Research Article

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Quantitative analysis of volatile compounds of four Chinese traditional liquors by SPME-GC-MS and determination of total phenolic contents and antioxidant activities

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Abstract: The aim of this work was to investigate the volatile compositions of four Chinese functional liquors. For this purpose, volatile compounds of four liquors were extracted with head-space solid-phase microextraction (HS-SPME) and analyzed with gas chromatography-mass spectrometry (GC-MS) along with the determination of odor activity value (OAV) and relative odor contribution (ROC). Sixty volatiles were tentatively identified and categorized into the following seven groups: alcohols, esters, fatty acids, carbonyl compound, hydrocarbons, phenols, and other components. The differences in chemical composition of volatile compounds were visualized with heat maps. Odorants were compared with different samples using a statistical analysis of Venn diagrams and a multivariate principal component analysis, and ethyl hexanoate, ethyl acetate, and ethyl octanoate were found to be the key odorants. Besides, abundant phenolic contents and high antioxidant ability of four Chinese functional liquors could potentially bring better health-boosting effects.

Keywords: volatile flavor compound, Chinese functional liquors, HS-SPME-GC-MS, PCA, total polyphenols contents, antioxidant capacities

Abbreviations

ABTS	2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
DPPH	1,1-diphenyl-2-picryl-hydrazyl
GAE	gallic acid equivalents
GC-MS	gas chromatography-mass spectrometry
HS-SPME	head-space solid-phase microextraction
OAV	odor activity value
PCA	principal component analysis
ROC	relative odor contribution
TEAC	trolox equivalent antioxidant capacity

1 Introduction

The Chinese functional wine, a combination of traditional Chinese wine and Chinese herbs, is produced by adding Chinese herbs into wine during its production. The following four Chinese functional liquors are dominant in the market: Kinmen-Kaoliang Liquor, Jin Liquor, highland barley wine, and Zhuyeqing Liquor. Kinmen-Kaoliang Liquor, which belongs to mild aromatic Chinese spirits and possesses the unique fragrance and mellow taste, is the representative of sorghum wine. Jin Liquor can improve both mental and physical fatigue caused by sub-health [1]. Highland barley, produced by fermentation of barley, is rich in amino acids, protein, dietary fiber, vitamins, and micro-elements [2]. It has a characteristic sweet taste and enables to reduce cholesterol and blood-lipid. Zhuyeqing Liquor delivers a full fragrance flavor based on a Fen wine distillate base. The liquor contains the extracts from bamboo leaves, chrysanthemum, angelica, and other herbs, and has the detoxicating and anti-aging capacity for humans [3].

Because of the presence of Chinese herbs, volatiles of Chinese functional liquor are most likely different

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compared to those of the non-functional liquor, which remains poorly understood. Aroma is a decisive factor to affect the quality and consumer acceptance of a liquor [4]. Therefore, unraveling the aroma profiles of Chinese functional liquors will be essential to understand their taste, nutrition, and popularity. Multiple methods have been used to extract the volatile compounds from wine; headspace solid-phase microextraction (HS-SPME) was found to be superior to other methods because of their simplicity, accuracy, and fast capacity [5]. Using this technique, Ivanova identified 30 representative wine volatile compounds from eight varietal wines in the Macedonian and Hungarian [6]. Xiao et al. used HS-SPME-GC-MS and electronic-nose to assess the aroma compounds and to determine odor descriptors of five typical Chinese liquors [7]. Eighty-six aroma compounds were identified, including 5 acids, 34 esters, 10 alcohols, 9 aldehydes, 4 ketones, 4 phenols, and 10 nitrous and sulfuric compounds. Odor activity value (OAV) and relative odor contribution (ROC) are the two main parameters to assess the contribution of aromatic compounds. OAV is the ratio of the real concentration of an individual compound and its olfactory threshold. ROC represents the ratio of the OAV percentage of each individual compound and the sum of the OAV of compounds that showed OAV >1 [8].

Polyphenols are the secondary metabolites of plants and have been demonstrated to be strong antioxidants in wine. In our previous study, it was found that the total polyphenols content could be 456 mg gallic acid equivalent (GAE)/L in gingko wine, and some typical Chinese liquors also contained 45–130 mg GAE/L total polyphenols. These bioactive compounds increase the HDL of high-density lipoprotein in blood, effectively reduce blood cholesterol, prevent atherosclerosis, and also inhibit platelet agglutination and prevent thrombosis [9].

The objective of the present work was to identify the volatile flavor compounds in four Chinese functional liquors and assess their *in vitro* antioxidant activity. Sixty volatile compounds were identified from the volatiles of four liquors GC-MS after HS-SPME extraction. The phenolic content, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) value of the liquor were also measured and compared with traditional Chinese wine.

2 Materials and methods

2.1 Wine samples

The four typical Chinese functional liquors, Kinmen-Kaoliang (L1), Jin (L2), Highland barley (L3), and Zhuyeqing (L4), were purchased from the local market. Their alcoholic contents were 45.0% (v/v), 45.0% (v/v), 35.0% (v/v), and 38.0% (v/v), respectively. To minimize the effects from different alcoholic contents, four wines were diluted with aqueous ethanol 50.0% (v/v) to reach a final alcoholic content of 12.0% (v/v).

