



Discrimination and characterization of the volatile profiles of five Fu brick teas from different manufacturing regions by using HS–SPME/GC–MS and HS–GC–IMS

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ABSTRACT

Although aroma is one of the most essential factors determining the quality of Fu brick tea (FBT), the aroma profiles of FBTs from different manufacturing areas are rarely investigated. The aroma profiles of FBTs manufactured in five typical provinces of China were comprehensively analyzed on the basis of headspace gas chromatography–ion mobility spectrometry (HS–GC–IMS), headspace solid-phase microextraction gas chromatography–mass spectrometry (HS–SPME–GC–MS), sensory evaluation, odor activity value (OAV), and relative odor activity value (ROAV). HS–GC–IMS and HS–SPME–GC–MS identified 63 and 93 volatile organic compounds (VOCs), respectively. Multivariate statistical analysis indicated that the FBTs from different production regions had remarkably varied aromas. HS–SPME–GC–MS revealed that 27 VOCs (OAV >1) contributed to the overall aroma of the samples, of which 15 key differential compounds can effectively distinguish the aroma profiles of different FBTs. FBT from Shaanxi manifested a strong floral and fruity aroma; that from Hunan had a floral, grassy, and pine-woody aroma; that from Guizhou presented a grassy and herbal aroma; that from Guangxi exhibited a sweet, floral, and minty aroma; and that from Zhejiang possessed various fruit flavors and floral fragrance. OAV analysis identified the biomarkers responsible for the variation in the aroma characteristics of diverse FBTs. These biomarkers included linalool, 6-methyl-5-hepten-2-one, α -ionone, hexanal, and ethyl hexanoate. Sensory evaluation demonstrated that the infusion color and aroma of FBT samples from different provinces also greatly varied. Network correlation analysis revealed that *Aspergillus* and *Eurotium* were the crucial microorganisms for the metabolism and formation of VOCs. These findings provide new insight into the VOCs and fragrance features of FBTs produced in different regions of China.

Abbreviations: FBT, Fu brick tea; HS–GC–IMS, headspace gas chromatography–ion mobility spectrometry; HS–SPME–GC–MS, headspace solid-phase microextraction gas chromatography mass spectrometry; VOCs, volatile organic compounds; PCA, principal component analysis; PLS-DA, partial least squares–discriminant analysis; HCA, hierarchical cluster analysis; VIP, variable importance in the projection.

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1. Introduction

Fu brick tea (FBT) is a type of Chinese dark tea that undergoes microbial fermentation during processing (Xu et al., 2011). It is gaining popularity worldwide due to its unique flavor and health effects. The main production areas of FBT in China are Hunan, Zhejiang, Guizhou, Guangxi, and Shaanxi Provinces (Chen et al., 2022). Piling, steaming, pressing, fermentation, and drying are the main steps in the manufacture of typical postfermented FBT and contribute to the formation of the distinct aroma features of FBT (Ling et al., 2010). Among these steps, microbial fermentation is thought to be the most important for developing the distinct flavors of FBT (Ling et al., 2010). Several previous works reported that this process involved a wide range of microorganisms, including fungi (e.g., *Eurotium*, *Cyberlindnera*, *Debaryomyces*, *Aspergillus*, and *Candida*) and bacteria (Xu et al., 2011; Li et al., 2019b; Xiao et al., 2022a). These microorganisms secrete a variety of extracellular enzymes (e.g., α -amylase, pectinase, polyphenol oxidase, cellulase, and protease) during the manufacturing of FBT (Ward et al., 2005). These enzymes decompose and convert protein, pectin, cellulose, and polyphenols for the formation of specific volatile organic compounds (VOCs). Alcohols, ketones, aldehydes, and heteroatomic compounds considerably accumulate in FBT during fermentation (Xu et al., 2007). Additionally, the aroma characteristics of FBT differ substantially in accordance with raw materials, processing conditions, and storage duration (Zheng et al., 2022), which may influence the microbial compositions of FBT that subsequently affect the flavor quality of the tea. For example, the production environment and processing conditions could jointly affect bacterial succession in dark tea (Yao et al., 2017). Tian et al. (2013) revealed that the bacterial community structure of Pu'er tea differed depending on storage conditions and that bacterial diversity was linked to aging time. Our previous study revealed that the fungal communities of FBT samples from different regions of China varied greatly and influenced the metabolites, antioxidant activity, and taste characteristics of the teas (Chen et al., 2022). However, only a few previous studies have focused on the dynamic changes in microbial composition and metabolites during the manufacturing of FBT. Therefore, the differences in the aroma profiles of FBT samples with different origins and their key differential VOCs remain poorly understood.

In recent years, many advanced techniques for food flavor analysis have been developed with the rapid evolution of science and technology. Headspace solid-phase microextraction gas chromatography–mass spectrometry (HS–SPME–GC–MS) combines the advantages of the high separation capacity of gas chromatography (GS) with the strong metabolite identification ability of mass spectrometry (MS) and has been broadly used on food samples for odor analysis and quality classification (Chen et al., 2021). However, GC–MS is not sensitive enough for low levels of VOCs, leading to the neglect of VOCs (Chen et al., 2021). Headspace–gas chromatography–ion mobility spectrometry (HS–GC–IMS) is a convenient scientific technique for food flavor analysis due to its rapid detection, lack of sample pretreatment requirement, excellent resolution, ease of operation, intuitive data visualization, ultrahigh sensitivity and analytical speed, and operation under atmospheric pressure. It combines the benefits of the high separation efficiency of GC with the fast response of IMS. In recent years, research on food flavor using HS–GC–IMS has increased (Xiao et al., 2022b). However, some VOCs have not been identified because of the lack of a complete database for HS–GC–IMS. Hence, a combination of HS–GC–IMS and HS–SPME–GC–MS could enable the comprehensive analysis of the VOCs in FBT samples.

Therefore, the objective of this study is to determine the variations in the VOCs of five FBTs from different regions of China through GC–IMS and GC–MS analyses and identify the key contributory VOCs to elucidate the characteristic aroma formation of FBT samples from different regions. This study could provide crucial information on the formation mechanism of the flavor characteristics of FBT samples from different regions of China.

2. Materials and methods

2.1. Reagents and tea samples

Sinopharm Chemical Reagent Beijing Co., Ltd. (Beijing, China) provided the C4–C9 *n*-ketones used in the GC–IMS study. Ethyl caprate was purchased from CATO Co., Ltd. (Guangzhou, China). All other reagents and compounds were of analytical grade. The FBT samples collected from five production areas in China were provided by Guangxi Jinhua Tea Co., Ltd. (GX); Hunan Anhua Yuntiange Tea Industry Co., Ltd. (HN); Guizhou Fanjin Tea Industry Co., Ltd. (GZ); Shaanxi Xianxi lamu Fu Tea Co., Ltd. (SX); and Zhejiang Wuyi luotuo Jiulong Brick Tea Co., Ltd. (ZJ). All of these FBT samples were produced by using traditional processing methods in the same year (2018). The obtained FBTs were ground, sealed, and frozen at -20°C before VOC analysis.

2.2. HS–GC–IMS analysis

The VOCs of FBT were analyzed by using a GC–IMS FlavourSpec instrument (Dortmund, Germany) equipped with an Agilent Technologies 490 micro gas chromatograph and a MXT-5 capillary column (15 m \times 0.53 mm). The temperature of the chromatographic column for separation was set as 60°C . The GC–IMS instrument was equipped with an automatic headspace injector (CTC Analytics AG, Zwingen, Switzerland) with a headspace sampling unit. A total of 1.5 g of sample was placed into a headspace sample vial and incubated for 15 min at 80°C . Then, 500 μL of headspace was injected by an injection syringe (splitless mode) heated to 85°C . The carrier gas (nitrogen, purity $\geq 99.99\%$) was delivered in programmed flow starting at the rate of 2 mL/min for 2 min. The rate was gradually increased to 100 mL/min over 18 min. The analytes were ionized by using a 3H ionization source in the IMS ionization chamber in positive ion mode. Then, the resulting ions were driven into a 9.8 cm drift tube that was conducted at a constant voltage (5 kV) and temperature (45°C). Nitrogen (purity $\geq 99.99\%$) was used as the drift gas (150 mL/min). The average of 12 scans was used to create each spectrum. The *n*-ketones C4–C9 were used to calculate the VOC retention index (RI). VOCs were identified by comparing the drift time and RI of the standard in the GC–IMS library (Dortmund, Germany), and the contents were estimated on the basis of the peak intensity in GC–IMS.

2.3. HS–SPME–GC–MS analysis

The GC–MS analysis of VOCs was performed by using an Agilent 7000D system (Agilent, Palo Alto, CA, USA). A HP-5MS capillary column (30 m \times 0.25 mm \times 0.25 μm , Agilent, USA) was used to separate the VOCs. The VOCs were extracted through headspace solid-phase microextraction (HS–SPME) as follows: 0.5 g of finely powdered FBT samples were placed in a headspace vial with a volume of 20 mL. Subsequently, the headspace vial was added with 0.5 g of sodium chloride, 5 mL of boiled ultrapure water, and 10 μL of ethyl caprate internal standard solution. The headspace glass bottle was pre-equilibrated on a heated plate (80°C) for 10 min, after which a 50/30 μm DVB/CAR/PDMS fiber (Supelco, Bellefonte PA, USA) was injected into the headspace vial for the extraction of VOCs for 50 min under the same conditions. Then, the fiber was desorbed in splitless injection mode at 250°C for 5 min in the injection port of the GC–MS. The following chromatographic separations for the column oven were carried out: The initial temperature was set at 40°C and maintained for 3 min, then increased to 80°C at the rate of $2^{\circ}\text{C}/\text{min}$, ramped to 90°C at the rate of $2^{\circ}\text{C}/\text{min}$, increased to 150°C at the rate of $3^{\circ}\text{C}/\text{min}$, and raised to 180°C at the rate of $5^{\circ}\text{C}/\text{min}$. The temperature was subsequently increased to 230°C at $15^{\circ}\text{C}/\text{min}$ and held for 2 min. Helium (99.999%) was used as the carrier gas at the flow rate of 1 mL/min. Mass spectrometry was carried out in electron impact ionization mode with a 70 eV electron energy and a mass scan range of 35–400 *m/z*. The temperature of the MS ion source was 230°C . The VOCs were identified by comparing the mass spectra of all detected

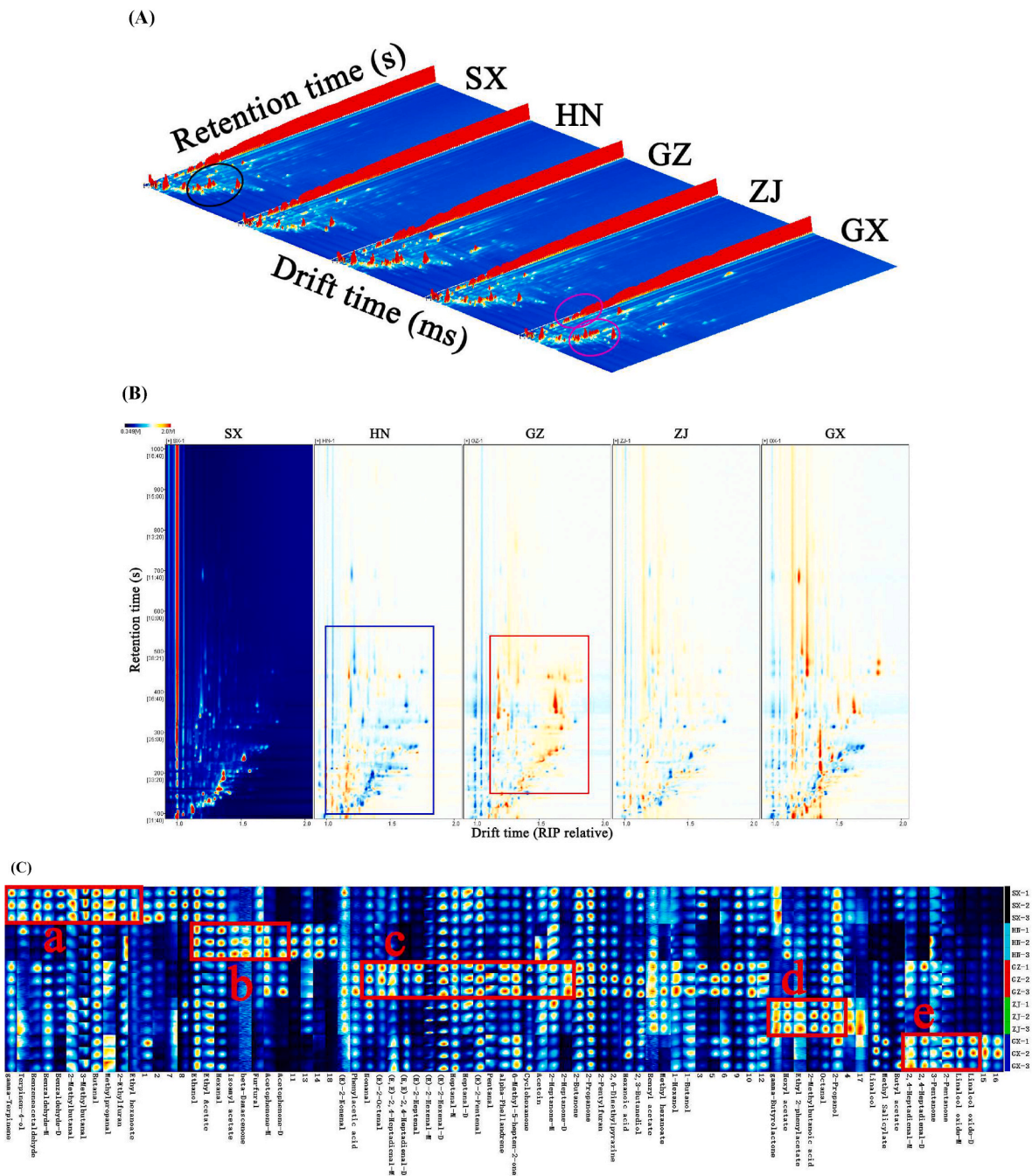


Fig. 1. (A) 3D-topographic and (B) 2D-topographic subtraction plots of the VOCs of different Fu brick tea. (C) VOCs fingerprint comparisons of different Fu brick tea.

metabolites with those in the NIST 17.0 library, Wiley MS library, and other public databases. Quantitative analysis was performed on the basis of the internal standard ethyl caprate.

2.4. Odor activity values and relative odor activity value analysis

Odor activity values (OAV) are widely used to assess the contributions of certain flavor compounds. Compounds with OAV > 1 are generally regarded as aroma-active compounds that greatly contribute to the overall aroma characteristics of a sample. OAV was calculated by using the ratio of the concentration (C) of each VOC to the odor threshold (OT) of the VOC in water (Zhong et al., 2022). OTs were obtained from earlier reports (Guo et al., 2022; Yang et al., 2022) and used in the following formula: $OAV = C/OT$, where OAV is the odor activity

value of the compound, C is the content of the compound, and OT is the odor threshold concentration of the compound in water.

