Supporting Information

Characterisation of the phytochemical and bioactivity profiles of raw tea, stale-aroma, and betelnut-aroma type of Liupao tea through GC/LC-MS-based metabolomics

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Text S1. Specific experimental procedures of different assays for *in vitro* bioactivities evaluation of LPT.

Antioxidant activities

OH- radical scavenging capacity, DPPH radical scavenging capacity, and ABTS radical scavenging capacity were carried out according to the method reported in our previous study [1].

OH- radical scavenging capacity

According to the manufacturer's protocols, OH- radical scavenging activity was assessed with a hydroxyl free radical assay kit (Nanjing Jiancheng Biotechnology Co., Ltd, Nanjing, China). The amount of H_2O_2 is proportional to the amount of OH- produced by the Fenton reaction. The OH- free radical scavenging rate was calculated using the following equation:

$$\text{OH- radical scavenging rate (\%)} = \left[1-\frac{(A_1-A_2)}{A_0}\right]\times 100 \quad (1)$$

A_0 and A_1 represent the absorbance of the OH- radical solution without samples and reagent II (blank) and with samples and reagent II, respectively, while A_2 represents the absorbance of the samples' solution with ethanol.

DPPH radical scavenging capacity

100 μL of the LPT extract at various concentrations was mixed with 100 μL of ethanol DPPH solution (0.1 mM). The reaction began for 30 min in the dark at 25℃. The absorbance of solutions at 517 nm was measured. The DPPH radical scavenging rate was calculated using the following equation:

$$\text{DPPH radical scavenging rate (\%)} = \left[1-\frac{(A_1-A_2)}{A_0}\right]\times 100 \quad (2)$$

A_0 and A_1 represent the absorbance of the DPPH radical solution with ethanol (control sample) and with samples, respectively, while A_2 represents the absorbance of the sample solution without the DPPH radical solution.

ABTS radical scavenging capacity

ABTS+ working solution was prepared by 12 h of reaction of ABTS (7 mmol) with potassium persulfate (140 mM) at room temperature in the dark. Furthermore, the solution was diluted with ethanol solution until the absorbance was 0.700 ± 0.005 at 734 nm before use [2]. The depolarization assay started by mixing 200 μL the diluted ABTS+ working solution with 40 μL different concentrations of LPT extract. The ABTS radical scavenging rate was calculated using the following equation:

$$\text{ABTS radical scavenging rate (\%)} = \left[1-\frac{(A_1-A_2)}{A_0}\right]\times 100 \quad (3)$$

A_0 and A_1 represent the absorbance of the ABTS+ working solution with ethanol (control sample) and with samples, respectively, while A_2 represents the absorbance of the sample solution without the ABTS+ working solution.

Anti-inflammatory activity

Inhibition of bovine serum albumin (BSA) denaturation

The anti-inflammatory activity of three types of LPT extract was evaluated by the inhibition of the albumin denaturation technique with minor modification [3]. To 0.5 mL of the LPT extract at various concentrations, 0.5 mL of BSA solution (0.2%) prepared in phosphate buffer saline (PBS, pH 6.8) was added. The mixture was reacted at 37℃ for 15 min and then incubated at 70℃ for 5 min. The turbidity at 660 nm was recorded. The percentage of inhibition of BSA denaturation was calculated using the formula given below:

$$\text{Inhibition rate of denaturation (\%)} = \left[\frac{(A_0-A_1)}{A_0}\right]\times 100 \quad (4)$$

A_0 and A_1 are the absorbance of BSA with PBS and with the samples, respectively.

Hypoglycemic activities

Inhibition of α-amylase

α-Amylase inhibitory activity was performed according to the reported method with minor modifications [4]. Briefly, 50 μL of α-amylase solution (1 U/mL, in 0.1 M PBS) and 50 μL of sample solutions at different concentrations were mixed and preincubated at 37°C for 10 min. 100 μL of 2% starch solution was then added. After 10 min, 200 μL of DNS reagent was added. The absorbance at 540 nm was determined after the mixture was reacted in a 100°C water bath for 5 min. The inhibition rate of α-amylase was calculated as follows:

$$\text{Inhibition rate (\%)} = \left[1-\frac{(A_2-A_3)}{(A_0-A_1)}\right]\times 100 \quad (5)$$

A_0 and A_1 denote the absorbance values of the control and the control blank, respectively, while A_2 and A_3 are the absorbance values of the sample and the sample blank, respectively.

