



Characterization of the key aroma compounds in three world-famous black teas

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Abstract

The volatile compounds of three world-famous black teas (Darjeeling, DJL, Keemun, KM, and Ceylon, CL) were extracted by stir bar sorptive extraction (SBSE), and analyzed by gas chromatography–olfactometry (GC–O), gas chromatography–mass spectrometry (GC–MS). The results indicated that 78, 76, and 69 volatile compounds were detected in the three tea infusions. And 9 sulfur compounds in black teas were identified by gas chromatography–flame photometric detection (GC–FPD). In addition, a total of 42 aroma compounds were perceived and 38 compounds were identified as important aroma compounds due to their high odor activity values (OAVs), such as 3-methylbutanal (OAV: 24–82), linalool (OAV: 24–64), geraniol (OAV: 2–97), β -ionone (OAV: 54–122), and *cis*-jasmone (OAV: 2–119). According to the results of aroma recombination and omission experiments, 2-methylbutanal, linalool, methyl salicylate and β -cyclocitral were confirmed to be the key aroma compounds in Darjeeling black tea, 3-methylbutanal, hexanal, β -myrcene, and methyl salicylate were the key aroma compounds in Keemun, while β -ionone, linalool, 2-methylbutanal, and salicylaldehyde were the key aroma compounds in Ceylon black tea.

Keywords Black tea · OAV · Omission experiment · Aroma recombination

Introduction

Tea, originating in China, is one of the world's three major non-alcoholic beverages along with coffee and cocoa. Owing to its unique taste and special aroma, tea has been popular for centuries [1]. Internationally, according to the degree of fermentation, tea can be classified into three main types: unfermented tea (green tea), semi-fermented tea (oolong tea), and fermented tea (black tea) [2]. Black tea is fermented, and the process of black tea production consists of four steps: withering, rolling, fermentation, and firing [3]. Besides, because black tea has an abundant aroma and a long cultural history, it is loved by consumers all over the world

and has become one of the most widely consumed teas [4]. Black tea can be divided into many types, according to the geographical origin [5], growth climate [6], degree of fermentation [7], and tea variety [3].

Although the aroma compounds in tea only account for 0.01% of the dry weight of tea leaves [8], aroma is one of the essential factors for sensory evaluation of black tea [9]. Different characteristics and styles of black tea are formed due to the different types, contents, ratios and interactions of aroma compounds. Among the numerous types of black teas, Keemun from China, Darjeeling from India, and Ceylon from Sri Lanka are honored as the top three of high-aroma black teas in the world, due to their rich and unique aroma qualities [10]. According to the literature, Darjeeling black tea has a musky scent, Keemun black tea shows a smoky smell, while Ceylon black tea presents a typical floral and sweet fragrance [11]. The aroma compounds in black tea have been extensively studied. For example, Magagna et al. [12] analyzed the volatiles in Ceylon black tea using two-dimensional gas chromatography–mass spectrometry (GC×GC–MS), and a total of 123 compounds were identified. The volatile compounds in Chinese congou black tea were investigated through aroma extract dilution analysis

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and odor active values, indicating that *cis*-3-hexen-1-ol, 1-octen-3-ol, *trans*-linalooloxide, linalool, benzyl alcohol, phenylethyl alcohol, valeraldehyde, hexanal, etc. make important contributions to the aroma of black tea [10]. However, the characteristic compounds and differences among the three world-famous black teas have not been studied systematically. The identification of characteristic aroma compounds can enrich the theoretical basis of tea flavor chemistry, enhance the comprehension of the chemical compounds which contribute to the tea's aroma quality, and provide a theoretical basis for quality identification among black tea specimen.

Since the twentieth century, gas chromatography–mass spectrometry (GC–MS) has developed rapidly to analyze the aroma compounds of tea. At present, more than 600 substances have been identified, including alcohols, aldehydes, acids, ketones, esters, sulfur, furans, lactones, etc. [13] Combining GC–MS with olfactometry can not only isolate and identify the aroma compounds in black tea, but also can sense the aroma characteristics of aroma compounds [14]. By GC–MS and GC–O (gas, chromatography–olfactometry), the volatile compounds in Hanzhong black tea were analyzed, indicating that linalooloxide, 1-octen-3-one, (E, E)-2,4-nonadienal, etc. were considered as the characteristic aroma compounds in tea infusion [15].

In particular, the identification of aroma compounds will vary greatly when different extraction methods are used, therefore, choosing appropriate extraction methods are important to the identification of aroma compounds in black tea. Commonly, the extraction methods applied in tea include simultaneous distillation extraction (SDE) [16], solvent-assisted flavor evaporation (SAFE) [17], and headspace solid phase micro-extraction (HS-SPME) [18, 19]. Since SDE specimens might introduce new substances under high temperature for a long time, it is unsuitable for extracting volatile compounds from tea. SAFE is carried out at a low temperature and low pressure, which will not destroy the original aroma, but it will consume significant volumes of solvents. SPME can quickly and easily absorb aroma compounds [20], but it is not suitable for aroma compounds with high boiling point. Noticeably, stir bar sorptive extraction (SBSE) is a new technique for enrichment of volatile and semi-volatile compounds from aqueous samples [21]. Besides, according to the literature, SBSE has the advantages of large extraction coating volume, high extraction capacity, high sensitivity, small sample amount, and environmental friendliness [22]. In recent years, SBSE has been widely used in the detection of aroma compounds in water, wine, fruits, etc. [23] Thus, SBSE might have great potential in the aroma analysis of black tea. Unfortunately, few people use SBSE to study the aroma of black tea, and there are few systematic comparisons of the characteristic aromas of the top three of high-aroma black teas in the world.

Therefore, the main purposes of this research were as follows: (1) to identify the volatile compounds of the three world-famous black teas by GC–MS and GC–FPD; (2) to determine the characteristic aroma compounds by gas chromatography–olfactometry (GC–O) and OAV; (3) to confirm the key aroma compounds by aroma recombination and omission experiments. The analysis of the characteristic aroma of these black teas will not only complement the flavor map of black tea, but also enable a more comprehensive understanding of the differences between them.

Materials and methods

Materials

Three kinds of black teas (Darjeeling black tea (DJL), Keemun black tea (KM) and Ceylon black teas (CL)) used in the experiment were purchased from tea trade (Redsunion Co., Ltd. (Qimen City, Anhui Province, China), Whittard Co., Ltd. (London, UK) and Basilur Co., Ltd. (Sri Lanka, India), respectively). Before the experiment, all black tea samples were stored in aluminum foil, and kept in a refrigerator at 4 °C. Each black tea sample was ground into powder using mortar before experiments.

Chemicals

The information about standard compounds for qualitative and quantitative analysis has been given in Supporting Information. All the standard compounds used were GC quality. N-alkanes (C₆–C₃₀) for linear retention index determination were purchased from Shanghai Titan technology Co., Ltd. (Shanghai, China). Pure water was obtained from Milli-Q purification system (Millipore, Bedford, MA, USA).

Stir bar sorptive extraction

The aroma compounds in black teas were extracted by Stir Bar Sorptive Extraction (SBSE) method, and an ACAR/PDMS twister (10 mm length and 0.5 mm thickness, Gerstel, Germany) was used. The SBSE extraction method was slightly modified on the basis of previous literature [24]. For each sample, 0.3 g of tea powder was weighted and poured into a 20 mL sample bottle, then 15 mL hot water (90 °C), 0.5 g NaCl and 5 µL internal standard solution 2-octanol (400 µg/mL in ethanol) were added. After 5 min of equilibration, the twister was immersed in the black tea infusion, and then the sample bottle was placed in a water bath magnetic stirrer (SHJ-4D, Yuhua, Tianjin, China), and extracted at 60 °C for 60 min with an agitation rate of 1250 rpm. Afterward, the twister was washed and dried with a lint-free tissue and then placed in the thermal desorption unit

(TDU, Gerstel, German) for subsequent GC–O and GC–MS analysis.

Thermal desorption

The parameters used for the thermal desorption unit (TDU, Gerstel, German) were: the desorption program was held at 40 °C for 1 min, and raised to 260 °C at a rate of 60 °C/min, then held for 5 min, in splitless mode. The cooled injection system (Gerstel CIS-4 PTV injector, German) was maintained at – 70 °C using liquid nitrogen (99.999%). Then the temperature of CIS-4 was raised to 260 °C at a rate of 12 °C/s and held for 5 min.

