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ORIGINAL PAPER

Comparison of different aroma-active compounds of Sichuan Dark brick tea (*Camellia sinensis*) and Sichuan Fuzhuan brick tea using gas chromatography–mass spectrometry (GC–MS) and aroma descriptive profile tests

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Abstract

Sichuan dark brick tea (*Camellia sinensis*) and Sichuan Fuzhuan brick tea have significantly different aroma characteristics although both of them have almost the same processing methods. Thus, these two types of tea were used as the research materials to determine the differences in their aroma compounds. The volatile compounds in the two types of tea were identified and quantified by headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME–GC–MS), results showed that they both had 37 common volatile compounds. Then the aroma-active components were identified by odour activity value (OAV). It was found that SFBT had 20 aroma-active components, of which β-ionone had the largest OAV (199547.72). SDBT has 21 aroma-active ingredients (including all 20 aroma-active components of SFBT), of which β-ionone again has the largest OAV (114800.66). Finally, the aroma profile differences between the two tea samples were studied by aroma profile tests, and the results showed that the main aroma differences of SDBT and SFBT were caused by β-ionone, epoxydihydrolinalool II, methyl salicylate, geranylacetone, nerolidol, benzaldehyde, benzyl acetate, nonanal, *trans*,*trans*-2,4-heptadienal and 1-octen-3-ol, in addition, defined SFBT's 'fungi flower aroma' and SDBT's 'aged fragrance' from the level of aroma monomer.

Keywords Dark tea · Aroma-active compounds · Odour activity value · Gas chromatography–mass spectrometry

Abbreviations

OAV	Odour activity value
GC–MS	Gas chromatography–mass spectrometry
SDBT	Sichuan Dark brick tea
SFBT	Sichuan Fuzhuan brick tea
HS-SPME	Headspace solid-phase microextraction
OT	Odour threshold
PCA	Principal component analysis

Practical application: The results of this study can be used to guide the development of new flavours and provide technical support for the process improvement of dark tea after fermentation.

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Introduction

Based on the sensory flavour and processing procedure, teas are classified as green, black, oolong, white, dark or yellow tea [10]. Sichuan Dark brick tea (SDBT) (*Camellia sinensis*) and Sichuan Fuzhuan brick tea (SFBT) are two important types of Chinese dark tea [31] which have 300 years of drinking history in China [68]. These two types of dark tea are generally distinguished by 'Jin Hua' (*Eurotium cristatum*) [22], which is generated by a unique "Fa-Hua" process used in the process of making the Fuzhuan brick tea, which promotes the reproduction and metabolism of the *Eurotium cristatum* [12], and a type of yellow mycelium ('Jin Hua') will be formed on the surface and inside of the tea. This also promotes the formation of the unique flavour quality of Fuzhuan brick tea [60].

SDBT and SFBT show features of "aged fragrance" and "fungi flower aroma", respectively, even though both of them are considered as dark tea and show the basic aroma characteristics of dark tea. To be more specific, the volatile

aroma components of SDBT and SFBT are significantly different, even though their raw fresh leaves are the same and the processing used for these two types of tea is similar (with only two different processing steps).

It was reported that the "aged fragrance" is slowly formed by oxidation during processing [73, 76], while the "fungi flower aroma" is formed by the special process called "Fa-Hua"—fermentation by *Eurotium cristatum* [42, 69, 71]. However, no study on the aroma components and odour characteristics of the aged fragrance and the fungi flower aroma has been reported, even though more than 600 aroma components have been found in tea [72].

It is very important to study the differences in the aroma components of SDBT and SFBT, not only because these differences have not yet been studied but also because the differences in these aroma components can reveal the secondary metabolism of *Eurotium cristatum* in the process of dark tea fermentation and the effects of these metabolites on the aroma quality of dark teas.

Over the past years, only the conventional sensory evaluation method has been used to characterize the aroma of tea [32, 33]. This method can distinguish the different types of tea but cannot determine the effect of a substance on the aroma. However, there are some new and efficient research methods which have emerged in recent studies on food flavour, such as odour activity value method [3, 11] and aroma descriptive profile test [26, 32, 33], which could evaluate the contributions of individual aroma compounds to the overall aroma profile in food flavour. These methods have enabled progress in the analysis of the main aroma components of foods [11, 58, 59]. But in terms of researches of tea aroma, recent studies have focused on green tea and black tea; there are only a minority of reports aiming to investigate the aroma compounds of dark tea, which remain to be fully characterized.

Therefore, to study the differences in the aroma components of SDBT and SFBT, and the secondary metabolism of *Eurotium cristatum* in the process of dark tea fermentation as well as the effects of these metabolites on the aroma quality of above dark teas, first, the headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME–GC–MS) method was used to study the aroma components of SDBT and SFBT, then the odour activity value (OAV) calculation and aroma profile test methods were used to study the aroma characteristics of "aged fragrance" and "fungi flower aroma".

Materials and methods

Tea samples

Fresh leaves were picked from a tea garden on Huaqiu mountain in Qionglai City, Sichuan Province (N30°23′, E103°17′), in June 2017. The tea trees were 5 years old, the picking standard was one bud and three leaves, and the tea cultivar was Hua Qiu No. 1. All the fresh leaves of the same batch were picked on 1 day (50–60 kg), and then the tea samples were immediately produced. The processing site was Huaqiu Tea Industry Co. Ltd. in Sichuan. The production process is shown in Fig. 1, and the specific process parameters are as follows:

Process parameters of SDBT

Fixing, which step that enzyme inactivation was achieved at high temperatures (200 °C for setting and 85 ± 2 °C for actual leaves temperature) and short time(110 ± 5 s), using a rotary continuous fixation machine.

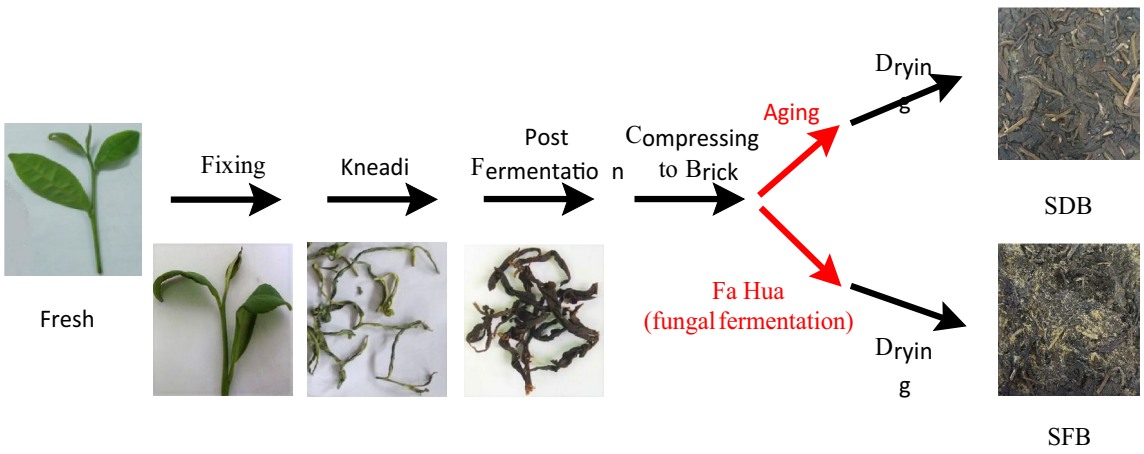

Fig. 1 Production process of SDBT and SFBT

Kneading, which step leaf cells were broken by a knead machine (55 ± 1r/min for rotation rate setting and the duration was 40 min).

Post-fermentation, which step that post-fermentation was achieved in a proving room (the moisture content of leaves was 55%, 45 °C for temperature setting and the duration was 22 h), then the tea material was pressed into brick shape with a mould (24 cm × 18 cm × 10 cm).

Ageing, which step that tea bricks were dried to 16% moisture content and then aged in a dry ventilated room (20 °C for ambient temperature and relative humidity was 55%) in 90 days.

Drying, which step that put aged tea bricks in a drying room and dried until the moisture content was less than 12% (temperature rose from 40 to 75 °C at a constant speed for 8 days).

Process parameters of SFBT

Fixing, kneading and post-fermentation were the same as SDBT.

Fa-Hua (fungal fermentation), which step that tea bricks were placed in a fermentation room (28 °C for ambient temperature and relative humidity was 80%) for natural fermentation for 10 days.

Drying, which step that put aged tea bricks in a drying room and dried until the moisture content was less than 12% (temperature rose from 40 to 75 °C at a constant speed in 12 days).

The tea samples were stored under vacuum at − 40 °C after manufacturing. The SDBT and SFBT samples which were used in the experiment are shown in Fig. 2.

