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Release effect of aroma compounds of Keemun black tea brewed with deuterium-depleted water with different deuterium content

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ABSTRACT

Keemun black tea (KBT) is one of the four famous black teas in the world. This work was the first to explore the interaction between deuterium-depleted water (DDW) and black tea. In this study, stir bar sorptive extraction (SBSE) and gas chromatography-mass spectrometry were used to determine the content of volatile substances in KBT in DDW containing deuterium (D) at a concentration of 40 parts per million (ppm), 70 ppm and the control at 150 ppm. Results indicated that there were 20 compounds (aroma activity value (OAVs) $\geq$ 1) in KBT which were identified as important substances, of which there were eight key aroma compounds (OAVs $\geq$10). Compared with the content and OAVs of aroma substances in natural water, water with a deuterium content of 40 ppm promoted the release of aroma substances, while the other water inhibited their release. This study is expected to provide theoretical guidance for the application of DDW among tea connoisseurs.

1. Introduction

Deuterium-depleted water (DDW), also called light water, the deuterium content of which is lower than that in water in nature (Rehakova et al., 2016). A mixture of H_2O and D_2O forms natural water, natural levels of deuterium in natural water range from approximately 145 ppm–150 ppm (Friedman, Redfield, Schoen, & Harris, 1964), so the water is called DDW when the deuterium concentration is less than 0.015%. With different ratios of deuterium and hydrogen, the physico-chemical properties of water will also change. D_2O is denser and more viscous than H_2O and has higher melting and boiling points. The deuterium bonds in D2O are stronger than the analogous hydrogen bonds in H2O. (Mladin, Ciobica, Lefter, Popescu, & Bild, 2014; Sen et al., 2009). Industrial quantities of DDW were obtained by repeated evaporation in vacuumed distillation towers and according to European Standards the deuterium content of water was decreased commensurate with the tray number of the distillation tower (Boros, Somlyai, Zs, Kovács, & Somlyai, 2021). Numerous studies showed that DDW can inhibit the growth and reproduction of pancreatic cancer cells (Boros et al., 2021) and breast cancer cells (Yavari & Kooshesh, 2019), etc. It can also be used as an ancillary drug in general anti-cancer treatment (Zhang, Gaetani, Chernobrovkin, & Zubarev, 2019). A study showed that surface tension and viscosity anomalies of water were caused by the change of deuterium concentration, which leads to the formation of water clusters of different sizes and quantities (Goncharuk, Kavitskaya, Romanyukina, & Loboda, 2013). Therefore, the surface tension of DDW differs from that of natural water, which may lead to different effects related to the release of aroma substances. As a type of water with great benefits to the human body, no researchers have yet studied the effect of its use when making tea on the release of aroma substances therein.

As a commonly consumed drink, tea has a unique taste and imparts significant benefits to the human body. Black tea has the strongest and most popular aroma compared to green tea or other types of tea (Magagna et al., 2017). Keemun black tea, native to Qimen County, Anhui Province, China, has a production history of more than 100 years. It is one of the four famous black teas in the world. KBT is loved by consumers all over the world for its unique Qimen aroma. At present, the aroma components of diverse kinds of black tea have been investigated, the most frequently-used methods for extracting the volatile aroma components of black tea were solid phase extraction (Xiaohua Chen et al., 2019), simultaneous distillation extraction (Joshi & Gulati, 2015), headspace solid phase micro-extraction (Kang et al., 2019), stir bar sorptive extraction (SBSE) (Shi, Wang, Dong, Zhu, & Lv, 2021; J. C. Zhu, Zhu, Wang, Niu, & Xiao, 2021) and solvent-assisted flavor evaporation

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Table 1
Physical properties of waters (T = 25 ± 0.04 °C).

NO.	Parameter	Deuterium-depleted water D/H = 40 ppm	Deuterium-depleted water D/H = 70 ppm	Distilled water D/H = 150 ppm	Relative error
1	Surface tension, mN/m	71.85	70.80	72.57	0.10%
2	Density, g/cm^3	0.995866	0.996443	0.997240	0.04%
3	Viscosity, mm^2/s	0.9178	0.8742	0.8856	0.17%

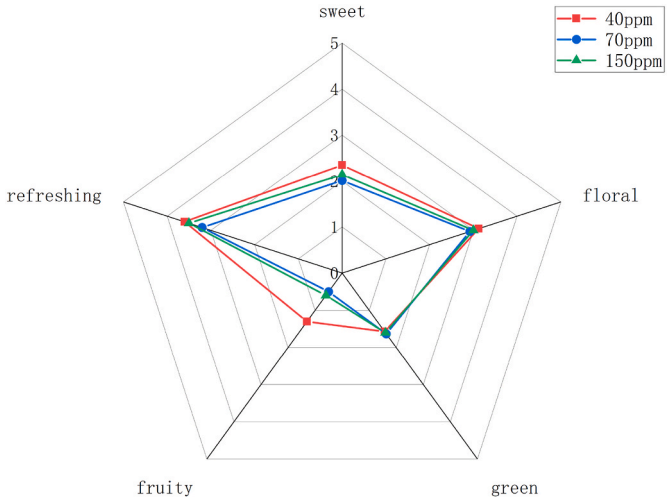

Fig. 1. Radar map of traditional sensory evaluation of five aromas of tea soup.

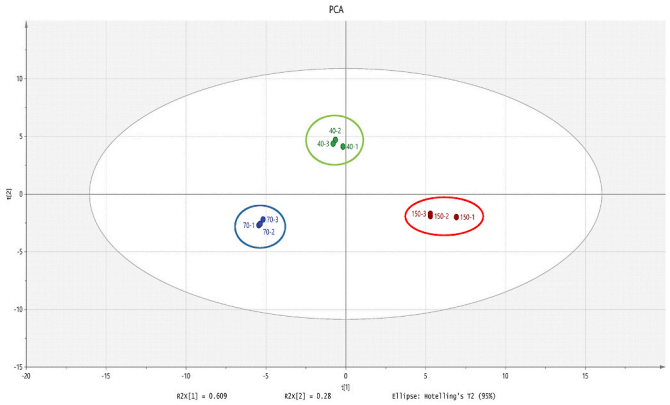

Fig. 2. Principal component analysis plots obtained from the electronic nose analysis data of the three tea soups.

