ± 0.03	40	578.03 ± 84.04	265.67 ± 10.61	127.52 ± 3.85	65.80 ± 0.91
(*E*)-2-nonenal	193.58 ± 37.78	74.07 ± 2.63	34.79 ± 8.33	29.55 ± 0.45	0.08	15.49 ± 2.98	5.93 ± 0.17	2.78 ± 0.54	2.36 ± 0.03
Methyl geranate	5.67 ± 1.52	0.76 ± 0.05	0.08 ± 0.01	0.03 ± 0.01	21.44	121.63 ± 32.53	16.33 ± 0.85	1.76 ± 0.10	0.55 ± 0.14

Note: RCBT, 'Rucheng baimaocha' black tea; KM, Keemun black tea; YH, Yingde black tea; DH: Dianhong tea.

%), (*E*)-2-nonenal (−12.50 %), and methyl geranate (−10.00 %). However, when all these compounds were omitted simultaneously, the decrease in the intensity of the minty-like aroma was most significant (−37.50 %). It was reported that (*E*, *Z*)-2,6-nonadienal with high FD factor and highest OAV in Meehan's mint was pinpointed as the key odorant of Meehan's mint (Dein et al., 2020). Additionally, (*E*)-2-nonenal was identified as AAC in Meehan's mint, and the differing olfactory perceptions between mint and RCBT may be related to variations in their concentrations (Dein et al., 2020). Methyl salicylate, on the other hand, was recognized as the most definitive aroma substance of Ceylon black tea and was associated with its minty aroma (Kang et al., 2019). The above results showed that these four compounds ((*E*, *Z*)-2,6-nonadienal, methyl salicylate, methyl geranate and (*E*)-2-nonenal) have a major influence on the minty-like property of RCBT.

To further verify the unique minty-like aroma of black tea made from the 'Rucheng Baiaocha' variety, we tested four key compounds associated with the minty attribute in black teas produced from three representative varieties, ranging from small to large-leaf types. As shown in Table 5, the content of the four key odorants contributing to the minty-like scent in RCBT was significantly higher than in black teas made from other varieties. Specifically, (*E*, *Z*)-2,6-nonadienal was identified only in KM and RCBT, with the latter exhibiting very high content and OAV. The content of (*E*)-2-nonenal in RCBT was more than twice that of the other three black tea. Furthermore, methyl salicylate concentrations were high in all four black teas but particularly high in RCBT, which may suggest that RCBT fresh leaves contain higher levels of methyl salicylate precursor substances. Notably, the levels of methyl geranate in RCBT was much greater compared to other black teas, and its OAV value was the only one exceeding 1. This result showed a strong correlation between the amount of methyl geranate and the 'Rucheng Baiaocha' variety, which was in accordance with earlier study trends on RCBT. In summary, the above results suggested that the minty-like aroma was associated with the uniqueness of the 'Rucheng Baiaocha' variety.

4. Conclusion

To sum up, this study systematically investigated the key odorants of RCBT through sensory evaluation, GC-O, OAV, aroma reconstitution and omission tests. Sensory evaluation revealed that RCBT possesses floral and sweet with unique minty-like aromatic attributes. A total of 26 AACs were identified. Among them, 12 key odorants, hexanal, (*Z*)-4-heptenal, benzeneacetaldehyde, linalool, (*E*, *Z*)-2,6-nonadienal, methyl salicylate, geraniol, citral, methyl geranate, trans-*β*-ionone, *β*-damascenone, and (*E*)-2-nonenal, were important for the aroma profile of RCBT. In particularly, four odorants, including methyl salicylate, (*E*, *Z*)-2,6-nonadienal, methyl geranate, and (*E*)-2-nonenal, proved to be the key odorants responsible for the unique minty-like aroma of the RCBT, with much greater concentrations and OAVs than those of other black tea varieties. However, further research is required to explore the synthesis of RCBT minty-like aroma substances in fresh leaves and their transformation during processing. In sum, this research offered a thorough understanding of the molecular foundation of RCBT odorants.

CRediT authorship contribution statement

Jian Ouyang: Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation. **Ronggang Jiang:** Methodology, Investigation. **Huimin An:** Methodology, Investigation. **Xingchang Ou:** Methodology. **Jing Wang:** Software, Methodology. **He Xie:** Investigation. **Wenjie Fu:** Investigation. **Jing Zhang:** Methodology. **Hongyu Chen:** Software. **Qi Liu:** Resources. **Juan Li:** Project administration. **Haitao Wen:** Resources. **Ligui Xiong:** Investigation. **Jian-an Huang:** Writing – review & editing, Supervision, Resources, Project administration. **Zhonghua Liu:** Writing – review & editing, Supervision, Resources.

Declaration of competing interest

The authors have explicitly stated and declared that they do not have any financial, personal, or professional conflicts or competing interests that could potentially influence or bias the results, conclusions, or interpretations presented in their study.

Data availability

Data will be made available on request.

Acknowledgements

This study was supported by funding from various grants, including the efficient utilization of 'Rucheng Baimaocha' resources (grant no. xiangcainongzhi,2022, 0016); Agriculture Research System of China of MOF and MARA (grant no. CARS-19); the exploration and application of genes for enhancing tea quality (grant no. 2021JC0007); key technology innovation and demonstration within Hunan hundred billion tea industry chain (grant no. 2021NK1020-4).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2025.102253.

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