GC–O analysis

In the GC–O experiment, a GC (7890A, Agilent, Santa Clara, CA) equipped with flame ionization detector (FID) and Olfactory Detector Port (ODP-3, Gerstel, Mulheim an der Ruhr, Germany) was used for aroma sniffing analysis. After chromatographic separation, the extracted volatile compounds were split between the FID and the GC–O sniffing port in a ratio of 1:1. The length between the splitter and the sniffing port was 107 cm. The chromatographic columns in GC–O experiment were HP-Innowax (60 m × 0.25 mm × 0.25 μm) and DB-5 (60 m × 0.25 mm × 0.25 μm). The chromatographic columns were purchased from Agilent (Santa, Clara, CA). Helium (99.999%) was used as the carrier gas, and the flow rate was 2 mL/min. The temperature program of oven was maintained at 40 °C for 6 min, then raised to 150 °C at a rate of 3 °C/min, and finally raised to 230 °C at a rate of 6 °C/min and kept it for 15 min. The splitless mode was adopted. The temperature of the injector was 250 °C, while the temperature of the FID detector was 280 °C.

The GC–O analysis was performed by an experienced sensory panel. The sensory panel consisted of 5 healthy and non-smoking members (two males and three females, with an average age of 24 years old) who performed GC–O sniffing. All of the panelists belonged to the School of Perfume and Aroma Technology of Shanghai Institute of Technology (Shanghai, China). They all had received aroma training at least 5 years. Before carrying out the formal experiment, the sensory panel was trained for odor characteristics and aroma intensity using standard compounds solution to be familiar with odor characteristics. During the training, group panelists were trained to unify the aroma intensity and description of the aroma. After training, five panelists performed GC–O sniffing on three tea samples. When an odor was perceived, sensory panelists recorded the retention time, aroma intensity (AI) and aroma characteristics. According to previous researches, each panelist scored the aroma intensity (AI) using an intensity scale from 0 to 10,

where “0” meant no aroma, “5” meant medium intensity, and “10” meant extremely strong [25]. Each panelist repeated the experiment three times, and the average scores of the intensity were taken as the aroma intensity of the aroma compounds.

GC–MS analysis

The aroma compounds in black teas were isolated and identified by GC–MS (7890-5977B, Agilent, Santa Clara, CA). The types of the chromatographic column and the temperature procedure of oven were consistent with GC–O. The temperature of injection port was set at 250 °C with split-less mode. The ion source, quadrupole mass filter, and default values of electron impact energy were 230 °C, 150 °C and 70 eV, respectively. Mass scanning range was 30–400 amu. The carrier gas was helium (99.999%) at a rate of 1 mL/min. The experiment was repeated in triplicate. The aroma substances were identified through matching the retention time of authentic standards, retention indices (RIs) and the MS in the NIST11 database. The RIs were calculated by retention time of the homogeneous series of alkanes (C₆–C₃₀) [26].

HS–SPME–GC–FPD analysis

HS–SPME was applied to extract the sulfur compounds in black teas. The tea infusions were the same as SBSE method. 6.5 g of tea infusion of each sample and 10 μL of internal standard solution (dipropyl disulfide, 4 mg/L) were added into screw-capped vials fitted with PTEE silicone septa. Then 75 μm carboxyl-poly-dimethyl siloxane (CAR-PDMS) fiber purchased from Supelco, Inc. (Bellefonte, PA, USA) was exposed to the headspace of the tea infusions in a water bath at 60 °C for 50 min. Then SPME fiber was inserted into the GC injector for desorption and analyzed at 250 °C for 5 min.

The sulfur compounds in black teas were identified by Agilent 7890A GC equipped with a flame photometric detector (FPD). The columns and oven temperature procedure were consistent with GC–MS. The GC was operated in constant flow mode at a rate of 1 mL/min with helium as carrier gas. The temperature of the FPD detector was 250 °C, while the PMT voltage was set to 500 V. The desorption time was 5 min and the injection port was operated in a splitless mode. Sulfur compounds in black tea samples were determined by comparing with retention time of authentic standards and retention indices (RIs). The experiment was carried out in triplicate.

Calibration of standard curves

According to previous literature, the standard curves of aroma compounds were made with a slight modification

[27]. The reconstituted sample consisting of all aroma compounds detected by GC–MS in the three specimens was dissolved in deionized water according to the concentration calculated by the internal standard concentration. And then the mixture was diluted with water according to the ratio of 1:2, 1:5, 1:10, 1:20, 1:50, 1:75 and 1:100. Afterward, 5 μL of the internal standard solution containing 2-octanol (400 $\mu\text{g}/\text{mL}$) was introduced to the diluted solution. Then each ratio of the solution was adsorbed by SBSE to construct the standard curve of aroma compounds and the analysis conditions were consistent with the aroma analysis of black tea. The calibration curves were established by the ratio of aroma compounds to internal standard. The abscissa was the ratio of the concentration of compound to internal standard, and the ordinate was the ratio of the peak area of compound to internal standard. All detected aroma compounds were quantified by the established standard curves.

Similarly, the standard curves of sulfur compounds were constructed the same as the non-sulfur compounds, except that the internal standard solution was changed to dipropyl disulfide (4 mg/L), and the extraction method was changed to HS–SPME.

Odor activity values (OAVs)

The odor activity value (OAV) was the ratio of the concentration of aroma compounds to its threshold. OAV method was often applied to evaluate the contributions of aroma compounds. In this experiment, the threshold values in water were collected from the literature [28].

Aroma recombination and electronic nose

To further confirm the key aroma compounds in the black tea samples were correctly identified and quantified, the aroma combination method was applied. According to the results of GC–MS, GC–O and GC–FPD, the aroma compounds with $\text{OAV} \geq 1$ or $\text{AI} > 0$ were dissolved in water as their quantitative concentration.

A total of 15 volunteers (7 men and 8 women, aged 22–28) were recruited. These panelists were also selected from the School of Perfume and Aroma Technology of Shanghai Institute of Technology (Shanghai, China). Sensory evaluation was conducted in sensory laboratory (25 $^{\circ}\text{C}$). A volume of 50 mL black tea infusion was placed in odorless plastic cup. Each panelists described the sensory properties of black tea infusions. Finally, panelists discussed their sensory results and adopted 8 descriptors: fruity, green, sweet, floral, mushroom-like, baked, caramel and woody. The intensity of these attributes was rated from 0 to 10, where “0” represented none, “5” represented moderate, and “10” is strongly perceivable [29]. Each panelist conducted each evaluation in triplicate, with 30 min rest after each test.

A HERACLES electronic nose (Alpha M.O.S., Toulouse, France) was applied to compare the difference between black tea infusion and aroma recombination model. 6.5 g of tea infusions or aroma recombination solutions were added into a 20 mL headspace vial. The parameters of the auto-sampler were: headspace heating for 20 min, headspace temperature was 50 $^{\circ}\text{C}$, the stirring speed was 500 rpm, the stirring opening time was 5 s, the stirring closing time was 2 s, and the washing time was 90 s. Sample headspace injection volume was 5000 μL , sampling speed was 125 $\mu\text{L}/\text{s}$, and injection temperature was 200 $^{\circ}\text{C}$. The electronic nose analysis was repeated three times.

Omission experiments

To further explore the contribution of the characteristic aroma compounds of black tea to the overall aroma, the omission experiments were adopted [26]. All aroma compounds with $\text{OAV} \geq 1$ or $\text{AI} > 0$ were selected for the omission experiments. The concentrations of all selected compounds were quantified by the standard curve. The differences between the omission model and aroma recombination model were evaluated through a triangle test according to the International Standard Method [30]. There were three bottles solution in each omission experiment. One bottle missed one aroma compound and the other two contained the whole aroma. The sensory panel of the omission experiment consisted of 15 members (7 males, 8 females, aged between 22 and 28), and each panelist was asked to select the different one from the three bottles. Each experiment was repeated three times.

Statistical analysis

The aroma intensity and concentration of volatile compounds in three black tea samples were evaluated by analysis of variance (ANOVA). All statistical analyses were conducted by Duncan’s multiple comparison tests in SPSS 21.0 software (SPSS Inc., Chicago, USA, 2019). The statistically significant level was 5% ($p < 0.05$). The radar map was drawn using Origin 19 software (Originlab Corporation, Northampton, Massachusetts, USA).