Chemicals

The benzyl alcohol, benzaldehyde, benzyl acetate, 6-methyl-5-hepten-2-one, methyl salicylate, *trans*-2,4-heptadienal, linalool, geraniol, β-ionone, 1-nonanal, nerolidol, hexyl hexanoate, 1-octen-3-ol, linalool oxide II, and indole standards were analytical reagents with at least 98% purity (Rhawn reagent, Shanghai, China), and ethyl decanoate (99.99% purity, used for quantitative analysis), C5–C16 normal paraffin standards (99.99% purity, used for RI calculation) was purchased from Sigma-Aldrich (Shanghai, China).

Equipment

HS-SPME was used to extract the aroma compounds. The SPME fibre was 50/30 μm DVB/CAR/PDMS whose length was 1 cm (Sigma-Aldrich, Shanghai, China), and the fibre penetration depth into the headspace was 2 cm. The size of the extraction bottle was 15 mL. The GC–MS was an Agilent 7890A/5975C-GC/MSD.

HS-SPME and GC–MS analysis

Extraction of aroma-active components

The HS-SPME method was chosen to isolate the aroma-active components; a tea sample (3.0 g) with 30 μg ethyl decanoate as an internal standard (10 mg/kg tea sample) was placed in an extraction bottle (15 mL volume). The sample was equilibrated in a thermostatic water bath for 10 min at 50 °C, and then sampled for 30 min in the head space. After that, SPME fibre was withdrawn and directly introduced to the GC–MS, and the process was repeated three times.

GC–MS instrument setup and analytical conditions

GC–MS analyses were performed on an Agilent 7890A/5975C-GC/MSD inert detector operating in EI mode in a 69.9 eV [75] chromatographic column: capillary-column chromatography DB-5 ms (30 m × 250 μm × 0.25 μm). The

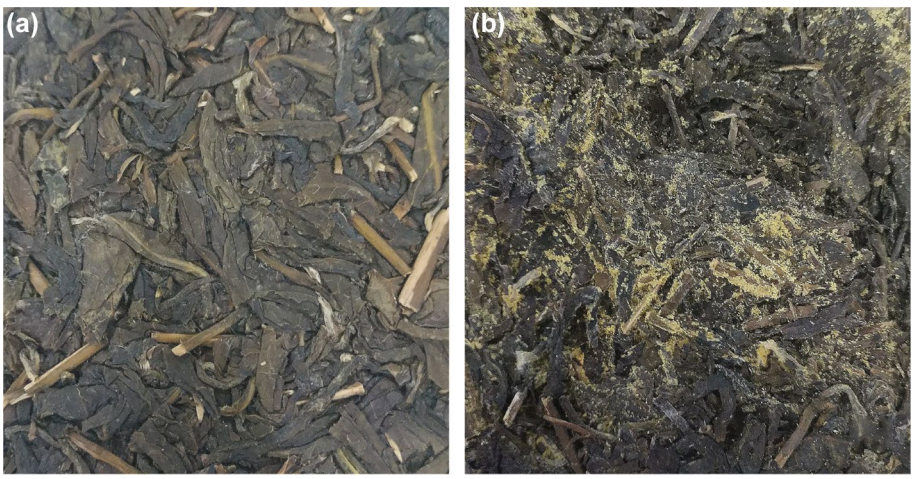

Fig. 2 The tea samples (SDBT and SFBT) which were used in the experiment. **a** Sichuan dark brick tea (SDBT), **b** Sichuan Fuzhuan brick tea (SFBT)

sampling was manual, no shunt was used, the sample was at a constant temperature, and the temperature of the injection port and GC–MS direct interface were 250 °C and 280 °C, respectively. Temperature programming: the column temperature was 50 ~ 250 °C; the starting column temperature was 50 °C which was held for 3 min, and then increased to 150 °C at a rate of 2 °C/min, held for 2 min, then increased to 250 °C at a rate of 2.5 °C/min and held for 4 min. Helium was used as a carrier gas at a flow rate of 1.0 mL/min. The ionic source temperature was 230 °C, the quadrupole temperature was 150 °C and the scanning quality range was set at 20 ~ 700 amu.

Qualitative and quantitative analysis of aroma compounds

Qualitative analysis: The NIST standard spectral library (https://www.nist.gov/) and retention indices (RIs) from other literatures were used to match ion mass spectra, and then completed the qualitative analysis of aroma components.

The RI calculation formula was

$$\mathrm{RI} = 100n + 100 \times \frac{\mathrm{RTx} - \mathrm{RTn}}{\mathrm{RTn} + 1 - \mathrm{RTn}} \quad (1)$$

where RI are the retention indices of analyte, n the number of carbon atoms of n-alkanes before the analyte, $\mathrm{RT_x}$ the retention time of the analyte, $\mathrm{RT_n}$ the retention time of n-alkanes before the analyte, $\mathrm{RT_{n+1}}$ is the retention time of n-alkanes after the analyte.

Quantitative analysis: the internal standard method was used to quantify the volatile flavour compounds in the tea aroma [17]. Ethyl decanoate was selected as the internal standard (10 mg/kg tea sample). The calculation formula was

$$W_\mathrm{i} = W_\mathrm{s} \times \frac{A_\mathrm{i}}{A_\mathrm{s}}, \quad (2)$$

where W_i is the analyte concentration in mg/kg, W_s the concentration of the internal standard in mg/kg, A_i the analyte area and A_s is the peak area of the internal standard.

Calculation method of odour activity value

Aroma description data were picked from the Leffingwell and Associates Threshold Value Database (http://www.leffingwell.com/) and the FEMA GRAS Database (https://www.femaflavour.org/) [1],and then data from other literatures and monographs were used to determine the odour threshold (OT) and odour quality values. The contents of the aroma-active compounds were analysed on a gas chromatography–mass spectrometry (GC–MS) system. In addition, OAV is defined as the ratio of concentration to odour threshold, and the calculation formula of OAV was [19]

$$\mathrm{OAV} = C/\mathrm{OT}, \quad (3)$$

where C was the concentration of a single volatile component, OT is the odour threshold of the same volatile component.

If the OAV was equal to or greater than one, then the single volatile component had some influence on the overall aroma, and the higher the OAV is, the higher the contribution to the overall aroma [3, 18]

Training the sniffers

This method was based on Jiancai Zhu and Ye Liu's method with some improvements [26, 32, 33, 80].

Sixteen assessors aged 23–28 years (half men, half women) were systematically trained for 4 months to recognize the aromas of substances naturally found in tea to define their aroma characteristics, detection thresholds and intensities. The training consists of two stages.

Stage 1: recognition training and intensity testing of single aroma components

Seventeen aroma compounds that are naturally found in dark tea with high frequency were identified (see Table 4 for details) and formulated as tenfold olfactory threshold water solutions. The test personnel were required to accurately identify the samples, and training was repeated to an accuracy of 95%.

Then the 17 aroma components were prepared into 0-fold (control check), 1-fold, 5-fold, 10-fold, 100-fold and 500-fold olfactory threshold aqueous solutions, and the test personnel were asked to sort the disordered solutions of a single aroma according to the order of intensity and scored them using a 7-point scoring method (above 7-points were 0, 0.5, 1, 1.5, 2, 2.5 and 3, respectively, in which 0-point is odourless and 3 points is the strongest solution). The training should be repeated to an accuracy of 99%.

Stage 2: mixed aroma recognition training and intensity testing

The above 17 aroma components were divided into 6 groups, floral, fruity, sweet, woody, fatty and mushroom earthy, and 2 aroma components were selected from each group. Then every two aromas are mixed together, and every pair was blended with each of the 12 aroma components (from a different group). It was required that the test personnel accurately distinguish the two types of aroma components (accuracy rate > 85%). At the same time, the tester needed to score the intensity of the aroma, the strength of the stronger

components of the aroma is scored on a scale of 1 and the strength of the weaker aroma on a scale of 0–1.

Preparation of the dark tea for sniffing

According to the sensory analyses and quantitative determinations of the sample aromas, six characteristic aromas were chosen to describe the aroma profile: floral (representation of linalool, geraniol and β-ionone), fruity (representation of benzaldehyde and hexyl hexanoate), sweet (representation of methyl salicylate), woody (representation of nerolidol), mushroom earthy (representation of 1-octen-3-ol) and fatty (representation of 1-nonanal and *trans*,*trans*-2,4-heptadienal).

The method is based on the method of And, C. S., Liu, Y, and Isleten, M. H with slight adjustment (4; Liu et al. 30, 31; 22). Two tea samples of 10.0 g were weighed into a triangulated flask (250 mL volume), heated for 10 min in a water bath at 50 ± 1 °C to ensure that the aroma diffuses into the bottle to saturate the space, and then immediately sniffed in a sensory panel room at 21 ± 1 °C. To overcome the memory effects of a single aroma compound, every compound was sniffed only once, and the sniffing trials were performed 10 min apart.