In this study, the concentration of VOCs obtained by GC-IMS was expressed by the relative content (%), therefore, relative odor activity values (ROAV) were performed to evaluate the specific contribution of each compound to the overall aroma, which was well reported in previous studies (Zhu et al., 2020; Zhong et al., 2022). The ROAV of a volatile compound (i) was calculated as $ROAV_i = (OAV_i/OAV_{max}) \times 100$ (Zhu et al., 2020). Given that $OAV_i = C_i/OT_i$, then $ROAV_i = (C_i/OT_i) \times (OT_{max}/C_{max}) \times 100$, where 'max' is the volatile compound, which has the highest value calculated by dividing the relative content compound by odor threshold concentration among the detected volatile compounds. OAV_{max} is the odor activity value of the above compound ('max') and is regarded as 100, C_{max} is the relative content of the

Table 1
GC–IMS integration parameters and peak intensity for the analysis of volatile components in Fu brick tea from 5 different places by HS–GC–IMS.

No	Compounds	Odor description ^a	CAS	Formula	MW	RI ^b	Rt ^c [sec]	Dt ^d [RIPrel]	peak intensity				
									SX	HN	GZ	ZJ	GX
<i>Esters</i>													
a1	Methyl Salicylate	Wintergreen, mint	119-36-8	C8H8O3	152.1	1232.8	684.21	1.20761	1175.06 ± 81.53b	636.96 ± 134.72b	1250.69 ± 242.36b	936.80 ± 72.61b	2324.23 ± 1132.39a
a2	Butyl acetate	Ethereal, solvent, fruity, banana	123-86-4	C6H12O2	116.2	804	210.85	1.62989	186.99 ± 29.99a	17.97 ± 1.76b	113.62 ± 25.15 ab	154.69 ± 82.83a	193.14 ± 91.08a
a3	Ethyl acetate	Ethereal, fruity, sweet, weedy, green	141-78-6	C4H8O2	88.1	605	139.544	1.34393	574.22 ± 76.32a	527.03 ± 64.43a	424.45 ± 23.48b	563.09 ± 38.98a	425.72 ± 7.03b
a4	Isoamyl acetate	Sweet, fruity, banana, solvent	123-92-2	C7H14O2	130.20	880.4	250.575	1.3078	133.94 ± 11.39b	287.66 ± 48.02a	111.61 ± 11.29bc	97.39 ± 23.21bc	78.12 ± 15.19c
a5	Ethyl hexanoate	Sweet, fruity, pineapple, waxy, green, banana	123-66-0	C8H16O2	144.20	1014.7	371.007	1.33039	669.26 ± 210.63a	299.64 ± 63.34b	359.33 ± 14.35b	271.03 ± 39.05b	328.42 ± 23.80b
a6	Hexyl acetate	Fruity, green, apple, banana, sweet	142-92-7	C8H16O2	144.20	1031	394.412	1.40906	122.76 ± 8.21b	370.00 ± 39.70a	121.70 ± 8.92b	367.00 ± 8.51a	129.77 ± 8.94b
a7	Benzyl acetate	Sweet, floral, fruity, jasmine, fresh	140-11-4	C9H10O2	150.20	1154.3	571.519	1.33512	139.64 ± 10.96b	167.08 ± 34.78 ab	193.30 ± 9.25a	152.31 ± 9.51b	95.64 ± 12.43c
a8	Ethyl 2-phenylacetate	Sweet, floral, honey, rose, balsam, cocoa	101-97-3	C10H12O2	164.20	1241.3	696.42	1.28639	161.70 ± 8.48c	243.43 ± 43.61 ab	217.98 ± 26.04bc	285.57 ± 9.65a	180.72 ± 47.83bc
a9	Methyl hexanoate	Ethereal, fruity, pineapple, apricot, strawberry, tropical, banana, bacon	106-70-7	C7H14O2	130.20	922.8	282.609	1.28394	60.71 ± 9.13b	48.12 ± 3.53b	106.67 ± 23.45a	111.65 ± 5.23a	43.28 ± 6.83b
<i>Aldehydes</i>													
a10	(E)-2-Nonenal	Fatty, green, cucumber, aldehydic, citrus	18829-56-6	C9H16O	140.20	1186	617.03	1.41242	260.39 ± 51.87a	231.18 ± 10.23a	252.30 ± 14.70a	235.73 ± 13.46a	263.37 ± 34.12a
a11	Nonanal	Waxy, aldehydic, rose, fresh, orris, orange, peel, fatty, peely	124-19-6	C9H18O	142.20	1109.2	506.74	1.47094	469.51 ± 32.15bc	384.80 ± 46.77cd	706.82 ± 94.96a	604.57 ± 111.66 ab	314.70 ± 56.94d
a12	(E)-2-Octenal	Fresh, cucumber, fatty, green, herbal, banana, waxy, leaf	2548-87-0	C8H14O	126.20	1057	431.77	1.33579	367.81 ± 9.41b	325.30 ± 18.26bc	648.49 ± 65.87a	277.04 ± 56.19c	270.53 ± 23.68c
a13	Benzeneacetaldehyde	Green, sweet, floral, hyacinth, clover, honey, cocoa	122-78-1	C8H8O	120.20	1041.8	409.92	1.25245	512.44 ± 129.86a	152.47 ± 61.14b	331.45 ± 149.15 ab	184.32 ± 40.42b	229.50 ± 50.78b
a14	(E,E)-2,4-Heptadienal–M	Fatty, green, oily, aldehydic, vegetable, cake, cinnamon	4313-03-5	C7H10O	110.20	1012.3	367.63	1.18994	1508.49 ± 239.20c	1019.71 ± 41.82d	2964.98 ± 43.84a	1419.67 ± 177.04c	2425.11 ± 247.65b
a15	(E,E)-2,4-Heptadienal–D	Fatty, green, oily, aldehydic, vegetable, cake, cinnamon	4313-03-5	C7H10O	110.20	1012.8	368.34	1.62748	1055.03 ± 108.37b	155.47 ± 21.21b	2503.27 ± 906.68a	778.98 ± 72.29b	2022.63 ± 572.62a
a16	2,4-Heptadienal-M	Green, pungent, fruity, spicy	5910-85-0	C7H10O	110.20	1001.1	351.60	1.19574	313.20 ± 44.30b	254.01 ± 11.66b	611.20 ± 81.76a	254.83 ± 68.50b	727.58 ± 61.76a
a17	2,4-Heptadienal-D	Green, pungent, fruity, spicy	5910-85-0	C7H10O	110.20	1001.1	351.60	1.63398	243.96 ± 21.11b	71.00 ± 18.31b	728.61 ± 400.33a	183.71 ± 35.06b	964.37 ± 232.53a
a18	Benzaldehyde-M	Strong, sharp, sweet, bitter, almond, cherry	100-52-7	C7H6O	106.10	960.5	314.48	1.15266	699.78 ± 108.97a	393.58 ± 18.96c	545.64 ± 41.05b	624.60 ± 26.81 ab	540.33 ± 65.70b
a19	Benzaldehyde-D	Strong, sharp, sweet, bitter, almond, cherry	100-52-7	C7H6O	106.10	960.5	314.48	1.47651	542.25 ± 103.35a	91.15 ± 3.38c	340.10 ± 60.05b	269.35 ± 24.59b	104.23 ± 12.93c
a20	(E)-2-Heptenal	Pungent, green, vegetable, fresh, fatty	18829-55-5	C7H12O	112.20	954.7	309.56	1.67558	103.33 ± 16.74b	64.63 ± 9.48b	562.20 ± 178.83a	114.36 ± 25.33b	141.61 ± 21.13b
a21	Heptanal-M	Fresh, aldehydic, fatty, green, herbal, wine-lee, ozone	111-71-7	C7H14O	114.20	905	267.52	1.33093	387.27 ± 87.39a	413.61 ± 52.12a	483.19 ± 23.58a	341.92 ± 79.73a	365.33 ± 74.94a
a22	Heptanal-D	Fresh, aldehydic, fatty, green, herbal, wine-lee, ozone	111-71-7	C7H14O	114.20	903.9	266.62	1.70083	730.83 ± 133.39a	316.14 ± 26.71b	758.55 ± 63.14a	583.74 ± 142.17a	236.01 ± 52.28b
a23	(E)-2-Hexenal-M	Green, banana, aldehydic, fatty, cheesy	6728-26-3	C6H10O	98.10	847.2	233.31	1.18472	1705.52 ± 19.21b	2007.49 ± 171.73a	1543.32 ± 44.42b	2065.85 ± 93.43a	1496.07 ± 125.87b
a24	(E)-2-Hexenal-D	Green, banana, aldehydic, fatty, cheesy	6728-26-3	C6H10O	98.10	847.2	233.31	1.52169	5268.13 ± 568.76b	4389.53 ± 363.82bc	6978.86 ± 485.37a	3514.24 ± 542.61c	4675.11 ± 682.86b
a25	Hexanal	Fresh, green, fatty, aldehydic, grass, leafy, fruity, sweaty	66-25-1	C6H12O	100.20	792.4	204.79	1.57116	1428.27 ± 142.16a	1716.64 ± 106.13a	1318.76 ± 51.31 ab	940.73 ± 372.23b	907.35 ± 246.07b

(continued on next page)

Table 1 (continued)

No	Compounds	Odor description ^a	CAS	Formula	MW	Ri ^b	Rt ^c [sec]	Dt ^d [RIPrel]	peak intensity				
									SX	HN	GZ	ZJ	GX
a26	(E)-2-Pentenal	Pungent, green, fruity, apple, orange, tomato	1576-87-0	C5H8O	84.10	748.2	185.89	1.36557	4555.79 ± 280.65b	2725.16 ± 56.78d	5146.09 ± 259.56a	3529.27 ± 167.78c	3544.20 ± 463.10c
a27	Pentanal	Fermented, bready, fruity, nutty, berry	110-62-3	C5H10O	86.10	696.5	164.86	1.43204	274.79 ± 17.32b	144.26 ± 90.46c	451.47 ± 64.38a	289.48 ± 18.43b	318.24 ± 49.81b
a28	2-Methylbutanal	Musty, cocoa, phenolic, coffee, nutty, malty, fermented, fatty, alcoholic	96-17-3	C5H10O	86.10	659	153.81	1.40576	1032.91 ± 9.28a	546.24 ± 50.11d	586.02 ± 25.56d	905.73 ± 30.39b	714.89 ± 118.48c
a29	3-Methylbutanal	Ethereal, aldehydic, chocolate, peach, fatty	590-86-3	C5H10O	86.10	637.4	148.10	1.41658	589.52 ± 14.64a	223.29 ± 21.65c	246.22 ± 13.97c	409.71 ± 34.27b	364.54 ± 112.84b
a30	Butanal	Pungent, cocoa, musty, green, malty, bready	123-72-8	C4H8O	72.10	590.1	135.622	1.29602	1268.41 ± 51.67a	1035.27 ± 60.71b	977.03 ± 11.15b	1008.86 ± 86.66b	963.78 ± 23.78b
a31	Methylpropanal	Fresh, aldehydic, floral, green	78-84-2	C4H8O	72.10	553.7	125.996	1.28365	1010.99 ± 21.48a	280.94 ± 26.82c	768.07 ± 63.53b	333.07 ± 89.66c	725.32 ± 94.53b
a32	Octanal	Aldehydic, waxy, citrus, orange, peel, green, herbal, fresh, fatty	124-13-0	C8H16O	128.20	1005.6	358.063	1.40123	164.99 ± 48.78b	191.11 ± 20.24b	281.01 ± 17.23a	321.15 ± 5.53a	148.73 ± 1.95b
a33	2-Furfural	Sweet, woody, almond, fragrant, baked, bread	98-01-1	C5H4O2	96.10	828.8	223.72	1.08249	144.24 ± 4.35a	182.62 ± 18.26a	89.84 ± 9.91b	68.19 ± 33.91b	55.41 ± 16.52b
<i>Alcohols</i>													
a34	Terpinen-4-ol	Pepper, woody, earth, musty, sweet	562-74-3	C10H18O	154.30	1159.5	578.93	1.2387	281.75 ± 33.70a	169.87 ± 75.73bc	105.09 ± 9.75c	215.72 ± 66.69 ab	168.07 ± 50.65bc
a35	Linalool	Citrus, floral, sweet, rose, woody, green, blueberry	78-70-6	C10H18O	154.30	1095.3	486.75	1.222	869.88 ± 108.43b	236.52 ± 24.28c	1126.92 ± 147.86b	1733.67 ± 183.43a	1711.42 ± 250.68a
a36	2,3-Butanediol	Fruity, creamy, buttery	513-85-9	C4H10O2	90.10	780.6	199.09	1.36248	2147.88 ± 502.00 ab	1472.48 ± 391.36b	3130.60 ± 287.90a	2545.50 ± 970.29 ab	2334.71 ± 766.84 ab
a37	Ethanol	Strong, alcoholic, ethereal, medical	64-17-5	C2H6O	46.10	451.1	98.9	1.05025	1655.17 ± 99.12a	1664.72 ± 146.53a	1080.75 ± 68.91c	1379.07 ± 126.22b	1523.98 ± 108.93 ab
a38	2-Propanol	Alcohol, musty, woody	67-63-0	C3H8O	60.10	565.2	129.051	1.21957	732.26 ± 6.61a	616.94 ± 18.23 ab	655.61 ± 70.72 ab	739.49 ± 30.38a	534.50 ± 169.82b
a39	Linalool oxide-M	Musty, camphor, fenchyl, alcohol	60047-17-8	C10H18O2	170.30	1066.4	445.364	1.26608	144.79 ± 27.49b	451.95 ± 135.88b	426.45 ± 63.36b	362.11 ± 12.92b	1824.81 ± 474.85a
a40	Linalool oxide-D	Musty, camphor, fenchyl, alcohol	60047-17-8	C10H18O2	170.30	1067.8	447.275	1.82189	95.34 ± 15.74b	133.13 ± 25.71b	229.11 ± 29.74b	134.29 ± 10.43b	729.04 ± 272.41a
a41	1-Hexanol	Ethereal, fusel oil, fruity, alcoholic, sweet, green	111-27-3	C6H14O	102.20	873.8	247.109	1.32584	198.94 ± 26.26bc	159.56 ± 11.06c	508.23 ± 40.91a	256.74 ± 41.00b	238.69 ± 52.96b
a42	1-Butanol	Fusel oil, sweet, balsam, whiskey	71-36-3	C4H10O	74.10	657.1	153.301	1.38066	291.58 ± 39.45a	310.22 ± 37.12a	465.09 ± 163.00a	376.08 ± 62.66a	443.71 ± 38.99a
<i>Ketones</i>													
a43	Acetophenone-M	Sweet, pungent, hawthorn, mimosa, almond, acacia, chemical	98-86-2	C8H8O	120.20	1061.4	438.11	1.18834	318.69 ± 10.41b	1228.10 ± 226.60a	1052.03 ± 148.12a	345.24 ± 13.67b	369.01 ± 135.56b
a44	Acetophenone-D	Sweet, pungent, hawthorn, mimosa, almond, acacia, chemical	98-86-2	C8H8O	120.20	1062.5	439.63	1.57705	41.67 ± 7.01b	605.84 ± 241.57a	588.74 ± 253.34a	45.21 ± 1.03b	51.50 ± 21.28b
a45	6-Methyl-5-hepten-2-one	Citrus, green, musty, lemongrass, apple	110-93-0	C8H14O	126.20	992.7	341.76	1.1794	1066.98 ± 26.36c	1338.92 ± 104.58b	2523.89 ± 210.16a	1287.07 ± 66.59bc	2394.50 ± 163.39a
a46	Cyclohexanone	Mint, cool	108-94-1	C6H10O	98.10	898.7	262.15	1.15266	912.42 ± 30.04 ab	782.86 ± 28.52b	1024.71 ± 182.43a	746.01 ± 41.23b	1007.87 ± 39.23a
a47	2-Heptanone-M	Fruity, spicy, sweet, herbal, coconut, woody	110-43-0	C7H14O	114.20	894.4	258.57	1.26111	248.68 ± 66.75 ab	338.97 ± 5.31a	285.33 ± 10.57a	267.86 ± 71.61a	165.05 ± 38.93b
a48	2-Heptanone-D	Fruity, spicy, sweet, herbal, coconut, woody	110-43-0	C7H14O	114.20	892.3	256.78	1.63695	121.01 ± 22.83c	94.64 ± 27.79cd	481.09 ± 55.70a	212.25 ± 5.00b	43.03 ± 6.79d
a49	Acetoin	Sweet, buttery, creamy, dairy, milky, fatty	513-86-0	C4H8O2	88.10	731.6	179.12	1.3393	692.06 ± 55.12b	269.84 ± 32.16c	930.64 ± 111.33a	880.67 ± 34.25b	653.03 ± 34.25b
a50	2-Butanone	Fragrant, fruit, pleasant	78-93-3	C4H8O	72.10	571.2	130.631	1.2481	2778.55 ± 159.22b	2077.00 ± 115.76c	3118.85 ± 67.77a	2142.26 ± 187.92c	2047.56 ± 159.61c
a51	2-Propanone	Solvent, ethereal, apple, pear	67-64-1	C3H6O	58.10	487.5	108.526	1.1229	9132.59 ± 616.63a	6907.91 ± 400.71bc	9473.44 ± 380.97a	7706.43 ± 461.39b	6855.33 ± 14.45c