Results and discussion

Identification and aroma intensity of aroma-active compounds in three world-famous black teas by GC–O

As a new sample preparation technique, SBSE had been successfully applied to the analysis of aroma profile in green tea [24]. GC–O was a method that combines GC

with the human nose, and had been widely used in the analysis of food flavor. It was generally believed that the aroma compounds perceived by GC–O were usually regarded as the key aroma compounds [31]. To explore the characteristic aroma compounds in these three high-aroma black teas, the volatile organic compounds were studied by SBSE-TDU/GC–O. As shown in Table 1, a total of 42 aroma compounds were perceived by GC–O, and their aroma intensity ranged from 0.3 to 7.9. These aroma substances were determined by RI, retention time of authentic standard, and aroma description. Among these aromatic compounds, there were 11 aldehydes, 11 alcohols, 5 ketones, 4 terpenes, 2 esters, 2 lactones, 2 pyrroles, 1 oxide, 1 acid, 1 indole, and 2 unknown compounds. Aldehydes and alcohols were the predominant aroma compounds in these black teas. Aldehydes were usually considered to impart “green, grassy, fatty, and citrus” notes, while alcohols generally presented “green, sweet, and alcoholic” notes. According to the results of GC–O, it could be seen that the floral, green, and sweet aromas had stronger intensities than other notes. Among these compounds, 2-methylbutanal (AI: 4.5–6.8), 2-methyl-2-butenal (AI: 4.3–6.2), *cis*-3-hexen-1-ol (AI: 4.2–6.7), (E, E)-2, 4-heptadienal (AI: 3.9–5.7), methyl salicylate (AI: 4.0–6.4), geraniol (AI: 0.5–7.3), and phenylethyl alcohol (AI: 5.8–7.9) presented the highest aroma intensities among volatile compounds. Therefore, these compounds might make significant contributions to the aroma and flavor of the three world-famous black teas.

In addition, a total of 39, 38, and 33 aroma compounds were perceived by GC–O in three tea specimens (DJL, KM and CL, respectively). Among the three world’s famous black teas, two compounds were unidentified. They all imparted a floral, green odor, but which specific substances they were remained unknown. Among the three types of black teas, phenylethyl alcohol had the highest aroma intensity. According to the analysis result of GC–O, phenyl alcohol was described as imparting a “sweet, rose-like” odor, which might relate to the floral note of black tea.

Among the three world-famous black teas, Keemun black tea had the highest aroma intensity and a unique “Keemun odor”, resembling flowers, fruits, and honey. In Keemun black tea, phenylethyl alcohol exhibited the strongest intensity (7.9), followed by geraniol (7.3), 2-methylbutanal (6.8), methyl salicylate (6.4), and phenylacetaldehyde (5.8).

In Darjeeling black tea, *cis*-3-hexen-1-ol demonstrated the highest intensity (6.7), followed by phenylethyl alcohol (6.3), (E, E)-2,4-heptadienal (5.7), methyl salicylate (5.4), and 2-methylbutanal (5.2). While in Ceylon black tea, 2-methyl-2-butenal exhibited the highest intensity (6.2), followed by phenylethyl alcohol (5.8), 2-acetylpyrrole (5.6), phenylacetaldehyde (5.2), and cinnamaldehyde (5.1). Acetophenone was only perceived in Ceylon black tea.

Ionone generally had a warm woody and violet-like floral odor. There were three isomers of ionone: α -ionone, β -ionone, and γ -ionone. Specifically, β -ionone was distributed in different teas. In this experiment, the odor of β -ionone was described as iris-like. The ionone in tea was produced by the oxidation of catechins and the degradation of carotenoids [32]. Among these world-famous black teas, Ceylon black tea had the strongest aroma intensity of β -ionone, while Darjeeling black tea had the weakest. In addition, dihydroactinidiolide was one of the characteristic aroma compounds in black tea, which was derived from β -ionone. β -Ionone could be oxidized to 5,6-epoxy- β -ionone, leading to the formation of saturated triol, which was then oxidized to dihydroactinidiolide by intramolecular cyclization [33].

Noticeably, 3-methylindole was described as elegant floral scent of jasmine in this experiment, which was widely present in black tea [34]. According to previous studies, 3-methylindole was also considered to be the characteristic aroma compound in Longjing tea. 3-Methylindole is also known as skatole, and its odor is concentration-dependent. At a high concentration, 3-methylindole would have a fecal, animal-like, unpleasant odor, while it would present a pleasant natural jasmine odor when diluted to a certain extent [27].

Although GC–O was widely applied in the aroma analysis of food, it also had some disadvantages: it relies mainly on the discriminant resolving power of the human nose, making the experimental results subjective. In addition, GC–O used air as the medium, which was different from its original water matrix. Therefore, other methods were required to further determine the characteristic aroma of black tea. To ensure the accuracy of the experimental results, OAV method was applied to further determine the key aroma compounds in these famous black teas in the world.

Quantitative analysis and OAVs of volatile compounds in three world-famous black teas

Subsequently, the aroma compounds of the three world-famous black teas were identified by SBSE-TDU/GC–MS. As shown in Table 2, a total of 89 aroma compounds were identified in the three tea samples, including 27 aldehydes, 16 alcohols, 13 ketones, 12 esters, 9 olefins, 4 acids, 3 lactones, and 5 others (indole, 3-methylindole, *cis*-linaloloxide, 2-acetylpyrrole, and 2,5-dimethylpyrazine). Besides, a total of 78, 76, and 69 volatile compounds were identified in three black tea samples (DJL, KM, and CL, respectively). Related quantitative information, such as the standard curve and evaluation index (R^2) of fitting regression of each aroma compounds, was summarized in the Supplementary Information. It could be seen from the quantitative results that *cis*-linaloloxide (32.68–364.49 $\mu\text{g}/\text{kg}$), (E, E)-2,4-heptadienal

Table 1 Aroma-active compounds identified by GC-O in three world-famous black teas