Sensory evaluations were carried out by a sensory panel. They were asked to score the aroma qualities of floral, fruity, sweet, woody, mushroom earthy and fatty on a scale from 0 to 3 (where 0 means no smell and 3 points was the strongest). The samples were analysed in triplicate by each panellist, and the average score was used to draw the aroma profile.

Statistical analysis

Excel 2016 and the SPSS 25 software package were used to analyse the data, and the Excel visual plug-in XLSTAT 2018 was used to draw tables.

Results and analyses

Identification with GC–MS and PCA analysis results of aroma compounds in SDBT and SFBT

The overall aroma characteristics of SDBT are pure added aged fragrance and long lasting, and those of SFBT are pure added fungi flower aroma, strong and long lasting (results from early research, not cited in this paper). Although both of them show dark tea's aromas, in accordance with traditional SDBT and SFBT, there are significant differences in their aroma characteristics under the premise that the materials and the production processes were both consistent, the differences between them may be caused by the unique technology "Fa Hua". For further study of the aroma differences between SDBT and SFBT, their aroma components' contents were detected by GC–MS.

According to Table 1, 37 volatile aroma components were detected in SDBT and SFBT. Among them, the components with the highest contents in SDBT were olivetol (15.55 mg/kg), linalool (14.01 mg/kg) and β-ionone (11.94 mg/kg), and those with the lowest contents were (E)-3,7-dimethylocta-1,3,6-triene (0.76 mg/kg), L-calamenene (0.99 mg/kg) and *cis*-3-hexenyl acetate (1.04 mg/kg). The components with the highest contents in SFBT were methyl salicylate (24.49 mg/kg), geranylacetone (21.73 mg/kg) and β-ionone (20.75 mg/kg), and those with the lowest contents were (E)-3,7-dimethylocta-1,3,6-triene (0.58 mg/kg), L-calamenene (0.74 mg/kg) and 2-acetyl pyrrole (0.84 mg/kg). Although the aroma components of SDBT and SFBT were highly consistent, their contents varied greatly. It is worth noting that the aroma components with the lowest contents in both SDBT and SFBT were (E)-3,7-dimethylocta-1,3,6-triene and L-calamenene.

The volatile aroma components in Table 1 were also analysed by PCA [24] (Fig. 4), and their detailed data are shown in Table 2. The results showed that the main sources of the difference between SDBT and SFBT were linalool, benzyl acetate, β-ionone, methyl salicylate, olivetol, geraniol, phenethyl alcohol, nerolidol, β-cyclocitral, (2,6,6-trimethyl-2-hydroxycyclohexylidene)acetic acid lactone, benzaldehyde and geranylacetone.

However, the PCA analysis did not take into account the threshold values of the aroma components. In other words, a low content of certain volatile aroma components may have a great impact on the overall aroma due to their low threshold values; on the contrary, a high content of other compounds may have little effect. Therefore, it is necessary to study further more about the contribution of each aroma component through OAV calculation.

Odour activity value (OAV) calculation and aroma description of the olfactory test results

Table 3 showed that there were 21 compounds whose OAVs are greater than 1 in SDBT. This result indicated that among the 37 detected components, only 21 components contributed to the overall aroma and were the aroma-active components of SDBT. According to the values of the aroma activity, from large to small, these components were β-ionone; linalool; benzyl acetate; 1-octen-3-ol; β-cyclocitral; *trans*,*trans*-2,4-heptadienal; geraniol; linalool oxide II; 1-nonanal; nerolidol; linalool oxide I; methyl salicylate; *cis*-3-hexenyl acetate; geranylacetone; hexyl hexanoate; benzaldehyde; 2,6,6-trimethyl-2-hydroxycyclohexylidene; indole; 6-methyl-5-hepten-2-one; (–)-alpha-terpineol and phenethyl alcohol. In terms of SFBT, there were 20 compounds

whose OAVs were greater than 1 in it; these components contributed to the overall aroma and were the aroma-active components of SFBT. According to the values of the aroma activity from large to small, they were β-ionone; linalool; benzyl acetate; β-cyclocitral; *trans*,*trans*-2,4-heptadienal; 1-octen-3-ol; linalool oxide II; linalool oxide I; methyl salicylate; 1-nonanal; nerolidol; geranylacetone; geraniol; *cis*-3-hexenyl acetate; 6-methyl-5-hepten-2-one; hexanoate; 2,6,6-trimethyl-2-hydroxycyclohexylidene; indole; benzaldehyde; (–)-alpha-terpineol and phenethyl alcohol.

There were 11 volatiles among the aroma-active components of SDBT and SFBT for which the difference of the OAV ratio is greater than 0.5. The OAVs of β-ionone (199547.72), geranylacetone (362.15), linalool oxide II (1262.68), linalool oxide I (855.99), methyl salicylate (408.24) and 6-methyl-5-hepten-2-one (32.07) in SFBT were 74, 363, 127, 103, 151 and 185% higher than those in SDBT, respectively. In SDBT, the OAVs of geraniol (1055.13), phenethyl alcohol (1.02), benzyl acetate (4574.33), benzaldehyde (28.60) and 1-octen-3-ol (2438.46) were higher by 274, 158, 92, 109 and 89% than those in SFBT, respectively.

Using OAV analysis, we can further explore whether and how the active components contribute to the overall aroma. However, the specific influences on the aroma profiles of SDBT and SFBT need to be further explored through the aroma profile test.