2.2 Chemicals and reagents

A C7-C30 *n*-alkane mixture, used for the determination of linear retention indices (RIs), was purchased from Supelco (Bellefonte, PA, USA). 2-Octanol used as internal standard was obtained from Dr. Ehrenstorfer (Augsburg, Germany). Folin-Ciocalteu reagent, DPPH, ABTS, trolox, gallic acid, and potassium persulfate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Water was obtained from a Milli-Q purification system (Millipore). Other chemicals and reagents were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). All reagents used were of analytical grade.

2.3 Extraction of volatile compounds

Volatile components of the liquors were extracted with HS-SPME method. Liquor samples (5 mL) were pipetted into 15 mL headspace vials, and 1 g sodium chloride and 50 µL 2-octanol (internal standard) were then added and mixed. The vial was sealed with a silicon septum, placed in 50°C water bath, and equilibrated for 15 min. A 75 µm DVB/CAR/PDMS solid-phase fiber (Supelco, Bellefonte, PA, USA) was then plugged into the headspace of the vial for 30 min. Later, the solid-phase fiber was immediately injected into the gas chromatography-mass spectrometry (GC-MS) injection port for desorption (5 min) in splitless mode and then analyzed [10]. Each sample was done in triplicates.

2.4 GC-MS conditions and quantitative analysis of wine volatiles

Volatile compounds were separated and identified on a 7890 gas chromatography coupled with a 5975 C mass selective detector (MS) (Agilent Technologies, USA), equipped with a HP-INNOWAX capillary column (60 m × 0.25 mm ID, 0.25 µm film thickness). The carrier gas helium was circulated at 1 mL/min in the constant flow mode. A split/splitless injector was used in the splitless

mode. The injected volume was 1 µL and the injector temperature was set at 250°C. The oven temperature program was set as follows: 50°C for 2 min; then 6°C/min ramps to 230°C and holding for 10 min. The transfer line and the ion source temperatures were set at 250°C. The ion energy for electron impact (EI) was 70 eV, and the chromatograms were obtained by recording a mass range of 30–450 *m/z*.

Tentative identification of the volatile compounds was achieved by comparing mass spectrum and RIs with the Nist05a.1 Database and Wiley7n.1 Database (Hewlett-Packard, Palo Alto, CA) and literatures. Some compounds were identified by injecting the authentic compounds into the GC-MS system, while the RI of the compounds was calculated using an *n*-alkane series under the same conditions according to Van Den Dool and Kratz equation [11]. Semiquantitative determinations were performed according to Xiao [10].

2.5 Determination of total phenolic content

The total phenolic content of the four Chinese functional liquors was measured by a modified colorimetric Folin–Ciocalteu's method [12,13]. Briefly, an aliquot (100 µL) of the diluted wines was pipetted into a 15 mL test tube with a cap, and Folin–Ciocalteu reagent (0.1 mL) was added and mixed. After 5 min, 10% Na_2CO_3 solution (w/v) (3 mL) was added, mixed, and heated at 75°C for 10 min before measurement of the absorbance at 760 nm using a UV-2350 spectrophotometer (UNICO (Shanghai) Instruments Co., Ltd, Shanghai, China). Gallic acid solutions with different concentrations (0–400 mg/L) were measured to obtain a calibration curve. The total phenolic was expressed as mg of GAE per liter of sample (mg GAE/L). Each sample was done in triplicates.

2.6 DPPH and ABTS free radical scavenging capacity

Diluted sample (1 mL) or aqueous ethanol (50.0%, v/v) (blank) was added into freshly prepared 0.04 mmol/L DPPH solutions (2 mL). The solutions were mixed and left at 30°C in dark for 30 min before measurement of absorbance at 515 nm using a spectrophotometer (UV-2350). A calibration curve was built with trolox at different concentrations (0–400 mg/L). The DPPH value of the samples was expressed as trolox equivalent antioxidant capacity (TEAC, mg/L). The percentage radical-scavenging activity (%SA) of DPPH was calculated using the equation:

$$\%SA = (A_{blank} - A_{sample})/A_{blank} \times 100\%. \quad (1)$$

The $ABTS^{\cdot}+$ cation was generated by mixing 7 mM $ABTS^{\cdot}+$ solution and 2.45 mM potassium persulfate solution and left at room temperature for 12–16 h in dark. It was then diluted with ethanol/water (50/50, v/v) to obtain an absorbance of 0.70 ± 0.02 at 734 nm before use. Liquor sample (1 mL) or aqueous ethanol (blank, 50.0%, v/v) was added into diluted $ABTS^{\cdot}+$ solution (2 mL), mixed, and incubated for 6 min at 37°C water bath. The decrease of absorbance at 734 nm was then measured. The percentage of inhibition (%I) was calculated using the following equation:

$$\%I = (A_{blank} - A_{sample})/A_{blank} \times 100\%. \quad (2)$$

The antioxidant activities of samples were expressed as TEAC values, defined as the concentration of standard trolox with the same antioxidant capacity as those of the samples.