Inhibition of α-glucosidase

A total of 50 μL of 50 mM phosphate buffer solution (PBS, pH 6.8), 20 μL of α-glucosidase solution (1 U/mL), and 20 μL of tea sample at varying concentrations were mixed and preincubated at 37°C for 5 min. Then 20 μL of 1 mM PNPG (in 0.1 M PBS) was added. After the mixture was reacted for 30 min at 37°C, the reaction was stopped by adding 50 μL of 0.2 M Na_2CO_3. The absorbance was measured at 405 nm [5]. The inhibition rate of α-glucosidase was calculated as follows:

$$\text{Inhibition rate (\%)} = \left[1-\frac{(A_2-A_3)}{(A_0-A_1)}\right]\times 100 \quad (6)$$

A_0 and A_1 denote the absorbance values of the control and the control blank, respectively, while A_2 and A_3 are the absorbance values of the sample and the sample blank, respectively.

Hypolipidemic activities

Inhibition of pancreatic lipase

The *in vitro* inhibition rate of pancreatic lipase of LPT extract was measured according to the previously reported method with some modifications [6]. Briefly, a mixture comprised of 875 μL

of Tris buffer (0.25 M, pH 7.4), 100 μL of pancreatic lipase solution (10 mM), and 20 μL of tea samples of various concentrations was preincubated for 5 min at 37 °C, followed by addition of 5 μL of the pNPB (10 mM). Subsequently, the mixture was reacted at 37 °C for 2.5 min. The absorbance of the final mixture was measured at 405 nm. The inhibition activities of pancreatic lipase of LPT were calculated using the following formula:

$$\text{Inhibition rate (\%)} = \left[1-\frac{(A_1-A_2)}{A_0}\right]\times 100 \quad (7)$$

A_0 and A_1 indicate the absorbance of the pancreatic lipase solution with tris buffer and with samples, respectively, while A_2 represents the absorbance of the sample solution without the pancreatic lipase solution.

Bile salt binding capacity

The determination of bile salt binding capacity was performed according to a method with appropriate adjustments [7, 8]. Briefly, the bile salts, including sodium taurocholate (STC, 0.5 mM) and sodium glycocholate (SGC, 0.5 mM) were dissolved in 0.1 M PBS (pH 6.8), respectively. Then, 0.4 mL of LPT extract was mixed with 0.8 mL of PBS solution and 0.8 mL of bile salt solution. The mixture was incubated at 37°C for 2 h and then centrifuged at 4000 rpm/min for 20 min. Next, 0.5 mL of the supernatant was added to 1.5 mL of 60% H_2SO_4 to react for 20 min in a 70°C water bath. The absorbance values were measured at 387 nm after the mixture was placed in an ice bath for 5 min. The bile acid salt binding rate was calculated using the following formula:

$$\text{Bile salt binding rate (\%)} = \left[1-\frac{(A_1-A_2)}{A_0}\right]\times 100 \quad (8)$$

A_0 and A_1 indicate the absorbance of the bile salt solution with PBS and with samples, respectively, while A_2 represents the absorbance of the sample solution without the bile salt solution.