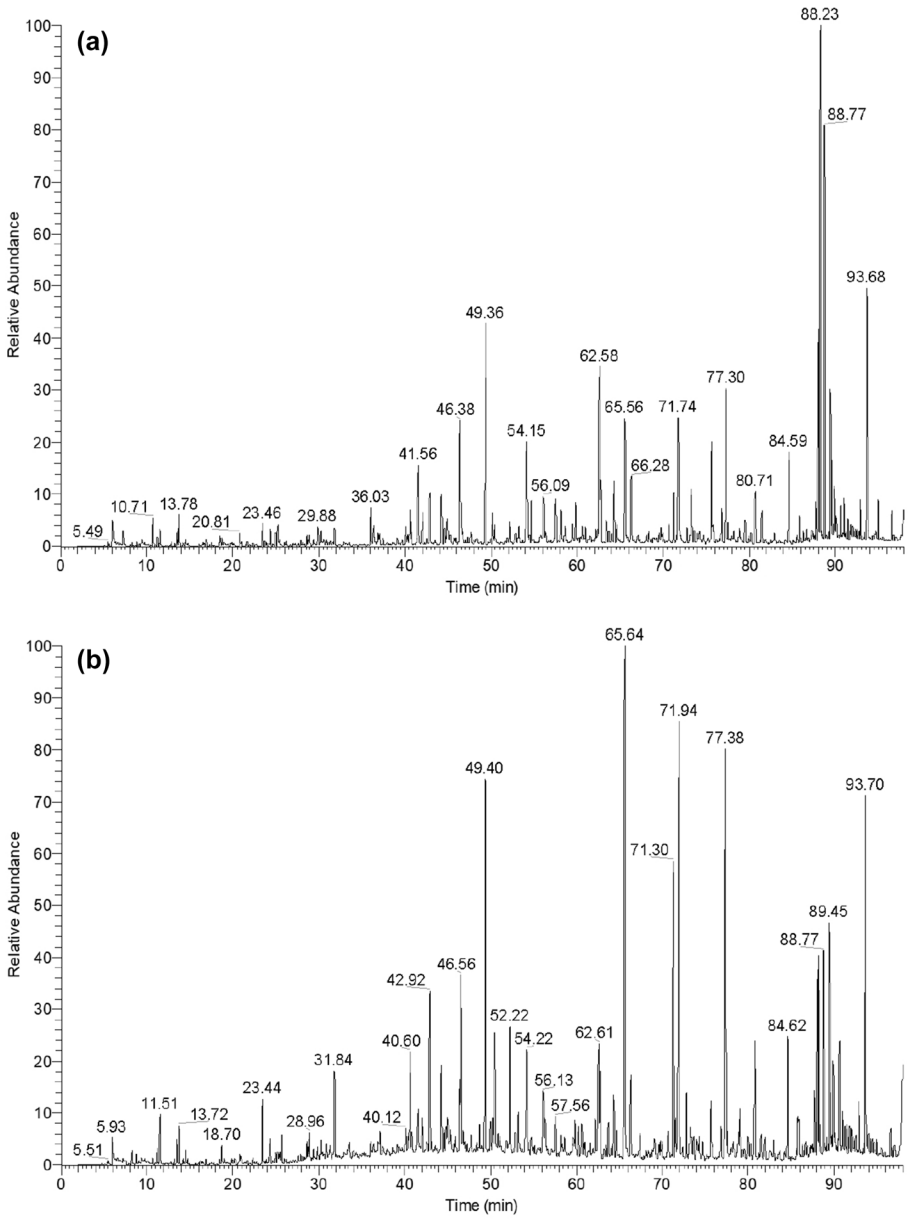

Fig. 3 Total ion chromatograms from SDBT (**a**) and SFBT (**b**) by GC–MS

Table 1 Aroma compounds detected in tea samples by GC–MS

No	Aroma compound	RIs (DB-5 ms)			RIs (HP-5 ms)		Identification[b]	Concn (mg/kg)	
		Calculate[a]	Reference	Literatures	Reference	Literatures		SDBT	SFBT
1	Linalool	1101	1101	[4]	1097	[7]	MS, RI	14.01	16.34
2	Benzyl acetate	1173	1172	[54]	1165	[50]	MS, RI	11.89	6.2
3	β-Ionone	1485	1485	[48]	1490	[6]	MS, RI	11.94	20.75
4	Methyl salicylate	1190	1191	[35]	1187	[56]	MS, RI	9.78	24.49
5	Olivetol	1753	1755	[61]	Null[c]	Null	MS, RI	15.55	4.83
6	Geraniol	1256	1256	[37]	1267	[25]	MS, RI	7.91	2.11
7	Phenethyl alcohol	1145	Null	Null	1121	[25]	MS	7.68	2.97
8	Nerolidol	1535	1534	[45]	1531	[53]	MS, RI	6.35	5.58
9	β-Cyclocitral	1227	Null	Null	1222.5	[38]	MS	8.17	7.04
10	(2,6,6-Trimethyl-2-hydroxycyclohexylidene) acetic acid lactone	1549	Null	Null	1538	[5]	MS	11.57	9.76
11	Benzaldehyde	964	964	[63]	961	[52]	MS, RI	8.58	4.11
12	7,11,15-Trimethyl-3-methylidene-hexadec-1-ene	1837	1836	[55]	1840	[29]	MS, RI	2.85	2.21
13	Farnesene	1524	1524	[51]	1507	[52]	MS, RI	4.12	3.28
14	Geranylacetone	1455	1456	[9]	Null	Null	MS, RI	4.69	21.73
15	Delta-cadinene	1484	1486	[39]	1519	[21]	MS, RI	1.27	1.49
16	*cis*-Hex-3-enyl hexanoate	1369	1369	77	Null	Null	MS, RI	4.6	2.81
17	Jasmone	1392	1391	[63]	1396	[30]	MS, RI	1.89	1.7
18	Indole	1290	1290	[27]	1293	[16]	MS, RI	1.86	1.58
19	2,3-Dihydro-2,2,6-trimethylbenzaldehyde	1174	1175	[79]	1178	[49]	MS, RI	4.51	4.16
20	7,11-dimethyl-3-methylene-(e)-10-dodec-atriene	1429	1429	[39]	1459	[6]	MS, RI	3.04	1.91
21	3,5-Octadien-2-one	1091	1098	[65]	1093	[2]	MS, RI	2.71	8.23
22	*trans*,*trans*-2,4-Heptadienal	1012	1012	[78]	1007	[29]	MS, RI	4.26	4.9
23	Linalool oxide II	1087	1086	[13]	1094	[25]	MS, RI	3.34	7.58
24	(–)-Alpha-terpineol	1199	Null	Null	1187	[57]	MS	1.56	1.95
25	(2E)-2-Hexenyl pentanoate	1289	Null	Null	Null	Null	MS	2.07	1.28
26	2-Acetyl pyrrole	1059	1059	[79]	1063.2	[38]	MS, RI	2.23	0.84
27	Cedrol	1598	1596	[15]	1607.9	[74]	MS, RI	1.98	2.14
28	*cis*-3-Hexenyl acetate	1009	1009	[62]	Null	Null	MS, RI	1.04	1
29	(E,E)3, 5-Octadien-2-one	1085	1098	[34]	Null	Null	MS, RI	1.59	5.97
30	Linalool oxide I	1067	1067	[8]	1078	[25]	MS, RI	2.53	5.14
31	L-Calamenene	1524	1522	[48]	1543	[25]	MS, RI	0.99	0.74
32	Benzyl alcohol	1057	1057	[65]	1034.4	[74]	MS, RI	1.54	1.16
33	1-octen-3-ol	983	983	[13]	986	[21]	MS, RI	4.88	2.58
34	1-Nonanal	1102	1102	[35]	1102	[6]	MS, RI	1.84	1.35
35	Hexyl hexanoate	1385	1384	[36]	1385	[50]	MS, RI	1.4	1.09
36	6-Methyl-5-hepten-2-one	986	986	[40]	988	[25]	MS, RI	1.13	3.21
37	(E)-3,7-Dimethylocta-1, 3, 6-triene	1045	1044	[4]	1050	[28]	MS, RI	0.76	0.58

MS mass spectrum comparison using NIST library, *RI* retention index in agreement with the literature value

[a]Calculate in RIs (DB-5 ms) column, Retention indices (RIs) on DB-5 ms column calculated against n-alkanes (C5–C16)

[b]Identification, means method of identification

[c]Null, no relevant references or data were found

The results of the panel training

Table 4 shows that after the first stage of identification training, the testers had the lowest recognizability (96%) for the four aroma components geraniol; linalool oxide I; *trans*,*trans*-2,4-heptadienal and 1-nonanal, and there were ten aroma components which reached 100% recognition rate. This result indicated that the identification of the single aroma components met the expected training target. After the first stage of intensity testing, the testers could correctly distinguish different concentration gradients of aroma components and provide aroma intensity scores. The training to judge the intensity of a single aroma component, thus, achieved the desired training objectives.

Figure 5 shows that after the second stage of the identification training, the testers' recognition of any 2 aroma components (60 groups) of different flavour types after mixing was at least 85.4%, with an average recognition of 94.8%, showing that it achieved the desired training purpose.

It is found that a mixture containing nerolidol (90.5%), 1-octen-3-ol (92.7%), geraniol (93.6%) and benzaldehyde (94.6%) had the lowest recognition rate. Among them, nerolidol and geraniol had the lowest aroma intensities, only 0.34 (1st) and 0.46 (2nd), respectively, and 1-octen-3-ol and benzaldehyde had the highest aroma intensities, reached 0.97 (1st) and 0.94 (2nd), respectively.

The aroma profile of SDBT and SFBT

The aroma profiles were obtained through a 1.2.5 aroma profile evaluation experiment, as shown in Fig. 6.

The results (Fig. 6) of the aroma profile evaluation test showed that SDBT's scores of floral, fruity, sweet, fatty, mushroom earthy and woody were 0.6, 1.2, 1.3, 1.8, 2.6 and 1.6, and SFBT's scores were 1.1, 1.3, 1.9, 1.4, 1.7 and 1.2, respectively. The SDBT was more prominent than the SFBT in the aspects of fatty, woody and mushroom earthy, while in the aspects of floral and sweet, SFBT was more obvious.

The connection between the aroma profiles' differences and aroma substance contents of the two tea samples, analysed by combining Fig. 3 with Table 3, is shown by the following:

First, as shown in Fig. 6, the two tea samples had similar basic aroma contours. Combined with that, as shown in Table 4, the SDBT and SFBT had 20 common active aroma components (the OAV of phenylethyl alcohol in SFBT is 0.4), and the highest OAV values in these two tea samples were both β-ionone (114800.66 and 199547.72, respectively), β-linalool and benzyl acetate followed. This result indicated that β-ionone, β-linalool and benzyl acetate had the greatest contributions to the aromas of SDBT and SFBT, and β-linalool, β-cyclocitral, *cis*-3-hexenyl acetate, hexyl hexanoate, (2,6,6-trimethyl-2-hydroxycyclohexylidene)acetic acid lactone, E-2-E-4-heptadienal, nonaldehyde, nerolidol, indole and terpene alcohol had similarly high OAVs in the two tea samples. Therefore, these results implied that both SDBT and SFBT showed analogical basic aroma characteristics of 'dark tea' because of above similar 13 aroma components.