(continued on next page)

Table 1 (continued)

No	Compounds	Odor description ^a	CAS	Formula	MW	RI ^b	Rt ^c [sec]	Dt ^d [RIPrel]	peak intensity					
									SX	HN	GZ	ZJ	GX	
a52	3-Pentanone	Ethereal, acetone	96-22-0	C5H10O	86.10	683	160.157	1.34757	1914.46 ± 42.33b	1750.00 ± 283.11b	2414.75 ± 101.02a	1884.24 ± 44.98b	2541.42 ± 160.98a	
a53	2-Pentanone	Sweet, fruity, ethereal, wine, banana, woody	107-87-9	C5H10O	86.10	694.1	163.847	1.36926	457.02 ± 17.56c	516.71 ± 129.12c	1087.87 ± 202.60b	498.76 ± 40.93c	1433.31 ± 135.39a	
a54	β-Damascenone	Apple, rose, honey, tobacco, sweet	23726-93-4	C13H18O	190.30	1402.4	927.741	1.39395	153.92 ± 4.91b	286.27 ± 35.11a	175.75 ± 18.52b	165.21 ± 36.86b	152.08 ± 4.84b	
<i>Acids</i>														
a55	Phenylacetic acid	Sweet, honey, floral, honeysuckle, sour, waxy, civet	103-82-2	C8H8O2	136.10	1283.1	756.40	1.32282	270.22 ± 13.42c	291.86 ± 21.38c	648.28 ± 97.98a	422.43 ± 9.12b	488.43 ± 30.37b	
a56	Hexanoic acid	Sour, fatty, sweat, cheese	142-62-1	C6H12O2	116.20	976.9	328.34	1.28785	543.01 ± 234.77 ab	288.09 ± 72.76b	821.92 ± 276.86a	387.34 ± 32.75b	698.78 ± 253.75 ab	
a57	2-Methylbutanoic acid	Pungent, acid, roquefort, cheese	116-53-0	C5H10O2	102.10	900.2	263.46	1.2072	219.82 ± 7.53b	321.47 ± 33.98b	251.36 ± 25.45b	595.58 ± 172.20a	176.94 ± 13.65b	
<i>Hydrocarbons</i>														
a58	γ-Terpinene	Oily, woody, terpene, lemon/lime, tropical, herbal	99-85-4	C10H16	136.20	1072.2	453.62	1.2236	856.25 ± 133.88a	235.52 ± 3.59c	771.72 ± 63.22a	609.94 ± 22.74b	494.37 ± 88.27b	
a59	α-Phellandrene	Citrus, herbal, terpene, green, woody, peppery	99-83-2	C10H16	136.20	1003.9	355.62	1.22694	273.03 ± 42.22d	363.60 ± 18.61c	598.04 ± 28.78a	215.12 ± 13.79d	430.51 ± 48.96b	
<i>Furans</i>														
a60	2-Pentylfuran	Fruity, green, earthy, beany, vegetable, metallic	3777-69-3	C9H14O	138.20	996.8	345.33	1.26111	579.36 ± 80.90c	770.08 ± 38.47b	1187.40 ± 20.42a	601.57 ± 20.32c	400.74 ± 18.12d	
a61	2-Ethylfuran	Chemical, beany, ethereal, cocoa, bread, malty, coffee, nutty	3208-16-0	C6H8O	96.10	683.3	160.22	1.32075	2419.96 ± 119.20a	1329.25 ± 106.35b	1662.85 ± 440.16b	2295.62 ± 81.35a	1529.12 ± 59.07b	
<i>Pyrazines</i>														
a62	2,6-Dimethylpyrazine	Ethereal, cocoa, nutty, roasted, roasted, meaty, beefy, brown, coffee, buttermilk	108-50-9	C6H8N2	108.10	933	291.22	1.13186	173.41 ± 11.90b	268.11 ± 20.52b	415.94 ± 79.96a	242.03 ± 58.98b	200.84 ± 32.40b	
<i>Lactones</i>														
a63	γ-Butyrolactone	Creamy, oily, fatty, caramel	96-48-0	C4H6O2	86.10	919.3	279.59	1.08284	235.22 ± 34.38a	127.43 ± 50.11b	79.99 ± 8.86b	224.18 ± 11.15a	141.47 ± 41.48b	

^a Odor descriptions were from FEMA database. Different small letters in the same row indicated significant difference ($p < 0.05$). D: Dimer, M: Monomer.

^b Represents the retention index in the capillary GC column.

^c Represents the retention time in the capillary GC column.

^d Represents the drift time in the drift tube.

compound ($'max'$), and OT_{max} is the odor threshold of the compound ($'max'$). OAV_i is the odor activity value of a compound, C_i is the relative content of the compound, and OT_i is the odor threshold of the compound.

A high ROAV is also indicative of the great contribution of a component to the overall flavor of the sample. Components with $ROAV \geq 1$ are generally considered as the key aroma compounds of the analyzed samples, whereas components with $0.1 \leq ROAV < 1$ have important modifying effects on the overall aroma of the samples (Zhang et al., 2020; Zhong et al., 2022).

2.5. Sensory evaluation

Ten well-trained panelists from Hunan Agricultural University (five females and five males, ages 22 years–50 years) evaluated the odor and taste qualities of FBT by using the Chinese Standard Methodology of Tea (GB/T 23776–2018) with minor modifications. All assessors were trained in the ability to identify, describe, and differentiate different aroma qualities. Each assessor completed 200 h of training on a specific tea infusion. After training, a discussion was held to determine accurate aroma characteristic terms to summarize the attributes of the five kinds of FBTs. Finally, five aroma attributes were selected to describe the overall aroma characteristics of the five FBTs: fungal floral, minty, floral, green, and woody. The color of the tea infusions was also evaluated. Briefly, 3.0 g of each FBT sample was placed in a teacup and brewed for 5 min with 150 mL of boiling water. Subsequently, the tea infusions were filtered into tea bowls and assessed by the panelists. Before the evaluation, all instructions were given to the panelists. Infusions of FBT from different regions were assigned three-digit numbers and presented in random order. During the examination of odor qualities, the panelists were given water to rinse their palates in between each sample. Panelists recorded scores for the sensory qualities of the FBT samples. High scores indicate strong intensities (10 = extremely high intensity, 5 = medium intensity, and 0 = none or unnoticeable intensity) in the sensory attribute descriptions of tea on a 10-point hedonic scale. The average sensory attribute scores were calculated on the basis of the scores recorded by the ten panelists.

2.6. Statistical analysis

All measurements were carried out at least three times, with the results reported as the mean \pm SD. SPSS 17.0 software was used to evaluate statistical significance ($p < 0.05$) by using one-way analysis of variance and Duncan's multiple range test (Chicago, IL, USA). SIMCA-P+ 12.0 software (Umea, Sweden) was used to perform principal component analysis (PCA), partial least squares–discriminant analysis (PLS–DA), and hierarchical cluster analysis (HCA). Graphical presentations, heat maps, and network plots were generated with Origin v8.5 (OriginLab Corporation, MA, USA), TTools version 0.655, and Cytoscape software (version 3.5.1) (<http://www.cytoscape.org/>). GC–IMS data were processed by using Reporter plug-ins, Laboratory Analytical Viewer software (Dortmund, Germany), dynamic PCA, and gallery plot analysis.

3. Results and discussion

3.1. Analysis of VOCs by HS–GC–IMS

3.1.1. Topographic plots of the HS–GC–IMS results of FBT samples from different regions

HS–GC–IMS was performed to elucidate the variations in the VOCs of different FBTs. Fig. 1A shows a three-dimensional (3D) topographic map of the VOCs of five samples. Although the VOCs of the five FBT samples were similar, several peaks were clearly different, indicating that the VOCs of the FBT samples from different regions differed drastically. An overhead view was conducted to observe the variations in VOCs with

increased clarity, and the result is shown in Fig. 1B. SX was used as the reference, and the spectra of HN, GZ, ZJ, and GX were obtained by subtracting the reference. Blue and red indicate lower and higher VOCs in the sample than in the reference, respectively. The majority of signals had a retention time of 100–800 s and a drift time of 1.0–2.0 s (relative RIP). The presence of more red spots in GZ and GX than in the reference suggested that VOCs were present in larger concentrations in these samples than in the reference. By contrast, HN and ZJ had numerous blue specks in the backdrop, indicating that they had lower VOC concentrations than the reference.

3.1.2. Differential VOCs of FBT samples

Supplementary Fig. 1 displays the qualitative analysis of VOCs, which were plotted in topographic plots (Fig. S1). A total of 63 VOCs were tentatively identified. They included nine esters, 24 aldehydes, nine alcohols, 12 ketones, three acids, two hydrocarbons, two furans, one pyrazine, and one lactone. The other 18 peaks were unidentifiable due to the limited data in the library databases. The detailed information on the 63 VOCs identified in the five FBT samples are listed in Table 1. The carbon chain of these identified VOCs were all within C2–C13, and several single compounds were seen to produce multiple spots or signals (dimer or monomer forms) owing to their varied levels and characteristics (Rodríguez-Maecker et al., 2017). These compounds included heptanal, (*E*)-2-hexenal, 2,4-heptadienal, linalool oxide, and 2-heptanone. The results of fingerprint analysis showed that the contents of VOCs in SX, HN, GZ, ZJ, and GX varied greatly in the gallery pot regions (a, b, c, d, and e). In Fig. 1C, each column represents a VOC, and each line represents a FBT sample. The SX sample had high concentrations of γ -terpinene, terpinen-4-ol, benzeneacetaldehyde, butanal, benzaldehyde-M, benzaldehyde-D, 2-methylbutanal, 3-methylbutanal, ethyl hexanoate, methylpropanal, and 2-ethylfuran as illustrated in box a. The HN sample showed high levels of ethanol, β -damascenone, hexanal, isoamyl acetate, ethyl acetate, furfural, acetophenone-M, and acetophenone-D as displayed in box b. The GZ sample showed high contents of phenylacetic acid, (*E*)-2-octenal, (*E,E*)-2,4-heptadienal-M, (*E,E*)-2,4-heptadienal-D, (*E*)-2-heptenal, (*E*)-2-hexenal-M, (*E*)-2-hexenal-D, pentanal, α -phellandrene, and 2-heptanone-D as depicted in box c. The ZJ sample had high concentrations of γ -butyrolactone, ethyl 2-phenylacetate 2-methylbutanoic acid, octanal, and 2-propanol as shown in box d. The GX sample displayed high concentrations of 2, 4-heptadienal-M, 2-pentanone, 3-pentanone, 2,4-heptadienal-D, linalool oxide-M, and linalool oxide-D as illustrated in box e. The peak intensities of these substances in the five samples of FBT were different.

Analyzing the VOC distributions revealed that aldehydes and ketones were the predominant groups in all FBT samples. Specifically, in these five FBT samples, aldehydes and ketones accounted for more than 57% of the total VOCs (Fig. S2A). Previous research has shown that ketones and aldehydes may be the key contributors to the distinctive scent of FBT (Li et al., 2019a). This result was in agreement with our current findings. In the tea samples, alcohols and esters each accounted for 28% of the total VOCs, followed by acids (5%), hydrocarbons (3%), furans (3%), pyrazine (2%), and lactone (2%). Aldehydes were the largest groups of VOCs present in the five FBT samples (Fig. S2B) as follows: SX (42.70%), HN (38.26%), GZ (42.56%), ZJ (37.18%), and GX (38.78%). SX had a larger quantity of aldehydes than the teas from other regions. Aldehydes are produced during the postfermentation stage via the oxidative deamination and decarboxylation of amino acids and the hydrolysis and oxidation of fatty acids (Xu et al., 2007). Nonanal may contribute to the differentiation of the diverse FBTs because it was present at low levels ($p < 0.05$) in GX and at comparable levels in the SX, HN, GZ, and ZJ samples. The concentrations of (*E*, *E*)-2,4-heptadienals (dimers and monomers) in GZ (7.80%) were higher than those in GX (7.64%), SX (4.44%), ZJ (4.25%), and HN (2.59%). (*E*)-2-hexenals, which confer a green flavor, were the most abundant aldehydes in all FBT samples and could help discriminate the characteristic aromas of diverse FBTs due to the variation in their levels.

Table 2
Concentration of volatile compounds among five aroma types of FBT by GC–MS.