(X. Chen et al., 2020). Compared with other methods, SBSE has the advantages of high sensitivity and excellent repeatability. The aroma substances identified by gas chromatography-mass spectrometer (GC-MS) include 1-octen-3-ol, linalool, phenylethyl alcohol, hexanal, benzeneacetaldehyde, methyl salicylate (Kang et al., 2019; Tao & Liu, 2019), etc. Studies have shown that, compared with the other three world-famous black teas, the key aroma compounds of Keemun black tea (KBT) were 1-octen-3-ol, linalool, hexamethylene alcohol, hexanal, phenylacetaldehyde, nonanal, etc., especially phenylacetaldehyde, which was proved to be unique (as an odorant) due to its significantly higher aroma activity value (OAV) in KBT (Kang et al., 2019). There were also findings that linalool, phenylacetaldehyde, 2-pentylfuran and methyl salicylate were key volatile compounds in the aroma of KBT, and the PLS model indicated that geraniol, linalool and salicylic acid methyl ester positively affected Keemun aroma, while (Z)-nerol, β-ionone, *cis*-3-hexenylisovalerate and hotrienol negatively affected Keemun aroma in a concentration-dependent manner (Su, He, Zhou, Li, & Zhou, 2022). The storage period and production area of KBT were also considered to exert a significant influence on its aroma and composition (Fang et al., 2019; Meng et al., 2021). In addition, the brewing conditions of black tea also have important effects on the release of its aroma components, such as water quality, temperature and brewing time (Tao & Liu, 2019). Therefore, brewing black tea with DDW may also affect the aroma of black tea.

In the long process of terrestrial evolution, organisms have adapted to the proportion of relatively stable hydrogen isotopes in natural water. Some studies have shown that deuterium can activate or inhibit biochemical processes in organisms (Syroeshkin, Antipova, Zlatska, Zlatskiy, & Goncharuk, 2018). Therefore, when the content of deuterium changes, the organism will change accordingly, so the ratio of protons, deuterium and tritium in water will affect the various indices of the organism in different ways (Goncharuk et al., 2018). In the process of brewing black tea with DDW, some substances in black tea will also undergo unknown changes under the influence of the deuterium content.

The purpose of this study was to evaluate the effects of DDW on the release of key aroma substances in KBT compared to that of natural water. Two types of DDW (D-concentration is 40 ppm and 70 ppm respectively) were used in this study, and distilled water (D-concentration is 150 ppm) was selected as the control sample. This study utilized SBSE and GC-MS to identify volatile compounds in different grades of KBT, and using multiple experimental methods, including odor activity values, combined with GC-O to screen the key aroma compounds of KBT, and the key aroma substances were accurately quantified by establishing a standard curve, so as to explore the different release effects of aroma substances with greater aroma contribution in DDW.

2. Material and methods

2.1. Samples

After pre-experiment, two types of DDW with the most significant effect were selected for formal experiment. Two types of water with different deuterium contents (40 ppm, 70 ppm) used in the study were procured from Shanghai Research Institute of Chemical Industry. Distilled water (in natural water, the deuterium concentration is about 150 ppm, this served as a control in the present research) was manufactured by Watsons Water Company, Inc. (Guangzhou, China). The requisite D-concentration for this experiment (40 ppm, 70 ppm) was prepared by mixing DDW with lower D-concentration with distilled water of 150 ppm D-concentration. Distilled water and DDW have no differences in physical (except small changes in mass) or chemical characteristics or in trace element composition, except the deuterium content, which excludes the multifactor influence in the system for all comparison groups. KBT was purchased from Anhui Keemun Black Tea Development Co., Ltd, which was a new tea produced in 2021. The KBT specimens were sealed and stored (at 5 °C) in the refrigerator for analysis. All types of water were kept in a clean, odor-free environment.

2.2. Chemicals

All reference compounds were purchased from commercial sources. Methylbutanal (≥98%), hexanal (≥97%), β-myrcene (≥90%, GC standard), D-limonene (≥95%), (E)-2-hexenal (≥98%), β-ocimene (≥98%), 1-pentanol (≥98.5%), 6-methyl-5-hepten-2-one (≥98%), 1-hexanol (≥98%), 2-nonanone (≥99%), furfural (≥98%), benzaldehyde (≥98.5%), 1-octanol (≥99%), 5-methyl furfural (≥99%),

Table 2
SBSE GC-O identified odor compounds in KBT with the Method of AI.