In addition, the results of the aroma profile test showed that the SFBT had more development in sweetness than SDBT (Fig. 6), while compared with Table 3, it was found that all sweet aroma components in SFBT with an OAV > 1

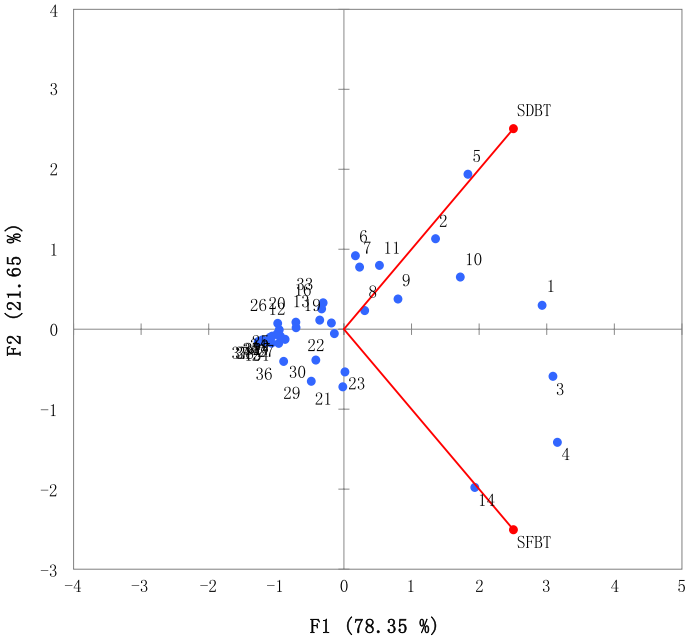

Fig. 4 PCA score plot depicting the distribution of aroma compounds detected by GC–MS analysis and sensory attributes in tea samples for the two principal components. Compounds represented from Table 1

Table 2 PCA scores of aroma compounds detected by GC–MS analysis in SDBT and SFBT for the first two principal components (F1 and F2)

No.	aroma compound	Factor scores		Contribution of the observations by PCA (%)	
		F1 (78.35%)	F2 (21.65%)	F1 (78.35%)	F2 (21.65%)
1	Linalool	2.931	0.299	14.821	0.559
2	Benzyl acetate	1.355	1.132	3.168	7.996
3	β-Ionone	3.093	−0.589	16.496	2.168
4	Methyl salicylate	3.158	−1.414	17.196	12.480
5	Olivetol	1.835	1.937	5.806	23.427
6	Geraniol	0.170	0.919	0.050	5.271
7	Phenethyl alcohol	0.232	0.776	0.093	3.762
8	Nerolidol	0.307	0.233	0.162	0.338
9	β-Cyclocitral	0.800	0.378	1.104	0.891
10	(2,6,6-Trimethyl-2-hydroxycyclohexylidene)acetic acid lactone	1.721	0.653	5.111	2.658
11	Benzaldehyde	0.525	0.799	0.475	3.981
12	7, 11, 15-Trimethyl-3-methylidene-hexadec-1-ene	−0.708	0.019	0.864	0.002
13	Farnesene	−0.358	0.114	0.222	0.081
14	Geranylacetone	1.936	−1.978	6.463	24.428
15	Delta-cadinene	−1.072	−0.174	1.983	0.190
16	*cis*-Hex-3-enyl hexanoate	−0.329	0.254	0.187	0.403
17	Jasmone	−0.938	−0.090	1.518	0.051
18	Indole	−0.956	−0.080	1.577	0.040
19	2,3-Dihydro-2,2,6-trimethylbenzaldehyde	−0.185	0.078	0.059	0.038
20	7,11-Dimethyl-3-methylene-(e)-10-dodecatriene	−0.711	0.087	0.873	0.047
21	3,5-Octadien-2-one	−0.017	−0.721	0.000	3.243
22	*trans*,*trans*-2,4-Heptadienal	−0.141	−0.055	0.034	0.019
23	Linalool oxide II	0.015	−0.534	0.000	1.782
24	(–)-Alpha-terpineol	−0.964	−0.177	1.604	0.196
25	(2E)-2-Hexenyl pentanoate	−0.956	−0.008	1.575	0.000
26	2-Acetyl pyrrole	−0.980	0.073	1.656	0.033
27	Cedrol	−0.870	−0.126	1.304	0.100
28	*cis*-3-Hexenyl acetate	−1.171	−0.157	2.364	0.153
29	(E, E)3, 5-Octadien-2-one	−0.483	−0.651	0.402	2.644
30	Linalool oxide I	−0.416	−0.386	0.299	0.929
31	L-Calamenene	−1.209	−0.134	2.522	0.113
32	Benzyl alcohol	−1.062	−0.087	1.947	0.047
33	1-Octen-3-ol	−0.308	0.330	0.164	0.681
34	1-Nonanal	−0.988	−0.057	1.683	0.020
35	Hexyl hexanoate	−1.095	−0.103	2.068	0.066
36	6-Methyl-5-hepten-2-one	−0.892	−0.403	1.373	1.013
37	(E)-3, 7-Dimethylocta-1, 3, 6-triene	−1.269	−0.155	2.777	0.151

were more prominent than those in SDBT, including linalool oxide II, linalool oxide I and methyl salicylate (Table 2), which had OAVs in SFBT of 1262.68, 855.99, 408.24, respectively; these 2 results, thus, were consistent. At the same time, the woody aroma of SDBT was more prominent than those of SFBT (Fig. 6), which was also consistent with the results in Table 3 that the OAV of nerolidol (423.28) and indole (18.63) in SDBT was greater than that in SFBT.

In terms of fruitiness, the scores of the two tea samples were close to each other (Fig. 6).The higher OAV values of phenylmethyl acetate (OAV = 4574.33), β-cyclocitral (OAV = 1633.31), hexyl hexanoate (OAV = 35.08), benzaldehyde (OAV = 28.60) and (2,6,6-trimethyl-2-hydroxycyclohexylidene)acetic acid lactone (OAV = 23.14) provided the SDBT with some fruit aromas such as apple peel and cherry. While SFBT also had prominent fruit aromas due

Table 3 Key aroma components' data (OAV > 1) from SDBT and SFBT by OAV calculation

Fragrant type[a]	Aroma components	OT (mg/kg)[b]	OAV		Ratio of the difference[c]	Aroma description[d]
			SDBT	SFBT		
Floral	β-Ionone	0.000104	114800.66	199,547.72	0.74	Floral, violet (a, b)
	Linalool	0.0015	9339.77	10,891.63	0.17	Coriander, floral, lavender, lemon, rose (a, b)
	Geraniol	0.0075	1055.13	281.97	2.74	Geranium, lemon peel, passion fruit, peach, rose (a, b)
	Phenethyl alcohol	7.5	1.02	0.40	1.58	Fruit, honey, lilac, rose, wine (a)
Fruit	Benzyl acetate	0.0026	4574.33	2386.53	0.92	Fruit (a, b)
	β-Cyclocitral	0.005	1633.31	1408.32	0.16	fresh,fruit (c)
	cis-3-Hexenyl acetate	0.0121	85.55	82.59	0.04	Fruit, prune (a)
	6,10-Dimethyl-5,9-undecadien-2-one	0.06	78.21	362.15	3.63	Fruit (a)
	Hexyl hexanoate	0.04	35.08	27.31	0.28	Apple peel, peach, plum (a, b)
	Benzaldehyde	0.3	28.60	13.71	1.09	Bitter almond, burnt sugar, cherry, malt, roasted pepper (a, b)
	(2,6,6-Trimethyl-2-hydroxy-cyclohexylidene)acetic acid lactone	0.5	23.14	19.52	0.19	lemon (c1)
Sweet	Linalool oxide II	0.006	555.94	1262.68	1.27	Leafy, earthy,sweet (stronger than LOI), floral, creamy (b, c2)
	Linalool oxide I	0.006	421.75	855.99	1.03	Leafy, earthy,sweet, floral, creamy (c2)
	Methyl salicylate	0.06	162.95	408.24	1.51	Almond, caramel, peppermint, sharp (a, b)
Mushroom earth	1-Octen-3-ol	0.002	2438.46	1289.80	0.89	Cucumber, earth, fat, floral, mushroom (a, b)
	6-Methyl-5-hepten-2-one	0.1	11.27	32.07	1.85	Citrus, mushroom, pepper, rubber, strawberry (a)
Fatty	*trans*,*trans*-2,4-Heptadienal	0.0035	1215.95	1401.02	0.15	Fat, nut (a,b)
	1-Nonanal	0.0035	525.81	385.11	0.37	Fat, floral, green, lemon (a, b)
	α-Terpineol	0.33	4.74	5.92	0.25	Anise, fresh, mint, oil (a)
Woody	Nerolidol	0.015	423.28	371.83	0.14	Fir, linoleum, pine (a, b)
	Indole	0.1	18.63	15.80	0.18	Burnt, mothball (a)

[a]The source of fragrant type: through the 1.2.2 test and reference FEMA GRAS database (https://www.femaflavor.org/)

[b]The references of odour threshold (OT):Leffingwell & Associates Database (http://www.leffingwell.com/), and compilations of odour threshold values in air, water and other media and compilations of flavour threshold values in water & other media (2011 editions)—by van Gemert [64] (http://www.thresholdcompilation.com/)

[c]Calculation formula of ratio of the difference: ratio of the difference = |HZ–FZ|/min {HZ, FZ}, bold font annotation with ratio of the difference > 0.5

[d]The source of aroma description: (a) FEMA GRAS data base (https://www.femaflavor.org/); (b) aroma characteristics test; (c1) Ortega-Heras et al. [47]; (c2) [67]

to the higher OAV of 6,10-dimethyl-5,9-undecadien-2-one (OAV = 362.15).