No	Compounds	CAS	Formula	Rt	Odor description	Content (ng/g)				
						SX	HN	GZ	ZJ	GX
<i>Esters</i>										
b1	Ethyl caprylate	106-32-1	C10H20O2	32.498	Apricot, brandy, fat, floral, pineapple	ND	ND	21.56 ± 6.18	ND	ND
b2	Methyl hexanoate	106-70-7	C7H14O2	13.332	Ester, fresh, fruit, pineapple	ND	ND	58.58 ± 0.72	ND	ND
b3	Methyl salicylate	119-36-8	C8H8O3	31.889	Almond, caramel, peppermint, sharp	568.55 ± 17.35	294.69 ± 30.48	606.82 ± 24.88	228.89 ± 3.47	622.63 ± 77.99
b4	Propionic anhydride	123-62-6	C6H10O3	4.025	Like acetaldehyde	ND	ND	ND	22.12 ± 12.87	49.50 ± 54.12
b5	Ethyl hexanoate	123-66-0	C8H16O2	18.196	Apple peel, brandy, fruit gum, overripe fruit, pineapple	91.78 ± 14.11	ND	ND	80.62 ± 2.65	ND
b6	Vinyl acrylate	2177-18-6	C5H6O2	6.163	–	ND	ND	4.40 ± 4.53	ND	ND
b7	2,2,4-Trimethyl-1,3-pentanediol diisobutyrate	6846-50-0	C16H30O4	52.276	–	ND	ND	ND	10.73 ± 3.58	ND
b8	Linalyl butyrate	78-36-4	C14H24O2	25.243	Bergamot, fruity, banana, berry	ND	72.23 ± 4.24	303.46 ± 19.66	ND	ND
<i>Aldehydes</i>										
b9	Benzaldehyde	100-52-7	C7H6O	15.317	Bitter almond, burnt sugar, cherry, malt, roasted pepper	508.83 ± 18.45	177.18 ± 16.88	555.84 ± 37.46	317.10 ± 27.60	536.52 ± 48.33
b10	3,3-Diethoxy-1-propyne	10160-87-9	C7H12O2	20.413	–	ND	ND	ND	24.59 ± 31.99	ND
b11	Heptanal	111-71-7	C7H14O	11.895	Citrus, fat, green, nut	ND	76.48 ± 4.94	217.91 ± 42.63	ND	432.04 ± 48.25
b12	Decanal	112-31-2	C10H20O	32.948	Floral, fried, orange peel, penetrating, tallow	ND	ND	30.65 ± 7.28	ND	25.43 ± 2.06
b13	2,3-Dihydro-2,2,6-trimethylbenzaldehyde	116-26-7	C10H14O	32.256	Fresh, herbal, phenolic, metallic, rosemary, tobacco, spicy	171.42 ± 12.55	175.16 ± 5.19	143.25 ± 10.34	177.84 ± 11.44	175.82 ± 12.32
b14	Benzeneacetaldehyde	122-78-1	C8H8O	20.908	Berry, geranium, honey, nut, pungent	49.87 ± 11.41	ND	ND	ND	ND
b15	(E)-2-Nonenal	18829-56-6	C9H16O	29.485	Fatty, green, cucumber, aldehydic, citrus	ND	ND	ND	ND	62.13 ± 5.79
b16	5-Ethylcyclopent-1-enecarboxaldehyde	36431-60-4	C8H12O	20.021	–	ND	ND	84.11 ± 27.11	ND	ND
b17	(E,E)-2,4-Heptadienal	4313-03-5	C7H10O	18.759	Fat, nut	ND	101.16 ± 4.97	399.23 ± 32.27	174.69 ± 21.66	1170.43 ± 103.67
b18	2,6,6-Trimethylcyclohex-2-en-1-carbaldehyde	432-24-6	C10H16O	26.092	–	ND	ND	ND	ND	84.00 ± 20.74
b19	2,6,6-Trimethyl-1-cyclohexene-1-carboxaldehyde	432-25-7	C10H16O	33.694	Tropical, saffron, herbal, clean, rose, oxide, sweet, tobacco, damascone, fruity	117.04 ± 6.72	190.27 ± 6.65	214.94 ± 7.04	132.46 ± 4.49	548.62 ± 27.45
b20	2,5-Dimethylbenzaldehyde	5779-94-2	C9H10O	30.449	–	ND	ND	ND	ND	39.27 ± 0.91
b21	Isophthalaldehyde	626-19-7	C8H6O2	30.405	–	22.79 ± 4.48	ND	ND	11.08 ± 5.98	34.43 ± 1.44
b22	Hexanal	66-25-1	C6H12O	6.995	Apple, fat, fresh, green, oil	373.51 ± 52.14	481.86 ± 15.88	521.05 ± 246.03	402.30 ± 76.54	1610.57 ± 182.86
b23	(E)-2-Hexenal	6728-26-3	C6H10O	9.285	Green, banana, aldehydic, fatty, cheesy	ND	ND	ND	ND	773.38 ± 66.13
<i>Alcohols</i>										
b24	2-Ethyl-1-hexanol	104-76-7	C8H18O	20.435	Citrus, fresh, floral, oily, sweet	ND	128.85 ± 15.11	74.63 ± 18.80	172.70 ± 21.94	ND
b25	1-Hexanol	111-27-3	C6H14O	10.506	Banana, flower, grass, herb	ND	125.94 ± 8.19	139.84 ± 18.01	ND	ND
b26	(S)-(+)-3-Methyl-1-pentanol	42072-39-9	C6H14O	10.409	–	ND	ND	159.30 ± 33.82	ND	ND
b27	Terpinen-4-ol	562-74-3	C10H18O	30.729	Pepper, woody, earth, musty, sweet	ND	ND	ND	ND	31.53 ± 5.70
b28	2,4,4-Trimethyl-2-cyclohexen-1-ol	73741-61-4	C9H16O	21.816	–	ND	103.68 ± 9.06	107.60 ± 4.15	ND	ND
b29	Linalool	78-70-6	C10H18O	25.154	Coriander, floral, lavender, lemon, rose	149.79 ± 45.98	ND	179.10 ± 19.21	360.10 ± 71.45	364.77 ± 124.02
<i>Ketones</i>										
b30	6-Methyl-5-hepten-2-one	110-93-0	C8H14O	17.202	Citrus, mushroom, pepper, rubber, strawberry	294.92 ± 32.29	571.02 ± 29.81	620.00 ± 31.96	177.45 ± 13.23	1181.21 ± 19.30
b31	2,6,6-Trimethyl-2-cyclohexene-1,4-dione	1125-21-9	C9H12O2	28.162	Floral	30.29 ± 3.65	72.80 ± 6.53	49.25 ± 1.89	40.50 ± 5.36	20.65 ± 2.48
b32	4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-3-en-2-one	1203-08-3	C13H18O	45.159	–	76.34 ± 33.10	85.14 ± 12.21	ND	ND	99.11 ± 4.20

(continued on next page)

Table 2 (continued)

No	Compounds	CAS	Formula	Rt	Odor description	Content (ng/g)				
						SX	HN	GZ	ZJ	GX
b33	α -Ionone	127-41-3	C13H20O	44.974	Violet, wood	100.26 \pm 19.76	310.32 \pm 162.35	386.17 \pm 86.12	174.03 \pm 11.52	813.81 \pm 40.52
b34	4,4,6-Trimethyl-2-cyclohexen-1-one	13395-73-8	C9H14O	23.437	–	ND	ND	ND	41.56 \pm 1.77	ND
b35	3-Nonen-2-one	14309-57-0	C9H16O	28.055	Fruity, berry, fatty, oily, ketonic, weedy, spicy, licorice	ND	ND	67.08 \pm 5.30	ND	ND
b36	4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-3-buten-2-one	14901-07-6	C13H20O	47.584	Floral, violet	282.09 \pm 108.33	ND	ND	ND	624.31 \pm 75.96
b37	6-Methyl-3,5-heptadiene-2-one	1604-28-0	C8H12O	25.457	Cinnamon, coconut, spice, woody, sweet, weedy	ND	ND	361.74 \pm 58.76	ND	ND
b38	6,10-Dimethyl-2-undecanone	1604-34-8	C13H26O	43.986	–	ND	ND	24.49 \pm 2.15	12.13 \pm 0.15	ND
b39	1-Penten-3-one	1629-58-9	C5H8O	5.558	Fish, green, mustard, pungent	ND	ND	ND	13.15 \pm 8.15	ND
b40	4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-butanone	17283-81-7	C13H22O	45.476	Earthy, woody, mahogany, orris, dry amber	39.76 \pm 9.15	23.07 \pm 5.25	46.31 \pm 15.40	38.38 \pm 10.38	30.60 \pm 6.41
b41	3,4,4-Trimethyl-2-cyclohexen-1-one	17299-41-1	C9H14O	23.443	–	ND	95.07 \pm 5.78	131.08 \pm 6.40	52.44 \pm 8.68	77.24 \pm 49.59
b42	<i>trans</i> -3-Nonen-2-one	18402-83-0	C9H16O	28.101	–	ND	ND	63.98 \pm 8.17	ND	37.49 \pm 6.93
b43	4-Methyleneisophorone	20548-00-9	C10H14O	33.543	–	ND	32.57 \pm 0.64	46.97 \pm 5.38	ND	12.27 \pm 5.07
b44	2,2,6-Trimethylcyclohexanone	2408-37-9	C9H16O	20.206	Floral	ND	ND	ND	ND	108.42 \pm 6.90
b45	4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-butanone	31499-72-6	C13H22O	44.430	Fruit	ND	55.45 \pm 5.53	ND	ND	ND
b46	(<i>E</i>)-6,10-Dimethylundeca-5,9-dien-2-one	3796-70-1	C13H22O	46.154	Fresh, green, fruity, waxy, rose, woody, magnolia, tropical	ND	32.19 \pm 1.41	80.07 \pm 78.21	ND	266.95 \pm 82.34
b47	3,5-Octadien-2-one	38284-27-4	C8H12O	24.573	–	ND	ND	355.70 \pm 159.60	114.12 \pm 10.82	297.63 \pm 28.96
b48	3,3,6-Trimethyl-1,5-heptadien-4-one	546-49-6	C10H16O	5.566	Fruity, fatty, mushroom	ND	ND	46.43 \pm 20.19	30.57 \pm 20.05	145.89 \pm 48.37
b49	(<i>R,S</i>)-5-Ethyl-6-methyl-3E-hepten-2-one	57283-79-1	C10H18O	28.501	–	31.32 \pm 3.16	34.09 \pm 6.94	105.26 \pm 7.36	46.12 \pm 2.09	47.64 \pm 7.38
b50	1-(2,3,6-Trimethylphenyl)-3-butanone	58720-40-4	C13H18O	51.515	–	52.94 \pm 35.56	62.98 \pm 44.27	58.30 \pm 22.38	49.59 \pm 16.54	ND
b51	Acetone	67-64-1	C3H6O	7.211	Pungent	ND	ND	56.28 \pm 13.14	ND	ND
b52	Isophorone	78-59-1	C9H14O	21.958	Cedarwood, spice	113.97 \pm 10.58	164.20 \pm 5.80	ND	ND	ND
b53	β -Ionone	79-77-6	C13H20O	47.615	Floral, violet	ND	40.92 \pm 7.22	181.62 \pm 126.57	172.52 \pm 129.06	700.34 \pm 15.87
b54	1-(4- <i>tert</i> -Butylphenyl)propan-2-one	81561-77-5	C13H18O	42.791	–	26.05 \pm 5.84	42.28 \pm 5.62	ND	ND	28.18 \pm 3.34
b55	Acetophenone	98-86-2	C8H8O	22.423	Almonds, flower, meat, must	93.99 \pm 9.91	665.33 \pm 26.28	636.03 \pm 14.15	71.38 \pm 6.69	89.40 \pm 5.92
<i>Hydrocarbons</i>										
b56	Styrene	100-42-5	C8H8	11.154	Sweet, balsam, floral, plastic	721.64 \pm 66.27	ND	ND	ND	ND
b57	1,3-Bis(1,1-dimethylethyl)benzene	1014-60-4	C14H22	35.929	–	315.95 \pm 30.34	96.40 \pm 56.12	189.61 \pm 22.43	ND	362.08 \pm 62.90
b58	1,4-Dimethyl-2,5-bis(1-methylethyl)-benzene	10375-96-9	C14H22	47.469	–	ND	ND	ND	ND	58.63 \pm 3.35
b59	1,3-Dimethyl-benzene	108-38-3	C8H10	10.010	Plastic	233.33 \pm 33.05	36.79 \pm 9.28	ND	ND	ND
b60	Toluene	108-88-3	C7H8	5.833	Sweet	86.48 \pm 16.74	ND	ND	ND	ND
b61	β -Himachalene	1461-03-6	C15H24	43.118	–	ND	ND	ND	ND	55.19 \pm 6.32
b62	Acenaphthylene	208-96-8	C12H8	45.586	–	ND	ND	35.62 \pm 6.09	ND	ND
b63	1,6,7-Trimethylnaphthalene	2245-38-7	C13H14	50.652	Earthy	ND	17.45 \pm 1.58	28.53 \pm 17.62	17.66 \pm 11.46	ND
b64	Ethylpentamethylbenzene	2388-04-7	C13H20	46.768	–	ND	ND	39.66 \pm 18.84	ND	ND
b65	Germacrene D	23986-74-5	C15H24	43.125	Woody, spice	ND	ND	ND	ND	58.34 \pm 5.77
b66	1,2,3,4-Tetrahydro-1,6,8-trimethylnaphthalene	30316-36-0	C13H18	41.426	–	81.07 \pm 101.63	ND	ND	ND	ND
b67	2,2',5,5'-Tetramethyl-1,1'-biphenyl	3075-84-1	C16H18	54.526	–	ND	ND	ND	11.92 \pm 7.73	ND

(continued on next page)

Table 2 (continued)