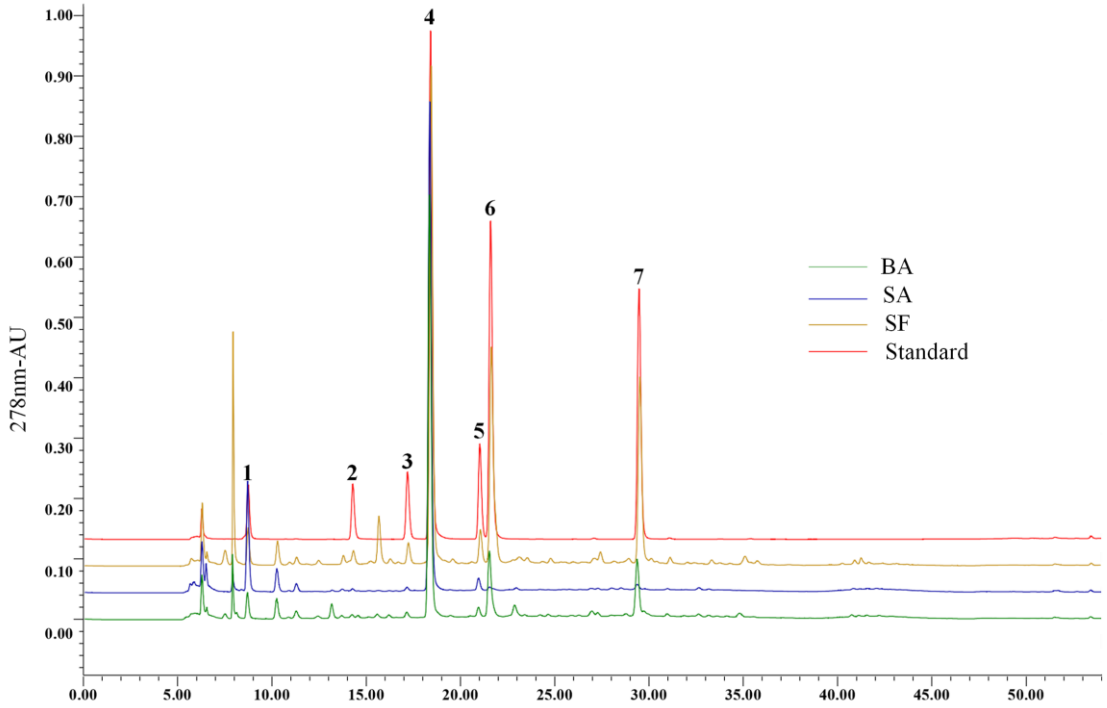
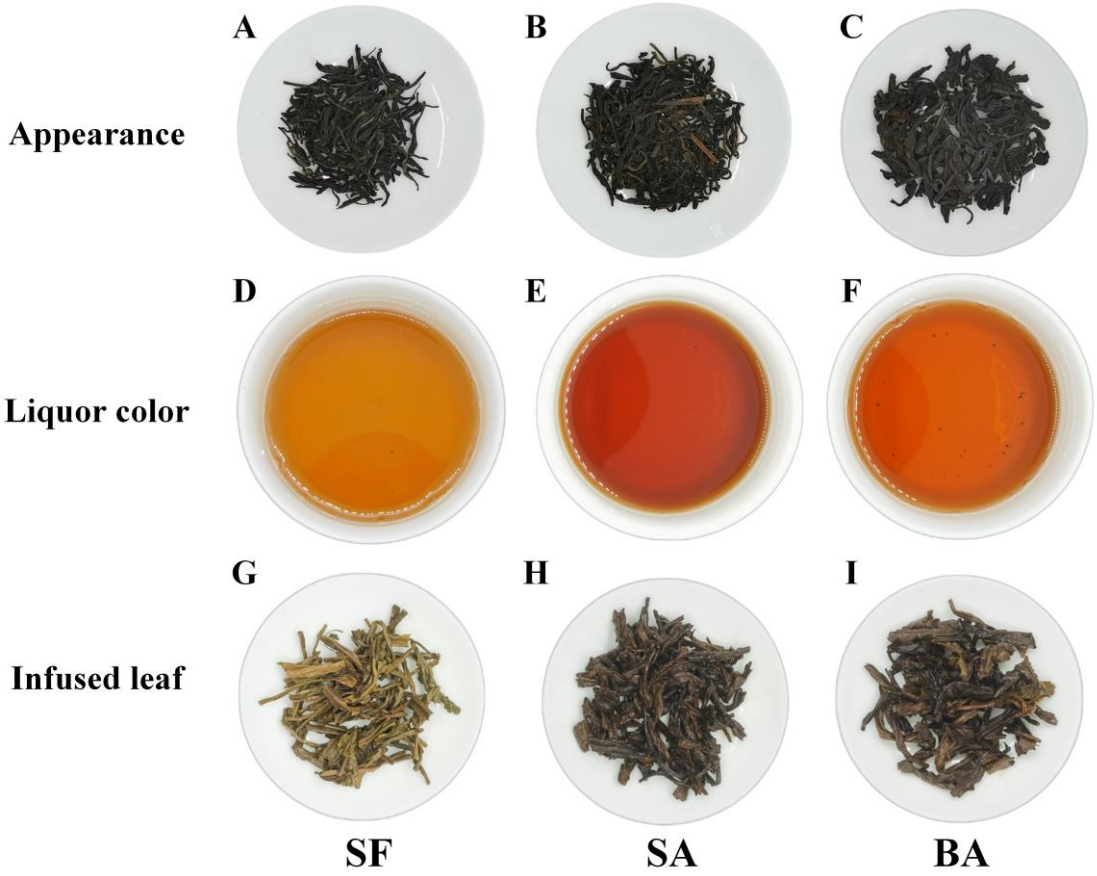