2.7 Statistical analysis

All measurements were done in triplicates and results were presented as mean ± standard deviation (SD) ($n = 3$). Linearity was studied by quantification of the correlation coefficients. The antioxidant capacity analysis was performed by analysis of variance using the software SAS V8 (SAS Institute Inc., Cary, NC). Statistical significance was declared at $P < 0.05$. Heat map visualization of data was performed using the TBtools v0.668375 (Toolbox for Biologists). Data was log-transformed dividing the values of relative peak areas of each volatile compound by mean to perform heat maps. The Venn diagram was generated with the web tool provided by the Bioinformatics and Systems Biology of Gent (URL: http://bioinformatics.psb.ugent.Be/webtools/Venn/).

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and discussion

3.1 Volatile compounds and OAV evaluation

Compositions of aroma compounds and their calculated RI values from the four functional liquors are listed in

Table 1: Volatile composition of four Chinese functional liquors (mg/L, $n = 3$) identified by GC-MS

		RIcal[a]	RIref[b]	OTS[c] (mg/L)	Concentration[d] (mg/L)				OAV[e]				ROC[f] (%)			
					L1	L2	L3	L4	L1	L2	L3	L4	L1	L2	L3	L4
Alcohols																
1	2-Butanol	1,016	1,017	3.300	—	9.43 ± 0.01	—	—	—	2.86	—	—	—	0.0040	—	—
2	Isobutanol	1,086	1,081	16.000	9.93 ± 0.02	10.11 ± 0.11	—	2.97 ± 0.12	0.62	0.63	—	0.19	—	—	—	—
3	Isoamyl alcohol	1,189; 1,199	1,214	2.800	84.76 ± 0.23	97.91 ± 0.05	43.1 ± 0.16	65.46 ± 0.11	30.27	34.97	15.39	23.38	0.0171	0.0495	0.0030	0.0108
4	1-Hexanol	1,327	1,387; 1,343	0.4251	4.26 ± 0.13	—	43.05 ± 0.32	7.00 ± 0.04	10.02	—	101.29	16.47	0.0057	—	0.0200	0.0076
5	Borneol	1,689	—	0.140	—	19.38 ± 0.03	—	1.53 ± 0.02	—	138.43	—	10.93	—	0.1959	—	0.0050
6	Phenylethyl alcohol	1,916	1,929; 1,907	1.12	2.08 ± 0.1	5.55 ± 0.01	—	1.61 ± 0.23	1.89	5.05	—	1.46	0.0011	0.0071	—	0.0007
7	α-Cadinol	2,195	2,231	NF	—	1.00 ± 0.06	—	—	—	—	—	—	—	—	—	—
8	*cis*-α-Santalol	2,355	—	NF	—	—	—	1.04 ± 0.34	—	—	—	—	—	—	—	—
Total					101.03	143.38	86.15	79.61								
Esters																
9	Ethyl acetate	878	900	12.33	111.7 ± 0.14	72.91 ± 0.24	199.38 ± 0.35	110.33 ± 0.19	9.08	5.93	16.21	8.97	0.0051	0.0084	0.0032	0.0041
10	Ethyl butyrate	1,030	1,042	0.024	13.9 ± 0.08	10.97 ± 0.39	33.75 ± 0.43	5.76 ± 0.02	695	548.50	1687.50	288.00	0.3929	0.7763	0.3334	0.1329
11	Ethyl 2-methylbutyrate	1,045; 1,063	1,041	0.0003	1.11 ± 0.17	—	—	0.40 ± 0.15	4269.23	—	—	1538.46	2.4133	—	—	0.7097
12	Ethyl isovalerate	1,058	1,055	0.0003	1.64 ± 0.21	—	—	0.52 ± 0.02	5466.67	—	—	1733.33	3.0902	—	—	0.7996
13	Isoamyl acetate	1,106	1,103	0.0301	9.6 ± 0.00	19.81 ± 0.02	11.58 ± 0.11	13.73 ± 0.29	320.0	660.33	386.00	457.67	0.1809	0.9346	0.0763	0.2111
14	Ethyl pentanoate	1,118	1,140	0.0051	4.18 ± 0.04	5.53 ± 0.00	44.43 ± 0.01	2.48 ± 0.04	836.0	1106.00	8886.00	496.00	0.4726	1.5653	1.7556	0.2288
15	Ethyl hexanoate	1,215	1,238	0.0003	48.11 ± 0.00	17.32 ± 0.10	124.25 ± 0.02	44.91 ± 0.16	160366.67	57733.33	414,167	149,700	90.6518	81.7088	81.8278	69.0616
16	Isopentyl isobutyrate	1,279	—	0.0003	—	—	14.19 ± 0.04	0.52 ± 0.10	—	—	—	47,300	—	—	—	21.8211
17	Ethyl 5-methylhexanoate	1,288	—	NF	—	—	2.91 ± 0.00	—	—	—	—	—	—	—	—	—