In terms of mushroom earthy, the score of the SDBT was much higher than that of the SFBT (Fig. 6), which was closely related to the high OAV of 1-octen-3-ol (OAV = 2438.46) (Table 3).

Additionally, the fatty aroma of the SDBT was more prominent than that of SFBT (Fig. 6), and this result was in accordance with the higher OAV of nonaldehyde in SDBT (OAV = 525.81) (Table 3), but it was contradictory to the OAV of (E,E)-2,4-hepadienal, which will be further elaborated in the discussion.

Different OAVs in β-ionone, geraniol and phenethyl alcohol caused the different floral scores between the SDBT and the SFBT (Fig. 6). The high OAV of β-ionone (OAV = 199547.72) in the SFBT was close to the sweet floral, and the high OAVs of Geraniol (OAV = 1055.13) and phenethyl alcohol (OAV = 1.02) were close to the

Table 4 Identification training and strength determination results of single aroma components

Fragrant type[a]	Aroma components	Correct recognition ratio[b] (%)	Aroma intensity scores at different olfactory threshold multiple[c]					
			0×	1×	5×	10×	100×	500×
Floral	β-Ionone	100.00	0.10	0.94	1.37	2.01	2.50	2.88
	Linalool	97.92	0.00	1.02	1.48	1.95	2.60	3.00
	Geraniol	95.83	0.10	1.01	1.47	1.97	2.44	2.99
	Phenethyl alcohol	100.00	0.00	1.03	1.49	2.02	2.48	2.98
Fruit	Benzyl acetate	100.00	0.00	1.01	1.61	1.96	2.50	3.00
	6,10-Dimethyl-5,9-undecadien-2-one	100.00	0.10	1.00	1.47	2.01	2.47	2.97
	Hexyl hexanoate	100.00	0.00	1.00	1.51	1.99	2.50	2.99
	Benzaldehyde	100.00	0.00	1.01	1.52	1.99	2.50	2.94
Sweet	Linalool oxide II	97.92	0.00	0.96	1.51	2.02	2.49	2.96
	Linalool oxide I	95.83	0.10	1.00	1.49	2.00	2.51	2.99
	Methyl salicylate	100.00	0.00	1.00	1.51	1.99	2.50	3.00
Mushroom earthy	1-Octen-3-ol	100.00	0.00	1.00	1.50	2.00	2.50	3.00
	6-Methyl-5-hepten-2-one	100.00	0.00	0.98	1.50	2.01	2.50	2.95
Fatty	*trans*,*trans*-2,4-Heptadienal	95.83	0.00	0.99	1.55	2.01	2.50	2.96
	1-Nonanal	95.83	0.00	0.99	1.52	1.98	2.50	2.99
Woody	Nerolidol	97.92	0.10	0.94	1.54	2.00	2.50	2.99
	Indole	100.00	0.00	1.05	1.59	2.00	2.50	2.98

[a]The source of fragrant type: through the 1.2.2 test and reference FEMA GRAS database (https://www.femaflavor.org/)

[b]Correct recognition ratio indicates the recognition accuracy of this aroma monomer by method 1.6, stage 1

[c]0×, 1×, 5×, 10×, 100×and 500×indicate 1-fold, 5-fold, 10-fold, 100-fold and 500-fold olfactory threshold aqueous solutions, respectively

green floral in the SDBT, which was consistent with the study of Ashu Gulati on Kangra orthodox black tea [26]. The floral score of the SDBT was 50% lower than that of the SFBT; this result was related to the masking of the green floral aroma-active components of linalool and geraniol by 1-octen-3-ol and benzaldehyde, which will be further elaborated in the discussion.

In summary, SDBT performed better than SFBT in the woody, mushroom earthy and fatty fragrances represented by nerolidol, 1-octen-3-ol and nonanal, respectively, which were the main components of the aroma characteristics of "aged fragrance". Compared with SDBT, SFBT showed better aspects of "floral", represented by β-ionone, and 'sweet', represented by linalool oxide II and methyl salicylate, which led to the formation of the aroma characteristics of "green floral fragrance".

Discussion

Causes of the main odour-active compound differences between the two types of dark tea

The results showed that the OAVs of β-ionone, geranylacetone, linalool oxide II, linalool oxide I, methyl salicylate and 6-methyl-5-hepten-2-one in SFBT are higher than those in SDBT. Therefore, it was considered that those substances mentioned above were the main active compounds that caused SFBT to show a stronger fungi flower aroma fragrance than SDBT. This difference was probably caused by the "Fa-Hua" (fungal fermentation) process.

β-ionone and geranylacetone were produced from the oxidization of β-carotene [14, 66] (Fig. 7). The evidence showed that there were high volumes of β-ionone and geranylacetone and lower volumes of β-cyclocitral and (2,6,6-trimethyl-2-hydroxy-cyclohexylidene)acetic acid lactone, from which can be deduced that the breaking of the double bonds of carbon between C9 and C10 and between C7 and C8 and C13 and C14 of β-carotene produced β-ionone and geranylacetone, respectively. This happened during the "Fa-Hua" (fungal fermentation) process and was caused by microorganism metabolism. In contrast, the SDBT had no "Fa-Hua" processing but rather had a drying process with high temperature. These processing characteristics may caused the β-carotene to break the C–C bond between C8 and C9 and produce (2, 6,6-trimethyl-2-hydroxy-cyclohexylidene) acetic acid lactone, and it may also caused the β-carotene to break the C = C bond between C7 and C8 and produce β-cyclocitral.

Geranylacetone, proved by experiments, originates from the degradation of carotenoid (Fig. 8) [23]. M Ibdah proved that geranylacetone was produced from the expressed

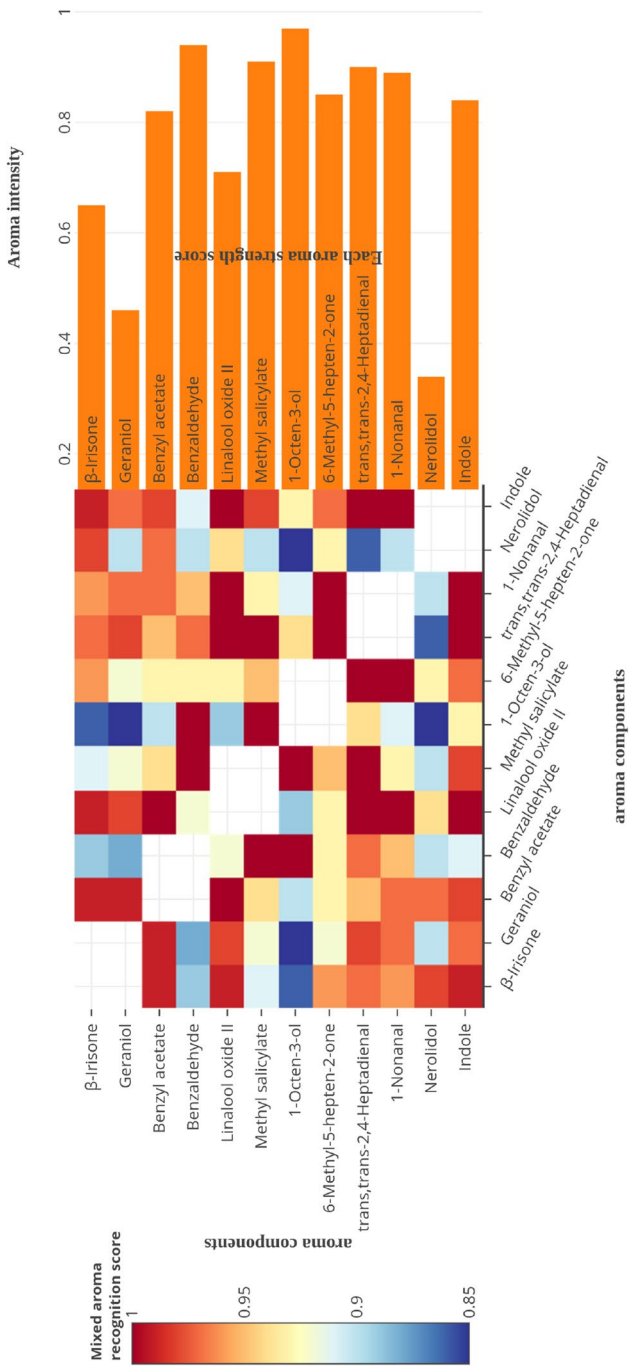

Fig. 5 Second stage: aroma monomer mixing test results

product of CmCCD1, which caused oxidative chemical degradation at the C9–C10 bond of the carbon chain of phytoene. Based on the above experimental results and the experimental data of this research, the following hypothesis was proposed: during the "Fa-Hua" (fungal fermentation) of SFBT, *Eurotium cristatum* caused the upregulated gene expression of the CmCCD1 gene family and produced secondary metabolites which helped to produce geranylacetone; however, this hypothesis needs further experimental proof.