Figure S1. HPLC profiles of SF, SA, BA, and standards by the UV with detected wavelength at 278 nm. Standards: peak 1, gallic acid; peak 2, (−)-epigallocatechin; peak 3, (+)-catechin; peak 4, caffeine; peak 5, (−)-epicatechin; peak 6, (−)-epigallocatechin gallate; peak 7, (−)-epicatechin gallate. Note: data for SA and standards were obtained from our previous work [9].



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118 **Figure S2.** Colour of tea leaves and infusions of SF, SA, and BA. (A-C) Appearance of tea

119 leaves of SF, SA, and BA, respectively, (D-F) Tea infusions of SF, SA, and BA, respectively.

120 The tea infusions were prepared by the addition of 3 g tea leaves in 150 mL of boiling water,

121 which was then brewed for 2 min, (G-I) Infused leaves of SF, SA, and BA, respectively.

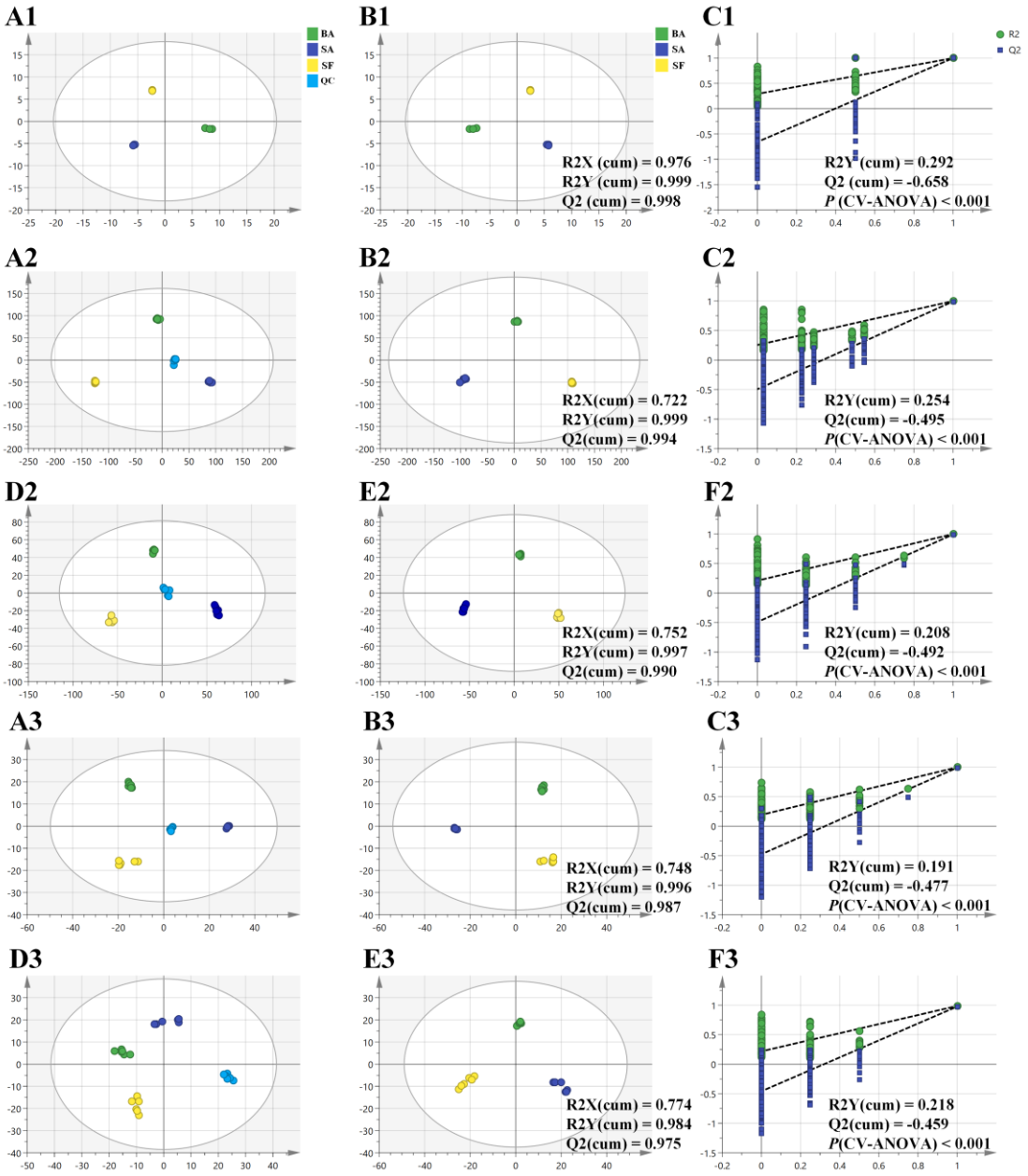

Figure S3. Multivariate statistical analyses of SF, SA, and BA. (A1-C1) PCA score plot, OPLS-DA score plot, and overfitting test plot among three groups by GC-MS analysis, respectively, (A2-F2) PCA score plot, OPLS-DA score plot, and overfitting test plot among three groups in ESI+ and ESI- modes by LC-MS-based metabolomics, respectively, (A3-F3) PCA score plot, OPLS-DA score plot, and overfitting test plot among three groups in ESI+ and ESI- modes by LC-MS-based lipidomics, respectively.

