



Characterization of volatile metabolites in Pu-erh teas with different storage years by combining GC-E-Nose, GC-MS, and GC-IMS

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ABSTRACT

Storage time is one of the important factors affecting the aroma quality of Pu-erh tea. In this study, the dynamic changes of volatile profiles of Pu-erh teas stored for different years were investigated by combining gas chromatography electronic nose (GC-E-Nose), gas chromatography-mass spectrometry (GC-MS), and gas chromatography-ion mobility spectrometry (GC-IMS). GC-E-Nose combined with partial least squares-discriminant analysis (PLS-DA) realized the rapid discrimination of Pu-erh tea with different storage time ($R^2Y = 0.992$, $Q^2 = 0.968$). There were 43 and 91 volatile compounds identified by GC-MS and GC-IMS, respectively. A satisfactory discrimination ($R^2Y = 0.991$, and $Q^2 = 0.966$) was achieved by using PLS-DA based on the volatile fingerprints of GC-IMS. Moreover, according to the multivariate analysis of VIP > 1.2 and univariate analysis of $p < 0.05$, 9 volatile components such as linalool and (E)-2-hexenal were selected as key variables to distinguish Pu-erh teas with different storage years. The results provide theoretical support for the quality control of Pu-erh tea.

1. Introduction

Tea is highly popular among the world's three major beverages, attracting many consumers with its pleasant aroma and taste. As a special microbial post-fermented tea variety, Pu-erh tea originates from Yunnan Province of China, and is famous for its unique stale flavor and mellow taste, as well as multiple health benefits such as anti-tumor, anti-oxidation, or lipid reduction (Yang, Wang, & Sheridan, 2018). The reported bioactive components of Pu-erh tea mainly include tea polyphenols, tea polysaccharides, gallic acid, caffeine and so on (Liu et al., 2021). Using leaves from *Camellia sinensis* var. *assamica* as raw material, Pu-erh tea is made through a series of special processes, including spreading, fixation, post-fermentation, shaping, and drying. And the post-fermentation is the key process to influence the quality of Pu-erh tea (Ma et al., 2022). In this process, the special environment of high humidity and temperature promotes the growth, propagation and fermentation of microorganisms, forming the special flavor of Pu-erh tea.

Aroma is one of the importance factors in the evaluation of Pu-erh tea quality, although it only accounts for 0.005–0.02% of the chemical substances in the tea. Tea aroma is a combination of various volatile components, and the differences in the types and contents of these substances may render different aroma types. Nowadays, instrumental analysis technologies have been increasingly used to characterize and quantify the volatile components. So far, more than 1000 volatile compounds have been found in Pu-erh tea (Wang et al., 2022). Electronic nose (E-nose) is one of the common intelligent sensory technologies, consisting of odor sensors and a pattern recognition algorithm. It can partly eliminate the subjectivity of human sensory evaluation and serve as its crucial supplement (Xu, Wang, & Zhu, 2021; Yu, Wang, Zhang, Yong, & Cong, 2008). As a new type of odor analyzer, gas chromatography electronic nose (GC-E-nose) exhibits the advantages of rapid separation and pattern recognition of the overall odor characteristics, making it an efficient and sensitive odor analysis technology (He et al., 2021; Li et al., 2022; Yang et al., 2020). Gas chromatography-mass spectrometry (GC-MS) has relatively matured over the years and is

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currently the most commonly used analysis technique for odorants in tea. It has the largest variety of columns for selection, and features the benefits of rapid separation with both qualitative and quantitative performances (Yin et al., 2022; Yun et al., 2021). As a new technology for the analysis of food flavors, gas chromatography-ion migration spectrometry (GC-IMS) combines the benefits of gas chromatography and ion migration spectrometry (IMS), and capitalizes on rapid, sensitive and simple pretreatment. Moreover, it supports detection and analysis in atmospheric pressure environments (Fan, Jiao, Liu, Jia, & Zhou, 2020; Feng, Wang, Ji, Min, & Yan, 2021). At present, using a single analysis technique may lead to failure to detect some important compounds. Meanwhile, the combination of multiple analysis technologies can provide more comprehensive, reliable and scientific information, and is becoming a popular research trend (Feng, Wang, Wang, Huang, & Kan, 2022; Qi, Ding, Pan, Li, & Fu, 2020; Wang et al., 2019).

In Pu-erh tea market, there is an old saying that “the older the better”, which means that Pu-erh tea with a longer storage time has a higher market value compared with newer tea (Wang, et al., 2018). Related studies also show that the quality of Pu-erh tea is positively correlated with the storage life (Lv et al., 2015), and long-term storage has a significant impact on the aroma. However, in order to pursue profits, fake and shoddy phenomena exist in the Pu-erh tea market, which seriously damages the interests of consumers. Therefore, it is necessary to understand the dynamic changes of volatile substances in Pu-erh tea during storage. In this study, the dynamic changes of volatile metabolites of Pu-erh tea with different storage years were comprehensively explored by combining GC-E-Nose, GC-MS and GC-IMS. Moreover, the key differential compounds and the key storage nodes responsible for Pu-erh tea with different storage periods were elucidated through multivariate statistical analysis. The results provide valuable information for distinguishing Pu-erh tea and also provide technical support for quality control of Pu-erh tea.

2. Materials and methods

2.1. Tea samples and chemicals

The experimental samples were provided by Yunnan Shuangjiang Mengku Tea Co., Ltd, mainly including one-storage-year sample (S1Y), two-storage-year sample (S2Y), three-storage-year sample (S3Y), and four-storage-year sample (S4Y). 20-mL headspace vials covered with 18 mm magnetic PTFE/silicone caps were purchased from Agilent Technologies Inc. (Palo Alto, CA, USA). A manual SPME fiber holder and fibers of divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS, 50/30 μm , 2 cm) were purchased from Supelco (Bellefonte, PA, USA). Ethyl decanoate was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd (Shanghai, China). The purified water was obtained by Hangzhou Wahaha Group Co., Ltd (Hangzhou, China).

2.2. Gas chromatography electronic nose analysis

The volatile fingerprints of Pu-erh tea samples with a different number of storage years were analyzed by GC-E-Nose (A M.O.S., Toulouse, France), as described in our previous work (Yang et al., 2020). In brief, 0.5 g of tea sample was weighed into a 20-mL sealed glass vial. High-purity helium was used as the carrier gas at a flow rate of 1 mL/min. 5000 μL of headspace gas was injected into the system at 200 $^{\circ}\text{C}$, with an injection pressure of 10 kPa. The volatile compounds were absorbed by an embedded odor concentrator (Tenax TA) at 20 $^{\circ}\text{C}$ for 27 s, with a split mode of 10 mL/min, and thermal desorption was performed at 240 $^{\circ}\text{C}$ for 35 s. The temperature was programmed as follows: the initial temperature was 50 $^{\circ}\text{C}$ for 5 s, increased to 80 $^{\circ}\text{C}$ at 0.1 $^{\circ}\text{C}/\text{s}$, then raised to 250 $^{\circ}\text{C}$ at 0.4 $^{\circ}\text{C}/\text{s}$ and held for 10 s. The acquisition time was 740 s. The detector temperature was 260 $^{\circ}\text{C}$, and the gain factor of two flame ionization detectors (FID) was 12.

2.3. Gas chromatography-mass spectrometry analysis

The volatile compounds of Pu-erh tea samples were analyzed by an Agilent 7890B GC system coupled with an Agilent 7000C series triple quadrupole system (Agilent Technologies, Palo Alto, CA, USA). A non-polar capillary column (DB-5MS, 30 m \times 0.25 mm \times 0.25 μm ; Agilent Technologies, Palo Alto, CA, USA) was applied for separation. In brief, 0.5 g of tea sample was placed in the 20-mL headspace vial, 5 μL of ethyl decanoate (100 mg/L, internal standard) was added, followed by 5 mL of pure water, and the vial was sealed. Then, the DVB/CAR/PDMS fiber was inserted into the headspace vial, and it was incubated at 60 $^{\circ}\text{C}$. After extraction for 60 min, the fiber was inserted into the GC-MS injector for thermal desorption (about 5 min). The initial column temperature was kept at 40 $^{\circ}\text{C}$ for 5 min, then increased to 160 $^{\circ}\text{C}$ at 4 $^{\circ}\text{C}/\text{min}$, held for 2 min, and finally increased to 270 $^{\circ}\text{C}$ at 10 $^{\circ}\text{C}/\text{min}$, holding for 12 min. High-purity helium (> 99.999%) was used as carrier gas with a flow rate of 1 mL/min. The splitless mode was selected. The mass spectrometry was operated in an electron ionization (EI) mode at 70 eV, with a mass range of 40–450 m/z . The temperature of ionization source and transmission line were 230 $^{\circ}\text{C}$ and 250 $^{\circ}\text{C}$, respectively.

The volatile compounds were determined by MassHunter Workstation Software Unknowns Analysis, and searched by the NIST 11 library according to the principle of similarity > 80%. The retention index (RI) of each compound was calculated according to the linear formula of n -alkanes (C7-C40) by comparing with the values in the literature (<https://webbook.nist.gov/chemistry/and> <https://www.flavornet.org/flavornet.html>). The volatile components were quantified using the following formula:

$$C_i = \frac{C_{ki} \cdot A_i}{A_{ki}} \quad (1)$$

where C_i denotes the concentration of volatile components to be measured, $\mu\text{g}/\text{L}$; A_i denotes the peak area of volatile components to be measured; C_{ki} denotes the concentration of ethyl decanoate (internal standard), $\mu\text{g}/\text{L}$; A_{ki} is the peak area of ethyl decanoate.

2.4. Gas chromatography-ion mobility spectrometry analysis

The volatile compounds in Pu-erh tea samples were detected by a GC-IMS instrument (Flavourspec®, G.A.S, Dortmund, Germany). Briefly, 1 g of tea sample was placed in a 20-mL headspace vial, and incubated at 60 $^{\circ}\text{C}$ for 20 min with an agitation speed of 500 rpm. Then, 500 μL of headspace gas was added into the injection port via a heated syringe at 85 $^{\circ}\text{C}$. A polar capillary column (WAX, 30 m \times 53 μm \times 1 μm , RESTEK Company, USA) was used for the separation. The column temperature was set at 60 $^{\circ}\text{C}$, and the ion mobility spectrum temperature was set at 45 $^{\circ}\text{C}$. High-purity nitrogen was employed as carrier gas at the following programmed flow: 2 mL/min for 2 min, raised to 10 mL/min within 10 min, then raised to 100 mL/min within 20 min and held for 20 min, with a drift gas flow rate of 150 mL/min.