No	Compounds	CAS	Formula	Rt	Odor description	Content (ng/g)				
						SX	HN	GZ	ZJ	GX
b68	1,7,7-Trimethylbicyclo[2.2.1]hept-2-ene	464-17-5	C10H16	34.217	–	ND	42.57 ± 1.23	29.75 ± 3.59	22.80 ± 4.88	104.81 ± 8.76
b69	α-Cedrene	469-61-4	C15H24	44.155	Woody, cedar, sweet, fresh	ND	38.93 ± 3.49	32.97 ± 0.28	16.95 ± 3.51	ND
b70	1,2,3,4-Tetrahydro-1,1,6-trimethylnaphthalene	475-03-6	C13H18	41.416	Fruit	ND	ND	125.62 ± 77.43	ND	41.25 ± 10.16
b71	Tridecane	629-50-5	C13H28	38.683	–	11.73 ± 2.11	ND	ND	ND	ND
b72	Tetradecane	629-59-4	C14H30	43.737	Mild, waxy	12.58 ± 1.00	ND	ND	ND	ND
b73	(Z)-3-Methyl-4-undecene	74645-87-7	C12H24	22.073	–	ND	ND	227.12 ± 24.23	84.81 ± 5.93	ND
b74	Acenaphthene	83-32-9	C12H10	47.169	–	ND	ND	9.87 ± 0.21	ND	ND
b75	Fluorene	86-73-7	C13H10	51.361	–	ND	ND	11.17 ± 2.00	ND	ND
b76	Naphthalene	91-20-3	C10H8	30.758	Pungent, dry, tarry	70.28 ± 3.28	168.29 ± 18.81	442.84 ± 23.54	66.07 ± 6.83	47.65 ± 8.00
b77	2-Methylnaphthalene	91-57-6	C11H10	38.822	Sweet, floral, woody	7.50 ± 1.37	56.23 ± 3.42	68.42 ± 4.10	11.88 ± 1.92	ND
b78	Biphenyl	92-52-4	C12H10	42.464	Pungent, rose, green, geranium	ND	ND	51.71 ± 6.75	9.61 ± 3.58	ND
b79	α-Terpinene	99-86-5	C10H16	19.091	Woody, terpene, lemon, herbal, medicinal, citrus	ND	ND	ND	22.77 ± 0.50	67.61 ± 35.05
<i>Furans</i>										
b80	Dibenzofuran	132-64-9	C12H8O	48.551	–	ND	ND	27.35 ± 5.52	ND	ND
b81	2-Acetyl-2-methyltetrahydrofuran	32318-87-9	C7H12O2	25.427	–	ND	ND	ND	22.05 ± 2.22	ND
b82	4-[(S)-1-Methylpropyl]-2,3-dihydrofuran	34379-54-9	C8H14O	16.253	–	100.45 ± 24.79	ND	56.49 ± 14.56	ND	ND
b83	5-Methoxy-6,7-dimethyl-1-benzofuran	35355-35-2	C11H12O2	46.777	–	ND	ND	35.60 ± 3.74	ND	ND
b84	2-Butylfuran	4466-24-4	C8H12O	25.743	Wet, hay	ND	ND	ND	ND	37.11 ± 4.63
<i>Lactones</i>										
b85	5,6,7,7a-Tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone	17092-92-1	C11H16O2	49.310	Musk, coumarin	66.49 ± 23.41	ND	60.82 ± 25.44	68.49 ± 7.73	100.04 ± 18.81
<i>Others</i>										
b86	Butylated Hydroxytoluene	128-37-0	C15H24O	48.736	Mild, phenolic, camphor	ND	ND	ND	11.85 ± 0.16	ND
b87	Dimethyl ether	115-10-6	C2H6O	3.109	Ethereal	ND	56.00 ± 20.40	ND	ND	ND
b88	Hexanoic acid	142-62-1	C6H12O2	20.445	Sour, fatty, sweat, cheese	ND	ND	179.31 ± 10.77	ND	ND
b89	3,4-Dimethoxytoluene	494-99-5	C9H12O2	35.191	–	ND	ND	21.56 ± 0.67	ND	ND
b90	1,2,3-Trimethoxybenzene	634-36-6	C9H12O3	39.456	–	25.59 ± 7.07	ND	ND	33.60 ± 12.69	ND
b91	Dimethylphosphinic fluoride	753-70-8	C2H6FOP	4.171	–	ND	ND	49.59 ± 22.75	ND	137.30 ± 92.04
b92	1,2-Dimethoxybenzene	91-16-7	C8H10O2	28.612	Earth, moss, wood	42.29 ± 3.19	30.82 ± 2.89	70.55 ± 6.72	66.62 ± 5.90	ND
b93	Precocene I	17598-02-6	C12H14O2	46.523	–	ND	ND	135.22 ± 103.55	ND	ND

* Odor descriptions were from FEMA database. ND, not detected.

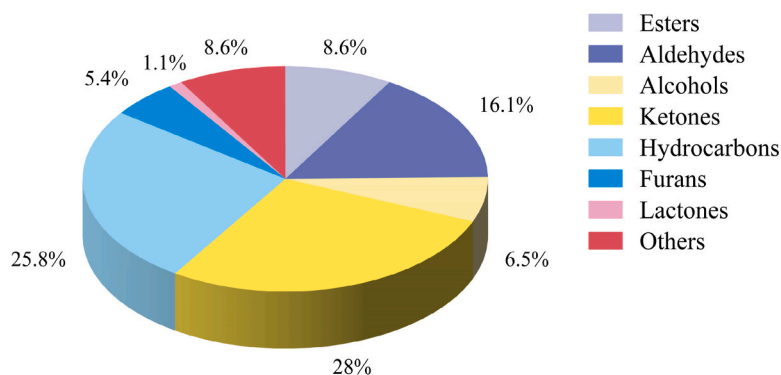
Ketones were the second largest group of VOCs and were present at comparable levels in the five FBTs (30.54%–35.70%). They were mainly composed of 2-butanone, 2-propanone, and 3-pentanone. Specifically, in the SX sample, 2-propanone and 2-butanone contents were the highest at 15.83% and 4.82%, respectively. The GX samples had the lowest 2-propanone and 2-butanone contents of 11.82% and 3.54%, respectively, and were remarkably different from other samples ($p < 0.05$). Therefore, 2-propanone and 2-butanone contribute to the identification of the geographical origin of FBTs. The amounts of 3-pentanone also differed in the different FBTs. The lowest 3-pentanone content was found in SX (3.33%), followed by GZ (3.45%), whereas the highest 3-pentanone level was detected in GX (4.39%).

The levels of alcohols in GZ (11.04%) were lower than those in SX (11.10%), HN (11.50%), ZJ (15.02), and GX (16.34%), and linalool,

linalool oxide, and 2,3-butanediol, which provided a floral flavor, were the most abundant compounds. Linalool and linalool oxide accounted for 0.52% and 0.28% of the VOCs in HN, respectively, and were present at greater concentrations in SX (1.50, 0.41%), GZ (1.61%, 0.94%), ZJ (3.36, 0.96%), and GX (2.95%, 4.40%). The GX sample had the highest contents of the monomers and dimers of linalool oxide ($p < 0.05$), which could help to distinguish the different FBTs.

Nine esters were identified and measured in the five FBTs. These esters were dominated by methyl salicylate and ethyl acetate. Methyl salicylate imparts the aroma of wintergreen oil and mint and has been reported to be one of the key VOCs contributing to the characteristic flavor of FBT samples (Li et al., 2020a, 2020b). Notably, organic acids were present at low levels in all FBT samples. We detected three organic acids and found that the content of 2-methylbutanoic acid was the

(A)



(B)

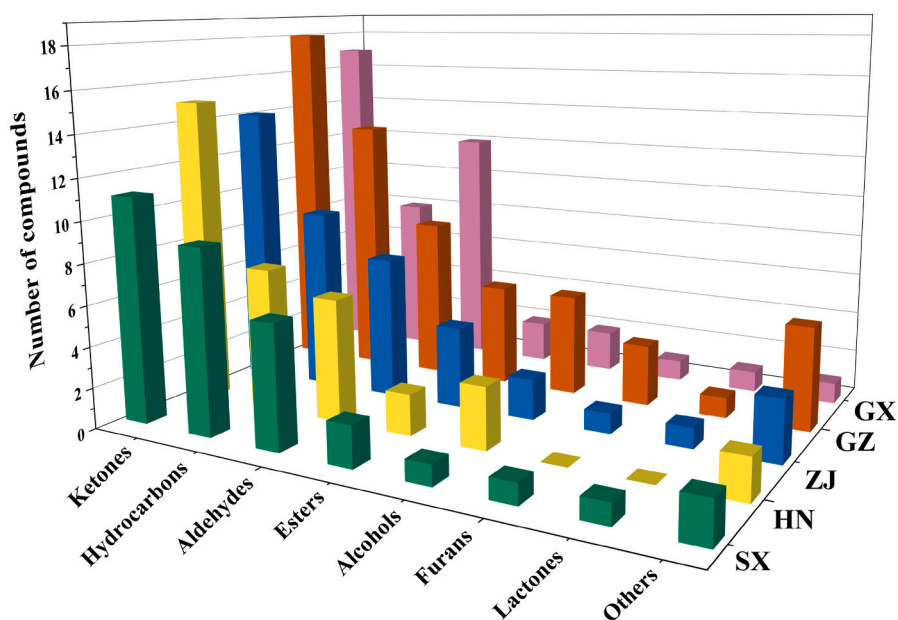


Fig. 2. (A) Classification of all VOCs, (B) category distributions of volatile organic compounds by GC-MS of five FBT samples.

highest in the ZJ sample at 1.16% ($p < 0.05$). This compound could help distinguish FBTs from ZJ from those from other production regions.

3.1.3. Odor profiles of FBTs from five different regions

The ROAVs of the 63 identified VOCs were determined to assess the role of these compounds in the aroma scent of the teas. A high ROAV indicates the great contribution to the scent qualities of FBT. A compound with $ROAV \geq 1$ was considered as a crucial volatile molecule, whereas a VOC with $0.1 \leq ROAV < 1$ plays a major part in modifying the overall flavor of a sample. A total of 20 key VOCs with $ROAV \geq 0.1$ were

found among the 63 VOCs (Table S1), which separated into two classes. The first group ($ROAV \geq 1$) included β -damascenone, isoamyl acetate (highest ROAV of 1.34 ± 0.07 in sample HN), (*E*)-2-nonenal (highest ROAV of 1.83 ± 0.29 in sample GX), 2-methylbutanal (highest ROAV of 1.34 ± 0.03 in sample SX), and linalool (highest ROAV of 10.27 ± 1.79 in sample GX). The second group ($0.1 \leq ROAV < 1$) included ethyl hexanoate, nonanal, (*E*)-2-octenal, benzeneacetaldehyde, (*E,E*)-2,4-heptadienal-M, (*E,E*)-2,4-heptadienal-D, heptanal-M, heptanal-D, hexanal, 3-methylbutanal, butanal, 2-pentylfuran, octanal, 1-hexanol, and methylpropanal. (*E,E*)-2,4-heptadienal, which provides a fatty, floral

odor, is also detected in stale tea and in tea with a sun-baked flavor due to photic oxidation. Microbial metabolism could contribute to the production of (*E,E*)-2,4-heptadienal during the fermentation of FBT (Xu et al., 2007). An early study reported that nonanal and heptanal are generated by the oxidation of the precursors oleic acid and palmitoleic (Ho et al., 2015) and contribute to fresh and greenish odors in tea infusions (Takeo and Tsushida, 1980). β -Damascenone had the highest ROAV value (ROAV = 100) and provide a strong contribution to the flavor characteristic of FBT. Given that β -damascenone has an apple-like flavor and a very low water threshold (0.002 ppb), it could easily affect the aroma attributes of teas. It results from the oxidation of neoxanthin by enzymes (Ho et al., 2015).

3.2. VOCs identified via GC–MS

3.2.1. Analysis of VOCs in FBT from five different production regions

The VOCs were also analyzed through GC–MS with HS–SPME to illuminate the flavor differences of diverse FBTs comprehensively. The representative total ion chromatograms of VOCs are illustrated in Fig. S3A, and Table 2 shows the detailed results of all VOCs in five FBT samples. A total of 93 VOCs were characterized through GC–MS. They included aldehydes (15), esters (8), alcohols (6), ketones (26), hydrocarbons (24), furans (5), lactone (1), and others (8). Similar to the results of GC–IMS, the peak areas and numbers of the VOCs in the five FBT samples greatly differed, indicating that the VOCs were drastically distinct. Fig. 2A and B illustrate the proportion and category distributions of the VOCs in the five FBT samples. These figures indicated that the number of VOCs varied among the five samples given that 33, 35, 58, 41, and 44 VOCs were identified in SX, HN, GZ, ZJ, and GX, respectively. The release of VOCs through the Maillard reaction, glycoside hydrolysis, carotenoid degradation, or lipid breakdown during processing resulted in either the reduction or generation of VOCs in distinct categories (Ho et al., 2015).

The FBT samples had 12 VOCs in common, and FBTs from different origins had unique VOCs. Among all the detected VOCs, six were detected only in SX, 10 only in GX, seven only in ZJ, 17 only in GZ, and two only in HN (Fig. S3B). Among these VOCs, benzeneacetaldehyde, styrene, toluene, 1,2,3,4-tetrahydro-1,6,8-trimethylnaphthalene, tridecane, and tetradeane were found only in SX samples. The VOCs unique to HN were 4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-2-butanone and dimethyl ether. The VOCs unique to GZ were ethyl caprylate, methyl hexanoate, vinyl acrylate, 5-ethylcyclopent-1-enecarboxaldehyde, (*S*)-(+)-3-methyl-1-pentanol, 3-nonen-2-one, 6-methyl-3,5-heptadiene-2-one, acetone, acenaphthylene, ethylpentamethylbenzene, acenaphthene, fluorene, dibenzofuran, 5-methoxy-6,7-dimethyl-1-benzofuran, hexanoic acid, 3,4-dimethoxytoluene, and precocene I. The VOCs unique to ZJ were 2,2,4-trimethyl-1,3-pentanediol diisobutyrate, 3,3-diethoxy-1-propyne, 4,4,6-trimethyl-2-cyclohexen-1-one, 2,2,5,5-tetramethyl-1,1-biphenyl, 2-acetyl-2-methyltetrahydrofuran, 1-penten-3-one, and butylated hydroxytoluene. The VOCs unique to GX were (*E*)-2-nonenal, 2,6,6-trimethylcyclohex-2-ene-1-carbaldehyde, 2,5-dimethylbenzaldehyde, (*E*)-2-hexenal, terpinen-4-ol, 2,2,6-trimethylcyclohexanone, 1,4-dimethyl-2,5-bis(1-methylethyl)-benzene, β -himachalene, germacrene D, and 2-butylfuran. These distinct VOCs could help discriminate FBTs from different regions.

GC–IMS and GC–MS displayed different identification capabilities for VOCs as previously demonstrated (Wang et al., 2018a). In the present study, GC–MS and GC–IMS measured aldehydes and ketones sensitively. Ketones could be generated by microbial enzymatic activity on lipids or amino acids, as well as Maillard reactions (Wang et al., 2018b). Although ketone molecules are often present, their aromatic contribution may be modest due to their high thresholds. Table 2 shows the distribution of VOCs in the five FBT samples. Ketones were the main VOCs in the five samples. A total of 11, 15, 18, 14, and 17 ketones were detected in SX, HN, GZ, ZJ, and GX, respectively, at the contents of 841.61 \pm 265.25, 2152.07 \pm 277.26, 2873.91 \pm 360.04, 894.76 \pm 173.06, and 3710.16 \pm 410.27 ng/g, respectively. The GX sample had

the highest content of 6-methyl-5-hepten-2-one (1181.21 \pm 19.30 ng/g) among the five samples. This compound may originate from the degradation of carotenoids in FBT (Takeo and Tsushida, 1980). α -Ionone and β -ionone contribute to the floral aroma of tea. GX had the highest contents of these two compounds (813.81 \pm 40.52 ng/g for α -ionone and 700.34 \pm 15.87 ng/g for β -ionone). Among all the ketones detected, 3-nonen-2-one with a fruity aroma (67.08 \pm 5.30 ng/g); 6-methyl-3,5-heptadiene-2-one with a woody, sweet aroma (361.74 \pm 58.76 ng/g); and acetone with a pungent scent (56.28 \pm 13.14 ng/g) were detected only in GZ samples. 4-(2,6,6-Trimethyl-2-cyclohexen-1-yl)-2-butanone (55.45 \pm 5.53 ng/g) with fruity aroma and 2,2,6-trimethylcyclohexanone (108.42 \pm 6.90 ng/g) with floral aroma were detected only in the samples of HN and GX, respectively. 4,4,6-Trimethyl-2-cyclohexen-1-one (41.56 \pm 1.77 ng/g) and 1-penten-3-one (13.15 \pm 8.15 ng/g) were detected only in ZJ. These compounds conferred fishy, green, mustardy, and pungent aromas.