Table S1. The conditions for HPLC analyses of GA, catechins, and caffeine.

Time (min)	Flow rate (mL/min)	Mobile phase A (%)	Mobile phase B (%)
0.00	0.4	10	90
25.00	0.4	25	75
30.00	0.4	25	75
40.00	0.4	50	50
42.00	0.4	100	0
47.00	0.4	100	0
47.01	0.4	10	90
54.00	0.4	10	90

Note: Analytical column: Agilent ZORBAX Eclipse Plus C18 column (250 mm × 4.6 mm, 5 μm) at 35°C temperature. The mobile phase A was CH_3OH and mobile phase B was H_2O, both of which contained 0.1% formic acid. UV detection wavelength: 278 nm. Injection volume: 10 μL.

Table S2. The conditions for GC-MS analysis.

Gas chromatographic conditions	
Carrier gas	Helium (purity>99.999%)
Inlet temperature	250°C
Carrier gas flow rate	1.0 mL/min
Injection volume	1 μL
Temperature program	
Time (min)	Temperature (°C)
0.00	50
2.00	50
7.00	100
32.00	300
34.00	300
Mass spectrometry conditions	
Ionization energy	70 eV
Ion source temperature	230°C
Scan range	40-600 *m/z*
Solvent delay time	3.0 min

Table S3. Chromatographic conditions for metabolomic analysis.

Time (min)	Flow rate (mL/min)	Mobile phase A (%)	Mobile phase B (%)
0.00	0.4	95	5
2.00	0.4	95	5
6.00	0.4	70	30
20.00	0.4	0	100
24.00	0.4	0	100
25.00	0.4	95	5

Note: Analytical column: Waters Acquity UPLC BEH C18 (1.8 μm, 2.1mm × 100 mm) with 45°C column temperature. The mobile phases: A was ultrapure water and B was acetonitrile, both of which contained 0.1% formic acid. Injection volume: 5 μL.