No	Compound	RI ^a		Aroma description ^c	Identification ^d	Aroma intensity					
		HP-Innowax	DB-5			DJL	SD ^e	KM	SD	CL	SD
1	2-Methylbutanal	945	638	Coffee, nutty	RI, Std, AD	5.2b ^e	0.17	6.8a	0.32	4.5c	0.13
2	3-Methylbutanal	948	630	Fatty, chocolate	RI, Std, AD	4.3ab	0.56	5.1a	0.63	3.9b	0.19
3	Hexanal	1076	778	Green, fatty	RI, Std, AD	2.9b	0.14	4.5a	0.26	1.5c	0.20
4	2-Methyl-2-butenal	1100	717	Green, fruity	RI, Std, AD	4.3c	0.25	5.2b	0.18	6.2a	0.58
5	β-Myrcene	1149	968	Terpene, spicy	RI, Std, AD	4.1b	0.31	5.3a	0.72	1.9c	0.26
6	(+)-Limonene	1173	1006	Citrus, orange	RI, Std, AD	2.0a	0.25	2.2a	0.64	2.0a	0.08
7	2-Heptanone	1177	869	Fruity, spicy, sweet	RI, Std, AD	1.7a	0.47	1.8a	0.32	2.2a	0.26
8	Heptanal	1198	880	Green, fatty,	RI, Std, AD	0.3b	0.15	2.2a	0.11	0.3b	0.08
9	(Z)-3,7-Dimethylocta-1,3,6,-triene	1214	1023	Herbal	RI, Std, AD	2.7	0.55	4.0	0.41	–	
10	Pentanol	1269	746	Oil, sweet	RI, Std, AD	2.8	0.22	2.3	0.56	–	
11	6-Methylhept-5-en-2-one	1318	963	Citrus, green	RI, Std, AD	2.3b	0.19	3.6a	0.61	3.8a	0.16
12	1-Hexanol	1353	850	Sweet, green	RI, Std, AD	2	0.84	–		1.5	0.22
13	Unknown 1	1354	– ^b	Green, floral	AD	– ^f		4.3	0.72	–	
14	cis-3-Hexen-1-ol	1363	837	Green, grass, fresh	RI, Std, AD	6.7a	0.72	4.8b	0.36	4.2b	0.86
15	trans-2-Hexen-1-ol	1398	847	Green, herbal	RI, Std, AD	4.5	0.55	–		3	0.89
16	cis-Linaloloxide	1433	1050	Sweet, woody	RI, Std, AD	4.7a	0.59	5.2a	0.63	2.6b	0.22
17	1-Octene-3-ol	1450	983	Mushroom, earthy	RI, Std, AD	1.4	0.34	0.5	0.02	–	
18	Furfural	1457	809	Woody, almond	RI, Std, AD	2.6b	0.41	5.3a	0.30	2.7b	0.35
19	Acetic acid	1464	621	Sour, vinegar	RI, Std, AD	3.4b	0.27	4.1a	0.44	3.6ab	0.18
20	(E, E)-2,4-Heptadienal	1478	988	Green, vegetable	RI, Std, AD	5.7a	0.73	5.4a	0.47	3.9b	0.46
21	2-Acetylfuran	1496	886	Sweet, coffee	RI, Std, AD	–		3.5	0.49	4.6	0.42
22	Benzaldehyde	1507	942	Almond	RI, Std, AD	4.5a	0.51	4.4a	0.30	3.6b	0.23
23	Linalool	1532	1077	Green, floral	RI, Std, AD	4.7a	0.32	4.1a	0.53	2.5b	0.17
24	5-Methyl furfural	1564	939	Sweet, caramel	RI, Std, AD	4.0b	0.31	5.5a	0.39	4.1b	0.36
25	Unknown 2	1592	–	Sweet, ocimene	AD	3.5	0.74	5.6	0.67	–	
26	1-Ethyl-2-pyrrolicarboxaldehyde	1595	1025	Roasted, smoky	RI, Std, AD	0.5	0.04	1.2	0.26	–	
27	Phenylacetaldehyde	1631	1021	Green, sweet, floral	RI, Std, AD	4.9a	0.78	5.8a	0.42	5.2a	0.29
28	Acetophenone	1636	1021	Sweet, almond	RI, Std, AD	–		–		3.1	0.56
29	α-Terpineol	1688	1172	Terpene, woody	RI, Std, AD	4.0a	0.62	2.4b	0.15	4.0a	0.53
30	Methyl salicylate	1765	1170	Wintergreen mint	RI, Std, AD	5.4a	0.51	6.4a	0.66	4.0b	0.65
31	Geraniol	1833	1225	Sweet, floral, rose	RI, Std, AD	4.6b	0.38	7.3a	0.67	0.5c	0.03
32	Nerol	1840	1200	Sweet, citrus	RI, Std, AD	2.3c	0.41	5.5a	0.27	4.4b	0.24
33	Benzyl alcohol	1871	1012	Floral	RI, Std, AD	2.2	0.19	3.1	0.58	–	
34	Phenylethyl alcohol	1905	1090	Rose, sweet	RI, Std, AD	6.3b	0.25	7.9a	0.32	5.8b	0.63
35	β-Ionone	1939	1450	Floral, orris	RI, Std, AD	2.1c	0.22	2.7ab	0.37	3.3a	0.48
36	2-Acetylpyrrole	1971	1043	Nut, yeast	RI, Std, AD	4.1b	0.26	4.5b	0.54	5.6a	0.43
37	γ-Nonalactone	2001	1332	Coconut, sweet	RI, Std, AD	2.7	0.28	3.0	0.34	–	
38	2-Formylpyrrole	2026	992	Sweet, coffee	RI, Std, AD	3.4	0.30	5.2	0.41	–	
39	Cinnamaldehyde	2046	1246	Sweet, spice	RI, Std, AD	3.2	0.47	–		5.1	0.32
40	Dihydroactinidiolide	2375	1502	Fruit, woody	RI, Std, AD	1.3b	0.19	1.6ab	0.14	2.0a	0.27
41	Coumarin	2483	1409	Sweet, hay, bean	RI, Std, AD	1.6b	0.22	2.9a	0.27	1.8b	0.09
42	3-Methylindole	2503	1360	Floral, jasmine	RI, Std, AD	1.1b	0.13	1.6a	0.25	1.0b	0.09

^aRetention index of compounds on HP-Innowax and DB-5 columns^bThe aroma compounds not identified on DB-5 column^cThe description of aroma compounds by sensory panel^dMethod of identification: RI: retention index; Std, confirmed by authentic standards; AD: aroma description^eValues with different Roman letters (a–c) in the same row are significantly different according to the Duncan test ($p < 0.05$)^fNot detected in samples^gThe standard deviation of aroma intensity of compounds

(64.29–102.87 µg/kg), benzaldehyde (60.66–267.13) µg/kg), linalool (145.18–385.8 µg/kg), methyl salicylate (181.65–340.64 µg/kg), geraniol (12.49–640.62 µg/kg), and phenylethyl alcohol (67.88–202.43 µg/kg) showed higher concentrations than other aroma compounds. As reported in the literature, linalool was an important aroma compound in black teas, such as Turkish black tea (70–241 µg/kg), Indian black tea (77–626 µg/kg) and Chinese black tea (12.7–2764 µg/kg) [35, 36]. Besides, geraniol and phenylethyl alcohol were present in high concentrations in most black tea infusions [10].

The qualitative and quantitative analysis of black tea (Table 2) indicated that the types and concentrations of aroma compounds in different black tea samples were different. The aroma compounds in black tea infusion depended on the glycoside aroma precursor in the black tea leaves, and were also related to the activity of glucosidase. These factors were all related to the origin of the black tea, the soil, climate, and the type of tea tree [37]. Tea leaves grown at high altitudes were prone to possess a richer aroma, and their tea infusions also had better quality [34]. Both Ceylon black tea and Darjeeling black tea plantations were located at high altitudes, especially Darjeeling black tea plantations, with an average altitude of 2100 m, endowing it with the characteristics of abundant fragrance, fresh taste, and a golden color.

The contribution of aroma compounds was not only affected by their concentrations, but also by their threshold. Generally, aroma compounds with $OAV \geq 1$ were considered to make a significant contribution to the overall aroma [26]. The quantitative data pertaining to of the top three of high-aroma black teas in the world allowed the calculation of OAVs (Table 3). A total of 37 aroma compounds with $OAV \geq 1$ were determined therein. In particular, 3-methylbutanal (OAV : 24–82), linalool (OAV : 24–64), 3-methylnonane-2,4-dione (OAV : 18–28), geraniol (OAV : 2–97), β -ionone (OAV : 54–122), *cis*-jasmone (OAV : 2–119), and (\pm)-dihydroactinidiolide (OAV : 54–122) had higher OAVs than other compounds. This indicated that these compounds might be the key aroma compounds of black tea. From Tables 2 and 3, the OAVs of low-concentration compounds were found to be not necessarily low. For instance, though the concentration of 3-methylnonane-2,4-dione among the three black tea samples was very low, its threshold was only about 0.00003 mg/kg, which resulted in its high OAV. In Darjeeling black tea, there were 27 aroma compounds with $OAVs \geq 1$, while there were 29 and 26 aroma compounds with $OAVs \geq 1$ in Keemun black tea and Ceylon black tea, respectively. In addition, the OAVs of aroma compounds showed significant differences in different tea infusions. For example, in Darjeeling black tea, linalool, β -ionone and 3-methylbutanal had the highest OAVs, *cis*-jasmone, geraniol and *cis*-4-heptenal had the highest OAVs in

Keemun black tea, while β -ionone, *cis*-jasmone, and 3-methylbutanal had the highest OAVs in Ceylon black tea.

Furthermore, the sulfur compounds in black tea were detected by sulfur-specific FPD. FPD is a detector which designed for the detection of phosphorus and sulfurs specially. It has high selectivity and sensitivity, only responds to phosphorus and sulfurs, and other elements have no or little interference with it [29]. As shown in Table 4, a total of 9 sulfur compounds were identified (the detailed quantitative information was listed in the Supporting Information). All sulfur concentrations were present at trace levels, and these sulfur compounds were not detected in GC–MS and GC–O. Among them, dimethyl sulfide (3.639–7.948 µg/kg), 2-methylthiophene (0.0292–1.658 µg/kg), and methionol (0.120–1.640 µg/kg) were present in higher concentrations than other sulfur compounds. Besides, the OAVs of dimethyl sulfide methionol, and 3-mercaptophexanol were greater than 1. Therefore, although these sulfur compounds in black tea samples were at trace levels, they were likely to be the key aroma compounds of black tea. Remarkably, the sulfur compounds in black tea were mainly produced by Strecker degradation [38], among which methionine played an important role in the formation of sulfur compounds. Methionol was generally regarded as the precursor of methanethiol. Besides, methanethiol was the direct precursor of many low-threshold sulfur compounds [39]. Dimethyl sulfide has been found in black tea, green tea, and oolong tea, and presented a garlic-like aroma [40]. According to the literature, methylmethionine sulfonium salt was considered to be the precursor of dimethyl sulfide [41].