Aldehydes constituted the second largest group of VOCs in the five FBT samples. Surprisingly, the variation in aldehyde content was similar to that in ketone content: the content of aldehydes was the largest in GX (4939.20 \pm 565.45 ng/g), followed by that in GZ (2015.99 \pm 349.23 ng/g). Benzaldehyde and benzeneacetaldehyde, which impart floral scents, were the crucial flavor compounds contributing to the characteristic aroma of tea (Ho et al., 2015; Wang et al., 2000). Benzaldehyde was found in all FBT samples, and its levels were highest in GZ (555.84 \pm 37.46 ng/g), whereas benzeneacetaldehyde was observed only in SX (49.87 \pm 11.41 ng/g). Interestingly, we identified diverse derivatives of benzaldehyde. For example, we tentatively characterized the methyl derivatives 2,3-dihydro-2,2,6-trimethylbenzaldehyde and 2,5-dimethylbenzaldehyde. The content of the former was highest in ZJ (177.84 \pm 11.44 ng/g), whereas the latter was detected only in GX (39.27 \pm 0.91 ng/g).

The GZ sample showed greater ester contents than the other samples, e.g., methyl salicylate, linalyl butyrate, methyl hexanoate, and ethyl caprylate, which impart fruity odors to tea. Methyl salicylate was the only ester identified in each FBT sample and was present at the concentrations of 568.55 \pm 17.35, 294.69 \pm 30.48, 606.82 \pm 24.88, 228.89 \pm 3.47, and 622.63 \pm 77.99 ng/g in SX, HN, GZ, ZJ, and GX, respectively. Hydrocarbons were present at the lowest levels in ZJ (181.15 \pm 29.91 ng/g) and the highest levels in SX (1408.97 \pm 124.52 ng/g). Compared with other compounds, hydrocarbons had less effect on the overall flavor of the teas due to their higher threshold values.

3.2.2. Key aroma active compounds forming the aroma characteristics of the five FBTs

Note that not all VOCs contribute to tea aroma. The sensory effects of VOCs are not only influenced by their level and odor attributes, they are also greatly associated with the odor threshold (Deng et al., 2021). In addition, a synergistic effect among different VOCs with diverse olfactory characteristics and ratios might have occurred and consequently influenced the types of aroma. OAV analysis is generally used to assess the contribution of VOCs to aroma, and high values represent great aroma contribution. Although many kinds of VOCs were detected in the five FBT samples, OAV analysis revealed that not all the VOCs contributed greatly to the flavor of FBT. The OAVs of 27 VOCs in five FBT samples were greater than 1. These VOCs consisted of three esters, eight aldehydes, two alcohols, eight ketones, five hydrocarbons, and a furan. These results are also shown in Table S2 β -Ionone, which had the highest OAV and provided a floral, violet odor, was found in HN, GZ, ZJ, and GX (58 46.08–100 048.11). Meanwhile, α -ionone had the maximum OAV value (215.29 \pm 10.72) in the GX sample and the minimum OAV value (26.52 \pm 5.23) in the SX sample. Previous literature (Wang et al., 2021) stated that the continuous postfermentation of dark tea caused the degradation of carotenoids, leading to the accumulation of α - and β -ionones as demonstrated by our findings. Ionones (including α - and β -forms) were reported to have a remarkable effect on the aroma of teas (Zhou et al., 2019), and the different ionone values of FBT samples could

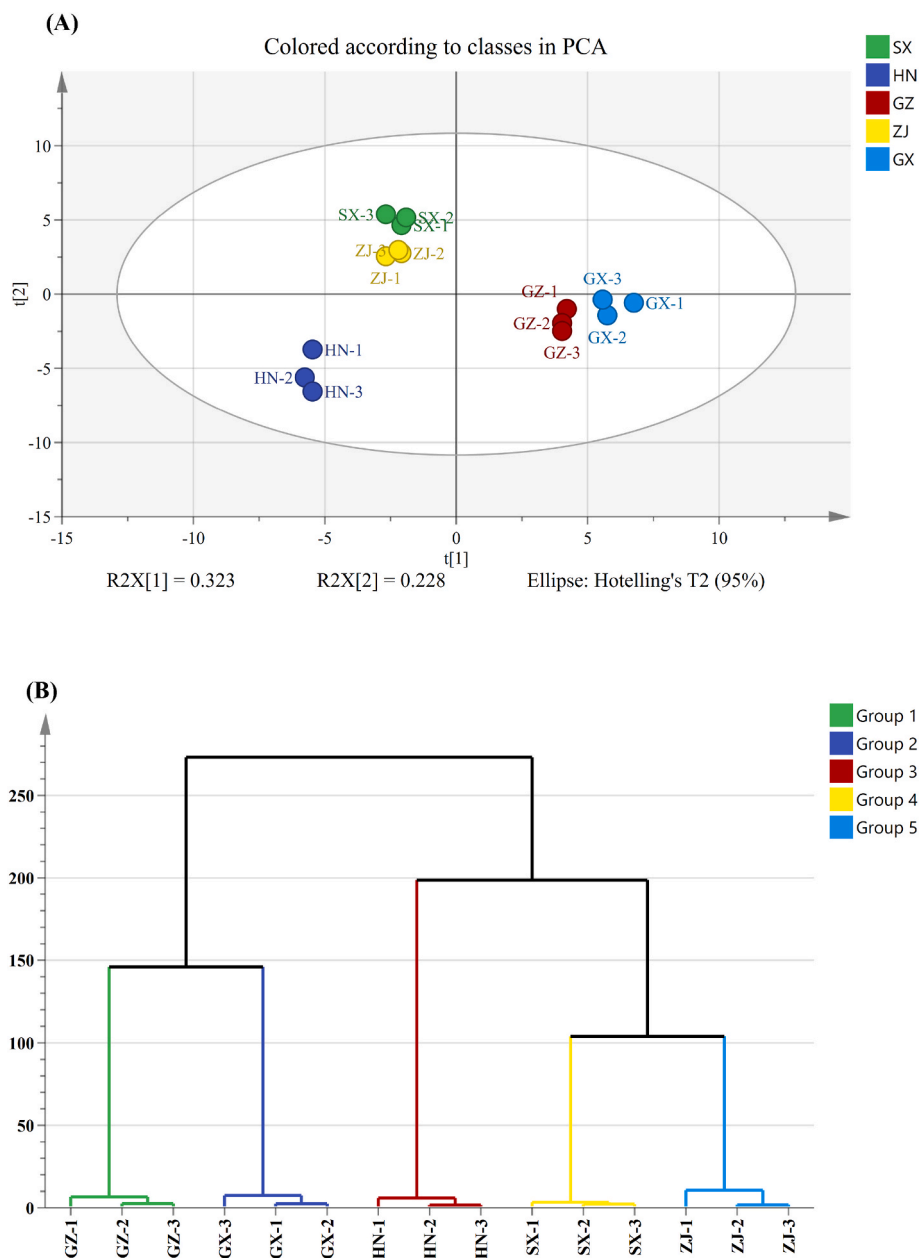


Fig. 3. Principal component analysis, hierarchical cluster analysis, partial least squares discriminant analysis, and variable importance in the projection in different manufacturing regions of FBT according to GC-IMS (A–D) and GC-MS (E–H).

be used to discriminate teas from diverse production regions.

In the SX sample, only linalool had OAV >600. VOCs with $100 > \text{OAV} > 10$ included methyl salicylate, 2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde, ethyl hexanoate, hexanal, α -ionone, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one, naphthalene, styrene, and 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-butanone. VOCs with $10 > \text{OAV} > 1$ included benzeneacetaldehyde, 6-methyl-5-hepten-2-one, 2-methylnaphthalene, acetophenone, and 2,6,6-trimethyl-2-cyclohexene-1,4-dione. The unique active aroma components of SX were benzeneacetaldehyde and styrene, which imparted floral and berry-like aromas.

The OAV of β -ionone in the HN sample exceeded 5000. Meanwhile, the VOCs in the HN sample with $100 > \text{OAV} > 10$ included 2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde, hexanal, 1-hexanol, α -ionone, heptanal, acetophenone, naphthalene, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-butanone, and 2-methylnaphthalene. The OAV scores of 2,6,6-trimethyl-2-cyclohexene-1,4-dione (floral) and

acetophenone (almond, floral, meaty, and musty aromas) in HN were higher than in SX, GZ, ZJ, and GX. Furthermore, heptanal, (*E,E*)-2,4-heptadienal, 1-hexanol, and 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-butanone, which were responsible for nutty, fatty, and green aromas, were lower in HN than in other samples. As a result, HN had a relatively weaker green odor characteristic than the other teas. Table S2 shows that 2,6,6-trimethyl-2-cyclohexene-1,4-dione and acetophenone (imparting floral and almond-like aromas) had the highest value in the samples and greatly contributed to discriminating the aroma of HN from that of teas from other regions.

The OAVs of β -ionone and linalool in the GZ sample were greater than 20 000 and 800, respectively. VOCs in the GZ sample with $500 > \text{OAV} > 100$ consisted of hexanal, α -ionone, and biphenyl; those with $100 > \text{OAV} > 10$ included methyl salicylate, heptanal, decanal, (*E,E*)-2,4-heptadienal, 2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde, 2-methylnaphthalene, naphthalene, 1-hexanol, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-

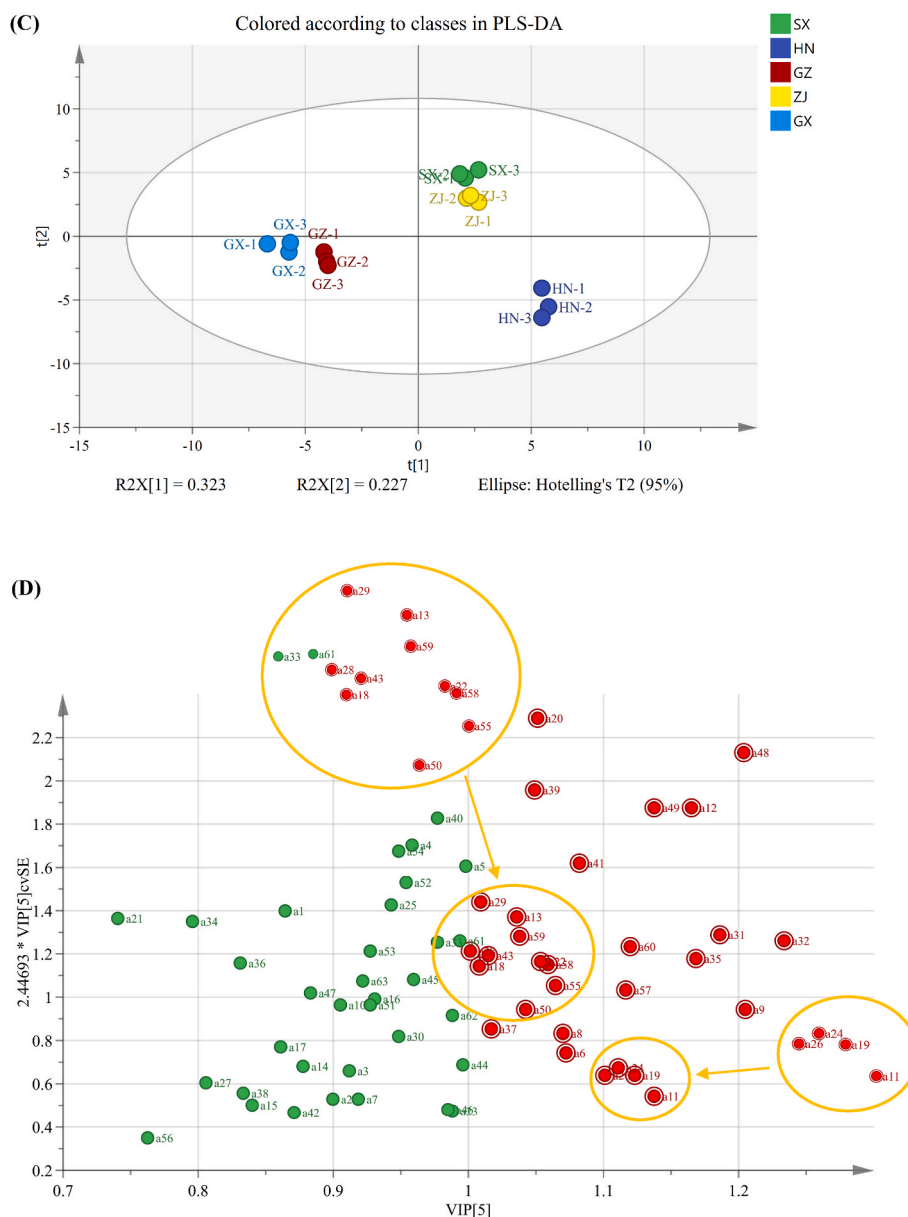


Fig. 3. (continued).

butanone, and 1,2,3,4-tetrahydro-1,1,6-trimethylnaphthalene; and those with $10 > \text{OAV} > 1$ included 6-methyl-5-hepten-2-one, ethyl caprylate, dibenzofuran, acetophenone and 2,6,6-trimethyl-2-cyclohexene-1,4-dione. In this sample, β -ionone showed the highest OAV. Furthermore, several VOCs showed higher OAV scores in GZ than in other FBTs. These VOCs included floral-woody and floral-fruity compounds, such as decanal, 1-hexanol, and 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-butanone, and fruity and sweet floral compounds, such as 2-methylnaphthalene and 1,2,3,4-tetrahydro-1,1,6-trimethylnaphthalene. The unique active aroma components in GZ included ethyl caprylate and dibenzofuran, which have oleaginous and fruity aromas.

In the ZJ sample, only β -ionone and linalool showed $\text{OAV} > 20\ 000$ and $\text{OAV} > 1600$; VOCs with $100 > \text{OAV} > 10$ included hexanal, (*E,E*)-2,4-heptadienal, ethyl hexanoate, 2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde, naphthalene, biphenyl, α -ionone, and 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-butanone; and those with $10 > \text{OAV} > 1$ included methyl salicylate, 6-methyl-5-hepten-2-one, 2-methylnaphthalene, acetophenone, and 2,6,6-trimethyl-2-cyclohexene-1,4-dione. All of these VOCs remarkably contributed to the formation of the aroma

characteristics of ZJ.

In the GX sample, β -ionone and linalool also had the highest OAV values that exceeded 100 000 and 1500, respectively; VOCs with $500 > \text{OAV} > 100$ included heptanal, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one, (*E*)-2-nonenal, hexanal, α -ionone, and 2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde; and VOCs with $100 > \text{OAV} > 10$ included methyl salicylate, (*E,E*)-2,4-heptadienal, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-butanone, 6-methyl-5-hepten-2-one, and 1,2,3,4-tetrahydro-1,1,6-trimethylnaphthalene. Notably, the OAVs of most active aroma compounds in GX were larger than those of the compounds in other samples. These compounds included methyl salicylate, heptanal, (*E,E*)-2,4-heptadienal, hexanal, linalool, and β -ionone. The unique active aroma components in GX were (*E*)-2-nonenal, (*E*)-2-hexenal, and 2,2,6-trimethylcyclohexanone, which imparted a fatty and green odor.