Table S4. Chromatographic conditions for lipidomic analysis.

Time (min)	Flow rate (mL/min)	Mobile phase A (%)	Mobile phase B (%)
0.00	0.3	70	30
6.00	0.3	40	60
13.00	0.3	0	100
19.00	0.3	0	100
22.00	0.3	70	30

Note: Analytical column: Waters Acquity UPLC HSS T3 (1.8 μm, 2.1mm × 100 mm) at 45°C column temperature. The mobile phase A was acetonitrile-water (60:40, v/v), and mobile phase B was isopropanol-acetonitrile (90:10, v/v), both of which contained 0.1% formic acid and 10 mM ammonium formate. Injection volume: 5 μL.

145 **Table S5.** Mass spectrometric conditions for lipidomic and metabolomic analyses.

Main parameters	**Values**
Capillary voltage	3500 V
Sheath gas flow rate	50 psi
Auxiliary gas flow rate	13 arb
Capillary temperature	320°C
Auxiliary gas heater temperature	420°C
Capillary temperature	320°C
Scan range	100-1200 *m/z*
Scan mode	Full scan (resolution of 70,000) and data-dependent MS/MS (resolution of 17,500)

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147 **Table S6.** PerMANOVA tests of SF, SA, and BA (permutation: 999).

Analysis	ESI mode	*p* value		
		SA *vs.* SF	**BA *vs.* SF**	**BA *vs.* SA**
GC-MS-based metabolomics	-	2.50E-02	2.30E-02	2.90E-02
LC-MS-based metabolomics	ESI+	1.00E-03	6.00E-03	2.00E-03
	ESI-	3.00E-03	1.00E-03	4.00E-03
LC-MS-based lipidomics	ESI+	6.00E-03	2.00E-03	3.00E-03
	ESI-	4.00E-03	3.00E-03	3.00E-03

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149 **Table S7.** Identified VOCs among the three types of LPT by GC-MS analysis.

NO.	Compounds	Odor description	OT (mg/kg)	RI (Theoretical)	RI (Calculated)	ID	CAS
Alcohols							
1	Phytol	floral, balsam, waxy [a]	0.64000 [A]	2111	2114	MS, RI	150-86-7
2	Geraniol	sweet-rose, waxy, citrus [a]	0.00110 [A]	1252	1254	MS, RI	106-24-1
3	Cis-Linalool Oxide	earthy, floral, sweet, woody [a]	0.00600 [B]	1077	1077	MS, RI	5989-33-3
4	Cyclobutanol	-	4.60000 [A]	-	-	MS	2919-23-5
5	Linalool	citrus, orange, lemon, floral, waxy [a]	0.00022 [A]	1102	1103	MS, RI	78-70-6
6	Nerol	sweet natural neroli citrus magnolia [a]	0.68000 [A]	1230	1230	MS, RI	106-25-2
7	Cedrol	woody, floral, cedar, musk [a]	0.00050 [C]	1611	1611	MS, RI	77-53-2
8	α-Terpineol	citrus, woody, lemon, lime [a]	0.35000 [C]	1191	1194	MS, RI	98-55-5
9	2,6-Dimethylcyclohexanol	-	-	1114	1115	MS, RI	5337-72-4
10	Epicedrol	-	-	1607	1611	MS, RI	19903-73-2
Aldehydes							
11	Benzeneacetaldehyde	floral-hyacinth [a]	0.00400 [D]	1042	1049	MS, RI	122-78-1
12	Benzaldehyde	fruity [c]	0.30000 [A]	968	966	MS, RI	100-52-7
13	β-Homocyclocitral	-	-	1254	1265	MS, RI	472-66-2
14	β-Cyclocitral	tropical saffron herbal, rose, tobacco [a]	0.00500 [A]	1227	1226	MS, RI	432-25-7
15	Safranal	fresh, herbal, phenolic, metallic, rosemary, tobacco, spicy [a]	-	1201	1205	MS, RI	116-26-7