3-Mercapto-1-hexanol possesses characteristic aroma of passion fruit and grapefruit, which might contribute to the fruity note of black tea. Among the black tea samples, the concentration of 3-mercaptop-1-hexanol ranged from 0.038 µg/kg in KM to 0.042 µg/kg in DJL. The different concentrations of 3-mercaptop-1-hexanol may arise from the different tree varieties, climate, planting conditions and soil environments. Although trace amounts of 3-mercaptop-1-hexanol were detected, the amounts were greater than its threshold. The results demonstrated that 3-mercaptop-1-hexanol was the characteristic compounds in black tea. The identification of sulfur compounds not only enriched the aroma profile of black tea, but provided a way of evaluating the quality of black tea.

Aroma recombination and E-nose

Aroma recombination was an essential part of flavor analysis, and it had been proved to be an important method for verification of key aroma compounds [42]. The volatile

Table 2 The concentration of volatile compounds detected by GC–MS in three world-famous black teas

No	Compounds	Quantitative ions		Identification ^b	Concentration (µg/kg)					
		RI ^a	DB-5		DJL	SD ^c	KM	SD	CL	SD
1	2-Methylbutanal	29, 41, 57	639	MS, RI, Std	77.46b ^c	2.56	88.09a	4.63	48.36c	3.17
2	3-Methylbutanal	41, 43, 44	948	MS, RI, Std	40.48b	3.23	90.56a	8.73	26.92c	2.76
3	Pentanal	29, 44, 58	977	MS, RI, Std	12.92	1.34	16.62	10.85	–	–
4	(+)-Camphene	79, 93, 121	1071	MS, RI, Std	0.69c	0.05	0.90b	0.05	1.70a	0.09
5	Hexanal	41, 44, 56	1077	MS, RI, Std	58.52b	3.27	92.57a	8.84	32.23c	3.12
6	2-Methyl-2-butanol	29, 55, 84	1101	MS, RI, Std	2.70c	0.09	4.78b	0.61	9.95a	0.46
7	δ-4-Carene	93, 121, 136	1142	MS, RI	1.55	0.12	6.37	0.35	–	–
8	β-Myrcene	41, 69, 93	1149	MS, RI, Std	82.43b	5.63	180.46a	12.51	60.59c	4.25
9	1-Penten-3-ol	29, 57	1165	MS, RI, Std	15.84a	1.12	10.87b	0.74	4.80c	0.20
10	(+)-Limonene	67, 68, 93	1173	MS, RI, Std	41.67ab	3.90	48.73a	3.92	37.41b	2.63
11	2-Heptanone	43, 58	1177	MS, RI, Std	8.18c	0.38	10.20b	1.03	15.30a	1.16
12	Methyl hexanoate	43, 74, 87	1192	MS, RI, Std	2.13	0.45	2.83	0.22	–	–
13	Heptanal	41, 44, 70	1198	MS, RI, Std	5.34b	0.37	8.71a	0.65	5.22b	0.44
14	(3E)-3,7-Dimethyl-1,3,6-octatriene	41, 79, 93	1199	MS, RI, Std	37.57	2.66	57.97	4.37	–	–
15	trans-2-Hexenal	39, 41, 42	1212	MS, RI, Std	55.01b	3.27	79.20a	6.30	20.25c	1.93
16	(Z)-3,7-Dimethylocta-1,3,6,-triene	41, 79, 93	1214	MS, RI, Std	15.02b	1.06	48.14a	3.84	3.22c	0.19
17	Ethyl hexanoate	29, 43, 88	1245	MS, RI, Std	– ^d	0.02	–	0.13	5.83	0.28
18	cis-4-Heptenal	29, 41, 68	1264	MS, RI, Std	0.62	0.02	2.35	0.13	–	–
19	2-Octanone	41, 43, 58	1264	MS, RI, Std	–	–	–	–	38.00	2.41
20	Pentanol	41, 42, 55	1269	MS, RI, Std	16.06	1.14	7.11	0.76	–	–
21	p-Cymene	91, 119, 134	1288	MS, RI, Std	10.56b	0.98	18.25a	1.92	18.38a	1.27
22	2,5-Dimethylpyrazine	39, 42, 108	1311	MS, RI, Std	1.60	0.02	4.39	0.20	–	–
23	6-Methylhept-5-en-2-one	41, 43, 69	1318	MS, RI, Std	28.14a	2.12	30.02a	2.61	31.19a	2.79
24	(4E,6Z)-2,6-Dimethylocta-2,4,6-triene	105, 121, 136	1330	MS, RI, Std	2.27c	0.16	20.28a	1.98	10.72b	1.06
25	1-Hexanol	41, 43, 56	1353	MS, RI, Std	28.77	1.25	7.67	0.66	–	–
26	cis-3-Hexen-1-ol	39, 41, 67	1382	MS, RI, Std	75.07a	5.59	39.28b	3.41	23.07c	1.74
27	2,4-Hexadienal	39, 41, 81	1393	MS, RI, Std	6.83b	0.41	8.46a	0.52	6.94b	0.45
28	trans-2-Hexen-1-ol	41, 57	1410	MS, RI, Std	24.82a	2.23	5.96c	0.44	21.34b	1.98
29	(2E)-2-Octenal	29, 41, 55	1418	MS, RI, Std	5.00b	0.37	5.40b	0.28	34.46a	2.64
30	cis-Linaloloxide	59, 43	1438	MS, RI, Std	223.91b	20.64	364.49a	32.68	110.01c	9.15
31	1-Octene-3-ol	57	1450	MS, RI, Std	6.32a	0.34	4.34b	0.31	2.16c	0.11
32	Furfural	39, 95, 96	1457	MS, RI, Std	7.00b	0.81	18.05a	0.75	4.71c	0.23
33	Acetic acid	43, 45, 60	1464	MS, RI, Std	28.24b	2.12	47.75a	2.91	12.88c	1.09
34	(E, E)-2,4-Heptadienal	81, 110	1478	MS, RI, Std	102.87a	9.54	92.32a	8.06	64.29b	4.44

Table 2 (continued)