There was an experimental evidence proving that epoxydihydrolinalool I, epoxydihydrolinalool II [67] and methyl salicylate originated from the enzymatic hydrolysis of their primeveroside precursor substances. Benzyl alcohol originated from the hydrolysis of β-D-glucoside and is oxidized to produce benzaldehyde, which shared the same pathway with phenethyl alcohol oxidation to produce phenylacetaldehyde. In many experiments on the addition of exogenous *Eurotium cristatum* into tea or a water extract of tea, the hydrolysis of primeverosides was commonly observed. Therefore, we considered that the vital activities of *Eurotium cristatum* increased the hydrolysis efficiency of β-primeverosides.

Considering all the presented information, we had some assumptions as follows: [1] The vital activities of *Eurotium cristatum* increase the hydrolysis efficiency of β-primeverosides, which offers the possibility of producing more β-primeverosidase or changing the environmental condition of the enzyme catalysis. This change causes volume increases in epoxydihydrolinalool I, epoxydihydrolinalool II and methyl salicylate which leads SFBT to have more such substances than SDBT (Fig. 9) [5]. When the SDBT was aged, the β-D-glucosidase hydrolysed the precursors of benzyl alcohol with glucoside and produced benzyl alcohol, which is further converted to benzaldehyde or benzyl acetate. This results in higher contents of benzaldehyde and benzyl acetate in SDBT than SFBT. (Fig. 10).

Geraniol and phenethyl alcohol can originate from the enzymatic hydrolysis of primeveroside precursor substances, but the analysis showed a contrary result. Geraniol and phenethyl alcohol had higher volumes in SDBT than SFBT. This difference may be rooted in the enzyme specificity variance of the β-primeverosides and β-D-glucosidase [20]. For instance, β-primeverosides had a lower hydrolytic activity to geraniol-6-O-α-L-arabinofuranose-β-bioside-D-glucopyranoside [46]. In addition, geraniol may undergo enzymatic isomerization and transform into linalool, and phenethyl alcohol may also oxidize to phenylacetaldehyde and phenylacetic acid. The above reactions may cause unpredictable changes in the geraniol and phenethyl alcohol contents.

Benzyl acetate and 1-octen-3-ol are present in high volumes in aged dark teas, such as aged Pu'er tea and aged Qing brick tea. This high volume is due to the same reason as discussed in the comparison between SDBT and SFBT. Benzyl acetate originates from the esterification of benzyl alcohol. 1-Octen-3-ol may be a secondary metabolite of fungi (Fig. 11) [70]. Xue inoculated microbes into wheat koji. 1-Octen-3-ol was detected in the fungi group. 1-Octen-3-ol follows the LOX pathway and is produced from the oxy-cracking of linoleic acid with the catalysis of lipoxygenase and hydroperoxide lyase [43, 44]. We speculated from the evidence above

Fig. 6 Aroma profile of SDBT and SFBT. SDBT's scores of floral, fruity, sweet, fatty, mushroom earthy and woody were 0.6, 1.2, 1.3, 1.8, 2.6 and 1.6, and SFBT's scores were 1.1, 1.3, 1.9, 1.4, 1.7 and 1.2, respectively

that a secondary metabolite of the fungi increases the catalytic efficiency and produces more 1-octen-3-ol, which leads to the conclusion that SDBT has more 1-octen-3-ol than SFBT.

Relationship between OAVs of main odour-active compounds and aroma states

Though linalool had a higher OAV than most odour substances, the tea did not show the characteristic aroma of linalool. The results of the mixed aroma recognition training and intensity tests also supported this finding (Fig. 5), i.e. the aroma intensity of the floral fragrance was lower than that of the other fragrances. Miettinen's research provided a clue to explain this finding—the volatilization of linalool and the sensitivity of the nose towards linalool will decrease when there are lipoids in the solution [41]. Lipoids exist in the fresh leaves, constituting 8% of the dry weight of the tea, including fats and glycerides. However, there is no clear clue about the volume of lipoids and their changes during the process. It is necessary to do more research about the influences of lipoids towards linalool.

Substances with a soil fragrance have a masking action towards those with a floral fragrance. 1-Octen-3-ol shows the

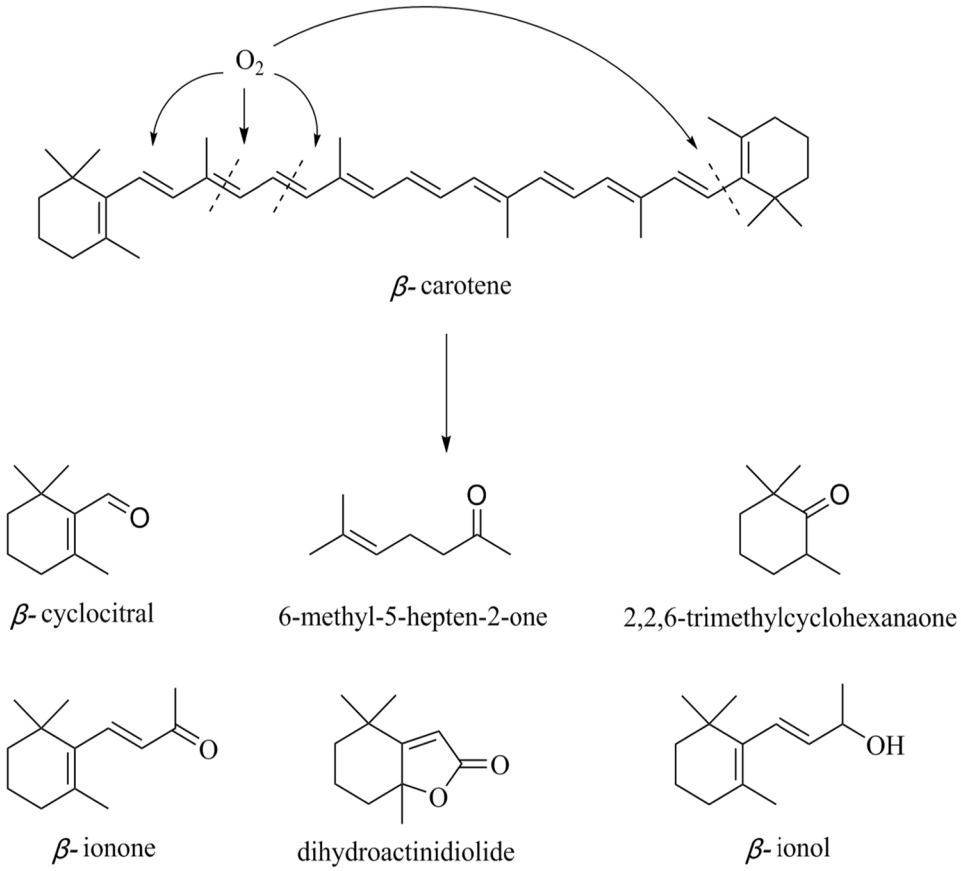

Fig. 7 Main degradation mechanism and major degradation products of β-carotene

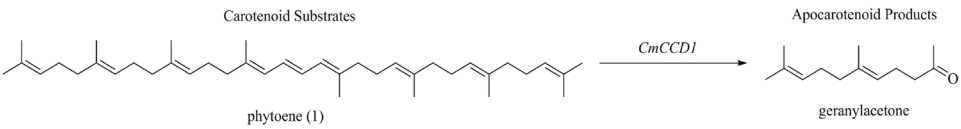

Fig. 8 Schematic diagram of degradation of carotenoids to produce geraniol acetone in melon [23]