Table 1: continued

		RIcal[a]	RIref[b]	OTS[c] (mg/L)	Concentration[d] (mg/L)				OAV[e]				ROC[f] (%)			
					L1	L2	L3	L4	L1	L2	L3	L4	L1	L2	L3	L4
18	Ethyl (*Z*)-hex-3-enoate	1,297	—	NF	—	—	3.21 ± 0.06	—	—	—	—	—	—	—	—	—
19	Ethyl hex-4-enoate	1,301	—	NF	—	—	2.86 ± 0.05	—	—	—	—	—	—	—	—	—
20	Propyl hexanoate	1,311	—	12.780	—	—	15.98 ± 0.00	—	—	—	1.25	—	—	—	0.0003	—
21	Ethyl heptanoate	1,311; 1,331	—	0.0021	3.55 ± 0.01	1.39 ± 0.03	159.53 ± 0.11	3.24 ± 0.08	1577.78	617.78	70902.2	1440.00	0.8919	0.87433	14.0083	0.6643
22	Ethyl lactate	1,323	—	1.4001	5.42 ± 0.00	5.63 ± 0.01	—	11.44 ± 0.00	3.87	4.02	—	8.17	0.0022	0.0057	—	0.0038
23	Isobutyl caproate	1,340	—	NF	—	—	4.00 ± 0.04	—	—	—	—	—	—	—	—	—
24	Ethyl octanoate	1,413	1,434	0.015	21.65 ± 0.03	50.72 ± 0.00	126.84 ± 0.03	135.93 ± 0.02	1443.33	3381.33	8456.00	9062.00	0.8159	4.7855	1.6707	4.1806
25	Isoamyl hexanoate	1,445; 1,458	—	0.320	—	—	7.93 ± 0.01	—	—	—	24.78	—	—	—	0.0049	—
26	Ethyl nonanoate	1,530; 1,535	—	12.000	—	—	5.21 ± 0.10	11.31 ± 0.04	—	—	0.43	0.94	—	—	0.0001	0.0004
27	Hexyl hexanoate	1,592	—	6.400	—	—	7.53 ± 0.11	—	—	—	1.18	—	—	—	0.0002	—
28	Ethyl decanoate	1,619; 1,637	1,639	0.023	—	21.53 ± 0.00	1.12 ± 0.02	71.13 ± 0.01	—	936.09	48.70	3092.61	—	1.3248	0.0096	1.4267
29	Diethyl succinate	1,663; 1,670	—	2.005	—	8.94 ± 0.03	—	4.23 ± 0.04	—	0.04	—	0.02	—	—	—	—
30	Ethyl benzoate	1,669	1,680	0.060	—	—	0.75 ± 0.01	5.69 ± 0.05	—	—	12.50	94.83	—	—	0.0025	0.0438
31	2-Phenethyl acetate	1,823	1,822	0.160	—	6.61 ± 0.00	—	—	—	41.31	—	—	—	0.05847	—	—
32	Ethyl dodecanoate	1,855; 1,840	1,839	0.400	—	—	—	0.60 ± 0.04	—	—	—	1.50	—	—	—	0.0007
33	Ethyl benzenepropanoate	1,897	—	NF	—	1.57 ± 0.05	—	—	—	—	—	—	—	—	—	—
34	Ethyl tetradecanoate	2,056	2,034	0.180	—	—	—	0.92 ± 0.00	—	—	—	5.11	—	—	—	0.0024
35	Ethyl hexadecanoate	2,261	2,260	2.000	—	—	—	5.31 ± 0.03	—	—	—	2.66	—	—	—	0.0012
36	Octyl adipate	1,889	1,892	NF	17.31 ± 0.01	—	—	—	—	—	—	—	—	—	—	—
Total					238.17	222.93	765.45	428.45								

Table 1: continued

		RIcal[a]	RIref[b]	OTS[c] (mg/L)	Concentration[d] (mg/L)				OAV[e]				ROC[f] (%)			
					L1	L2	L3	L4	L1	L2	L3	L4	L1	L2	L3	L4
Acid																
37	Butyric acid	1,604	1,639	1.400	—	—	16.75 ± 0.00	23.57 ± 0.07	—	—	11.96	16.84	—	—	0.0024	0.0078
38	Hexanoic acid	1,865	1,871	3.0001	12.02 ± 0.23	1.98 ± 0.11	34.8 ± 0.03	—	4.01	0.66	11.60	—	0.0023	0.0009	0.0023	—
39	Octanoic acid	2,055	2,051	0.5001	2.09 ± 0.02	0.87 ± 0.24	1.56 ± 0.10	4.16 ± 0.00	4.18	1.74	3.12	8.32	0.0024	0.0025	0.0006	0.0038
Total					14.11	2.85	53.11	27.73								
Carbonyl compound																
40	Acetaldehyde	627	741	0.010	16.03 ± 0.00	42.98 ± 0.01	1.44 ± 0.06	11.17 ± 0.10	1603.00	4298.00	144.00	1117.00	0.9061	6.0829	0.0285	0.5153
41	Isovaleraldehyde	916	924	0.006	—	5.59 ± 0.06	—	—	—	931.67	—	—	—	1.31857	—	—
42	Hexanal	1,073	1,083	0.009	0.65 ± 0.03	—	—	0.93 ± 0.00	65.00	—	—	93.00	0.0367	—	—	0.0429
43	2-Octanone	1,272	1,275	0.050	7.91 ± 0.01	9.18 ± 0.05	19.48 ± 0.00	8.43 ± 0.06	158.20	183.60	389.60	168.60	0.0894	0.2599	0.0770	0.0778
44	3-Nonanone	1,339	—	0.033	1.00 ± 0.06	—	—	—	30.30	—	—	—	0.0171	—	—	—
45	2-Nonanone	1,371	1,388	0.200	0.28 ± 0.03	—	38.68 ± 0.09	—	1.40	—	193.40	—	0.0008	—	0.0382	—
46	Nonanal	1,376	1,396	0.260	0.43 ± 0.08	—	—	2.51 ± 0.03	1.65	—	—	9.65	0.0009	—	—	0.0045
47	2-Decanone	1,486	1,491	0.008	—	—	3.48 ± 0.03	—	—	—	419.28	—	—	—	0.0828	—
48	Furfural	1,453	1,477	0.770	4.91 ± 0.07	6.08 ± 0.04	20.87 ± 0.00	—	6.38	7.90	27.10	—	0.0036	0.0112	0.0054	—
49	(*E*)-Cinnamaldehyde	2,072	—	0.750	—	2.12 ± 0.04	—	—	—	2.83	—	—	—	0.0040	—	—
50	Benzylidenemalonaldehyde	2,078	—	NF	—	1.25 ± 0.06	—	—	—	—	—	—	—	—	—	—
Total					31.21	67.20	83.95	23.04								
Hydrocarbons																
51	Camphene	1,057	1,077	1.860	—	4.75 ± 0.05	—	—	—	2.55	—	—	—	0.0036	—	—
52	β-Myrcene	1,140	1,170	0.100	—	0.69 ± 0.03	—	1.32 ± 0.00	—	6.90	—	13.20	—	0.0098	—	0.0061