NO.	Compounds	RI[a]		AI[c]			Aroma Description	Identification[b]
		Innowax	DB-5	40 ppm	70 ppm	150 ppm		
1	2-methylbutanal	925	646	3.4 ± 0.2	1.9 ± 0.1	3.0 ± 0.3	Musty, malty	AD, RI, STD
2	3-methylbutanal	929	659	1.2 ± 0.3	1.3 ± 0.2	–	Chocolate, fatty	AD, RI, STD
3	Hexanal	1076	802	3.3 ± 0.3	1.8 ± 0.3	1.7 ± 0.3	Green, grass, leafy	AD, RI, STD
4	(E)-2-Methyl-2-butenal	1087	754	4.0 ± 0.2	3.7 ± 0.3	1.8 ± 0.3	Green, fruit	AD, RI, STD
5	β-Myrcene	1170	995	6.0 ± 0.5	4.9 ± 0.3	5.1 ± 0.5	Woody, peppery	AD, RI, STD
6	D-Limonene	1188	1031	3.1 ± 0.3	2.9 ± 0.1	2.4 ± 0.3	Citrus, orange, fresh	AD, RI, STD
7	2-Heptanone	1190	901	–	–	2.2 ± 0.3	Fruity, woody	AD, RI, STD
8	(E)-2-Hexenal	1216	857	2.3 ± 0.3	1.8 ± 0.4	3.0 ± 0.3	Green	AD, RI, STD
9	β-Ocimene	1237	–	–	–	1.4 ± 0.1	Green, woody	AD, RI, STD
10	1-Pentanol	1246	769	3.7 ± 0.3	1.8 ± 0.4	2.1 ± 0.2	Oil, sweet	AD, RI, STD
11	6-Methyl-5-hepten-2-one	1321	977	1.9 ± 0.1	1.0 ± 0.2	1.2 ± 0.1	Citrus, peppery	AD, RI, STD
12	1-Hexanol	1334	864	–	–	2.5 ± 0.1	Fruity, green	AD, RI, STD
13	2-Nonanone	1356	1082	–	1.7 ± 0.3	1.7 ± 0.2	Fresh, green, earthy	AD, RI, STD
14	(Z)-3-Hexen-1-ol	1363	867	–	1.0 ± 0.0	2.6 ± 0.2	Fresh, green, herbal	AD, RI, STD
15	(E,E)-2,4-Hexadienal	1380	–	2.8 ± 0.3	–	2.2 ± 0.3	Green, spicy	AD, RI, STD
16	1-Nonanal	1402	1096	3.5 ± 0.1	–	–	Rose, orange, fatty	AD, RI, STD
17	1-Octen-3-ol	1422	983	–	1.1 ± 0.1	–	Mushroom, earthy, green	AD, RI, STD
18	1-Heptanol	1437	977	3.5 ± 0.2	2.9 ± 0.3	3.3 ± 0.3	Musty, leafy	AD, RI, STD
19	Furfural	1443	842	4.3 ± 0.3	5.7 ± 0.3	1.9 ± 0.2	Sweet, woody	AD, RI, STD
20	2-Ethyl-1-hexanol	1479	1039	4.0 ± 0.4	3.6 ± 0.3	3.2 ± 0.1	Citrus, fresh	AD, RI, STD
21	(E,E)-2,4-Heptadienal	1486	1006	3.0 ± 0.3	1.1 ± 0.1	1.0 ± 0.2	Fatty, green	AD, RI, STD
22	(3E,5E)-3,5-Octadien-2-one	1492	1102	–	5.2 ± 0.3	3.0 ± 0.1	Fruity, green	AD, RI, STD
23	Benzaldehyde	1520	995	4.7 ± 0.3	3.1 ± 0.4	1.2 ± 0.2	Almond, bitter	AD, RI, STD
24	Linalool	1544	1092	5.2 ± 0.4	6.9 ± 0.5	5.6 ± 0.1	Rose, woody, citrus	AD, RI, STD
25	1-Octanol	1551	1081	3.7 ± 0.3	2.8 ± 0.4	2.7 ± 0.2	Green, orange	AD, RI, STD
26	5-Methyl furfural	1562	977	6.4 ± 0.4	2.4 ± 0.6	3.3 ± 0.2	Caramel, maple	AD, RI, STD
27	γ-Butyrolactone	1618	913	3.4 ± 0.2	2.0 ± 0.5	2.7 ± 0.1	Creamy, fatty	AD, RI, STD
28	Benzeneacetaldehyde	1643	1046	6.7 ± 0.3	4.1 ± 0.4	5.6 ± 0.2	Green, sweet	AD, RI, STD
29	1-Nonanol	1662	–	–	1.0 ± 0.0	–	Fatty, floral	AD, RI, STD
30	Ethyl benzoate	1681	–	–	–	1.0 ± 0.1	Musty, wintergreen	AD, RI, STD
31	α-Terpineol	1702	1196	7.4 ± 0.3	3.0 ± 0.4	3.2 ± 0.3	Floral, terpenic	AD, RI, STD
32	Benzyl acetate	1724	1165	3.3 ± 0.2	3.7 ± 0.3	2.5 ± 0.2	Floral, fruity	AD, RI, STD
33	Citral	1733	1183	3.3 ± 0.2	2.8 ± 0.3	2.2 ± 0.2	Sharp, lemon, sweet	AD, RI, STD
34	Methyl salicylate	1769	1204	3.6 ± 0.3	4.8 ± 0.3	6.6 ± 0.4	Wintergreen, mint	AD, RI, STD
35	Nerol	1775	1228	1.3 ± 0.2	1.5 ± 0.1	1.1 ± 0.2	Sweet, natural, citrus	AD, RI, STD
36	Geraniol	1833	1237	3.4 ± 0.1	3.2 ± 0.4	3.2 ± 0.3	Sweet, floral, citrus	AD, RI, STD
37	Geranylacetone	1854	1449	4.9 ± 0.4	4.7 ± 0.3	–	Green, fruity	AD, RI, STD
38	Benzyl alcohol	1882	1034	3.3 ± 0.3	5.5 ± 0.3	6.3 ± 0.2	Floral, phenolic	AD, RI, STD
39	Phenylethyl alcohol	1897	1113	6.2 ± 0.3	4.4 ± 0.5	4.6 ± 0.2	Floral, rose	AD, RI, STD
40	β-Ionone	1953	1493	3.2 ± 0.1	2.3 ± 0.3	3.0 ± 0.0	Woody, floral	AD, RI, STD
41	2-Acetyl pyrrole	1968	1065	0.9 ± 0.1	3.6 ± 0.4	1.7 ± 0.3	Nut, bready	AD, RI, STD
42	gamma-Nonanolactone	2214	–	–	4.8 ± 0.3	–	Coconut, creamy	AD, RI, STD
43	Benzoic acid	2445	1189	3.2 ± 0.3	0.9 ± 0.3	2.3 ± 0.3	Balsam	AD, RI, STD

[a] Retention index of compounds on DB-5 and DB-Wax columns.
[b] Method of identification: AD, aroma descriptor; RI, retention index; STD, confirmed by authentic standards.
[c] The aroma intensity was evaluated by GC-O.

benzeneacetaldehyde (≥95%), ethyl benzoate (≥98%), α-terpineol (≥97%), benzyl alcohol (≥99%), phenylethyl alcohol (≥98%) and gamma-nonanolactone (≥98%) were purchased from Meryer (Shanghai) Chemical Technology Co., Ltd. Benzoic acid (≥99%) and (E)-2-methyl-2-butenal (≥95%, GC standard) were obtained from TCI (Shanghai) Chemical Industry Development Co., Ltd. 3-methylbutanal (≥98%), 2-heptanone (≥99%), (Z)-3-hexen-1-ol (≥99%), (E,E)-2,4-hexadienal (≥95%), 1-nonanal (≥95%), 1-octen-3-ol (≥98%), 1-heptanol (≥99%), 2-ethyl-1-hexanol (≥99%), (E,E)-2,4-heptadienal (≥98%), (3E,5E)-3,5-octadien-2-one (≥97%), linalool (≥98%), γ-butyrolactone (≥96%), 1-nonanol (≥98%), benzyl acetate (≥99%), citral (≥95%), methyl salicylate (≥99%), nerol (≥95%), geraniol (≥98%), geranylacetone (≥98%), β-ionone (≥96%), 2-acetyl pyrrole (≥98%), N-alkanes (C_6 to C_30) and ethyl decanoate (≥98%) were purchased from Titan Technology (Shanghai, China).

2.3. Extraction of volatile compounds

The experimental conditions were established with reference to the literature (Shi et al., 2021; Su et al., 2022) with some adjustments. Accurate 1 g of a tea and NaCl (0.3 g) were infused with 50 mL freshly boiled water (DDW with a deuterium content of 40 ppm, 70 ppm and 150 ppm, respectively), and filtered after brewing for 5 min. Some 10 g of tea sample, 20 μL of ethyl decanoate (50 μg/mL) as an internal standard, and stirring bar (10 mm/0.5 mm, ACAR/PDMS, 24 μL capacity, Gerstel, Germany) were placed in a 20 mL sealed vial. The stirring bar was adopted to stir at 900 rpm at 30 °C. After 90 min of extraction, the bar was removed from the sample vial and gently wiped with a clean lint-free tissue to remove any water, followed by loading it into a pyrolysis pipette (180 mm length, 4 mm OD, 3 mm ID, Gerstel) for a GC-MS analysis. The thermos-desorption system (TDS3, GERSTEL, Germany) was operated in the solvent vent mode for the first 3 min and splitless mode thereafter. The transfer capillary temperature was kept constant at 280 °C. Desorption temperature was programmed from 40 °C (equilibrium for 1 min) to 260 °C (held at this temperature for 5 min) at 60 °C/min under a helium flow (75 mL/min).