The RI of the volatile compounds in Pu-erh tea samples was calculated using n -ketones C4–C9 as external references. The volatile compounds were qualitatively characterized by comparing the RI and drift time with those of standard substances in the user-built database, as well as the NIST library and IMS database. The preliminary choice and analysis of the GC-IMS data was performed by Laboratory Analytical Viewer (LAV). The fingerprints and differential spectrograms were analyzed by the Gallery Plot plug-in and the Reporter plug-in, respectively.

2.5. Statistical analysis

All tea samples were tested three times in parallel. Partial least squares discriminant analysis (PLS-DA) and orthogonal PLS-DA (OPLS-DA) were performed using SIMCA-P 14.1 software (Umetrics, Sweden).

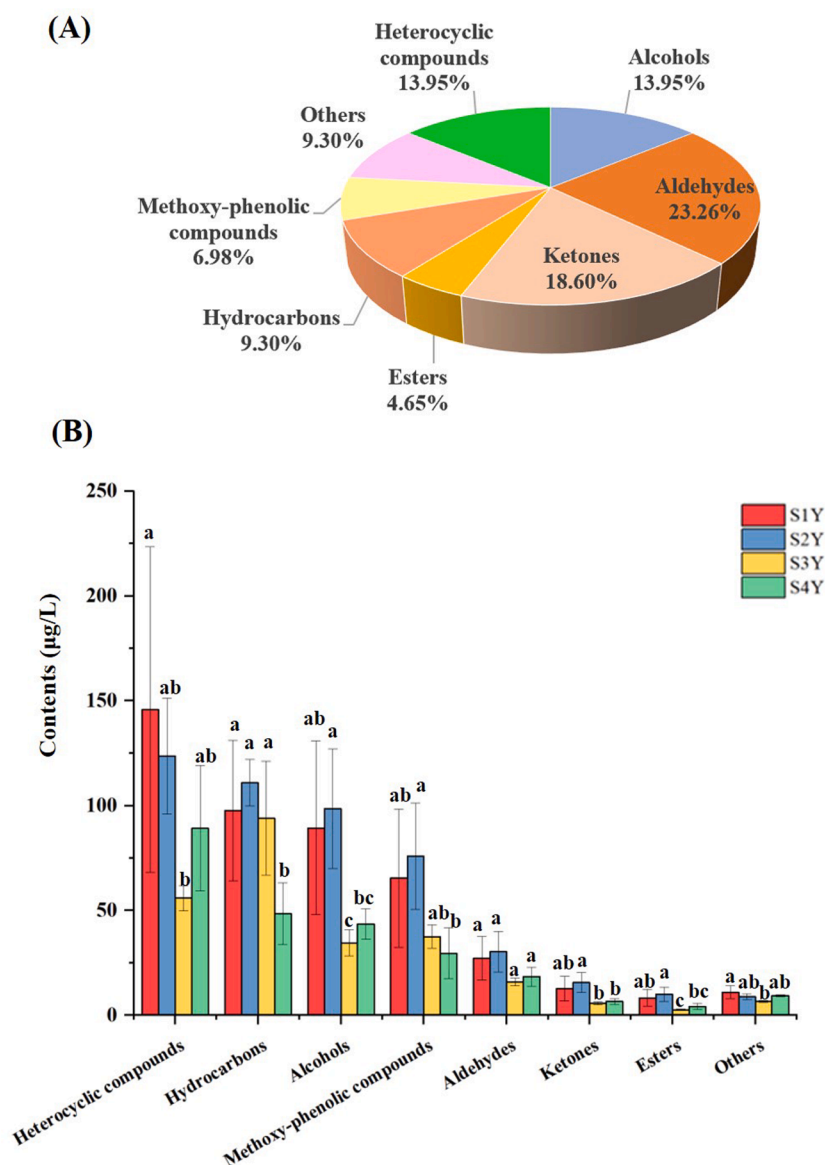


Fig. 1. The volatile compounds in Pu-erh teas with different storage years obtained from GC-MS. (A) Proportion of different classes of volatile compounds; (B) Content comparison of different classes of volatile compounds. Values with different letters are significantly different ($p < 0.05$).

The significance levels among Pu-erh tea samples with different storage years were analyzed by one-way analysis of variance (ANOVA) using SPSS statistics 24.0 software (SPSS Inc., Chicago, IL, USA). The pie chart, histogram and box-plots were plotted by Origin software (Originlab, USA).

3. Results and discussion

3.1. The volatile fingerprints analyzed by gas chromatography electronic nose

Traditional electronic noses are mostly based on metal oxide sensors, which are easily polluted by different samples and subsequently fail to obtain complete and accurate information by each sensor. As a new odor analysis technique, GC-E-Nose combines the advantages of gas chromatography and electronic nose, with the merit of sensitive detection and rapid analysis. The instrument contains two columns (weak-polarity column MXT-5 and medium-polarity column MXT-1701), which can effectively separate volatile components with different polarity (Yang et al., 2021). In this study, the odor information of Pu-erh teas stored for

a different number of years was analyzed and collected by GC-E-Nose. The radar maps obtained from MXT-5 and MXT-1701 columns in parallel were shown in Fig. S1. Each peak represents a specific volatile component, and the peak area represents the level of the volatile component. It could be seen that the peak intensities of volatile compounds detected by the MXT-5 column were greater than those by MXT-1701 column in all samples. Moreover, the peak intensities showed different trends with the extension of storage time, indicating that storage time had an important impact on the volatile profiles of Pu-erh teas.

Next, PLS-DA was conducted to further characterize the changes during the storage process. As a supervised discriminant method, PLS-DA can effectively solve the high correlation between variables. As illustrated in Fig. S2A, good model parameters ($R^2Y = 0.992$, $Q^2 = 0.968$) were obtained, indicating that the model had an excellent explanatory ability and predictive ability. The score of PLS-DA analysis revealed that the samples could be divided into four clusters based on the volatile fingerprints resulting from four different storage times. Specifically, S1Y, S2Y and S3Y could be clearly distinguished by the principal component one (PC1), while the difference between S4Y and

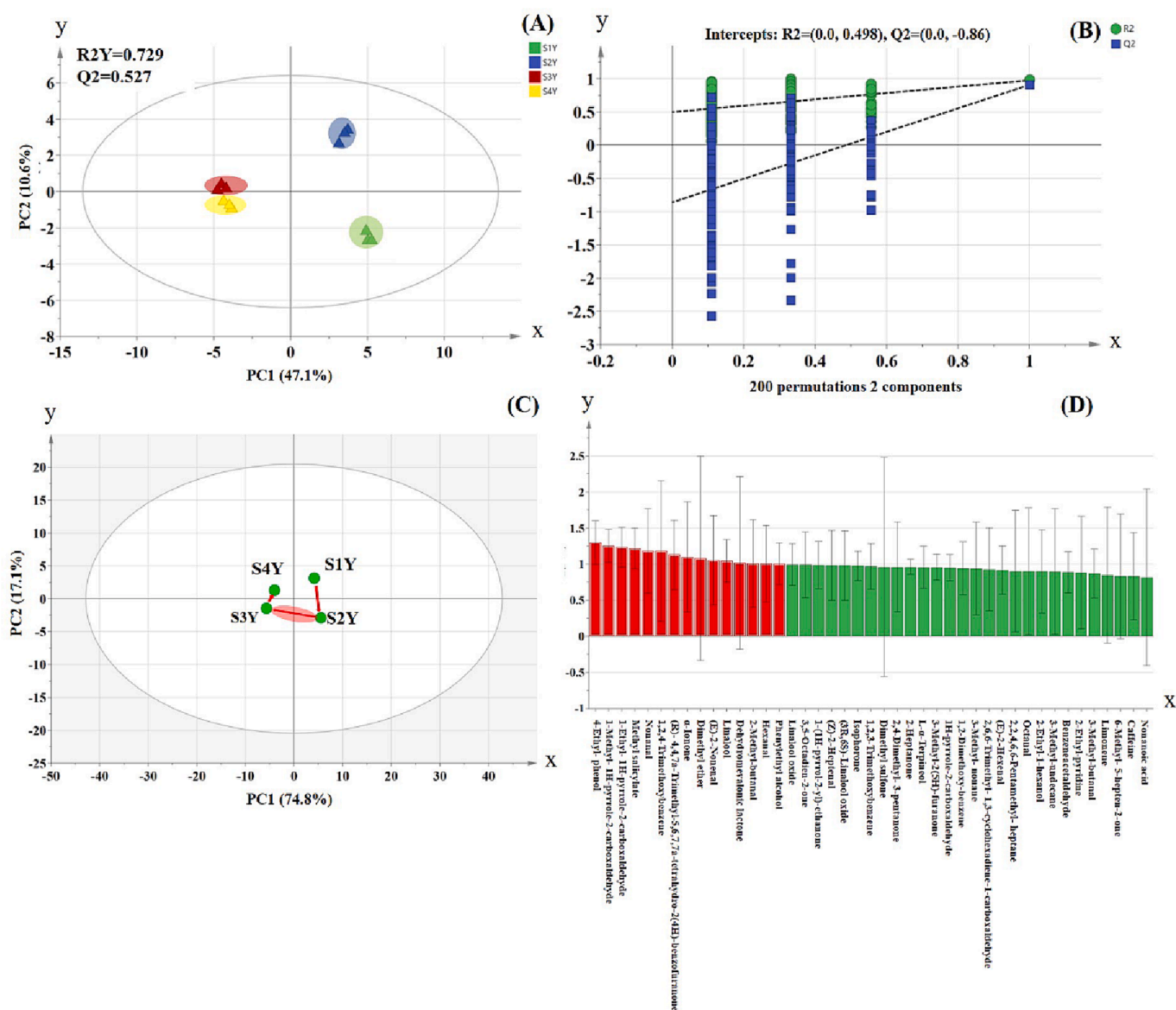


Fig. 2. The OPLS-DA results of Pu-erh teas with different storage years by using GC-MS. (A) Scores plots of OPLS-DA ($R^2Y = 0.729$, $Q^2 = 0.527$); (B) Cross-validation plot by 200 permutation tests ($R^2 = 0.498$, $Q^2 = -0.86$); (C) The metabolic trajectory plot; (D) The red part represents 15 key compounds with $VIP > 1$.

other samples could be separated by the principal component two (PC2). In order to assess the robustness of the model, 200 permutation tests were conducted. The criteria for model validity are as follows: The regression line of the Q2-points intersects the vertical axis (on the left) at, or below zero. The parameters ($R^2 = 0.396$, $Q^2 = -0.342$) indicated that the model was robust and showed no overfitting (see Fig. S2B). Based on the above analysis, GC-E-Nose combined with multivariate statistical analysis could quickly distinguish Pu-erh teas stored for a different number of years.