Table 1: continued

		RIcal[a]	RIref[b]	OTS[c] (mg/L)	Concentration[d] (mg/L)				OAV[e]				ROC[f] (%)			
					L1	L2	L3	L4	L1	L2	L3	L4	L1	L2	L3	L4
53	D-Limonene	1,177	—	1.200	—	4.43 ± 0.00	—	65.97 ± 0.00	—	3.69	—	54.98	—	0.0052	—	0.0254
54	(−)-Calamenene	1,840	1,837	NF	—	1.39 ± 0.02	—	—	—	—	—	—	—	—	—	—
55	Dodecane	1,170	—	10.000	—	—	—	2.66 ± 0.01	—	—	—	0.27	—	—	—	0.0001
56	*O*-Cymene	1,249	—	NF	—	—	—	8.01 ± 0.04	—	—	—	—	—	—	—	—
Total					0	11.26	0	77.96								
Phenols																
57	Eugenol	2,191	2,172	0.150	—	0.40 ± 0.09	—	—	—	2.67	—	—	—	0.0038	—	—
Total					0	0.40	0	0								
Others																
58	L-Alanine	553	—	710.000	—	12.12 ± 0.03	—	—	—	0.02	—	—	—	0.00002	—	—
59	*N*-Ethyl-1,3-dithioisoindoline	912	—	0.500	—	—	3.33 ± 0.05	—	—	—	6.66	—	—	—	0.0013	—
60	Indane	1,371	1,370	0.010	—	—	2.28 ± 0.15	—	—	—	228.00	—	—	—	0.0451	—
Total					0	12.12	5.61	0								

NF: not found.

[a] Linear RIs calculated of unknown compounds on a HP-INNOWAX capillary column (60 m × 0.25 mm × 0.25 μm) with a homologous series of *n*-alkanes (C7–C30) obtained from literatures. [b] The theoretical retention index obtained from the flavor net database (i), in the literature. [c] OTS, odor threshold measured in 10–12% (v/v) ethanol obtained from literatures [14–18]. [d] Values are the mean ± SD. [e] Odor activity value. [f] Relative odor contribution.

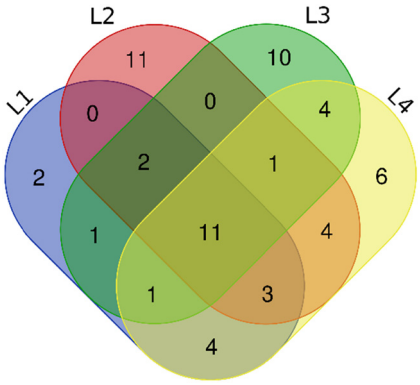

Figure 1: Venn diagrams for comparison of volatile components in four functional liquors.

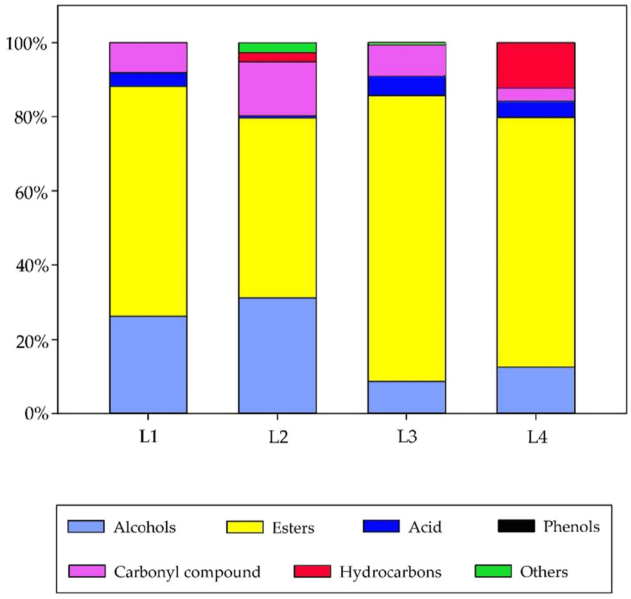

Figure 2: The proportion of various kinds of aroma substances in four functional liquors.