No	Compounds	Quantitative ions		RI ^a	Identification ^b		Concentration (µg/kg)					
		HP-Innowax	DB-5		MS, RI, Std	DJL	SD ^c	KM	SD	CL	SD	
35	2-Acetylfuran	1496	886	1496	MS, RI, Std	0.88c	2.80b	0.04	2.80b	0.03	3.45a	0.22
36	3,5-Octadiene-2-one	1502	1046	1502	MS, RI, Std	33.00b	38.90a	2.41	38.90a	3.64	6.41c	0.53
37	Decanal	1503	1193	1503	MS, RI, Std	15.42a	0.70c	1.03	0.70c	0.01	3.27b	0.17
38	Benzaldehyde	1507	942	1507	MS, RI, Std	126.27b	267.13a	10.56	267.13a	24.63	60.66c	5.09
39	Linalool	1532	1077	1532	MS, RI, Std	385.8a	246.37b	25.67	246.37b	21.07	145.18c	13.35
40	Linalyl acetate	1547	1220	1547	MS, RI, Std	22.25	–	1.84	–	–	–	–
41	(E,E)-3,5-Octadien-2-one	1556	1069	1556	MS, RI, Std	15.69	33.26	1.74	33.26	2.74	–	0.20
42	5-Methyl furfural	1564	939	1564	MS, RI, Std	1.81c	6.04a	0.12	6.04a	0.45	3.54b	2.27
43	(-)-Cedrene	1582	1396	1582	MS, RI, Std	–	–	–	–	–	29.03	0.32
44	1-Ethyl-2-pyrrolicarboxaldehyde	1595	1025	1595	MS, RI, Std	14.56b	42.79a	1.44	42.79a	3.01	5.92c	0.02
45	β-Cyclocitral	1598	1193	1598	MS, RI, Std	15.42	–	1.06	–	–	0.48	–
46	Hotrienol	1602	1080	1602	MS, RI, Std	40.48	30.87	3.10	30.87	2.22	–	3.55
47	Ethyl furoate	1614	1030	1614	MS, RI, Std	–	–	–	–	–	42.75	0.06
48	Terpinen-4-ol	1616	1158	1616	MS, RI, Std	6.97a	7.68a	0.32	7.68a	0.66	1.10b	3.20
49	Phenylacetaldehyde	1631	1021	1631	MS, RI, Std	44.43b	113.11a	2.90	113.11a	10.27	49.97b	6.09
50	Ethyl decanoate	1631	1368	1631	MS, RI, Std	–	–	–	–	–	79.37	5.66
51	Acetophenone	1637	1050	1637	MS, RI, Std	–	–	–	–	–	67.77	2.74
52	Ethyl benzoate	1647	1145	1647	MS, RI, Std	2.65c	11.57b	0.14	11.57b	0.89	27.89a	5.02
53	Salicylaldehyde	1682	1027	1682	MS, RI, Std	–	–	–	–	–	55.31	1.25
54	α-Terpineol	1688	1172	1688	MS, RI, Std	24.69a	15.08c	1.71	15.08c	1.21	21.21b	0.43
55	(Z)-Citral	1688	1209	1688	MS, RI, Std	6.33b	18.43a	0.52	18.43a	1.63	5.27b	2.08
56	Citral	1712	1240	1712	MS, RI, Std	9.78b	31.15a	0.53	31.15a	3.06	28.31a	1.37
57	Benzyl acetate	1714	1136	1714	MS, RI, Std	3.53c	6.02b	0.21	6.02b	0.44	22.63a	0.03
58	3-Methylnonane-2,4-dione	1719	1220	1719	MS, RI, Std	0.85a	340.64a	0.04	0.53c	0.03	0.64b	17.05
59	Methyl salicylate	1765	1170	1765	MS, RI, Std	237.76b	12.65	20.81	340.64a	32.71	–	–
60	Geranyl acetate	1766	1348	1766	MS, RI, Std	10.35	12.65	1.14	12.65	1.23	–	–
61	α-Ionone	1822	1393	1822	MS, RI, Std	4.89b	5.26b	0.28	5.26b	0.37	16.95a	1.13
62	Geraniol	1833	1225	1833	MS, RI, Std	129.69b	640.62a	10.73	640.62a	55.49	12.49c	1.44
63	Nerol	1840	1200	1840	MS, RI, Std	8.74c	19.94a	0.41	19.94a	1.32	15.78b	1.37
64	Ethyl 3-phenylpropanoate	1862	1322	1862	MS, RI, Std	1.11	–	0.09	–	–	31.60	2.31
65	Benzyl alcohol	1871	1012	1871	MS, RI, Std	16.59b	122.99a	1.50	122.99a	10.30	7.10b	0.25
66	Guaiacol	1875	1062	1875	MS, RI, Std	–	–	–	–	–	15.03	1.61
67	Phenylethyl alcohol	1905	1090	1905	MS, RI, Std	99.91b	202.43a	6.81	202.43a	19.46	67.88c	6.29
68	δ-Octalactone	1918	1254	1918	MS, RI, Std	–	23.86	–	23.86	2.18	–	–
69	γ-Octanolactone	1919	1230	1919	MS, RI, Std	–	–	–	–	–	68.91	5.88

Table 2 (continued)

No	Compounds	Quantitative ions	RI ^a	Identification ^b		Concentration (µg/kg)						
				HP-Innowax	DB-5	DJL	SD ^c	KM	SD	CL	SD	
70	4-Methyl-2-phenyl-2-pentenal	43, 91, 103	1919	1338	MS, RI, Std	8.41	0.93	12.83	1.15	–	–	–
71	2-Phenyl-2-butenal	115, 117, 146	1929	1242	MS, RI, Std	13.41	1.76	26.33	2.34	–	–	–
72	β-Ionone	43, 177	1938	1450	MS, RI, Std	10.72b	0.92	11.79b	1.01	24.47a	2.13	2.13
73	cis-Jasmone	79, 110, 164	1944	1367	MS, RI, Std	0.56c	0.02	30.89a	2.48	22.37b	1.94	1.94
74	Heptanoic acid	60, 73	1948	1053	MS, RI, Std	–	–	16.12	1.79	–	–	–
75	(E)-3-Hexenoic acid	41, 55, 60	1955	978	MS, RI, Std	4.95	0.20	3.49	0.31	–	–	–
76	2-Acetylpyrrole	39, 66	1970	1043	MS, RI, Std	9.44c	0.06	27.57b	2.07	34.46a	3.11	3.11
77	β-Ionone epoxide	43, 123	1973	1454	MS, RI, Std	14.31b	1.34	16.97a	1.35	10.57c	0.89	0.89
78	γ-Nonalactone	85	2001	1332	MS, RI, Std	17.93	1.88	24.72	2.02	–	–	–
79	2-Formylpyrrole	66, 94, 95	2026	992	MS, RI, Std	1.42	0.12	17.53	1.16	–	–	–
80	2,5-Dimethyl-4-hydroxy-3-(2H)-furanone	129	2028	1055	MS, RI, Std	0.34c	0.01	0.52b	0.01	0.66a	0.03	0.03
81	Cinnamaldehyde	103, 131, 132	2033	1246	MS, RI, Std	5.61	0.34	–	–	41.64	4.46	4.46
82	Eugenol	164	2138	1324	MS, RI, Std	4.94b	0.21	5.81a	0.24	1.70c	0.10	0.10
83	(2)-7-Decen-5-olide	55, 71, 99	2274	1460	MS, RI, Std	13.23	0.88	44.42	4.01	–	–	–
84	Methyl dihydrojasmonate	83	2288	1609	MS, RI, Std	11.45b	0.83	13.93a	0.95	13.25a	0.82	0.82
85	(±)-Dihydroactinidiolide	43, 109, 111	2376	1502	MS, RI, Std	39.26a	3.34	34.95a	3.26	40.03a	3.54	3.54
86	Coumarin	118, 146	2431	1409	MS, RI, Std	6.26c	0.41	27.55a	2.68	10.69b	1.09	1.09
87	Indole	89, 90, 117	2456	1265	MS, RI, Std	4.44b	0.21	8.59a	0.77	3.61b	0.38	0.38
88	3-Methylindole	130, 131	2503	1360	MS, RI, Std	2.34b	0.17	4.13a	0.33	0.82c	0.03	0.03
89	Vanillin	81, 151, 152	2566	1367	MS, RI, Std	11.99a	1.03	2.62b	0.10	2.10b	0.15	0.15

^aRetention index of compounds on HP-Innowax and DB-5 columns^bMethod of identification: RI: retention index; Std, confirmed by authentic standards; AD: aroma description^cValues with different Roman letters (a–c) in the same row are significantly different according to the Duncan test ($p < 0.05$)^dNot detected in samples^eThe standard deviation of aroma intensity of compounds

Table 3 The aroma-active compounds with OAVs ≥ 1 in three world-famous black teas