3.2. Analysis of Pu-erh teas with different storage years by GC-MS

3.2.1. The volatile components analyzed by GC-MS

A total of 43 volatile components were identified by GC-MS, which were classified into 8 categories, including 10 aldehydes, 8 ketones, 6 alcohols, 6 heterocyclic compounds, 4 hydrocarbons, 3 methoxyphenolic compounds, 2 esters, and 4 other compounds. The detailed information of volatile components was listed in Table S1. Among them, the four component types with higher proportions were aldehydes (23.26%), ketones (18.60%), alcohols (13.95%) and heterocyclic

compounds (13.95%) (see Fig. 1A). In addition, the contents of volatile component categories varied between different storage stages (see Fig. 1B). The content of heterocyclic compounds in S1Y (145.78 $\mu\text{g/L}$) was the highest, followed by S2Y (123.68 $\mu\text{g/L}$), S4Y (89.21 $\mu\text{g/L}$), and S3Y (55.88 $\mu\text{g/L}$). Heterocyclic compounds such as furan, pyrrole and their derivatives have been reported to be generated by Maillard reaction during the manufacturing process of fixation and drying (Yang, Xie, et al., 2022). It is worth mentioning that 2-ethyl-pyridine, which has been detected only in Pu-erh tea, provided green and grassy odors (Ma et al., 2021).

Four hydrocarbons were identified in four storage years of Pu-erh tea, one of which was unsaturated hydrocarbon, which played an important role in tea aroma, while saturated hydrocarbons had little effect on tea aroma. The contents of hydrocarbon components were in the order of S2Y (110.90 $\mu\text{g/L}$) > S1Y (97.67 $\mu\text{g/L}$) > S3Y (93.94 $\mu\text{g/L}$) > S4Y (48.48 $\mu\text{g/L}$), and those in S4Y were significantly lower than other groups ($p < 0.05$).

The contents of alcohols in S2Y were the highest with up to 98.51 $\mu\text{g/L}$, followed by S1Y (89.35 $\mu\text{g/L}$), S4Y (43.58 $\mu\text{g/L}$) and S3Y (35.54 $\mu\text{g/L}$). Linalool was reported to contribute significantly to tea aroma (Mao

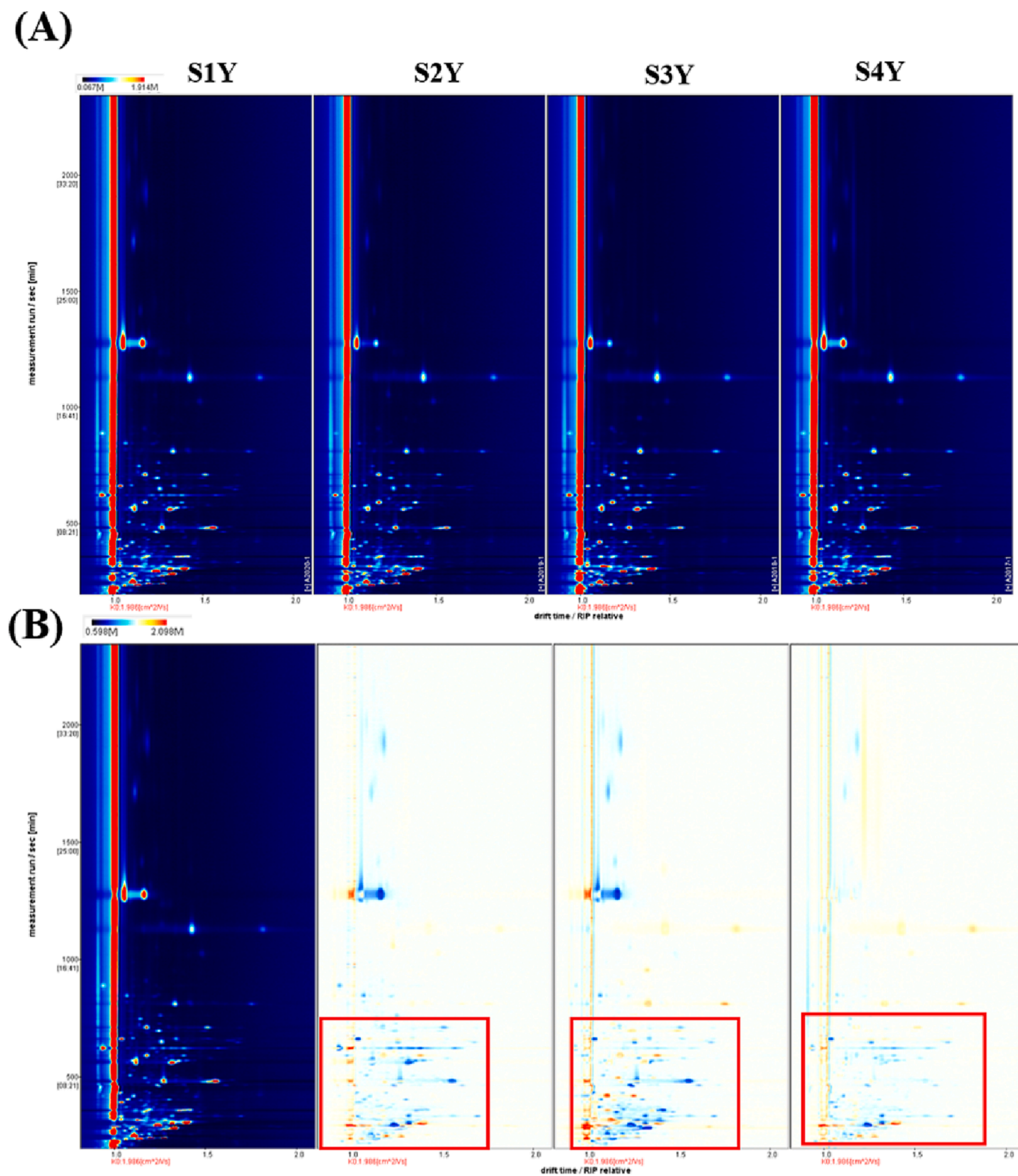


Fig. 3. Fingerprints of Pu-erh teas with different storage years obtained from GC-IMS. (A) Topographic plots; (B) Difference comparison plots (S1Y as the reference).

et al., 2018; Pang et al., 2019). The concentration of linalool was the highest in S1Y (4.59 $\mu\text{g/L}$), significantly higher than S3Y (0.63 $\mu\text{g/L}$) and S4Y (1.32 $\mu\text{g/L}$) ($p < 0.05$). Phenylethyl alcohol was an important volatile component released from the hydrolysis reaction of glycosides or shikimate synthesis pathway, and was linked to the aromas of sweet flowers, fresh bread and rose (Su, Xia, Gao, Dai, & Zhang, 2010). Its content reached the maximum level in S2Y (37.00 $\mu\text{g/L}$) and dropped to

its lowest level in S3Y (3.65 $\mu\text{g/L}$). L- α -terpineol, contributing to the tree, lilac and woody odors, decreased gradually with the number of storage years.

Methoxy-phenolic compounds played an important role in the unique “stale” aroma of Pu-erh tea, which were the special compounds different from green tea, black tea, and others (Lv, Zhang, Yang, Shi & Lin, 2015). These volatile components originated from microbial and

Table 1

The information of identified volatile compounds by GC-IMS.