Table 1. A total of 61 volatile compounds were tentatively identified and categorized into the following seven groups: esters (28), alcohols (8), fatty acids (3), carbonyl compound (including aldehydes and ketones) (11), hydrocarbons (7), phenols (1), and other compositions (3). The relatively low standard deviations obtained for most compounds confirmed the authenticity and validity of the volatile profile of the four liquors. The threshold values for 12 compounds remained unknown and only five compounds with OAVs <1 were found. In other words, most of the volatile compounds contributed significantly to the overall aroma of the liquor.

As shown in Figure 1, the similarity and difference of volatile compounds in four functional liquors were analyzed using Venn diagram. From left to right, the common compounds and different compounds of L1, L2, L3, and L4 were grouped. There were 60 different components including 1 acid (octanoic acid), 1 alcohol (isoamyl alcohol), 1 aldehyde (acetaldehyde), and 7 esters (ethyl acetate, ethyl butyrate, isoamyl acetate, ethyl valerate, ethyl hexanoate, ethyl heptanoate, and ethyl octanoate). L1, L2, L3, and L4 have 2, 11, 10, and 6 components respectively and large difference in taste was observed between L2 and L3.

Esters were shown to be the dominant group in the four liquors accounted for 61.9, 48.5, 76.9, and 67.3% of total amount of volatile compounds for L1, L2, L3, and L4, respectively (Figure 2). Esters are important family of aroma compounds in liquors. They are commonly formed by esterification of alcohols and acids followed by dehydration. In general, esters were divided into two categories: acetate esters and ethyl esters. Ethyl esters of fatty acids were produced during the alcoholic fermentation and endowed the fruity aromas. Ethyl hexanoate, ethyl acetate, and ethyl octanoate were the major components in ester group of the four samples. Their presence contributed to the pleasant, fruity fragrance notes of the liquors. Ethyl octanoate generated pineapple, pear, and sweet fruit aroma was reported to be the most abundant compound in white wines [14]. Other dominant compounds in ester groups were ethyl butyrate, isoamyl acetate, ethyl pentanoate, ethyl heptanoate, ethyl lactate, and ethyl decanoate. Their presence commonly contributed to the fruity bouquet notes of the alcoholic drinks. Ethyl lactate produced rum, fruit, and cream aroma. Hexyl hexanoate with apple peel and peach aroma were detected in L3 (7.53 mg/L) with the highest concentration. Ethyl nonanoate with floral and fruity aroma were found only in L3 (5.21 mg/L) and L4 (11.3 mg/L).

There were 21 esters with OAVs >1 (Table 1). Four of them (ethyl hexanoate, ethyl pentanoate, ethyl heptanoate, and ethyl octanoate) showed high values of OAV over 1,000, especially ethyl hexanoate, which correlates with a green apple odor. Welke and coworkers studied the volatile compounds of Chardonnay wine using HS-SPME-GC × GC/TOFMS and found that esters were the large class and ethyl hexanoate had the highest OAV and ROC values [15], which is consistent with the present findings.

Eight alcohols were detected in the functional liquors and served as one of the decisive factors for the flavor of liquors. Isoamyl alcohol was the most abundant alcohol in all samples (from 43.1 to 97.9 mg/L). It contributed to a whiskey, malt, and burned aroma. 1-Hexanol contributed herbaceous, grass, and woody aroma to liquors [14]. Isobutanol was the abundant alcohol next to 1-hexanol, with a "wine, solvent, bitter" odor. 2-Butanol and α-cadinol were identified only in L2 liquor with a low

concentration 9.43 mg/L and 1.00 mg/L, respectively. 2-Butanol gave nuances of "fruity and wine," which is vinous in character. α-cadinol has a "herbaceous and woody" odor, but the detection thresholds of the compounds are unavailable, thereby their contribution to the whole aroma is uncertain.

Acids were responsible for fruity, cheese, fatty, and rancid notes. Short-chain acids had the aroma of sour and rancid, and could suppress and cover other aroma in liquors, so appropriate concentration of them in liquor was preferred. Hexanoic and octanoic acid produced a cheese flavor at low concentrations and harsh and rancid odors at high concentrations [16]. Butyric acid contributed a cheese aroma at low concentrations while yielding rancid and sweat odors at high concentrations. It was only detected in L3 (16.8 mg/L) and L4 (23.6 mg/L), which might cause cheese flavors.

Seven aldehydes and four ketones were identified, all of them showed OAVs >1. Aldehyde compounds, formed from unsaturated fatty acids, also can be considered as products of lipoxygenase catalysis. Acetaldehyde and hexanal were responsible for grass and tallow fat aroma, furfural for bead almond and sweet aroma, and nonanal for fatty-floral aroma [17]. Ketones can be formed by condensation of activated fatty acids. 2-Octanone, which exhibited fruity and floral notes, 2-nonanone and 2-decanone were detected with higher OAVs, the former contributed to fruity, floral, and herbal notes. Only eugenol was detected in four liquors. It has been indicated that phenols often have spicy and smoky-clove-like odors.