Hydrocarbon							
16	Neo-allo-ocimene	-	-	1838	1833	MS, RI	7216-56-0
17	Limonene	citrus [a]	0.03400 [A]	1030	1032	MS, RI	5989-27-5
18	1,1-Diphenylpropane	-	-	-	1608	MS	1530-03-6
19	Fluorene	-	-	1584	1589	MS, RI	86-73-7
20	1,6,7-Trimethylnaphthalene	-	-	1572	1568	MS, RI	2245-38-7
Ketones							
21	Hexahydrofarnesyl acetone	oily, herbal, jasmin, celery, woody [a]	-	1837	1841	MS, RI	502-69-2
22	Isophorone	cooling, woody, sweet, green, camphoraceous, fruity, musty [a]	11.00000 [A]	1127	1127	MS, RI	78-59-1
23	Geranylacetone	fresh green, fruity, waxy, rose, woody [a]	0.06000 [A]	1453	1454	MS, RI	3796-70-1
24	Farnesyl acetone	flower, ether [b]	-	1927	1921	MS, RI	1117-52-8
25	β-Ionone	woody, floral, berry [a]	0.00020 [D]	1491	1494	MS, RI	79-77-6
26	α-Ionone	woody, floral [a]	0.00040 [D]	1429	1431	MS, RI	127-41-3
27	Dihydro-β-ionone	woody, floral [a]	0.03100 [C]	1444	1443	MS, RI	17283-81-7
Methoxybenzenes							
28	Elemicin	spice, flower [b]	-	1554	1555	MS, RI	487-11-6
29	2-Methoxy-4-vinylphenol	spicy, powdery clove, woody, smoky, amber [a]	0.01202 [A]	1319	1320	MS, RI	7786-61-0
30	3,5-Dimethoxytoluene	-	-	1274	1271	MS, RI	4179-19-5
31	3,4-Dimethoxytoluene	-	-	1246	1239	MS, RI	494-99-5
32	1,2,3-Trimethoxy-5-methylbenzene	musty, earthy [a]	0.00445 [G]	1407	1401	MS, RI	6443-69-2
33	1,2,3-Trimethoxybenzene	stale, musty [d]	0.00075 [C]	1315	1315	MS, RI	634-36-6

34	1,2-Dimethoxybenzene	sweet, creamy, vanilla, musty [a]	0.00317 [G]	1150	1147	MS, RI	91-16-7
Phenols							
35	4-Propoxyphenol	-	-	-	1569	MS	18979-50-5
Ester							
36	Ethyl palmitate	waxy, fruity, creamy, milky, balsamic, greasy, oily [a]	2.00000 [A]	1984	1985	MS, RI	628-97-7
37	Methyl stearate	-	-	2125	2124	MS, RI	112-61-8
38	Ethyl linolenate	-	-	2169	2163	MS, RI	1191-41-9
39	Methyl linolenate	-	-	2100	2103	MS, RI	301-00-8
Furans							
40	2,5-Diformylfuran	-	100.00000 [E]	1084	1092	MS, RI	823-82-5
41	Dihydroactinidiolide	musk, coumarin [d]	0.00210 [F]	1539	1541	MS, RI	17092-92-1
Fatty acids							
42	Palmitic acid	waxy, creamy fatty [a]	1.00000 [F]	1958	1956	MS, RI	57-10-3
Purines							
43	Caffeine	-	-	1842	1852	MS, RI	58-08-2

Note: OT: odour thresholds in water, which were obtained from several reported literature: [A] reference[10]; [B] reference[11]; [C] reference[12]; [D] reference[13]; [E] reference[14]; [F] reference[15]; [G] reference[16]. Odour description was obtained from [a] http://www.thegoodscentscompany.com/; [b] http://www.flavornet.org/; [c] reference[13]; [d] reference[12]. The data were presented as the mean ± standard deviation (SD) (n = 3). Different letters in the same row indicate significant differences between the two groups (p<0.05). ND means the compounds were not detected.

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