2.4. Determination of physical properties of DDW and distilled water

Three types of water (40 ppm, 70 ppm, and 150 ppm) were prepared for the detection of surface tension, density and viscosity. Density and viscosity of water was measured by Viscometer Falling ball (Lovis 2000

Table 3
Identification concentration of important aroma compounds (OAV ≥1) in Keemun Black Tea.

NO.	Compounds[a]	identification	quantitative ions	Standard curve						Concentration[b](μg/kg)			RSD[c]		
				40 ppm	R^2	70 ppm	R^2	150 ppm	R^2	40 ppm	70 ppm	150 ppm	40 ppm	70 ppm	150 ppm
1	2-methylbutanal	RI, MS, STD	57	y = 0.5803x-0.0368	0.9969	y = 0.2843x+0.0033	0.9990	y = 0.4638x+0.0014	0.9999	32.13	20.23	28.40	3.00%	7.81%	6.69%
2	Hexanal	RI, MS, STD	44	y = 0.1901x+0.0037	0.9912	y = 1.1376x-0.0013	0.9940	y = 1.0526x+0.0073	0.9998	836.50	57.45	150.54	1.16%	9.06%	1.32%
3	(E)-2-Methyl-2-butenal	RI, MS, STD	84	y = 0.2824x+0.0018	0.9994	y = 0.2750x+0.0214	0.9934	y = 0.6014x+0.0122	0.9976	71.18	7.49	14.73	1.15%	8.26%	7.20%
4	β-Myrcene	RI, MS, STD	93	y = 0.2212x-0.2271	0.9897	y = 0.6011x+0.0813	0.9974	y = 0.2906x+0.0923	0.9940	1619.40	156.15	782.23	1.18%	7.73%	8.61%
5	(E)-2-Hexenal	RI, MS, STD	41	y = 0.1332x+0.0056	0.9992	y = 0.8139x+0.0038	0.9845	y = 0.2861x+0.0293	0.9810	457.97	25.38	371.64	7.06%	8.57%	3.64%
6	β-Ocimene	RI, MS, STD	93	y = 0.1002x-0.0018	0.9846	y = 1.1748x-0.1199	0.9969	y = 0.4439x-0.1048	1.0000	193.91	21.89	82.39	1.16%	7.10%	6.66%
7	1-Pentanol	RI, MS, STD	42	y = 0.0766x+0.0073	0.9949	y = 0.3816x+0.0283	0.9871	y = 0.2929x-0.0017	0.9997	547.93	17.82	163.62	5.43%	8.90%	2.60%
8	1-Hexanol	RI, MS, STD	56	y = 0.2080x+0.0041	0.9965	y = 1.1929x+0.0005	0.9997	y = 0.6096x-0.0136	0.9995	125.13	5.38	31.38	6.92%	10.92%	6.10%
9	(E,E)-2,4-Hexadienal	RI, MS, STD	81	y = 0.2721x+0.0036	0.9967	y = 0.4772x-0.0811	0.9877	y = 1.0347x-0.0177	0.9970	71.79	44.64	19.79	7.28%	8.95%	3.00%
10	1-Octen-3-ol	RI, MS, STD	57	y = 0.5915x+0.0025	0.9967	y = 0.9764x-0.0138	0.9994	y = 0.7128x+0.0097	0.9928	22.82	19.53	9.38	10.10%	3.71%	9.89%
11	(E,E)-2,4-Heptadienal	RI, MS, STD	81	y = 0.6859x-0.0388	0.9849	y = 0.5475x-0.0130	0.9999	y = 1.1763x-0.2151	0.9650	63.58	14.53	62.22	9.92%	9.36%	8.72%
12	(3E,5E)-3,5-Octadien-2-one	RI, MS, STD	95	y = 0.8235x+0.0039	0.9982	y = 0.8563x+0.0102	0.9899	y = 1.1172x-0.1264	0.9935	65.55	15.61	46.56	8.87%	6.93%	9.69%
13	Linalool	RI, MS, STD	71	y = 0.4480x-0.1446	0.9880	y = 1.1656x-0.0454	0.9946	y = 0.4452x+0.0269	0.9844	271.02	34.16	137.43	5.77%	8.43%	3.13%
14	Benzeneacetaldehyde	RI, MS, STD	91	y = 1.15689x-0.2115	0.9695	y = 2.8315x-0.5850	0.9578	y = 2.1595x-0.5968	0.9999	30.15	36.69	55.72	8.41%	7.84%	6.75%
15	Citral	RI, MS, STD	69	y = 0.1974x+0.0007	0.9899	y = 0.1316x+0.0038	0.9905	y = 0.1874x+0.0089	0.9902	117.16	36.37	69.00	5.80%	9.30%	7.31%
16	Methyl salicylate	RI, MS, STD	120	y = 0.7411x+0.0560	0.9875	y = 1.7675x-0.3438	0.9811	y = 0.9103x-0.0352	0.9948	200.89	66.75	148.03	8.49%	6.79%	2.00%
17	Geraniol	RI, MS, STD	69	y = 0.2914x-0.2878	0.9876	y = 0.6836x-0.1951	0.9941	y = 0.3013x-0.0881	0.9936	1087.67	159.34	427.42	7.36%	4.43%	1.79%
18	Benzyl alcohol	RI, MS, STD	79	y = 0.2488x-0.1465	0.9849	y = 0.6695x-0.0188	0.9936	y = 0.0593x+0.0212	0.9895	4685.58	110.99	2312.49	2.66%	8.40%	4.76%
19	Phenylethyl alcohol	RI, MS, STD	91	y = 0.0917x+0.0899	0.9976	y = 0.4988x+0.0359	0.9891	y = 0.1700x+0.0547	0.9901	2816.16	149.36	864.66	4.80%	3.64%	4.52%
20	β-Ionone	RI, MS, STD	177	y = 0.115x-0.0174	0.9420	y = 0.8395x-0.0738	0.9732	y = 0.3339x+0.0032	0.9880	84.55	17.53	9.70	9.26%	7.10%	7.43%

[a] Annotation: The identification methods of aroma compounds were MS, RI, and Std. MS, mass spectrometry; RI, retention index of each compound on HP-Innowax and DB-5 column. Std, pure authentic standards.
[b] average concentration of triplicates.
[c] RSD, relative standard deviation.