No.	Compounds	MW	RI	Rt (sec)	Dt (RIP relative)	Peak intensities				VIP
						S1Y	S2Y	S3Y	S4Y	
1	α -Terpineol	154.3	1732.7	2106.033	1.20441	916.79±86.87a	787.49±43.13a	693.19 ±157.71a	826.66 ±186.80a	0.513
2	Terpinen-4-ol	154.3	1640.5	1724.156	1.22738	677.44 ±125.11b	512.10±21.80b	536.33 ±193.81b	1714.64 ±128.26a	1.528
3	Linalool	154.3	1560.3	1448.81	1.23197	626.81±86.80b	357.45±25.29c	377.93±93.12c	841.12 ±125.22a	1.247
4	1-Octen-3-ol	128.2	1483.8	1227.125	1.16532	146.80±10.35a	120.85±22.63a	108.86±34.08a	122.69±34.00a	0.514
5	1-Hexanol-M	102.2	1367.4	953.192	1.33114	135.22±21.90b	129.86±14.59b	417.49±16.48a	136.04±25.25b	0.902
6	Cis-2-Penten-1-ol	86.1	1335.2	888.778	0.94607	1193.26 ±41.73a	634.45±37.19d	951.52±41.92c	1062.05 ±61.99b	1.068
7	Pentanol-M	88.1	1264	763.941	1.25615	481.23±18.44a	260.16±6.87c	502.74±21.88a	396.10±8.02b	1.080
8	Pentanol-D	88.1	1265	765.514	1.50954	38.63±5.91a	30.37±0.64a	45.18±13.68a	34.08±8.37a	0.642
9	Linalool oxide-M	170.3	1488.8	1240.392	1.26991	384.63±12.12a	229.51±39.05b	234.45±27.10b	286.24±38.83b	0.858
10	3-Methyl-1-butanol-M	88.1	1218.7	695.497	1.23944	1399.08 ±61.66b	1160.16 ±117.05c	1848.55 ±42.53a	1359.35 ±105.71b	0.968
11	3-Methyl-1-butanol-D	88.1	1220.3	697.857	1.49005	270.52±21.28b	181.69±21.56c	456.28±19.55a	263.87±42.19b	0.983
12	1-Penten-3-ol-M	86.1	1175.4	622.845	0.94022	4314.71 ±28.71a	2897.27 ±72.23d	4075.46 ±78.39b	3789.26 ±62.27c	1.081
13	1-Penten-3-ol-D	86.1	1175.4	622.845	1.37749	925.03±53.89a	228.76±5.40d	586.31±47.24b	435.03±32.79c	1.071
14	1-Butanol-M	74.1	1158.8	589.405	1.18231	1971.69 ±19.70c	2468.66 ±62.70b	2641.35 ±10.39a	1647.28 ±22.06d	1.186
15	1-Butanol-D	74.1	1159.4	590.599	1.38052	726.20±36.47c	1188.76 ±66.29b	1507.61±9.44a	459.17±18.22d	1.096
16	2-Methyl-1-propanol-M	74.1	1107.6	497.36	1.17483	685.80±4.17b	716.84±27.41b	1186.50 ±24.16a	623.00±22.79c	0.912
17	2-Methyl-1-propanol-D	74.1	1106.9	496.136	1.37214	71.56±0.10b	55.60±4.99c	146.90±7.87a	55.98±5.90c	0.941
18	1-Propanol-M	60.1	1051.5	419.614	1.11146	992.23±22.46b	716.07±31.25c	2709.60±8.61a	593.69±6.23d	0.947
19	1-Propanol-D	60.1	1051	419.002	1.25548	140.85±1.77b	116.28±7.97c	878.15±23.87a	97.59±5.75d	0.913
20	2-Butanol-M	74.1	1036.4	401.196	1.14949	354.42±8.78b	301.43±18.01c	481.32±14.39a	373.26±32.58b	1.022
21	2-Butanol-D	74.1	1035.6	400.236	1.32496	556.31±21.35a	305.37±19.83c	314.58±18.19c	429.54±14.20b	0.889
22	Ethanol	46.1	940.8	314.289	1.13231	1849.63 ±135.44b	1513.90 ±66.66c	4030.40 ±128.67a	1617.91 ±96.26c	0.930
23	tert-Butanol	74.1	920.5	300.274	1.32561	3267.28 ±47.26a	2213.70 ±43.10b	2236.94 ±103.04b	2340.16 ±109.88b	1.048
24	3-Methyl-3-buten-1-ol	86.1	1271.2	775.495	1.19675	102.32±10.49a	82.97±4.46ab	78.87±6.88b	87.38±15.00ab	0.689
25	2-Octanol-M	130.2	1444.2	1126.068	1.42754	5971.70 ±218.94c	6459.46 ±182.54b	7028.30 ±49.89a	7163.88 ±88.84a	1.398
26	2-Octanol-D	130.2	1444.8	1127.445	1.82345	2232.43 ±196.08c	2502.23 ±156.57b	3119.17 ±55.23a	3293.79 ±66.69a	1.465
27	(Z)-3-Hexenol-M	100.2	1400.7	1024.621	1.23628	158.11 ±15.42ab	146.34 ±11.35ab	138.72±19.77b	205.88±57.08a	1.043
28	Benzaldehyde-M	106.1	1547.6	1409.475	1.15618	805.31±44.09a	611.61±16.46b	337.58±66.66c	602.55±84.46b	0.883
29	(E)-2-Hexenal-M	98.1	1230	712.018	1.18096	2675.08 ±73.38b	2070.46 ±97.25d	2221.99 ±12.14c	2856.72 ±50.21a	1.222
30	(E)-2-Hexenal-D	98.1	1229.5	711.231	1.51929	1664.03 ±134.33b	830.08±59.34d	1006.26 ±21.76c	1963.53 ±74.65a	1.236
31	Octanal	128.2	1294.4	813.603	1.4117	398.76±10.09b	464.70±3.75a	408.61±28.69b	423.13±16.48b	0.947
32	2-Hexenal-M	98.1	1212.7	686.98	1.18122	160.16±6.85b	125.28±5.57d	136.87±0.81c	176.39±6.92a	1.284
33	3-Methyl-2-butenal	84.1	1211.8	685.666	1.0961	254.46±4.45a	210.13±20.36b	196.12±9.33b	235.50±14.18a	0.765
34	Heptanal-M	114.2	1194.9	662.154	1.33514	1038.49 ±48.05b	944.42±40.27c	615.05±9.68d	1149.87 ±10.27a	0.971
35	Heptanal-D	114.2	1194.9	662.154	1.6966	129.72±17.51b	101.01±11.49c	50.42±7.64d	159.97±10.50a	1.032
36	Hexanal-M	100.2	1097.4	480.831	1.26412	3966.58 ±32.58a	3410.04 ±25.56c	3185.27 ±83.70d	3857.74 ±36.20b	0.885
37	Hexanal-D	100.2	1097.4	480.831	1.56224	5269.79 ±199.40a	2602.96 ±78.30c	2012.88 ±181.70d	4496.21 ±106.80b	0.881
38	Pentanal-M	86.1	1002	362.304	1.18998	881.65±29.01b	1003.66±4.13a	682.49±19.83c	1002.14 ±11.39a	1.010
39	Pentanal-D	86.1	1002	362.304	1.42435	1337.48 ±79.50a	867.26±96.35c	227.50±17.13d	1040.09 ±11.26b	0.836
40	3-Methylbutanal	86.1	924.9	303.246	1.40472	7051.17 ±225.79c	10244.57 ±13.56a	7523.77 ±292.31b	10120.71 ±169.31a	1.189
41	Butanal	72.1	885.2	277.318	1.28078	1443.48 ±64.23a	932.78±78.56c	672.93±38.07d	1131.46 ±21.32b	0.838
42	Propanal	58.1	812.2	235.229	1.14226	6943.95 ±180.98a	5142.64 ±254.44c	4118.84 ±167.61d	5930.79 ±72.08b	0.832
43	2-Methylpropanal	72.1	825.5	242.395	1.28209	974.80±69.79b	1313.53 ±39.92a	840.02±86.11b	1292.87 ±100.41a	1.067
44	(Z)-2-Pentenal	84.1	1126	528.659	1.11217	106.12±6.09a	65.81±5.06c	69.79±5.04c	90.22±2.10b	0.890
45	cis-4-Heptenal	112.2	1253.9	748.091	1.15001	646.98±39.65a	269.03±17.04c	285.42±5.75c	496.75±4.65b	0.898
46	2-Methylbutanal	86.1	953.2	323.183	1.40723	271.42±50.41a	271.19±46.82a	49.29±6.39b	273.98±43.84a	0.856
47	(E)-2-Pentenal	84.1	1147.6	568.039	1.36016	1194.84 ±61.48a	520.60±37.51d	637.84±7.46c	959.88±40.35b	0.935

(continued on next page)

Table 1 (continued)