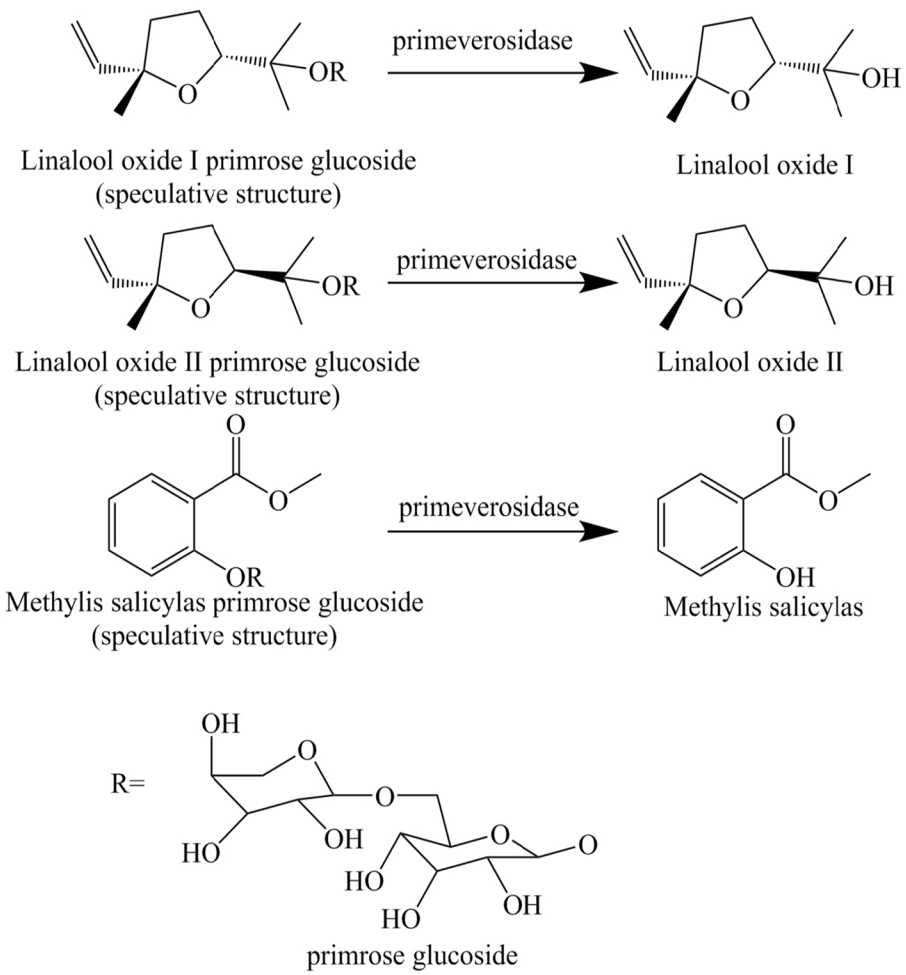

Fig. 9 Probable enzymatic hydrolysis pathway of primeverosides

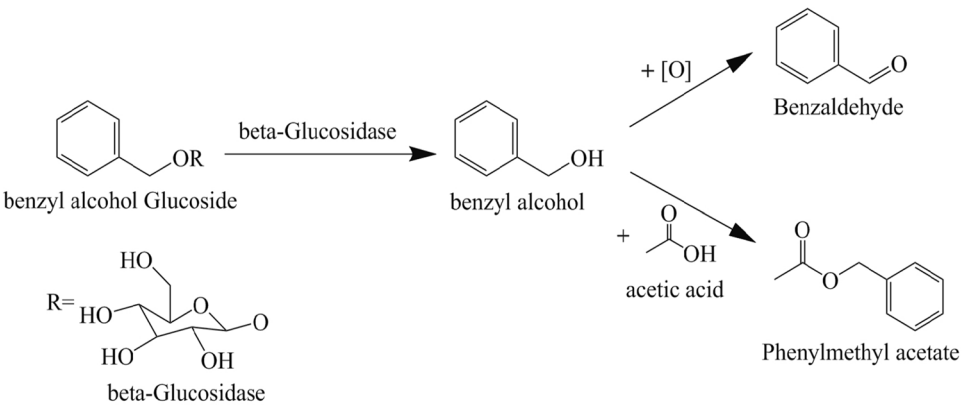

Fig. 10 Probable metabolism pathway of benzyl alcohol

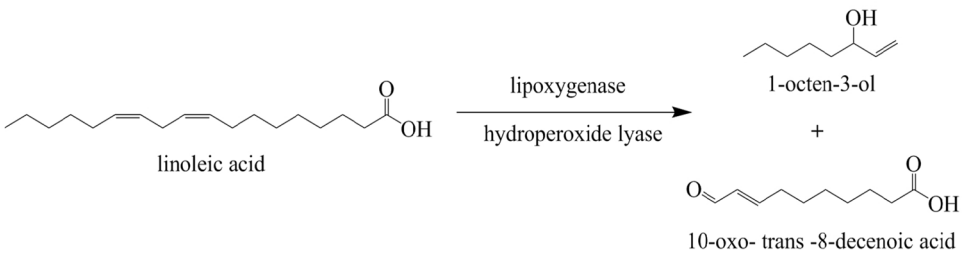

Fig. 11 Probable metabolism pathway of 1-octen-3-ol in tea

same relationship towards linalool and geraniol. The mixture of these three substances with a volume of ten times the threshold shows the main odour of 1-octen-3-ol rather than the other two. In Fig. 5, 1-octen-3-ol also has the highest aroma intensity (0.97), and when 1-octen-3-ol is mixed with β-ionone and geraniol, the correct recognition rate was only 0.86 and 0.85, respectively, and the result was consistent with the above hypothesis. This result means that 1-octen-3-ol has a higher aroma intensity than the other two with the same OAV, which is supported by Zhao's experiment [77]. This may be the main reason for SDBT lacking a floral fragrance. SDBT has a stronger oily fragrance than SFBT. This is mainly caused by nonanal (OAV = 525.81). However, the opposite was observed for *trans*,*trans*-2,4-heptadienal, which has a high OAV but does not show a characteristic aroma. This is caused by high volume of 1-octen-3-ol that enhances the intensity of the oily fragrance.

Among all the odour-active compounds, only epoxydihydrolinalool I and epoxydihydrolinalool II are described as having creamy aromas. Additionally, an olfactory experiment shows that only a slight creamy aroma was detected in SFBT. However, no such aroma was detected in SDBT, even though such odour-active compounds are present. This result might be caused by the difference in the odour-active rates, which can lead to changes in or the masking of the fragrance profile.

The experiments on mixtures of two single aroma substances showed that benzaldehyde, 1-octen-3-ol, methyl salicylate, *trans*,*trans*-2,4-heptadienal and nonanal showed stronger aromas that can easily be recognized (Fig. 6). The above five aroma components' intensities are 0.94, 0.97, 0.91, 0.90 and 0.89. This result indicates that those substances mentioned above provide greater contributions to the tea aroma.

Conclusions

There were 20 odour-active compounds among the volatile compounds of SFBT, while the number in SDBT was 21. 20 substances were the same in both teas, including β-ionone; linalool; benzyl acetate; β-cyclocitral; *cis*-3-hexenyl acetate; geranylacetone; hexyl hexanoate; benzaldehyde; (2,6,6-trimethyl-2-hydroxycyclohexylidene)acetic acid lactone; epoxydihydrolinalool I; epoxydihydrolinalool II; methyl salicylate; 1-octen-3-ol; 6-methyl-5-hepten-2-one; *trans*,*trans*-2,4-heptadienal; nonanal; α-terpineol; nerolidol and indole. These substances constituted the basic fragrance of dark tea.

What caused the 'fungi flower aroma' and 'aged fragrance' is the volume and OAV differences of those above odour-active compounds. 'Fungi flower aroma' showed a floral fragrance represented by β-ionone, a sweet fragrance represented by epoxydihydrolinalool II and methyl salicylate, and a fruity fragrance represented by geranylacetone. 'Aged fragrance' showed a fragrance of wood that is represented by nerolidol, a fruity fragrance represented by benzaldehyde and benzyl acetate, an fatty fragrance that is represented by nonanal and *trans*,*trans*-2,4-heptadienal, and a soil fragrance that is represented by 1-octen-3-ol.

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Author contributions CN was responsible for the sorting of the experimental data and proofreading and wrote the manuscript. XZ was responsible for the design, implementation of the tests and collection of raw data. LH revised the manuscript critically for important intellectual content. YG, XZ, CW were responsible for some experiments. XD provided design ideas, ensured the smooth progress of the tests and revised the outline and the manuscript.

Compliance with ethical standards

Conflict of interest There is no conflict of interest in this manuscript, which has been approved by all authors for publication. On behalf of my co-authors, I declare that this study is an unpublished original study and that it is not considered to be published in whole or in part elsewhere. All the authors listed approved the declaration.

Compliance with ethics requirements The study was approved by the local ethics committee in China.

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