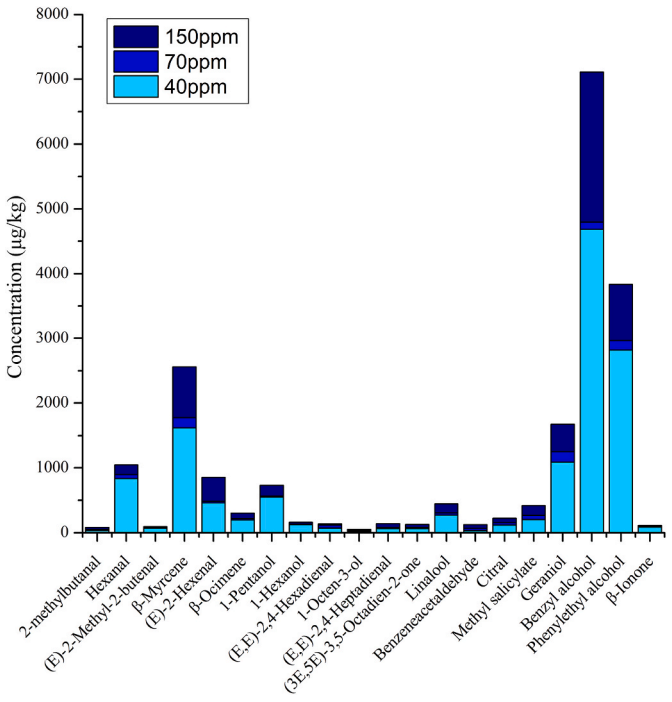

Fig. 3. Concentrations of 20 substances of KBT in three types of water.

M/ME). Surface tension of water was determined by pendant drop method on Surface Tensiometer (LAUDA Scientific GmbH,LSA-100).

2.5. GC-MS analysis

GC–MS (7890 GC-5973C MSD, Agilent, Santa Clara, California, USA) was used in this study to determine the volatile compounds of KBT brewed with three different types of waters and a quantitative analysis of key aroma compounds. The two chromatographic columns used in the present study were an HP-Innowax (60 m × 0.25 mm × 0.25 μm) and DB-5 (60 m × 0.25 mm × 0.25 μm) devices. The flow rate of the GC carrier gas (helium, 99.999%) was 2 mL/min. The heating program was set as follows: the initial temperature was 40 °C which was held for 3 min, the temperature was increased to 120 °C at a rate of 4 °C/min, and then to 230 °C at a rate of 10 °C/min and held for 10 min. The scan range of ions was set to 30 to 400. The inlet temperature was 250 °C, the ion source temperature was 230 °C, and the quadrupole temperature was 150 °C. The analysis was conducted three times. Experimental data were screened and identified in NIST11 and NIST17 database, then the RI value of the aroma substance was calculated based on the retention time of the n-alkane standard (C_6 to C_{30}) (Xu et al., 2019), facilitated the screening of certain of the aroma substances.

2.6. GC-O analysis

GC-O analysis required the use of an Agilent 7890A chromatograph equipped with a flame ionization detector (FID) and sniff detection port (ODP-3, Gerstel, Germany) (coefficients equal to those used in GC-MS). The aroma substances flowing from the end of the capillary tube of the chromatograph are distributed between the FID and the sniffing port in a 1:1 ratio. The sniffing port temperature increases to 250 °C and remains unchanged thereafter. The length from the Y-shaped separator to the sniffing port is 1070 mm. The moist air flows into the sniffing port at a rate of 50 mL/min, which can remove the residual aroma of the sniffing port and moisten the nasal cavity of the sensory person, so as to obtain more accurate sensory data. The two column parameters and temperature program used in the experiment are the same as those used during GC-MS.

The experiment was conducted by six professional sensory panelists (two males and four females aged between 22 and 25) each of whom came from the scientific research laboratory of the School of Perfume and Aroma Technology of Shanghai Institute of Technology (Shanghai, China), with more than four years of professional sensory experience each. Before the formal experiment, it is necessary to train the sensory personnel on the aroma intensity assessment protocol; we prepare 1-butanol with a total of 10 intensity levels of 1–10 as the intensity reference, and the concentrations are 10 ppm, 20 ppm, 40 ppm, …, 5120 ppm, among which “1” implies that the aroma is very weak, “5”

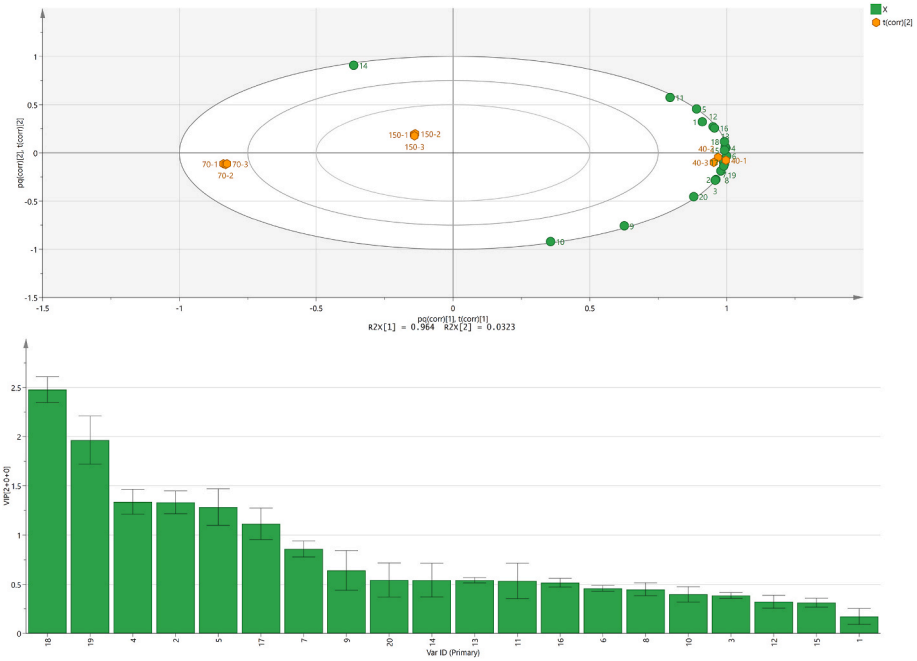

Fig. 4. OPLS-DA (the 20 numbers in the figure correspond to the 20 compounds in Table 2 in order).

Table 4
Odor Threshold and OAV in KBT of three kinds of waters.