No.	Compounds	MW	RI	Rt (sec)	Dt (RIP relative)	Peak intensities				VIP
						S1Y	S2Y	S3Y	S4Y	
48	(E, E)-2,4-Heptadienal	110.2	1515.9	1315.616	1.20425	281.87±13.10a	128.98±27.16c	93.85±9.50d	170.06±17.17b	0.896
49	Nonanal	142.2	1401.2	1025.804	1.48396	417.54±15.94b	524.57±29.35a	345.36±43.82c	565.34±14.71a	1.134
50	Acrolein	56.1	876.1	271.653	1.06297	553.02±18.99b	595.75	648.71±34.81a	601.99	0.720
							±37.97ab		±49.83ab	
51	Phenylacetaldehyde	120.2	1763.9	2253.641	1.25786	422.38±37.21a	435.22	328.95	398.85	0.438
							±120.94a	±100.89a	±137.05a	
52	(E)-2-Nonenal	140.2	1536.7	1376.348	1.42632	311.67±40.46b	296.25±24.27b	466.36±53.55a	191.92±47.18c	1.055
53	6-Methyl-5-hepten-2-one	126.2	1347.3	912.554	1.17885	445.64±13.15b	409.21±33.16c	592.57±39.88a	495.55±46.21b	1.022
54	Acetoin	88.1	1296.9	818.043	1.06398	222.39±12.57a	156.13±8.54b	171.50±13.30b	164.36±8.17b	1.054
55	1-Hydroxy-2-propanone-M	74.1	1311.3	844.024	1.06166	1030.97	461.91±6.60c	467.06±25.38c	794.54±69.05b	0.874
						±105.36a				
56	1-Hydroxy-2-propanone-D	74.1	1312.4	845.956	1.23458	181.43±13.39a	69.12±10.26d	114.11±4.40c	135.82±7.50b	0.977
57	2-Heptanone-M	114.2	1190.7	655.402	1.26445	751.27±8.88a	740.56±40.21a	772.17±26.28a	751.88±23.30a	0.475
58	2-Heptanone-D	114.2	1191.4	656.818	1.63184	189.21±8.15a	185.11±21.54a	197.15±13.58a	220.49±7.71a	1.276
59	4-Methyl-3-penten-2-one-M	98.1	1149.8	572.088	1.12027	1972.90	1398.77	1964.99	1788.93	1.095
						±33.28a	±42.22c	±42.75a	±33.87b	
60	4-Methyl-3-penten-2-one-D	98.1	1149.5	571.569	1.44526	105.19±7.07a	53.25±5.82b	69.06±10.42b	63.52±13.32b	1.008
61	Cyclopentanone	84.1	1143.8	560.766	1.33168	4054.91	1650.08	4607.57	3471.27	1.107
						±266.69b	±236.75d	±49.98a	±15.12c	
62	2-Hexanone	100.2	1120	518.174	1.20363	47.55±3.93a	34.62±7.21b	32.15±1.36b	28.96±4.77b	1.171
63	1-Penten-3-one	84.1	1040.4	405.998	1.07955	842.23±17.81a	545.77±39.12c	479.72±10.97d	698.50±11.31b	0.850
64	3-Pentanone	86.1	1026.6	389.673	1.11268	48.69±2.25b	30.75±5.55c	62.17±3.38a	28.75±3.83c	1.078
65	2-Pentanone	86.1	996.1	356.062	1.35809	1633.97	987.37	1783.70	1390.23	1.061
						±64.07b	±108.53d	±80.02a	±38.67c	
66	Diacetyl	86.1	990.8	351.741	1.17035	515.56±4.17a	522.79±33.59a	258.52±5.78b	526.02±16.94a	0.903
67	2-Butanone	72.1	911.4	294.123	1.24643	10524.34	4082.73	5227.79	4747.29	1.110
						±128.99a	±198.99c	±365.65b	±394.37b	
68	Acetone	58.1	835.7	248.029	1.11513	12345.51	9996.29	10125.02	10478.78	0.990
						±191.31a	±150.45b	±324.65b	±315.39b	
69	2-Octanone-M	128.2	1292.8	810.848	1.33785	2122.51	2272.53	2956.14	2636.06	1.162
						±73.56d	±59.58c	±54.33a	±34.20b	
70	2-Octanone-D	128.2	1292.9	811.056	1.75355	606.67±23.90d	715.42±28.82c	1314.67	995.73±3.26b	1.124
								±33.39a		
71	Acetophenone	120.2	1688.4	1912.864	1.18817	1835.94	594.15±98.55c	453.61±35.23c	780.75±73.20b	0.997
						±89.17a				
72	3-Methyl-2-pentanone	100.2	1066.6	438.779	1.1815	149.12±6.92a	158.76±11.10a	130.71±2.26b	149.31±5.76a	0.885
73	4-Methyl-2-pentanone	100.2	1023.3	385.955	1.17961	197.75±7.12b	187.63±3.47b	141.34±5.12c	223.90±12.66a	1.036
74	Cyclohexanone	98.1	1293.7	812.495	1.15813	151.05±1.34b	212.93±24.99a	143.67±12.07b	111.56±17.29c	1.377
75	2-Acetylpyridine	121.1	1636.1	1707.766	1.11714	2905.60	2044.02	1087.18	2090.82	0.892
						±157.15a	±222.31b	±60.31c	±79.38b	
76	2-Pentylfuran	138.2	1239.5	726.179	1.25197	199.76±14.48a	118.85±6.70c	200.05±7.91a	180.86±7.07b	1.102
77	2-Isopropyl-3-methoxy-pyrazine	152.2	1453.2	1148.289	1.26683	477.11±16.63b	404.89±29.97c	471.64±17.71b	540.69±32.55a	1.388
78	2,3-Dimethylpyrazine	108.1	1349.4	916.75	1.10501	195.63±13.70a	177.66±10.44a	181.01±17.83a	181.83±17.20a	0.504
79	Pyrazine	80.1	1194.3	661.31	1.04571	2514.41	1126.42	1287.32	1418.29	1.018
						±129.77a	±75.24c	±3.46bc	±120.03b	
80	2-Ethylfuran	96.1	966.5	333.015	1.04519	1107.34	717.73±20.40d	1332.35	847.17±31.81c	1.050
						±30.78b		±27.88a		
81	2-Methylpyrazine	94.1	1274.1	780.036	1.10196	169.30±4.92a	113.31±19.37b	154.27±5.80a	167.24±9.99a	1.113
82	3-Ethylpyridine	107.2	1384.1	988.417	1.09814	134.44±10.41a	117.85±9.98a	107.14±19.02a	132.59±18.92a	0.601
83	Tetrahydrofuran	72.1	876.1	271.653	1.22321	235.62±13.74a	175.30	150.89±11.73c	191.79±19.95b	0.814
							±19.23bc			
84	γ-Butyrolactone	86.1	1705.8	1986.389	1.08958	850.14±21.59a	544.97±29.36c	432.93±93.62c	706.90±74.51b	0.805
85	Butyl acetate-M	116.2	1084.3	462.466	1.24108	1062.30	735.66±46.24b	92.23±8.93c	752.19±48.65b	0.879
						±13.91a				
86	Ethyl butanoate	116.2	1060.9	431.445	1.19857	200.09±21.21a	103.01±2.75c	77.37±4.50d	138.85±8.94b	0.856
87	Methyl butanoate-M	102.1	1013	374.308	1.16299	540.00±36.75a	379.12±42.70b	351.44±27.66b	494.58±20.86a	0.844
88	Methyl butanoate-D	102.1	1013	374.308	1.45625	65.58±5.17a	44.91±2.97c	52.24±9.25bc	58.38±2.03ab	0.852
89	Diethyl acetal	118.2	899.6	286.441	1.02924	1029.63	1628.08	2491.08	1551.06	0.873
						±43.25c	±70.54b	±68.86a	±28.69b	
90	Ethyl Acetate	88.1	893.6	282.6	1.34091	7195.51	6830.01	860.52±44.66d	6475.90	0.902
						±115.37a	±273.88b		±147.24c	
91	Methyl acetate	74.1	848.4	255.234	1.19997	444.96±32.32b	551.25±10.97a	280.33±27.08c	543.01±33.28a	0.994
92	Propyl acetate	102.1	989	350.357	1.47598	124.47±5.61a	105.65±13.29a	18.57±2.81b	107.79±13.95a	0.862
93	Ethyl formate	74.1	826.2	242.791	1.19639	1285.79	1355.00	1072.92	1419.77	0.991
						±22.45b	±21.15ab	±59.81c	±76.40a	
94	Dihydro-5-methyl-2(3H)-furanone	100.1	1735.3	2117.807	1.12836	1125.28	811.94±45.26b	879.04±59.49b	845.06±99.08b	0.991
						±102.29a				
95	1-Methoxy-2-propyl acetate	132.2	1237.2	722.756	1.14162	276.83±22.58b	196.56±19.55c	315.56±12.63a	214.67±11.11c	1.032
96	Butyl acetate-D	116.2	1086.2	465.09	1.61886	155.82±6.25a	62.98±7.12b	11.41±3.15c	71.65±8.94b	0.942
97	Ethyl propanoate	102.1	965.8	332.52	1.46303	122.03±1.75a	69.65±1.52b	46.66±6.38c	64.16±4.47b	1.075
98	2-Methylbutyl acetate	130.2	1153.3	578.852	1.28631	195.13±6.82a	108.41±5.11b	61.41±6.60d	74.54±6.75c	1.213
99	β-Pinene	136.2	1132.5	540.212	1.21515	54.10±5.53b	54.10±7.16b	105.40±3.18a	58.82±8.13b	0.891
100	α-Pinene	136.2	1030.7	394.474	1.18876	352.84±14.90b	391.60±8.87a	319.14±6.47c	391.30±8.77a	1.034

(continued on next page)

Table 1 (continued)

No.	Compounds	MW	RI	Rt (sec)	Dt (RIP relative)	Peak intensities				VIP
						S1Y	S2Y	S3Y	S4Y	
101	α -Phellandrene	136.2	1166.8	605.393	1.2376	304.40 \pm 8.35b	357.29 \pm 27.02a	295.79 \pm 16.93b	300.27 \pm 27.12b	1.029
102	Camphene	136.2	1093.4	475.029	1.18993	329.80 \pm 5.09d	458.30 \pm 10.61a	438.27 \pm 10.82b	374.15 \pm 10.34c	0.928
103	Acetic acid-M	60.1	1501.5	1275.22	1.05656	19181.60	14537.82	14055.54	18929.03	0.986
						\pm 204.69a	\pm 527.90b	\pm 197.53b	\pm 32.50a	
104	Acetic acid-D	60.1	1501.5	1275.22	1.15696	11114.35	3845.19	3401.38	10261.99	0.968
						\pm 772.25a	\pm 422.38c	\pm 165.49c	\pm 45.16b	
105	p-Xylene	106.2	1143	559.416	1.07488	331.37 \pm 10.68a	316.96 \pm 5.87b	239.45 \pm 5.03d	296.83 \pm 1.55c	0.976
106	2-Butoxyethanol	118.2	1413.8	1054.134	1.23657	176.47 \pm 31.23b	462.00 \pm 24.13a	99.29 \pm 17.51c	117.95 \pm 26.90c	1.284
107	2-Methylthiophene	98.2	1060.9	431.445	1.03783	1068.94	785.84 \pm 16.33c	704.49 \pm 11.54d	929.95 \pm 21.80b	0.841
						\pm 44.87a				
108	Thiophene	84.1	1027.4	390.633	1.03905	1830.97	1396.30	2202.56	1343.00	1.094
						\pm 24.50b	\pm 54.68c	\pm 22.24a	\pm 23.44c	
109	Dimethyl sulfide-M	62.1	788.5	222.988	0.95914	510.05 \pm 9.89a	307.37 \pm 15.73b	302.63 \pm 15.81b	323.94 \pm 11.72b	1.065

Note: MW: Molecular weight; RI: Retention index; Rt: Retention time; Dt: Drift time; RIP: Reactive ion peak; VIP: Variable importance in projection; Suffix M represented the monomer of volatile compound and suffix D represented the dimer of volatile compound; S1Y, S2Y, S3Y and S4Y represented four Pu-erh samples with different storage years, respectively; Values with different letters in a row indicated significant differences using Duncan's multiple comparison tests ($p < 0.05$).

thermal degradation during the pile fermentation process. In this study, the contents of methoxy-phenolic compounds in Pu-erh tea gradually decreased with the rising number of storage years, but reached the highest level in S2Y (75.93 μ g/L). Among them, 1,2,3-trimethoxybenzene and 1,2,4-trimethoxybenzene have been reported to be the crucial odorants (Lv et al., 2012; Pang et al., 2019).

As for aldehydes, their contents reached the maximum level in S2Y (30.32 μ g/L), while they reached the minimum level in S3Y (15.94 μ g/L). For example, benzeneacetaldehyde with sweet and rose aromas was reported to be an essential product of the shikimate pathway (Chen et al., 2019). Hexanal and (E)-2-hexenal was reported to contribute to green and grass fragrances, which were usually formed by the degradation of fatty acids (Yang, Baldermann, & Watanabe, 2013).

Usually, ketones make an essential contribution to the aroma of tea because of their low threshold values. For example, 3-methyl-2(5H)-furanone has a strong caramel flavor in tea (Lv et al., 2014). α -Ionone with a relatively low odor threshold (0.4 μ g/L in water) was described as presenting sweet and floral odors (Zhai, Zhang, Granvogl, Ho, & Wan, 2022). Among the four storage years of Pu-erh tea, S2Y possessed the highest content of ketones (15.63 μ g/L). The others followed the order of S1Y (12.75 μ g/L) > S4Y (6.85 μ g/L) > S3Y (5.82 μ g/L).

The content of esters in S2Y (10.06 μ g/L) was the highest, followed by S1Y (8.27 μ g/L), S4Y (4.20 μ g/L) and S3Y (2.59 μ g/L). Methyl salicylate is recognized as a vital volatile compound by providing sweet and floral fragrances, and is derived from the oxidation of α -linolenic acid or hydrolysis reaction of β -Primeveroside (Wang et al., 2011). (R)-5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone with sweet, coumaric and coconut fragrance, reached the highest level in S2Y (9.00 μ g/L) and dropped to the lowest in S3Y (2.27 μ g/L).