Six hydrocarbons are listed in Table 1, and D-limonene belongs to terpene compounds giving sweet, citrus-, and lemon-like notes. The olfactory detection threshold of L-alanine was far more than its content in L2 liquor. Therefore, its contribution to the wine flavor was negligible. As the odor detection thresholds of the major part of these compounds have not been determined, their contribution to four liquors aroma remains unknown.

To intuitively perceive the different contents of aroma compounds in four functional liquors, a heat map (Figure 3) was generated based on the data in Table 1. After logarithmic conversion of the data, we can compare the expression of the same substance in different samples or the expression of different substances in the same sample. The concentrations of the flavor substances in four functional liquors were log-transformed. As many flavors do not appear in the samples (for 0 has not logarithmic), the expression for the entire data matrix for each

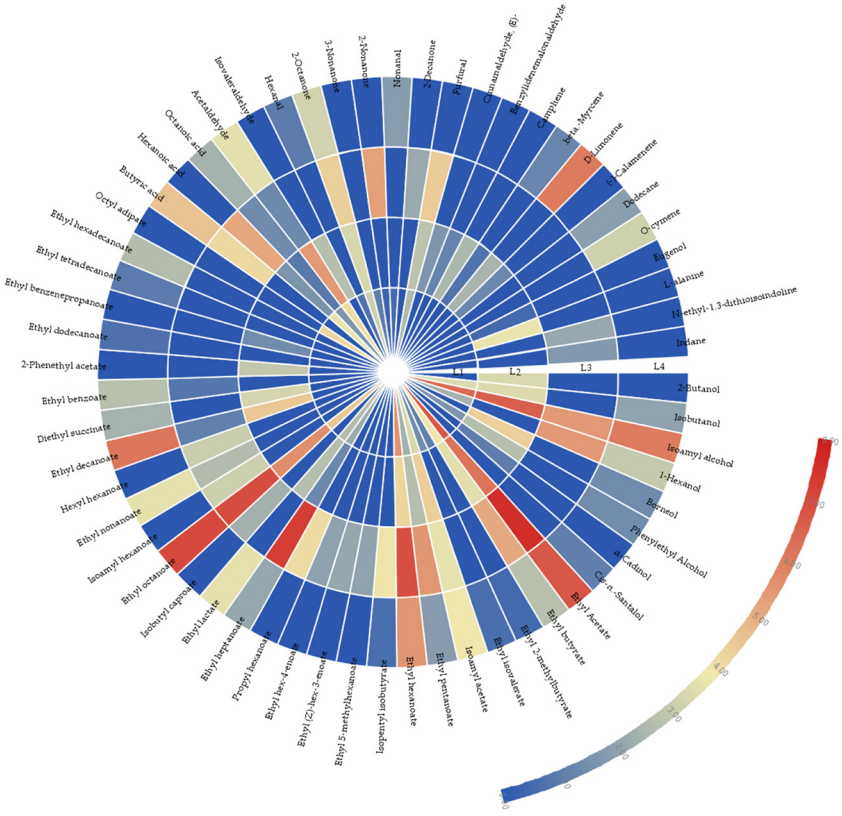

Figure 3: Heat map analysis of aroma compounds in four functional liquors.

value *x* was set. log scale base is 2, and log width is 1. As can be seen in Figure 3, color coding was devised based on the scale from blue to red with the relative intensity decreasing from high to low (0.00–8.00), which enables to distinguish different samples.

ROC that represented the contribution percentage of each volatile compound to aroma is also shown in Table 1. Ethyl hexanoate showed the highest contribution to final aroma of wine (ROC = 90.7, 81.7, 81.8, and 69.1%, respectively). It was reported that ethyl hexanoate was also the key compound to the odor of Cabernet Sauvignon and Chardonnay wines from China [18]. The total ROC of esters was above 90.0%. These results confirm the decisive role of esters on the aroma of four liquors.

3.2 Principal component analysis (PCA)

To unravel the similarities of these four samples from the aroma component varieties and concentrations and also characterize the key volatile compounds of each Chinese functional liquor, the remaining 33 components (OAVs >1) in Table 1 were subjected to PCA. Principle Component 1 (PC1) is 45.6% and Principle Component 2 (PC2) is 23.6%. According to the analysis of score plot (a) and loading plot (b), the distribution of the four samples was widely dispersed, especially L3 and L4. It showed that the content of flavor components in different functions liquors differed remarkably, and the body style was also inconsistent (Figure 4).