NO	Compounds	Threshold (μg/kg)			OAV		
		40 ppm	70 ppm	150 ppm	40 ppm	70 ppm	150 ppm
1	2-methylbutanal	8.847	14.748	10.859	3.632	1.372	2.615
2	Hexanal	8.422	13.756	7.860	99.327	4.176	19.154
3	(E)-2-Methyl-2-butenal	5.493	8.997	5.326	12.959	0.832	2.766
4	β-Myrcene	20.441	25.966	21.928	79.224	6.014	35.673
5	(E)-2-Hexenal	74.370	91.770	70.146	6.158	0.277	5.298
6	β-Ocimene	42.121	53.827	45.572	4.604	0.407	1.808
7	1-Pentanol	157.761	178.608	146.893	3.473	0.100	1.114
8	1-Hexanol	9.513	19.324	10.950	13.154	0.278	10.950
9	(E,E)-2,4-Hexadienal	12.826	15.787	13.690	5.597	2.828	1.446
10	1-Octen-3-ol	3.969	5.333	4.530	5.749	3.662	2.071
11	(E,E)-2,4-Heptadienal	52.893	73.621	66.665	1.202	0.197	0.933
12	(3E,5E)-3,5-Octadien-2-one	6.155	9.078	7.892	10.651	1.720	5.899
13	Linalool	17.620	24.946	19.861	15.382	1.369	6.920
14	Benzeneacetaldehyde	13.999	19.378	6.904	2.154	1.893	8.071
15	Citral	56.234	64.565	67.608	2.083	0.563	1.021
16	Methyl salicylate	60.298	100.300	60.814	3.332	0.666	2.434
17	Geraniol	31.398	70.081	46.989	34.642	2.274	9.096
18	Benzyl alcohol	191.690	494.311	347.536	24.443	0.225	6.654
19	Phenylethyl alcohol	72.444	91.201	87.096	38.874	1.638	9.928
20	β-Ionone	12.677	13.677	13.274	6.670	1.282	0.731

denotes that the aroma intensity is moderate, and "10" represents that the aroma intensity is very strong (Atanasova, Langlois, Nicklaus, Chabanet, & ETIÉVANT, 2010; Y. Niu, Deng, Xiao, & Zhu, 2021; J. Zhu, Niu, & Xiao, 2020). After two weeks of training, the sensory personnel became familiar with the aroma intensity and the experiment officially started. During GC-O analysis, the sensory personnel recorded the retention time, scored the aroma intensity (AI), and described the odor characteristics when they detected the odor. Each experiment was performed by two members in turn to avoid olfactory fatigue. Panelists repeated the experiment three times.

2.7. Standard curves

In this study, aroma compounds in three waters were conducted quantitatively analyzed by establishing a standard curve method. A stock solution containing all the standards was prepared using ethanol as a solvent and diluted with water with three different deuterium contents to seven standards of different concentrations. During the experiment, 10 g of standard solution, 0.1 g of NaCl and 20 μL of ethyl decanoate (50 μg/mL) were added to a 20 mL sample bottle. The extraction conditions were the same as those described in Section 2.3, and each experiment was repeated three times. The abscissa represents the ratio of the compound concentration to the internal standard concentration, and the ordinate is the ratio of the compound peak area to the internal standard peak area, and the standard curve was fitted (Seo & Baek, 2005; Wu, Pan, Qu, & Duan, 2009).

2.8. OAV calculation and sensory evaluation

The OAV is the ratio of the concentration of volatile aroma substances to the threshold of the substance, which can evaluate the contribution of aroma substances to the overall aroma of black tea (Caliari, Burin, Rosier, & Bordignonluiz, 2014).When the OAV >1, the aroma substances can be smelt. The larger the OAV, the greater its contribution to the aroma, allowing for the screening of key volatile aroma substances. In this experiment, three different water substrates with different deuterium contents were configured to measure the threshold of substances, and the release effects of three water substrates were observed. The threshold of substance was determined by a three-point matching method (Michael, Martin, Monika, Anja, & Michael, 2008). There are 14 sensory panelists (seven men and seven women, and their average age is 24 years); the panelists work in the research studio of the Perfume and Aroma Technology of Shanghai Institute of Technology (Shanghai, China), and have all undergone professional training.

KBT and boiling water were mixed in a 1:50 ratio, and 5 min later, the tea was filtered. The sensory panel would undertake traditional sensory assessments of the three teas obtained by KBT, rating them on a scale of 0–5 for floral, fruity, sweet, green, and light scents.

2.9. Statistical analysis

Results from three types of water were initially analyzed using the function of electronic nose V12.44 (Alpha M.O.S.,Toulouse, France) to confirm whether there were significant differences between them. SPSS v26.0 software (SPSS, Chicago, IL, USA) was used to analyze the quantitative results of volatile aroma compounds from three different waters by single factor analysis of variance. Origin 9.0 software (OriginLab Corporation, Northampton, MA, USA) was used to create line and column charts. The correlation between deuterium content and volatile aroma compounds in water was revealed by a clearer and more accurate principal component analysis of electronic nasal data using SIMCA software. Orthogonal partial least squares discriminant analysis (OPLS-DA) was conducted using SIMCA 14.1 (Umetrics, Sweden).

3. Results and discussion

3.1. Differences in physical properties of three types of waters

The density, viscosity and surface tension of water with varying amounts of deuterium at the temperature of 25 °C are shown in Table 1. It occurred that the physical properties of the DDW differ from the distilled water having natural isotope composition. The difference of surface tension (at the water-air interface) is the most significant among the three types of water. In terms of the parameters of viscosity and density, the DDW is close to the distilled water. Because that the density of the DDW and distilled water to a great extent is determined by the concentration of heavy isotopes of oxygen (Goncharuk et al., 2013).