3.3. Differential VOCs in FBTs from different regions

PCA and HCA were conducted on the VOCs identified by using GC-IMS to further reveal the similarities and differences in VOCs among

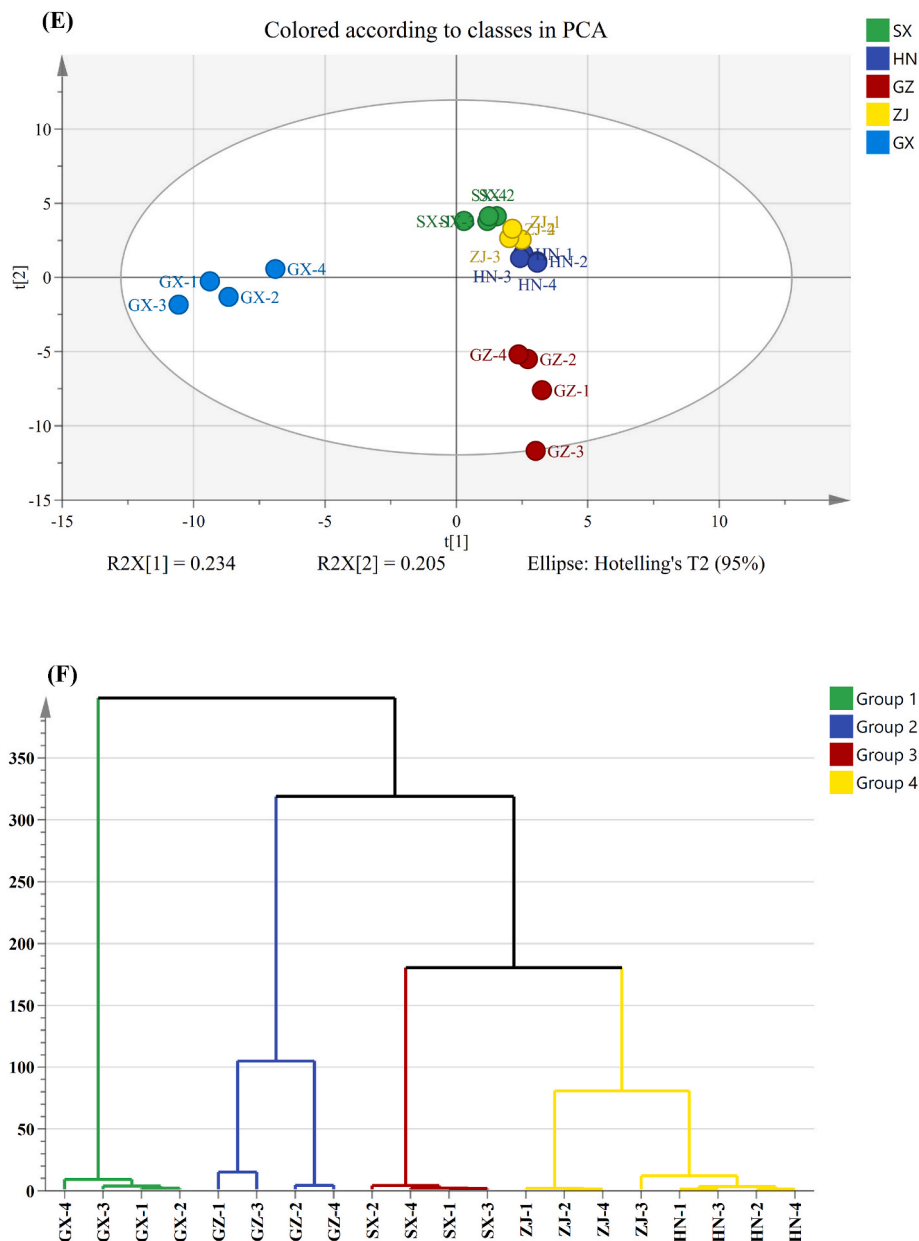


Fig. 3. (continued).

the FBT samples. The results are shown in Fig. 3. The PCA plot constructed on the basis of GC–IMS results (Fig. 3A) showed clear separation among the FBTs from five different sources, implying that the VOCs of tea samples differed dramatically. The HN samples clustered in the bottom left corner, samples from ZJ and SX clustered in the upper left corner, and samples from GX and GZ were located in the right area. HCA also classified the FBT samples into three groups (HN, SX–ZJ, and GZ–GX) (Fig. 3B) in agreement with the PCA results. The PLS–DA model was further constructed to clarify the differential VOCs that were responsible for distinguishing FBTs with various origins, and the results are presented in Fig. 3C. A total of 29 VOCs were identified as differential metabolites on the basis of the common screening criteria of variable importance in the projection (VIP) > 1 and $p < 0.05$ (Fig. 3D). Notably, the levels of these differential VOCs varied drastically among the FBT samples. Among them, nine differential VOCs were found with the highest concentration in the SX sample; four kinds of VOCs were present at the highest concentration in the HN sample; and six, eight, and two discriminatory VOCs had the highest contents in the GZ, ZJ and

GX samples, respectively (Table 1). In addition, the discriminatory aldehyde compounds, such as benzeneacetaldehyde, heptanal, and 3-methylbutanal, might greatly contribute to discriminating the aroma of SX from that of FBTs from other regions, and differential heteroatomic compounds and ketones, such as 2-pentylfuran and acetophenone, might differentiate HN from the other FBT samples. In addition, the discriminatory aldehyde compounds, including (*E*)-2-octenal, (*E*)-2-hexenal (dimer), and (*E*)-2-heptenal, might differentiate GZ from other teas. Discriminatory ester and alcohol compounds, including methyl hexanoate, ethyl 2-phenylacetate, and linalool, were might be responsible for distinguishing ZJ from the other samples. Discriminatory VOCs in the GX sample included phenylacetic acid and linalool oxide (monomer) might differentiate it with others samples.

The PCA plot and HCA and PLS–DA analysis of VOCs were also obtained from the results of GC–MS and displayed in Fig. 3E–G. These results showed that the production area had a great influence on the flavor profiles of the FBT samples. In accordance with the screening criteria for VIP > 1.0 and $p < 0.05$, 37 VOCs were screened as

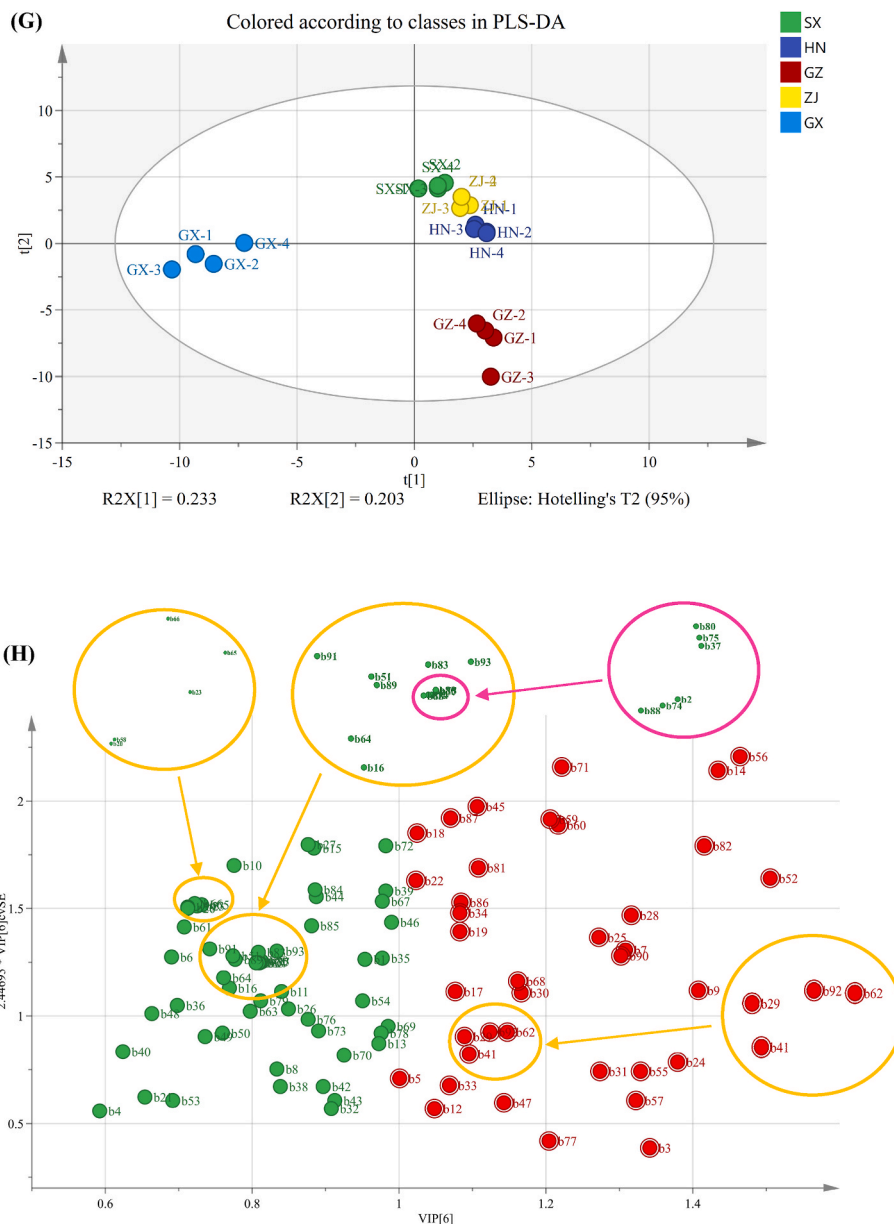


Fig. 3. (continued).

discriminating metabolites (Fig. 3H). A large value of VIP is indicative of the great difference between the groups of the aroma compounds and is important for distinguishing the aroma characteristics of diverse FBTs. Considering the lack of systematic comparative research on FBTs from different regions, we systematically compared the aroma attributes of FBTs from five different production regions. On the basis of the OAV results, 15 VOCs were considered as differential metabolites (OAV >1, $p < 0.05$, VIP >1) (Fig. 4). Three VOCs, namely, styrene, benzeneacetaldehyde, and ethyl hexanoate, were identified as discriminatory aroma components and regarded as the characteristic compounds in the SX sample. Among the five FBT samples, the SX sample had the greatest levels of these VOCs. Styrene, which imparts a sweet, balsamic, and almost floral aroma, has a crucial effect on improving the flavor of tea and forming the unique floral aroma characteristic of FBT. A previous study reported that styrene is a key odorant in Qingzhuan, Liubao, and Tibet teas (Takeo and Tsuchida, 1980). In this study, styrene may have contributed to the differentiation of FBT samples from different sources because it was detected only in the SX sample. Benzeneacetaldehyde, a type of phenylalanine-derived volatile in tea, generally has floral, fruity,

or sweet scents (Yang et al., 2013). Aromatic amino acid decarboxylases decarboxylate phenylalanine to produce phenylethylamine, which is then converted into benzeneacetaldehyde by amine oxidase, dehydrogenase, or transaminase (Tieman et al., 2006). Benzeneacetaldehyde has been reported to be an important fragrant compound of Keemun black tea (Su et al., 2022). The increase in its level might contribute to the floral and fruity aroma characteristics of black tea. In this study, benzeneacetaldehyde was detected only in the SX sample and could be considered as the key VOC for distinguishing SX from the teas from the other four regions. Therefore, the aroma characteristics of SX could be described as strongly floral and fruity. Two VOCs, including 2,6,6-trimethyl-2-cyclohexene-1,4-dione and acetophenone, could be regarded as discriminatory VOCs for the Hunan sample. These discriminatory aroma compounds had the highest content in the HN sample and could be used as biomarkers to distinguish this sample from other samples. In particular, acetophenone was considered to be the basic volatile of the aroma characteristics of FBT, and its production is related to microorganisms in the fermentation process (Lv et al., 2014). Li et al. (2020a) reported that Hunan FBT has high acetophenone content and that acetophenone

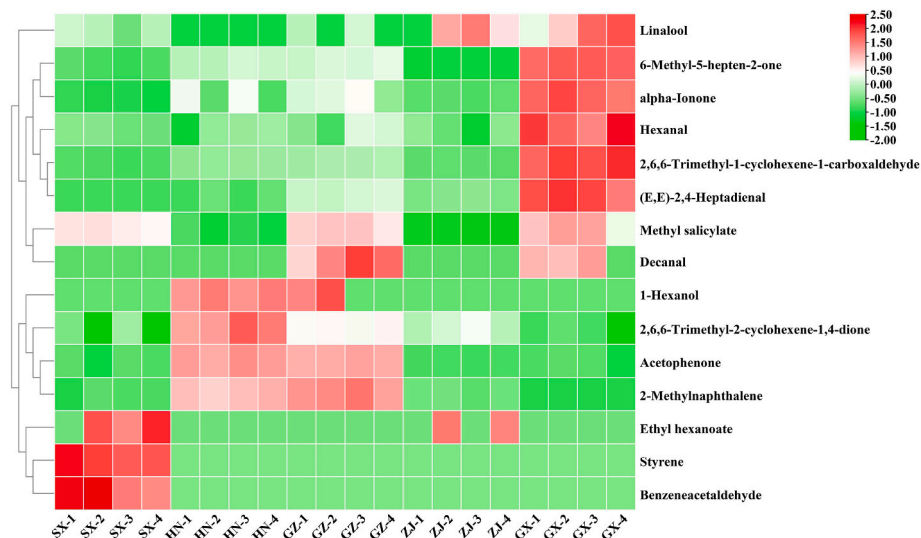


Fig. 4. Heatmap analysis for the discriminatory volatile compounds among the five sources of FBT.

provides floral, woody, and green attributes, which could promote the formation of the fungal floral aroma characteristics of Hunan FBT. In this study, acetophenone might contribute to the discrimination of HN samples from the other FBT samples. Three volatiles were identified as differential VOCs for the FBT from Guizhou: 1-hexanol, 2-methylnaphthalene, and decanal. The three compounds displayed higher concentrations in the GZ sample than in the other samples. 1-Hexanol has been well-documented to be an important contributor to the flavor and aroma of various kinds of teas (Howard, 1979) and generally imparts strong grassy odors. In earlier investigations, decanal was identified as the primary fragrance molecule that forms the green characteristic of postfermented dark tea (Shi et al., 2019). 1-Hexanol and decanal are produced through the degradation of unsaturated fatty acids in tea and were important to forming the unique green aroma characteristics of the GZ sample. In addition, 2-methylnaphthalene, the main contributor to green, pungent, and herbal-like aromas, could be formed by microbes (Tanguler et al., 2017). Li et al. (2022) reported that 2-methylnaphthalene is a vital VOC contributing to the aroma characteristics of Liupao tea with a betel-nut type aroma. Therefore, the GZ sample was characterized by a grassy and herbal aroma, whereas its floral aroma was weak. For GX, seven VOCs were identified as differential metabolites, including (E,E)-2,4-heptadienal, α -ionone, methyl salicylate, linalool, 2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde, hexanal, and 6-methyl-5-hepten-2-one. GX had the highest levels of these VOCs among all samples. Methyl salicylate, a vital contributor to the flavor quality of tea, confers the characteristics of fresh, faint gingery, grass, milky, and minty odors and could be generated through enzymatic hydrolysis (e.g., β -glucosidase and β -primeveroside) (Celik et al., 2016). This metabolite contributes to distinguishing semifermented tea from fully fermented tea (Wang et al., 2008) and is also considered as the most crucial contributor to the formation of the aroma characteristic profiles of FBT (Li et al., 2020a, 2020b). 6-Methyl-5-hepten-2-one, α -ionone, and 2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde are important aromatic compounds that impart sweet, floral, or fruity aromas (Kang et al., 2019; Joshi and Gulati, 2015) and may be related to the degradation of carotenoids during the fermentation of FBT (Li et al., 2018). Linalool is generated by the hydrolysis of the geranyl pyrophosphate precursor by microbial glycosidase that is produced during FBT processing (Ho et al., 2015). In this study, these VOCs may contribute to discriminating the source of FBT samples and to forming the unique sweet, floral, minty aroma characteristic of the GX sample. However, no VOCs were found to clearly distinguish the ZJ sample from the other samples, and only ethyl caproate with a fruity aroma and linalool with a floral aroma were

existed higher levels in this sample.