The above results indicated that the contents of volatile components of Pu-erh samples stored for a different number of years varied considerably, that is, storage time had a great influence on the flavor of Pu-erh tea.

3.2.2. Multivariate statistical analysis

OPLS-DA analysis was conducted to determine the volatile components causing the aroma differences of Pu-erh tea among the four storage years. As shown in Fig. 2A, a clear separation could be observed through a dependable OPLS-DA model. Specifically, S1Y and S2Y were located in the fourth and first quadrants, respectively while S3Y and S4Y were located in the second and third quadrants, respectively. And the S3Y and S4Y samples almost coincided, indicating that their volatile metabolites were relatively similar. The model parameters ($R^2Y = 0.729$, $Q^2 = 0.527$) showed that it had good interpretation and prediction performance. In addition, 200 iterations of permutation test showed no overfitting ($R^2 = 0.498$, $Q^2 = -0.86$) (see Fig. 2B). The metabolic trajectory plot can reflect the changing trend of metabolites. Generally, the greater

the distance between the two, the more drastic the change of metabolites. As shown in Fig. 2C, the distance between S3Y and S2Y was the furthest, indicating that the storage time of 3 years was a critical transition point. Variable Importance in Projection (VIP) was employed to evaluate the influence strength and explanatory ability of each variable on the classification and discrimination. The variable was considered to play an important role when VIP exceeded 1. In this study, a total of 15 compounds with VIP > 1 were screened out, which were mainly aldehydes and esters (see Fig. 2D). The representative volatile compounds mainly included 4-ethyl-phenol, 1-methyl-1H-pyrrole-2-carboxaldehyde, 1-ethyl-1H-pyrrole-2-carboxaldehyde, methyl salicylate, nonanal, 1,2,4-trimethoxybenzene, (R)-2(4H)-5,6,7,7a-tetrahydro-4,4,7a-trimethyl-benzofuranone, α -ionone, dimethyl ether, (E)-2-nonenal, linalool, dehydromevalonic lactone, 2-methyl-butanal, hexanal, and phenylethyl alcohol.

3.3. Analysis of Pu-erh teas with different storage years by GC-IMS

3.3.1. The topographic plots in Pu-erh tea with different storage years

As a new analytical technique, GC-IMS was applied to obtain the global IMS information of Pu-erh tea samples, with the advantages of extraordinary sensitivity, high separation ability, easy operation, and visualization of flavor substances (Guo, Schwab, Ho, Song, & Wan, 2021; Yang, Zhu, et al., 2022). This technique could separate and identify ionized compounds at ambient pressures based on the difference of migration rates in an electric field. The topographic plots obtained from GC-IMS analysis in this study were shown in Fig. 3A. The vertical coordinate represents the retention time of gas chromatography, and the horizontal coordinate represents the ion migration time. The red vertical line at abscissa 1.0 represents the reactive ion peak (RIP) after normalization. Each point on both sides of the RIP peak represents a volatile component, and the color represents the content of a volatile component. White color represents a lower content while red represents a higher content. It could be found that the types of volatile components of the different storage samples were similar, mainly reflected in the content difference.

In order to observe the differences more clearly, the spectral diagram of S1Y was chosen as the reference, and the other samples were deducted as the reference. Red signified that the content of a volatile component was higher than the reference, whereas blue signified that the content of a volatile component was lower than the reference. As can be seen in Fig. 3B, most of the signal points were shown as a retention time range of 0 to 1500 s and a drift time range of 1.0 to 2.0. The concentrations of some volatile compounds increased significantly with the increase in storage years and reached the highest level in S3Y. This phenomenon indicates that the contents of volatile components of Pu-erh tea were significantly different based on the storage time.

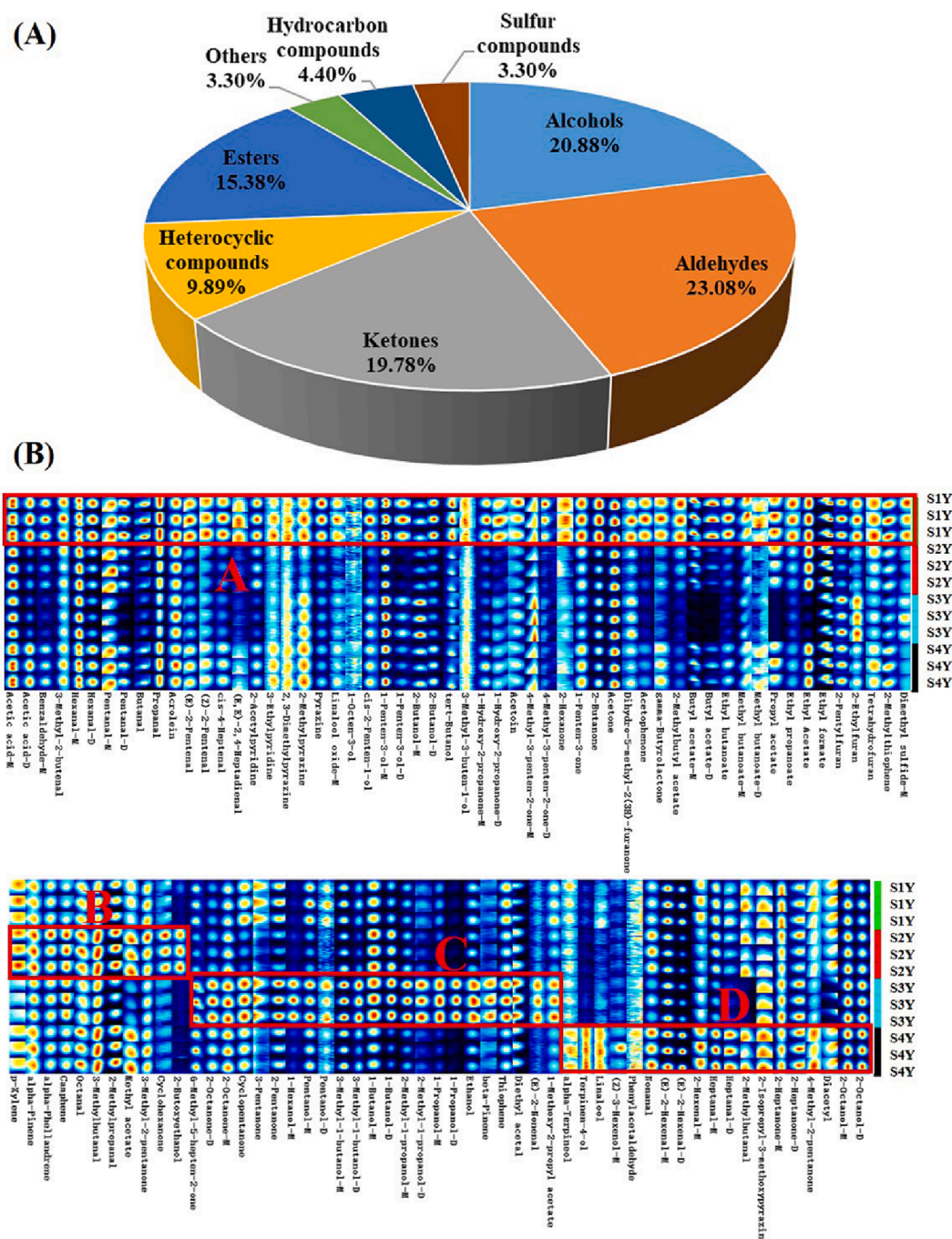


Fig. 4. The information of volatile compounds in Pu-erh teas with different storage years by using GC-IMS. (A) Proportion of different classes of volatile compounds; (B) Volatile fingerprints generated by Gallery Plot.

3.3.2. Volatile compounds in Pu-erh teas stored for different periods

In order to further observe the variation regularity in specific volatile compounds throughout the storage process, the volatile components were qualitatively characterized by comparing the retention time and drift time with those of the authentic reference compounds. A total of 91 known volatile substances (corresponding to 109 peak signals) were identified, which were divided into 8 categories, including 19 alcohols, 21 aldehydes, 18 ketones, 9 heterocyclic compounds, 14 esters, 4 hydrocarbons, 3 sulfur compounds, and 3 other compounds (Table 1). Aldehydes (23.08%), alcohols (20.88%) and ketones (19.78%) were the main volatile components, consistent with the results of GC-MS (Fig. 4A). It is worth mentioning that some single volatile compounds might produce multiple signals of their dimers in the ionization region

due to the concentrations of volatile components.

In order to directly reflect the content difference of volatile components in Pu-erh teas with different storage years, the volatile fingerprints were constructed and analyzed. Each column was presented on behalf of a volatile compound in different samples, and each row represented the signal peak of one sample. The color was on behalf of the content of a volatile component, and the brighter the color, the higher content. As shown in Fig. 4B, the fingerprint could be divided into four regions of A, B, C and D. The volatile components in region A reached the maximum concentration in S1Y, and were dominated by heterocyclic compounds and esters (such as pyrazine, 2-methylpyrazine, 2-methylthiophene, 2-ethylfuran, 3-ethylpyridine, ethyl acetate, propyl acetate and ethyl propanoate, etc). Alkenes (such as α -pinene, camphene and