3.2. Sensory evaluations

The overall flower and fruit notes in KBT were obvious, imparting its unique Qimen aroma. The tea made with water with a deuterium content of 40 ppm had a harmonious and silky aroma with a little sweet aroma compared to the tea in natural water, and the tea in another DDW had a lighter aroma and more astringency. The five aromas of KBT

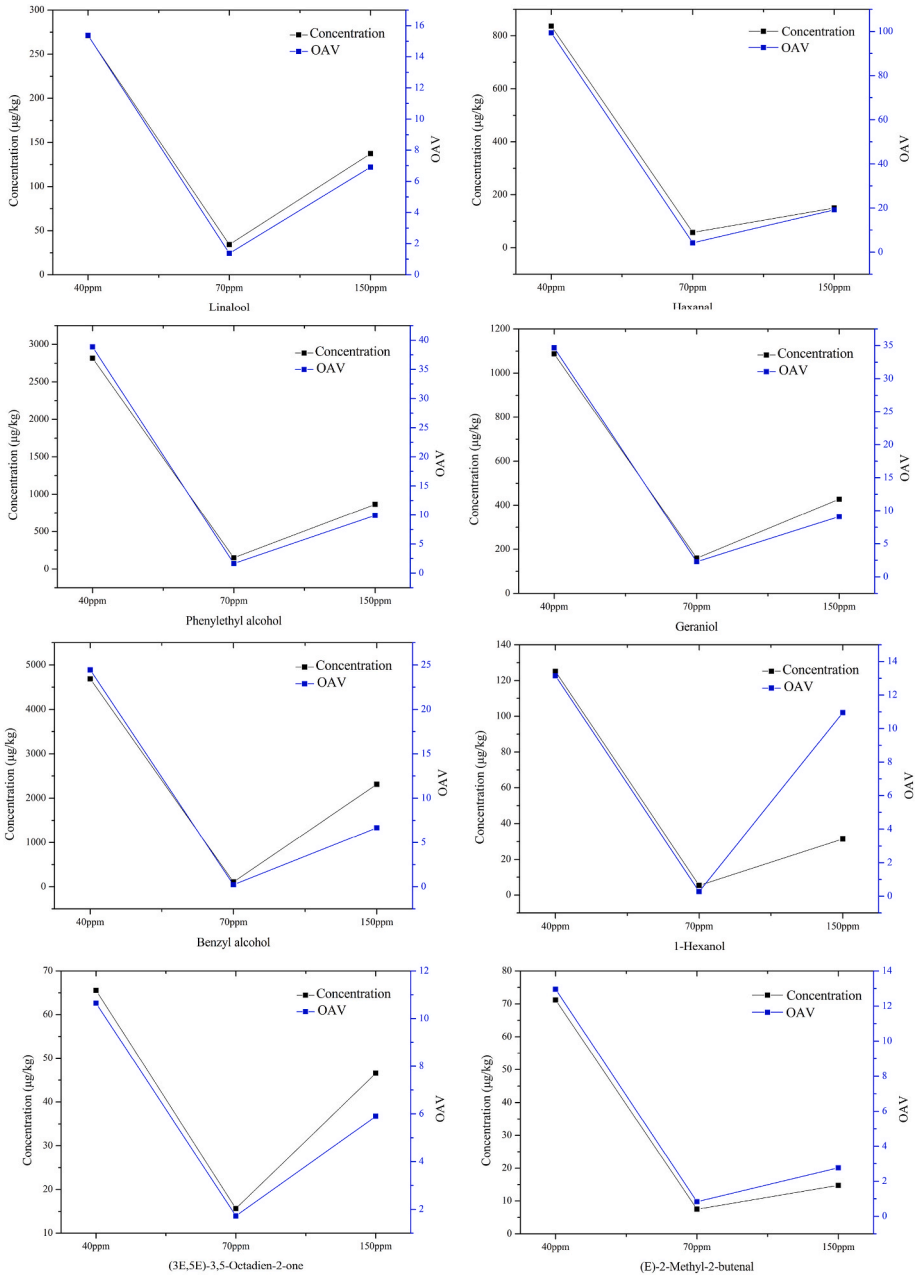

Fig. 5. Content and OAV comparison chart of 8 key aroma compounds of Keemun black tea brewed by three kinds of water with different deuterium content.

brewed by water with three different deuterium contents were sensually graded (Fig. 1). Traditional sensory assessments showed that each of the three tea has a smaller difference in flavor. The intensity of the five aroma notes of the tea in the first type of water was almost the highest, especially the intensity of fruity and sweet aromas was more prominent. Results from GC-O showed aroma release of furfural, hexanal, β-myrcene, 1-pentanol, benzeneacetaldehyde, etc. could be promoted, resulting in changes in the overall aroma of black tea. The release of aroma substances in water with a deuterium content of 70 ppm was weaker than that of natural water in overall aroma, indicating that deuterium was not only the cause of the volatilization of aroma substances, but also warrants further study.

3.3. Principal component analysis

In this study, three types of KBT were studied by electronic nose rapid gas chromatography, and the results were analyzed by principal component analysis. As can be seen from Fig. 2, the error between the repeated experiments of these three types of tea was small, and the difference between groups was significant; the date were therefore deemed to represent an effective analysis. That R^2X [1] = 0.609, showed that there were significant differences in the first principal component of the three groups of data. In the second principal component, the tea (at 40 ppm) differed from the other two samples, bSut that at 70 ppm cannot be distinguished from that at a deuterium content of 150 ppm. The sum of R^2X [1] and R^2X [2] was 0.889 (approaching 1), indicating that there were significant differences among the three groups of samples, and further indicating that the deuterium content does affect the aroma released from black tea.

3.4. Quantitative analysis and OAVs of key aroma compounds of KBT brewed with water at three different deuterium contents

3.4.1. GC-O analysis and GC-MS qualitative analysis

SBSE and GC-O analyses yielded a total of 43 compounds, including 15 alcohols, 13 aldehydes, 5 esters, 3 olefins, 6 ketones, and 2 other compounds (Table 2). Among them, the aroma intensity of β-myrcene (4.9–6.0), D-limonene (2.4–3.1), furfural (1.9–5.7), (3E,5E)-3,5-octadien-2-one (3.0–5.2), linalool (5.2–6.9), 5-methyl furfural (2.4–6.4), benzeneacetaldehyde (4.1–6.7), α-terpineol (3.0–7.4), methyl salicylate (3.6–6.6), benzyl alcohol (3.3–6.3), and phenylethyl alcohol (4.4–6.2) were higher, and these aroma substances impart flower, woody and sweet notes (as a whole). Black tea has a faster rate of aroma formation and a higher aroma concentration than other teas (Feng et al., 2019). β-Myrcene, one of the substances with the highest average aroma intensity, plays an essential role in the formation of aroma in Congfu black tea and is an important aroma compound in black tea (Mao et al., 2018).

From the aroma intensity values in Table 2, the release effect of aroma substances differed when using water with different deuterium contents. β-Myrcene and D-limonene had higher AIs in the first type of water, suggesting that they were better released in water with a deuterium content of 40 ppm. The fact that some substances had no aroma intensity means that such water suppresses its volatilization effect, and the aroma emitted cannot be detected by the human body, which is lower than the threshold of human detection (Z. Xiao, Chen, Niu, & Zhu, 2021).