3.4. Sensory evaluation

Sensory evaluation was performed to identify the flavor differences in FBT samples from different sources. The results are presented in Fig. 5. The color of diverse FBT infusions varied. Specifically, the colors of the ZJ and HN samples were dark, whereas those of the GZ and SX samples were light. Five aroma characteristics (fungal floral, green, floral, woody, and minty) were used to describe the flavor attributes of FBT (Fig. 5B). The results indicated that among the samples, the HN sample showed the highest intensity of fungal floral and woody aromas; the GX sample showed the highest intensity of green and minty aromas; and the GZ sample presented lower intensities of floral aroma than the other samples. The results showed that the color and aroma characteristics of the FBT samples differed by production region likely due to the differences in climate, processing, and fermentation time, as well as the different microorganisms involved in their production. Correlation analysis was further conducted to investigate the relationships between sensory attributes and VOCs (OAV > 1). The data are shown in Fig. 5C. The results revealed that methyl salicylate was closely associated with fungal floral and floral attributes ($p < 0.05$); 2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde, 6-methyl-5-hepten-2-one, (E,E)-2,4-heptadienal, and α -ionone were found to be greatly related to the floral attribute ($p < 0.05$); and 2-methylnaphthalene and decanal were highly related to the green attribute ($p < 0.05$). Consistent with a previous study (Lv et al., 2014), this work found that ethyl hexanoate and styrene were related to the mint attribute ($p < 0.05$).

3.5. Association between microorganisms and volatile compounds in FBTs from different regions

Fungal communities have been reported to be a critical contributor to the drastic effects on the aroma compounds of postfermented FBT (Li et al., 2020a). In our previous study, we investigated the fungal communities of diverse FBT samples and found them to be considerably different (Chen et al., 2022). Spearman connection analysis was conducted to clarify the differences in fungal communities (at the genus level) leading to the variation in VOCs in different FBT samples, and the result is shown in Fig. 6. The important VOCs with $VIP_{\text{value}} \geq 1.0$ in GC-MS analysis were selected for correlation analysis. The results indicated that 19 fungal genera were significantly correlated with 30 VOCs ($p < 0.05$), including three esters, five aldehydes, four alcohols, six

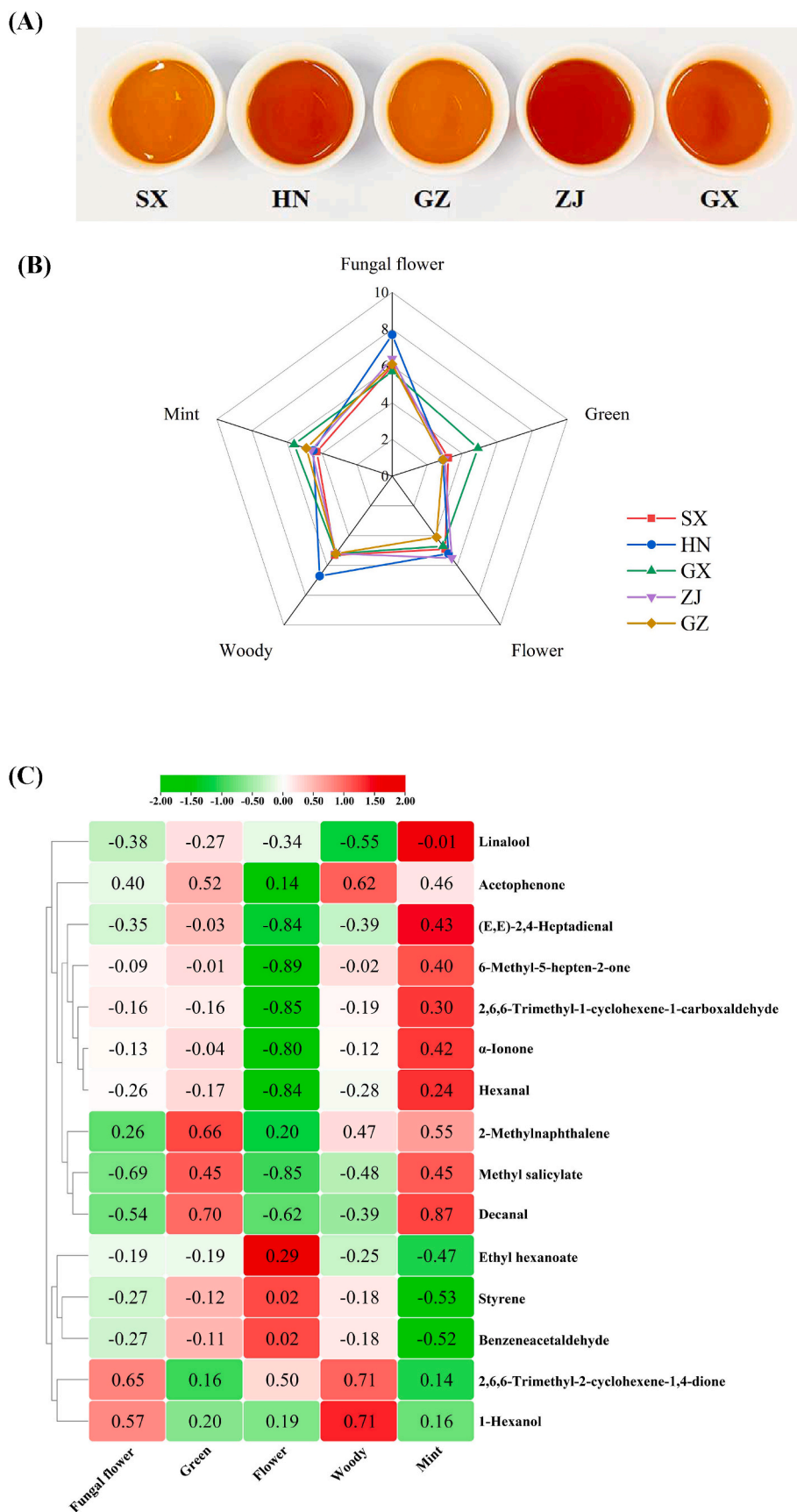


Fig. 5. (A) Color of tea infusions, (B) radar of sensory aroma and (C) the correlation results between the sensory aroma and the key volatile components.

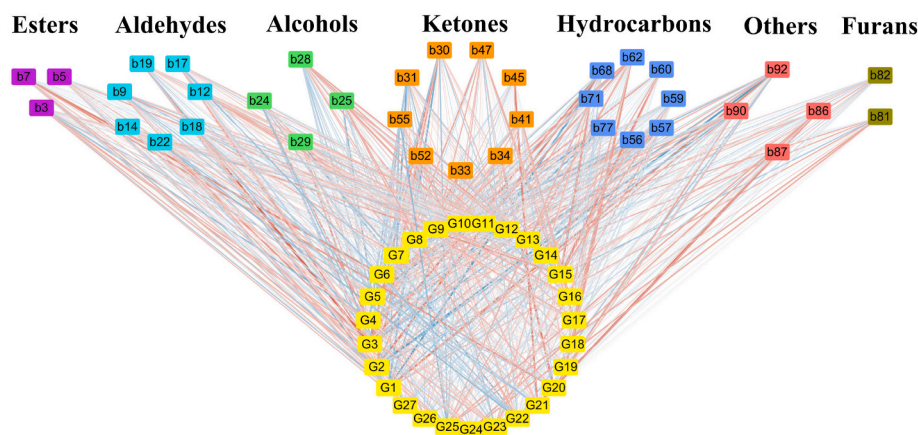


Fig. 6. Network correlation between fungal community and volatile flavor compounds. The abbreviations of fungi are shown in our previous study (Chen et al., 2022). G1: *Eurotium*, G2: *Aspergillus*, G3: *Thermoascus*, G4: *Monascus*, G5: *Saccharomycopsis*, G6: *Debaryomyces*, G7: *Saccharomyces*, G8: *Udeniomyces*, G9: *Marasmius*, G10: *Alternaria*, G11: *Penicillium*, G12: *Chytridiomycota_unclassified*, G13: *Saccharomycetales_Incertae_sedis_unclassified*, G14: *Nectriaceae_unclassified*, G15: *Chaetothyriales_unclassified*, G16: *Mortierella*, G17: *Sporobolomyces*, G18: *Eurotiomycetes_unclassified*, G19: *Monosporascus*, G20: *Walleria*, G21: *Agaricomycetes_unclassified*, G22: *Fungi_unclassified*, G23: *Thermomyces*, G24: *Quambalaria*, G25: *Spizellomycetaceae_unclassified*, G26: *Malassezia*, G27: *Ascomycota_unclassified*.

ketones, eight hydrocarbons, and four others. Specially, *Aspergillus* and *Eurotium* were the most important fungi in the fermentation of FBT (Xu et al., 2011). Consistent with our study, a previous work had also reported that these two fungi greatly contribute to aroma formation in FBT (Li et al., 2020a). It has also been reported that *Aspergillus* and *Eurotium* could produce extracellular enzymes, such as polyphenol oxidases, pectinases, and cellulases, to convert tea polyphenols into other active aroma compounds that are primarily responsible for the development of the unique flavor of tea (Chen et al., 2021; Xiao et al., 2022b). In this study, we noted that *Aspergillus* was closely positively correlated ($p < 0.05$) with 2,6,6-trimethylcyclohex-2-ene-1-carbaldehyde, 6-methyl-5-hepten-2-one, alpha-ionone, 1,3-bis(1,1-dimethylethyl)benzene, and 1,7,7-trimethylbicyclo[2.2.1]hept-2-ene. 6-Methyl-5-hepten-2-one and alpha-ionone might be formed through the oxidative decomposition of beta-carotene and the oxidative condensation of carotenoids (Waché et al., 2003) under the action of oxidases produced by *Aspergillus* (Chen et al., 2021). In this study, we discovered that two of the representative methoxyphenolic compounds, namely, 1,2,3-trimethoxybenzene and 1,2-dimethoxybenzene, were greatly positively correlated with *Eurotium* ($p < 0.05$). Methoxyphenolic compounds are thought to be the methylation products of gallic acid and tannins, which are metabolized by microorganisms (Du et al., 2013; Xu et al., 2016). Methoxyphenolic compounds have been reported as the main typical compounds in dark tea, and their accumulation during fermentation could effectively improve the coarse and old taste of raw dark tea and impart a mellow aroma to fermented dark tea (Cao et al., 2018). Notably, *Thermoascus* was significantly positively correlated with (*E,E*)-2,4-heptadienal, hexanal, linalool, 6-methyl-5-hepten-2-one, alpha-ionone, and 1,3-bis(1,1-dimethylethyl)benzene, implying that this fungus plays important role in the aroma characteristics of FBT. These results demonstrated that microorganisms provide great contributions to the formation of the characteristic aroma of FBT and that the variation in VOC profiles may be caused by the differences in the fungi present in FBT samples from different production regions.

4. Conclusion

In this study, we systematically analyzed the aroma characteristics of FBTs originating from five regions of China through GC-IMS, GC-MS, OAV, ROAV, and sensory analysis. A total of 63 and 93 VOCs were identified and quantified through GC-IMS and GC-MS, respectively. GC-MS and GC-IMS revealed that aldehydes and ketones were the major VOCs in the five samples. PCA, PLS-DA, and HCA results demonstrated that FBT samples from different production regions could be clearly separated, implying that their aroma characteristics greatly varied. GC-IMS found high levels of 2-propanone and (*E*)-2-pentenal in all FBT samples. The OAV and PCA based on GC-MS results showed that the key aroma components of FBT were beta-ionone, linalool, 2,6,6-trimethyl-1-

cyclohexene-1-carboxaldehyde, heptanal, alpha-ionone, and hexanal. The OAV of these aroma substances in some samples exceeded 100. Therefore, these compounds provided a great contribution to the aroma characteristics of the FBT samples. Fifteen key differential VOCs (i.e., linalool, 6-methyl-5-hepten-2-one, alpha-ionone, hexanal, 2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde, (*E,E*)-2,4-heptadienal, decanal, methyl salicylate, 1-hexanol, 2,6,6-trimethyl-2-cyclohexene-1,4-dione, acetophenone, 2-methylnaphthalene, ethyl hexanoate, styrene, and benzeneacetaldehyde) could discriminate the FBT samples from different regions. Sensory analysis also noted that the color and aroma of the infusions of the FBT samples from five regions differed and that methyl salicylate was significantly correlated with fungal floral and floral attributes ($p < 0.01$). In addition, the correlation analysis between VOC and fungal data showed that fungi played a crucial role in the formation of the characteristic aroma of FBT samples from different regions. *Aspergillus* and *Eurotium* were important beneficial fungi in FBT. The results of our work demonstrated that the VOCs and aroma characteristics of FBT samples from different regions of China differed greatly. These differences contributed to the classification of FBT samples from differing production regions and could also offer strong evidence for FBT quality identification.

CRedit authorship contribution statement

Yu Xiao: Project administration, Formal analysis, Methodology, Supervision, Funding acquisition, Conceptualization, Data curation, Validation, Writing – original draft, Writing – review & editing, Investigation, Resources. **Yuxin Huang:** Methodology, Data curation, Validation, Writing – original draft, Investigation. **Yulian Chen:** Writing – original draft, Investigation. **Leike Xiao:** Investigation. **Xilu Zhang:** Investigation. **Chenghongwang Yang:** Investigation. **Zongjun Li:** Resources. **Mingzhi Zhu:** Writing – review & editing. **Zhonghua Liu:** Project administration, Supervision, All authors have read and agreed to the published version of the manuscript. **Yuanliang Wang:** Writing – review & editing, Supervision, Resources.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.crfs.2022.09.024>.

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