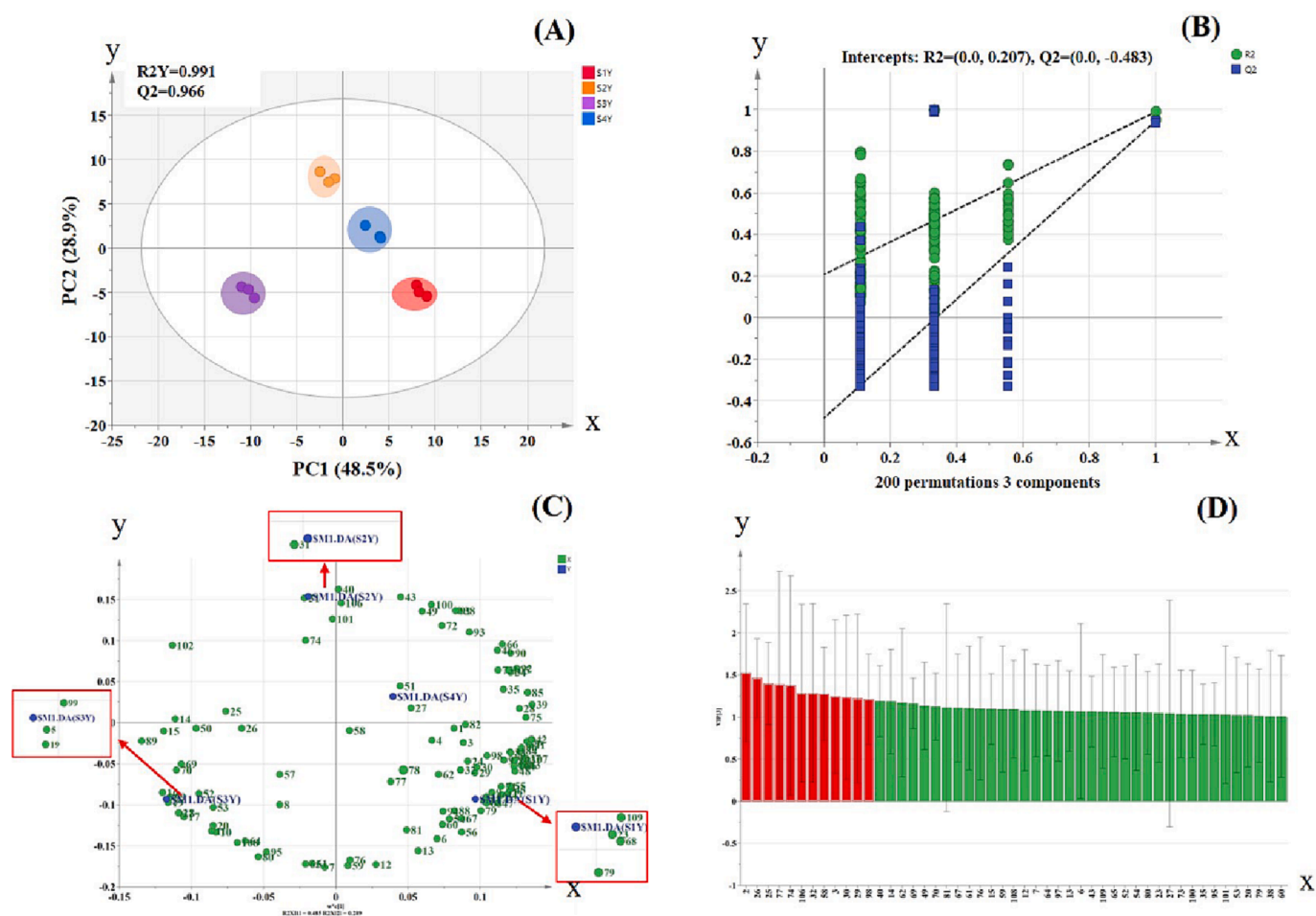


Fig. 5. The PLS-DA results of Pu-erh teas with different storage years by using GC-IMS. (A) Scores plots of PLS-DA ($R^2Y = 0.991$, $Q^2 = 0.966$); (B) Cross-validation plot by 200 permutation tests ($R^2 = 0.207$, $Q^2 = -0.483$); (C) Loading plot of PLS-DA; (D) The red part represents key differential compounds with $VIP > 1.2$, and compound numbers corresponded to Table 1.

α -phellandrene) dominated in B region, and reached the highest level in S2Y. In region C, ketones and alcohols were the main compounds, reaching the highest content in S3Y. The typical compounds included 6-methyl-5-hepten-2-one, cyclopentanone, 2-pentanone, pentanol monomer and dimer, and 1-butanol monomer and dimer, etc. Aldehydes were the main odorants in D region. The representative compounds were 2-methylbutanal, nonanal, phenylacetaldehyde and heptanal monomer and dimer, which increased slowly with the passage of storage time and reached their highest concentration in S4Y.

3.3.3. Multivariate statistical analysis

The fingerprint only roughly distinguished the volatile components in Pu-erh teas with different storage years. It was hard to precisely establish which volatile compounds made the difference. PLS-DA could effectively distinguish between the observed values in different groups and found the importance variables that led to the differences among all groups. The similarities and differences between samples could be visualized by the score plot of the model. As shown in Fig. 5A, the four groups (S1Y, S2Y, S3Y and S4Y) achieved a good separation. The fitting parameters of the PLS-DA model ($R^2Y = 0.991$, $Q^2 = 0.966$) indicated that it had strong explanatory and predictive ability. In addition, 200 replications of permutation test were performed to evaluate the robustness of the PLS-DA model. As presented in Fig. 5B, the intercept between the Q2 regression line was less than 0, proving that the model was reliable and there was no overfitting ($R^2 = 0.207$, $Q^2 = -0.483$). The key compounds responsible for the aroma profile differences between Pu-erh tea samples with different storage years were further

analyzed by a load diagram (Fig. 5C). For example, *tert*-butanol (No.23), acetone (No.68) and dimethyl sulfide monomer (No.109) that appeared in higher concentrations in S1Y than other tea samples, have been reported to produce camphor, apple, pear, and creamy or vegetable odors. Octanal (No.31), with a citrus orange and green odor, presented high content in S2Y. Some volatiles such as 1-hexanol monomer (No.5), 1-propanol dimer (No.19), and β -pinene (No.99) had higher concentrations in S3Y than other groups, with a contribution to fruity, green, musty, and woody aromas. (Z)-3-Hexenol monomer (No.27) and phenylacetaldehyde (No.51), which occurred in higher concentrations in S4Y than others, provided green and floral aromas. To characterize the key differential compounds obtained from different storage years, the VIP was investigated. In this model, a total of 49 variables with $VIP > 1$ were screened out, and alcohols (10), ketones (12) and aldehydes (8) were more abundant (Fig. 5D). In addition, based on double variable criterion of one-way ANOVA ($p < 0.05$) and $VIP > 1.2$, nine key substances were picked out to distinguish the tea samples with different storage durations. The box-plots of the above nine key volatile components were shown in Fig. S3. The contents of nine key compounds could be divided into three groups. Group I was dominated by 2-octanol monomer and dimer (No.25 and No.26), with fresh grassy and earthen aroma. Its content increased gradually with the extension of storage time. The volatile compounds including cyclohexanone (No.74) and 2-butoxyethanol (No.106) in group II reached the highest level in S2Y, and declined sharply after S3Y. The volatile components in group III decreased gradually and reached the lowest level during storage for two or three years (S2Y or S3Y), and then increased sharply after S4Y. These

representative compounds included linalool (No.3), (E)-2-hexenal (monomer and dimer) (No.29 and No.30), 2-hexenal monomer (No.32), 2-methylbutyl acetate (No.98), terpinen-4-ol (No.2) and 2-isopropyl-3-methoxypyrazine (No.77). Linalool was an important monoterpene alcohol which was widely exists in teas. It was mainly derived from the hydrolysis reaction of β -glucoside and β -primrose glucoside in tea (Ho, Zheng, & Li, 2015). As stereoisomers, (E)-2-hexenal and 2-hexenal were important volatile compounds in fresh leaves, contributing to the green and grassy aromas (Guo, Ho, Wan, Zhu, Liu, & Wen, 2021). Terpinen-4-ol was reported to present woody and earthy odor and was detected in oolong tea (Guo, Schwab, Ho, Song, & Wan, 2021). 2-Isopropyl-3-methoxypyrazine had a lower threshold (0.0039 $\mu\text{g/L}$ in water), and was reported as an important heterocyclic compound with earthy and pea-like odor (Flaig, Qi, Wei, Yang, & Schieberle, 2020).

4. Conclusions

In this study, the aroma profiles of Pu-erh teas stored for a different number of years were comprehensively characterized by combining GC-E-nose, GC-MS and GC-IMS. GC-E-nose achieved the rapid differentiation of Pu-erh teas with different storage years ($R^2Y = 0.992$, $Q^2 = 0.968$). A total of 43 volatile components were identified by GC-MS, while 91 volatile substances were detected by GC-IMS. A satisfactory discrimination was achieved by using PLS-DA based on the volatile fingerprints obtained from GC-IMS, with robust model parameters ($R^2Y = 0.991$, and $Q^2 = 0.966$). In addition, 9 flavor compounds were considered as important variables that caused the aroma differences of Pu-erh teas with different storage years, including linalool, (E)-2-hexenal, 2-hexenal, 2-methylbutyl acetate and terpinen-4-ol, cyclohexanone, 2-butoxyethanol, 2-octanol, and 2-isopropyl-3-methoxypyrazine. The overall information of volatile components of Pu-erh tea was retained to the greatest extent by the combination of GC-E-nose, GC-MS and GC-IMS, and the use of multivariate statistical analysis realized the rapid distinction of Pu-erh teas with different storage periods.

CRedit authorship contribution statement

Yuting Rong: Resources, Conceptualization, Methodology, Investigation. **Jialing Xie:** Methodology, Software, Validation, Writing – original draft. **Haibo Yuan:** Formal analysis, Visualization. **Lilei Wang:** Software. **Fuqiao Liu:** Resources. **Yuliang Deng:** Data curation. **Yongwen Jiang:** Funding acquisition, Supervision, Project administration. **Yanqin Yang:** Conceptualization, Methodology, Supervision, 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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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fochx.2023.100693>.