3.4.2. Quantitative analysis of GC-MS

In the present study, SBSE was used to study the key volatile aroma components of KBT brewed in three kinds of water. Based on the above GC-O results, the key aroma components ≥1 in AI were quantitatively analyzed and the key aroma compounds (OAVs ≥1) that contributed significantly to overall aroma were selected from all aroma components. As can be seen from Table 3, a total of 20 aromatic aroma compounds contributing significantly to aroma were selected, of which alcohols and aldehydes were higher, accounting for 77.86%, 69.58% and 78.28%, respectively, of the three waters. The contents of β-myrcene (0.14–1.60), geraniol (0.15–1.13), benzyl alcohol (0.10–4.82), and phenylethanol (0.15–2.83) were the highest, and there were significant differences among the three kinds of water ($P < 0.05$). Then combining the results with their detection thresholds and aroma activity values, the aroma compounds in KBT which contributed greatly to the overall aroma could be determined. There have been many studies (Meng et al., 2021; Yunwei Niu et al., 2022; Su et al., 2022; Zuobing Xiao, Cao, Zhu, Chen, & Niu, 2022) on black tea showing that linalool, methyl salicylate, β-myrcene, phenylethanol were the basic aroma-active compounds in Congou black tea.

Fig. 3 shows more directly the difference of the content of aroma substances in the three types of water according to the quantitative results of GC-MS. The aforementioned studies have proven that the change of the deuterium content does have an effect on the release of aroma compounds. As can be seen from Fig. 3, the 20 substances showed the same trend, the release effect of the aroma substances in water with a deuterium content of 40 ppm was the best, but the release effect of the aroma substances in water with a deuterium content of 70 ppm was suppressed, and the content was much lower than that in ordinary water. Therefore, from the quantitative results of GC-MS, it can be concluded that the key aroma compounds in KBT have the best release effect DDW at 40 ppm, especially alcohols such as geraniol, benzyl alcohol, and phenethyl alcohol; however, the release effect of aromatic compounds was suppressed in DDW at 70 ppm, indicating that the release effect of aroma compounds did not increase simply with the decrease of the deuterium content in water.

OPLS-DA is a new multivariate statistical method for accurately identifying key variables affecting a population by regression modelling multiple variables to multiple independent variables (Bylesjö et al., 2006), and it was used to screen further the key volatile compounds that affect the aroma characteristics of KBT (Su et al., 2022). The fitting parameters R^2Y and Q^2 in the OPLS-DA diagram shown in Fig. 4 were 0.996 and 0.992, respectively, indicating accuracy in model establishment. When the variable importance in projection (VIP) value is greater than 1, this variable can be used as the key variable of the discriminating model. According to the VIP values, there were six substances with VIP >1, namely benzyl alcohol (18), phenylethyl alcohol (19), β-myrcene (4), hexanal (2), (E)-2-hexenal (5), and geraniol (17).

The difference between DDW and normal water is that the isotope ratio of hydrogen in the water has changed the response in terms of human olfactory activity. When the isotope ratio of hydrogen is changed, it reacts with the substances in black tea under the action of hot water, which affects the aroma substances. The changes of its contents and the physico-chemical changes still need further study.

3.4.3. Measurement results of detection threshold and OAVs

The odor activity value of an aroma substance is the ratio of its content to the threshold value, which can reflect the contribution of this volatile substance to the overall aroma of KBT. The larger the OAV, the greater its contribution. The OAVs of these substances are shown in Table 4. Twenty substances with OAVs ≥1 in three types of water with different deuterium contents were screened and their detection thresholds were obtained. It can be seen from Table 4 that the detection thresholds of the same substance in the three types of water were also different. The human olfactory sense is less accurate than that of machine, but sensor-based thresholds also differ, further illustrating the impact of the deuterium content on aroma release.

Substances with OAVs ≥10 in Table 4 include hexanal, (E)-2-methyl-2-butenal, 1-hexanol, (3E,5E)-3,5-octadien-2-one, linalool, geraniol, benzyl alcohol and phenylethyl alcohol, these substances are considered as the key aroma substances in KBT and contribute greatly to the overall aroma, but in ordinary water, only hexanal, β-myrcene and 1-hexanol have OAVs ≥10. In the water with a deuterium content of 70 ppm, the OAVs of all substance were less than 10, which meant that in the DDW of 40 ppm, the number of compounds with OAVs ≥10 was the greatest, therefore this DDW promoted most of the release of aroma substances.

The line charts based on the quantitative results and OAV results are shown in Fig. 5, which are eight key aroma substances with OAVs ≥10. The ordinate represents the content and OAV. Water with a deuterium content of 70 ppm has the least amount of eight substances, which is lower than the deuterium content of normal water, implying that water with a deuterium content of 70 ppm inhibits the release of aroma substances to a certain extent. It may be that when the deuterium content is 70 ppm, the boiling water reacts with the black tea to generate an unknown substance, which will combine with the aroma substances to inhibit their release; while the 40 ppm DDW promotes the release of the aroma substances. The specific mechanism by which the change of the deuterium content will lead to the change in the release effect of aroma substances is still unknown, and more in-depth research is needed in the future.

4. Conclusion

In this study, the release effects of key aroma substances in KBT were identified in three waters with different deuterium contents (40 ppm, 70 ppm, 150 ppm), using qualitative and quantitative analyses based on SBSE and GC-MS. The key aroma substances detected with OAVs ≥10 include hexanal, (E)-2-methyl-2-butenal, 1-hexanol, (3E,5E)-3,5-octadien-2-one, linalool, geraniol, benzyl alcohol, and phenylethyl alcohol. These substances are considered to make a greater contribution to the aroma of KBT. The influence of the deuterium content on the aroma of black tea was confirmed by traditional sensory and electronic nose rapid gas-phase analysis. The release of most of the key aromatic aroma substances in black tea was determined by the standard curve quantification and OAV method, with a deuterium content at 40 ppm, which produced

the best release of its Qimen aroma. Water with a deuterium content of 70 ppm inhibited the release of aroma substances, negatively affecting the overall aroma of KBT. The effects of the deuterium content in water on the surface tension and viscosity of water may be the reason for this phenomenon. This study further explored the composition of volatile aroma substances in KBT and introduced DDW into food science for the first time. However, in this experiment, the study of DDW is not comprehensive. Due to the limitations of experimental conditions, few types of DDW were selected, which cannot fully show the effect of DDW on the aroma of black tea. And the mechanism by which DDW affects aroma substances in black tea is unclear and requires further study.

CRediT authorship contribution statement

Yunwei Niu: Conceptualization, Methodology, Funding acquisition, Writing – original draft, and, Supervision. **Yue Zhang:** Conceptualization, Visualization, Writing – original draft, Formal analysis, and, editing. **Zuobing Xiao:** Writing – review & editing, Funding acquisition, and, Supervision. **Jiancai Zhu:** Writing – review & editing, Data curation. **Fengmei Zhang:** Methodology, and, Writing – review & editing. **Feng Chen:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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