No	Compound	OT ($\mu\text{g}/\text{kg}$) ^a	OAV ^b		
			DJL	KM	CL
1	2-Methylbutanal	0.003	26	29	16
2	3-Methylbutanal	0.0011	37	82	24
3	Pentanal	0.005	3	3	–
4	Hexanal	0.005	12	19	6
5	β -Myrcene	0.015	5	12	4
6	(+)-Limonene	0.034	1	1	1
7	Heptanal	0.0028	2	3	2
8	(3E)-3,7-Dimethyl-1,3,6-octatriene	0.034	1	2	–
9	(Z)-3,7-Dimethylocta-1,3,6,-triene	0.034	<1	1	<1
10	Ethyl hexanoate	0.005	– ^c	–	1
11	<i>cis</i> -4-Heptenal	0.000025	25	94	–
12	p-Cymene	0.0114	<1	2	2
13	<i>cis</i> -3-Hexen-1-ol	0.05	2	<1	<1
14	(2E)-2-Octenal	0.003	2	2	11
15	<i>cis</i> -Linaloloxide	0.32	<1	1	<1
16	(E,E)-2,4-Heptadienal	0.056	2	2	1
17	Linalool	0.006	64	41	24
18	β -Cyclocitral	0.003	5	–	<1
19	Phenylacetaldehyde	0.0063	7	18	8
20	Ethyl decanoate	0.005	–	–	16
21	Acetophenone	0.065	–	–	1
22	Salicylaldehyde	0.03	–	–	2
23	Citral	0.028	<1	1	1
24	3-Methylnonane-2,4-dione	0.00003	28	18	21
25	Methyl salicylate	0.04	6	9	5
26	α -Ionone	0.00378	1	1	4
27	Geraniol	0.0066	20	97	2
28	Guaiacol	0.0016	–	–	9
30	Phenylethyl alcohol	0.14	<1	1	<1
31	γ -Octanolactone	0.0179	–	–	4
32	β -Ionone	0.0002	54	59	122
33	<i>cis</i> -Jasmone	0.00026	2	119	86
34	γ -Nonalactone	0.0079	2	3	–
35	Eugenol	0.0025	2	2	<1
36	Methyl dihydrojasmonate	0.013	<1	1	1
37	(\pm)-Dihydroactinidiolide	0.0021	19	17	19
38	3-Methylindole	0.00041	6	10	2

^aThe odor threshold (OT) of volatile compounds in water referred in the literature [27]

^bThe odor activity values of volatile compounds in black tea infusions

^cThe compounds not detected in samples

compounds identified by GC-O and the OAV calculation were commonly regarded as the key aroma compounds. To verify the accuracy of qualitative and quantitative results, aroma recombination was conducted in this experiment. The 59 aroma compounds with OAV ≥ 1 or AI > 0 were mixed according to concentrations calculated by standard curves. Sensory evaluations between the aroma recombination models and original black tea models were

conducted. The sensory attributes used in this analysis were eight main aromas: sweet, woody, floral, caramel, green, fruity, baked and mushroom-like. The aroma profiles of the aroma recombination models and original black tea models were shown in Fig. 1, which indicated that there were slight differences between the recombination models and original samples. For example, the original model was sweeter and more baked in DJL than in recombination

Table 4 Concentration and OAVs of sulfur compounds detected in three world-famous black teas

No	Compound	RI ^a		Identification ^b	OT ($\mu\text{g}/\text{kg}$) ^c	Concentration ($\mu\text{g}/\text{kg}$) ^c				OAV ^d			
		HP-Innowax	HP-5			DJL	SD	KM	SD	CL	SD		
1	Methanethiol	701	<600	Std, RI, FPD	0.2	0.152a ^e	0.019	0.105b	0.009	0.048c	0.004	<1	<1
2	Dimethyl sulfide	751	<600	Std, RI, FPD	0.3	5.432b	0.384	3.639c	0.243	7.948a	0.587	18	12
3	1-Propanethiol	855	626	Std, FPD	3.1	0.129	0.011	– ^f	–	0.024	0.002	<1	<1
4	2-Methylthiophene	1098	797	Std, RI, FPD	885	0.363b	0.015	1.658a	0.028	0.0292c	0.012	<1	<1
5	2,5-Dimethylthiophene	1155	871	Std, RI, FPD	0.5	0.027	0.001	–	–	–	–	<1	–
6	2-Isopropyl-4-methylthiazole	1352	1032	Std, RI, FPD	8	0.070	0.005	0.070	0.007	–	–	<1	<1
7	Methionol	1720	999	Std, RI, FPD	0.128	0.440b	0.024	1.640a	0.021	0.120c	0.014	3	12
8	3-Mercaptohexanol	1874	1127	Std, RI, FPD	0.013	0.042	0.003	0.038	0.001	–	–	3	3
9	Benzothiazole	1977	1243	Std, RI, FPD	71	0.016	0.002	0.016	0.000	–	–	<1	<1

^aRetention index of compounds on HP-Innowax and HP-5 columns^bMethod of identification: RI: retention index; Std, confirmed by authentic standards; FPD: flame photometric detector^cThe odor threshold (OT) of sulfur compounds in water referred in the literature [26, 27]^dThe odor activity values of sulfur compounds in black tea infusions^eValues with different Roman letters (a–c) in the same row are significantly different according to the Duncan test ($p < 0.05$)^fNot detected in samples

model. In KM, the original model was sweeter and fruitier than its recombination model. In CL, the original model was sweeter and greener than recombination. The analysis of the eight sensory attributes between original black tea samples and aroma recombination models showed that aroma characteristics of the black tea recombination model were similar to the original sample. Therefore, the results confirmed the accuracy of identification and quantification of the key aroma compound in black teas.

Besides the sensory evaluation, the electronic nose was also applied to assess the differences between recombination and original samples. The electronic nose was a device that imitated the human sense of smell [43]. Its principle was based on the response signal generated by the interaction between aroma compounds and sensors [44]. Through the application of an electronic nose, the verification of aroma recombination could avoid innate human subjectivity, rendering the results more objective. As shown in Fig. 2, the three figures represented the profile of aroma compounds the three black tea specimens and their aroma recombination. From the comparison results between the aroma profiles of original black tea infusions and profiles of aroma recombination, we could see that the aroma peaks of aroma recombination models were almost the same as those of the black tea infusions, which confirmed the accuracy of the qualitative and quantitative results. However, some peaks were slightly different from those of the black tea infusions. This might be due to the lack of unknown compounds, or the complex tea matrix containing tea polyphenol and other non-volatile compounds, which would affect the release of aroma compounds. Overall, the qualitative and quantitative results were almost identical to those of the original black tea infusions; the figures indicated that the aroma recombination models could successfully simulate the aroma of the top three of high-aroma black teas in the world.

Omission experiment

Omission experiments are often used to evaluate the contribution of aroma compounds to overall aroma [45, 46]. The omission experiments were designed to explore the importance of aroma compounds in black tea to the overall aroma. In the omission experiments, compounds with $OAV \geq 1$ or $AI > 0$ were selected for the omission experiments to explore their influence on the overall aroma. Noticeably, there were many non-volatiles compounds in tea infusions, which might affect the release of volatile organic compounds. Furthermore, the interaction between the aroma compounds might cause masking effect or synergistic effect, resulting in the change of actual OAVs [47]. In addition, the results of GC–O were perceived through air, while the thresholds in air were different from those in water. Therefore, it was necessary to use omission experiments to verify the characteristic aroma compounds in black tea. In the omission experiments, a total of 59 aroma compounds were recombined, and one aroma compound was omitted in each omission experiment. There were 50, 49, and 47 omission models in three black tea samples (DJL, KM, and CL, respectively), and the results of omission experiments were shown in Table 5.

Among the aldehydes of Darjeeling black tea, the omission models of 2-methylbutanal, 3-methylbutanal, benzaldehyde, phenylacetaldehyde, β -cyclocitral, and *cis*-4-heptenal showed extremely significant differences ($p \leq 0.1\%$). Among the alcohols, the omission experiments of linalool, geraniol, and phenylethyl alcohol exhibited extremely significant differences ($p \leq 0.1\%$). In addition, after removing β -ionone, methyl salicylate, and β -myrcene, the overall aroma also showed significant differences ($p \leq 0.1\%$). Similarly, in Keemun black tea, when 2-methylbutanal, 3-methylbutanal, pentanal, hexanal, *cis*-4-heptenal, and *cis*-jasnone were omitted, most of the sensory panel members reached correct judgments. While in Ceylon black tea, the results of omission experiments showed significant differences ($p \leq 0.1\%$) when omitting 2-methylbutanal, (2E)-2-octenal, salicylaldehyde, linalool, β -ionone, etc.