References

- Chen, X., Chen, D., Jiang, H., Sun, H., Zhang, C., Zhao, H., ... Xu, Z. (2019). Aroma characterization of Hanzhong black tea (*Camellia sinensis*) using solid phase extraction coupled with gas chromatography–mass spectrometry and olfactometry and sensory analysis. *Food Chemistry*, 274, 130–136.
- Fan, X., Jiao, X., Liu, J., Jia, M., & Zhou, Z. (2020). Characterizing the volatile compounds of different sorghum cultivars by both GC-MS and HS-GC-IMS. *Food Research International*, 140(4), Article 109975.
- Feng, D., Wang, J., Ji, X.-J., Min, W.-X., & Yan, W.-J. (2021). HS-GC-IMS detection of volatile organic compounds in yak milk powder processed by different drying methods. *LWT*, 141, Article 110855. <https://doi.org/10.1016/j.lwt.2021.110855>
- Feng, X., Wang, H., Wang, Z., Huang, P., & Kan, J. (2022). Discrimination and characterization of the volatile organic compounds in eight kinds of huajiao with geographical indication of China using electronic nose HS-GC-IMS and HS-SPME-GC-MS. *Food Chemistry*, 375, Article 131671. <https://doi.org/10.1016/j.foodchem.2021.131671>
- Flaig, M., Qi, S. C., Wei, G., Yang, X., & Schieberle, P. (2020). Characterisation of the key aroma compounds in a Longjing green tea infusion (*Camellia sinensis*) by the sensomics approach and their quantitative changes during processing of the tea leaves. *European Food Research and Technology*, 246(12), 2411–2425. <https://doi.org/10.1007/s00217-020-03584-y>
- Guo, X., Ho, C. T., Wan, X., Zhu, H., Liu, Q., & Wen, Z. (2021). Changes of volatile compounds and odor profiles in Wuyi rock tea during processing. *Food Chemistry*, 341(Pt 1), Article 128230. <https://doi.org/10.1016/j.foodchem.2020.128230>
- Guo, X., Schwab, W., Ho, C. T., Song, C., & Wan, X. (2021). Characterization of the aroma profiles of oolong tea made from three tea cultivars by both GC-MS and GC-IMS. *Food Chemistry*, 376, Article 131933. <https://doi.org/10.1016/j.foodchem.2021.131933>
- He, X., Yangming, H., Gorska-Horczynczak, E., Wierzbicka, A., & Jelen, H. H. (2021). Rapid analysis of Baijiu volatile compounds fingerprint for their aroma and regional origin authenticity assessment. *Food Chemistry*, 337, Article 128002.
- Ho, C.-T., Zheng, X., & Li, S. (2015). Tea aroma formation. *Food Science and Human Wellness*, 4(1), 9–27. <https://doi.org/10.1016/j.fshw.2015.04.001>
- Li, Y., Fei, C., Mao, C., Ji, D., Gong, J., Qin, Y., ... Lu, T. (2022). Physicochemical parameters combined flash GC e-nose and artificial neural network for quality and volatile characterization of vinegar with different brewing techniques. *Food Chemistry*, 374, Article 131658.
- Liu, J. Y., He, D., Xing, Y. F., Zeng, W., Ren, K., Zhang, C., ... Xing, X. H. (2021). Effects of bioactive components of Pu-erh tea on gut microbiomes and health: A review. *Food Chemistry*, 353, Article 129439. <https://doi.org/10.1016/j.foodchem.2021.129439>
- Lv, H. P., Zhang, Y., Yang, T., Shi, J., & Lin, Z. (2015). Key aroma constituents affecting the quality of ripened Pu-erh tea. *Modern Food Science and Technology*, 31, 394–399. <https://doi.org/10.13982/j.mfst.1673-9078.2015.12.058>
- Lv, H. P., Zhong, Q. S., Lin, Z., Wang, L., Tan, J. F., & Guo, L. (2012). Aroma characterisation of Pu-erh tea using headspace-solid phase microextraction combined with GC/MS and GC-olfactometry. *Food Chemistry*, 130(4), 1074–1081.
- Lv, S., Wu, Y., Li, C., Xu, Y., Liu, L., & Meng, Q. (2014). Comparative analysis of Pu-erh and fuzhuan teas by fully automatic headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry and chemometric methods. *Journal of Agricultural and Food Chemistry*, 62(8), 1810–1818. <https://doi.org/10.1021/jf405237u>
- Lv, S., Wu, Y., Wei, J., Lian, M., Wang, C., Gao, X., & Meng, Q. (2015). Application of gas chromatography-mass spectrometry and chemometrics methods for assessing volatile profiles of Pu-erh tea with different processing methods and aging years. *RSC Advances*, 5(107), 87806–87817. <https://doi.org/10.1039/c5ra15381f>
- Ma, W., Shi, Y., Yang, G., Shi, J., Ji, J., Zhang, Y., ... Lv, H. (2022). Hypolipidaemic and antioxidant effects of various Chinese dark tea extracts obtained from the same raw material and their main chemical components. *Food Chemistry*, 375, Article 131877. <https://doi.org/10.1016/j.foodchem.2021.131877>
- Ma, W., Zhu, Y., Shi, J., Wang, J., Wang, M., Shao, C., ... Lv, H. (2021). Insight into the volatile profiles of four types of dark teas obtained from the same dark raw tea material. *Food Chemistry*, 346, Article 128906. <https://doi.org/10.1016/j.foodchem.2020.128906>
- Mao, S., Lu, C., Li, M., Ye, Y., Wei, X., & Tong, H. (2018). Identification of key aromatic compounds in Congou black tea by partial least-square regression with variable importance of projection scores and gas chromatography-mass spectrometry/gas chromatography-olfactometry. *Journal of the Science of Food and Agriculture*, 98(14), 5278–5286. <https://doi.org/10.1002/jsfa.9066>
- Pang, X., Yu, W., Cao, C., Yuan, X., Qiu, J., Kong, F., & Wu, J. (2019). Comparison of potent odorants in raw and ripened Pu-Erh tea infusions based on odor activity value calculation and multivariate analysis: understanding the role of pile fermentation. *Journal of Agricultural and Food Chemistry*, 67(47), 13139–13149. <https://doi.org/10.1021/acs.jafc.9b05321>
- Qi, H., Ding, S., Pan, Z., Li, X., & Fu, F. (2020). Characteristic volatile fingerprints and odor activity values in different citrus-tea by HS-GC-IMS and HS-SPME-GC-MS. *Molecules*, 25(24), 6027. <https://doi.org/10.3390/molecules25246027>
- Su, E., Xia, T., Gao, L., Dai, Q., & Zhang, Z. (2010). Immobilization of beta-glucosidase and its aroma-increasing effect on tea beverage. *Food and Bioprocess Processing. Transactions of the Institution of Chemical Engineers, Part C*, 88(2/3).
- Wang, C., Li, J., Wu, X., Zhang, Y., He, Z., Zhang, Y., ... Liu, Z. (2022). Pu-erh tea unique aroma: Volatile components, evaluation methods and metabolic mechanism of key odor-active compounds. *Trends in Food Science & Technology*, 124, 25–37. <https://doi.org/10.1016/j.tifs.2022.03.031>
- Wang, K., Liu, F., Liu, Z., Huang, J., Xu, Z., Li, Y., ... Yang, X. (2011). Comparison of catechins and volatile compounds among different types of tea using high

- performance liquid chromatograph and gas chromatograph mass spectrometer, International. *Journal of Food Science & Technology*, 46(7), 1406–1412. <https://doi.org/10.1111/j.1365-2621.2011.02629.x>
- Wang, T., Li, X., Yang, H., Wang, F., Kong, J., Qiu, D., & Li, Z. (2018). Mass spectrometry-based metabolomics and chemometric analysis of Pu-erh teas of various origins. *Food Chemistry*, 268, 271–278.
- Wang, X., Rogers, K. M., Li, Y., Yang, S., Chen, L., & Zhou, J. (2019). Untargeted and targeted discrimination of honey collected by *Apis cerana* and *Apis mellifera* based on volatiles using HS-GC-IMS and HS-SPME-GC-MS. *Journal of Agricultural and Food Chemistry*, 67(43), 12144–12152. <https://doi.org/10.1021/acs.jafc.9b04438>
- Xu, M., Wang, J., & Zhu, L. (2021). Tea quality evaluation by applying E-nose combined with chemometrics methods. *Journal of Food Science and Technology*, 58(4), 1549–1561.
- Yang, C. S., Wang, H., & Sheridan, Z. P. (2018). Studies on prevention of obesity, metabolic syndrome, diabetes, cardiovascular diseases and cancer by tea. *Journal of Food and Drug Analysis*, 26(1), 1–13. <https://doi.org/10.1016/j.jfda.2017.10.010>
- Yang, Y., Hua, J., Deng, Y., Jiang, Y., Qian, M. C., Wang, J., ... Yuan, H. (2020). Aroma dynamic characteristics during the process of variable-temperature final firing of Congou black tea by electronic nose and comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry. *Food Research International*, 137, Article 109656.
- Yang, Y., Xie, J., Chen, J., Deng, Y., Shen, S., Hua, J., ... Jiang, Y. (2022). Characterization of N, O-heterocycles in green tea during the drying process and unraveling the formation mechanism. *Food Control*, 139, Article 109079. <https://doi.org/10.1016/j.foodcont.2022.109079>
- Yang, Y., Zhu, H., Chen, J., Xie, J., Shen, S., Deng, Y., ... Jiang, Y. (2022). Characterization of the key aroma compounds in black teas with different aroma types by using gas chromatography electronic nose, gas chromatography-ion mobility spectrometry, and odor activity value analysis. *LWT*, 163, Article 113492. <https://doi.org/10.1016/j.lwt.2022.113492>
- Yang, Y. Q., Rong, Y. T., Liu, F. Q., Jiang, Y. W., Deng, Y. L., Dong, C. W., & Yuan, H. B. (2021). Rapid characterization of the volatile profiles in Pu-erh tea by gas phase electronic nose and microchamber/thermal extractor combined with TD-GC-MS. *Journal of Food Science*, 86(6), 2358–2373.
- Yang, Z., Baldermann, S., & Watanabe, N. (2013). Recent studies of the volatile compounds in tea. *Food Research International*, 53(2), 585–599. <https://doi.org/10.1016/j.foodres.2013.02.011>
- Yin, X., Huang, J. a., Huang, J., Wu, W., Tong, T., Liu, S., . . . Zhang, S. (2022). Identification of volatile and odor-active compounds in Hunan black tea by SPME/GC-MS and multivariate analysis. *LWT*, 164, 113656. doi: 10.1016/j.lwt.2022.113656.
- Yu, H., Wang, J., Zhang, H., Yong, Y., & Cong, Y. (2008). Identification of green tea grade using different feature of response signal from E-nose sensors. *Sensors and Actuators B Chemical*, 128(2), 455–461.
- Yun, J., Cui, C., Zhang, S., Zhu, J., Peng, C., Cai, H., ... Hou, R. (2021). Use of headspace GC/MS combined with chemometric analysis to identify the geographic origins of black tea. *Food Chemistry*, 360, Article 130033. <https://doi.org/10.1016/j.foodchem.2021.130033>
- Zhai, X., Zhang, L., Granvogl, M., Ho, C. T., & Wan, X. (2022). Flavor of tea (*Camellia sinensis*): A review on odorants and analytical techniques. *Comprehensive Reviews in Food Science and Food Safety*, 21(5), 3867–3909. <https://doi.org/10.1111/1541-4337.12999>