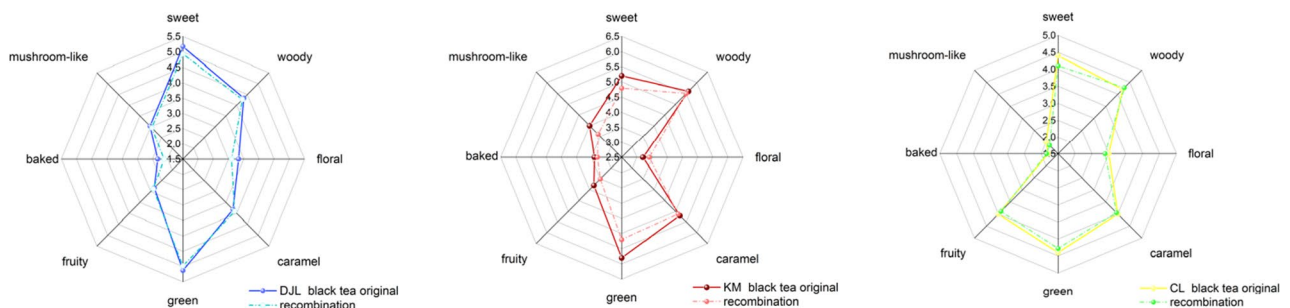


Fig. 1 Comparison of aroma profiles between original black tea infusions and the aroma recombination models by sensory panel

Table 5 Omission experiments results of each aroma-active compound (OAV ≥ 1 or AI > 0)

No	Compound ^a	<i>n</i> ^b		
		DJL	KM	CL
1	Aldehydes			
1-1	2-Methylbutanal	15*** ^c	14***	12***
1-2	3-Methylbutanal	12***	15***	11**
1-3	Pentanal	4=	13***	– ^d
1-4	Hexanal	9*	15***	8=
1-5	2-Methyl-2-butenal	2=	7=	3=
1-6	Heptanal	10**	12***	11**
1-7	(2E)-2-Octenal	6=	10**	12***
1-8	Furfural	8=	5=	3=
1-9	(E,E)-2,4-Heptadienal	9*	8=	6=
1-10	Benzaldehyde	12***	9*	10**
1-11	5-Methyl furfural	7=	5=	4=
1-12	1-Ethyl-2-pyrrolecarboxaldehyde	6=	7=	–
1-13	Citral	6=	8=	8=
1-14	β -Cyclocitral	13***	–	–
1-15	Phenylacetaldehyde	10**	12***	8=
1-16	Salicylaldehyde	–	–	12***
1-17	γ -Nonalactone	2=	8=	–
1-18	2-Formylpyrrole	5=	6=	–
1-19	Cinnamaldehyde	7=	–	8=
1-20	<i>cis</i> -4-Heptenal	12***	14***	–
2	Alcohols			
2-1	Pentanol	8=	4=	–
2-2	1-Hexanol	9*	–	5=
2-3	<i>cis</i> -3-Hexen-1-ol	10**	7=	8=
2-4	<i>trans</i> -2-Hexen-1-ol	7=	–	4=
2-5	1-Octene-3-ol	8=	5=	–
2-6	Linalool	14***	13***	13***
2-7	α -Terpineol	6=	3=	7=
2-8	Geraniol	12***	14***	9*
2-9	Nerol	3=	5=	7=
2-10	Benzyl alcohol	4=	4=	3=
2-11	Phenylethyl alcohol	12***	14***	11**
2-12	Guaiacol	–	–	11**
2-13	Eugenol	10**	11**	–
3	Ketones			
3-1	2-Heptanone	8=	5=	6=
3-2	6-Methylhept-5-en-2-one	7=	9*	10**
3-3	2-Acetylfuran	–	5=	9*
3-4	Acetophenone	–	–	10**
3-5	3-Methylnonane-2,4-dione	7=	4=	6
3-6	α -Ionone	5=	3=	11**
3-7	β -Ionone	12***	12***	15***
3-8	<i>cis</i> -Jasmone	4=	13***	12***
3-9	Coumarin	9*	10**	8=
4	Esters			
4-1	Ethyl hexanoate	–	–	10**
4-2	Ethyl decanoate	–	–	12***

Table 5 (continued)

No	Compound ^a	<i>n</i> ^b		
		DJL	KM	CL
4-3	Methyl salicylate	13***	15***	11**
4-4	γ -Octanolactone	–	–	12***
4-5	Methyl dihydrojasmonate	–	9*	8=
4-6	Dihydroactinidiolide	10**	8=	11**
5	Terpenes			
5-1	β -Myrcene	12***	15***	12***
5-2	(+)-Limonene	10**	12***	10**
5-3	(Z)-3,7-Dimethylocta-1,3,6,-triene	10**	12***	–
5-4	p-Cymene	–	4=	3=
6	Acids			
6-1	Acetic acid	2=	3=	3=
7	Sulfur compounds			
7-1	Dimethyl sulfide	12***	12***	14***
7-2	Methionol	8=	11**	–
7-3	3-Mercaptohexanol	10**	11**	–
8	Others			
8-1	2-Acetylpyrrole	3=	5=	8=
8-2	3-Methylindole	8=	6=	4=
8-3	<i>cis</i> -Linaloloxide	10**	13***	11**

^aThe aroma-active compounds with OAV ≥ 1 or AI > 0

^bCorrect judgment numbers for evaluating the difference of aroma by triangle tests

^cThe odor threshold (OT) of sulfur compounds in water referred in the literature. Significance: ***, 0.1% significant level ($p \leq 0.1\%$); **, 1% significant level ($p \leq 1\%$); *, 5% significant level ($\leq 5\%$); =, no significant difference

^dNot aroma-active compound in samples

The results of most of the omission experiments were consistent with the results of GC-O and OAVs. Notably, there were some differences between the results of the omission experiments and OAVs. The OAVs of α -ionone, (E, E)-2,4-heptadienal, (2E)-2-octenal, γ -nonalactone, 3-methylnonane-2,4-dione, 3-methylindole, and methionol in black tea were greater than 1. However, they did not appear important in omission experiments. On the contrary, the OAVs of *cis*-3-hexen-1-ol and benzaldehyde were less than 1, but they played important roles in the omission experiments. This might be because the thresholds of aroma compounds were determined in water when calculating OAVs, but the omission experiments were conducted in mixed aroma solutions, which meant that the thresholds might have changed. Besides, there might be perceptual interactions among aroma compounds, which affected the thresholds of the aroma compounds [48].

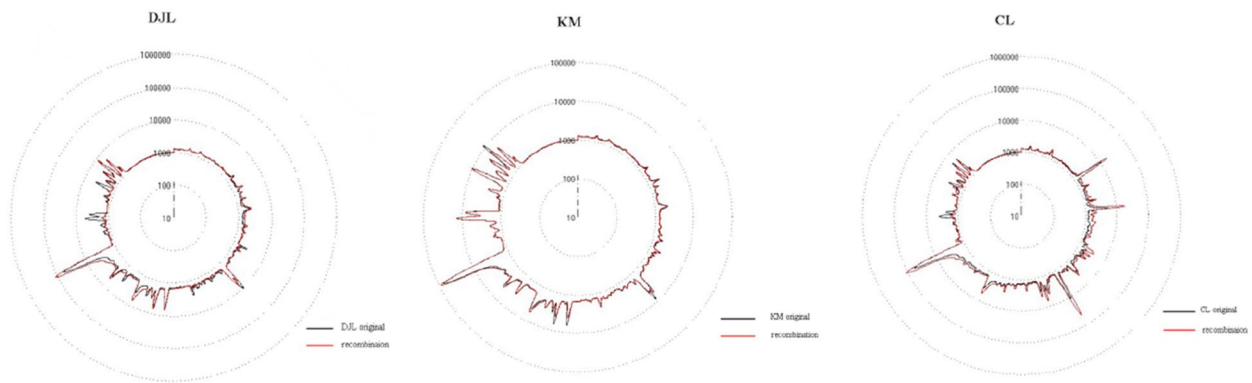


Fig. 2 Comparison of aroma profiles between original black tea infusions and the aroma recombination models by electronic nose

Conclusion

The characteristic aroma compounds in three world-famous black teas were comprehensively analyzed by SBSE/GC-O and OAV methods. A total of 98 volatile compounds were identified, and 87, 83, and 74 aroma compounds were determined in Darjeeling, Keemun, and Ceylon black tea, respectively. In this study, sulfur compounds in black tea were detected for the first time. The comprehensive analysis of the black tea would complement the aroma map of tea. The contributions of characteristic aroma compounds to overall aroma were evaluated through omission experiments. The comprehensive identification of the characteristic aroma compounds of black tea not only provides theoretical guidance to quality control of black tea, but also forms a fundamental basis for recombination and simulation of the black tea aroma.

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Declarations

Conflict of interest Authors have declared no conflict of interests.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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