The distribution position of highland barley wine (L3) was located in the positive axis of PC1 and was correlated with a greater abundance of esters (including isopentyl isobutyrate (X7), ethyl lactate (X9), ethyl decanoate (X12), ethyl benzoate (X13), ethyl dodecanoate (X15)), and a hydrocarbon. Conversely, Kinmen-Kaoliang Liquor (L1), Jin Liquor (L2), and Zhuyeqing Liquor (L4) were located in the negative axis of PC1, revealing a greater abundance of carbonyl compounds (2-nonanone (X23) and furfural (X26)), an alcoholic substance (1-hexanol (X2)) and hexanoic acid (X19), and a lower abundance of the compounds was present in L3. On the positive axis of PC2, Zhuyeqing Liquor (L4) was observed, which was correlated with a higher abundance of esters (propyl hexanoate (X8), isoamyl hexanoate (X10) and hexyl hexanoate (X11)), acids (butyric acid (X18) and hexanoic acid (X19)), and carbonyl compound (furfural (X26)). On the contrary, Kinmen-Kaoliang Liquor (L1) and Jin Liquor (L2) were found to correlate with the compounds in greater abundance, such as alcohols (2-butanol (X1), borneol (X3) and phenylethyl alcohol (X4)), carbonyl compounds (isovaleraldehyde (X20), 3-nonanone (X22) and (*E*)-cinnamaldehyde (X27)), and ester (2-phenethyl acetate (X14)). Particularly, 3-nonanone (X22) was more abundant for L2. These observations were consistent with the Venn results, and different types of liquor had a great effect on the distribution of flavor compounds.

3.3 Total phenolic content and antioxidant activity

In recent years, an increasing number of studies have shown the role of polyphenols in the antioxidant activity of red and white wines [19]. The antioxidant capacity of

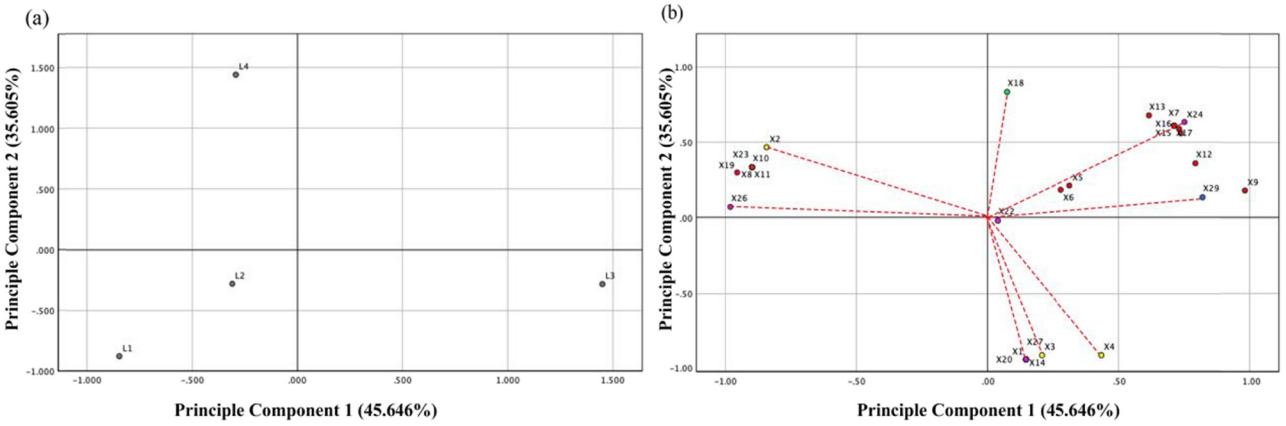

Figure 4: PCA. Score plot (a) and loading plot (b) of PC1 and PC2, from volatile compound in the L1, L2, L3, and L4. Different colored dots represent different kinds of compounds: yellow (alcohols), red (esters), green (acids), blue (hydrocarbons), and purple (carbonyl compounds).

Table 2: Total phenolic content and antioxidant activity of four functional liquors and three typical white wines

Name	TP* (mg GAE/L)	DPPH# (TE)	ABTS# (TE)
L1	261 ± 1.1[a]	148 ± 6.3[ac]	96.8 ± 0.6[ac]
L2	275 ± 2.9[b]	161 ± 2.1[b]	102 ± 1.4[b]
L3	232 ± 0.7[a]	118 ± 2.2[ac]	75.5 ± 0.3[ad]
L4	120 ± 4.3[b]	79.6 ± 2.4[bc]	55.8 ± 0.7[c]
Luzhou Laojiao	50.9 ± 2.1[d]	72.0 ± 1.0 [cd]	33.8 ± 1.2 [cd]
Maotai Wangzi	125 ± 3.3[c]	77.1 ± 6.2[dc]	43.6 ± 2.0[c]
Haizhilan	48.8 ± 4.2[d]	85.7 ± 5.3[bc]	11.2 ± 1.0 [cd]

Values are means of triplicate replicates ± SD, and different letters represent significantly different among the data in the same column ($P < 0.05$). *Total phenols expressed as gallic acids equivalents. #DPPH and ABTS expressed as mg/L trolox equivalents.

polyphenols correlates with the extent of hydroxylation and conjugation.

As shown in Table 2, the total phenolic contents of four liquors were 261 ± 1.1 mg GAE/L (L1), 275 ± 2.9 mg GAE/L (L2), 232 ± 0.7 mg GAE/L (L3), and 120 ± 4.3 mg GAE/L (L4). They were significantly ($P < 0.05$) higher than those of the typical Chinese white liquors.

The capacities of free radical scavenging of four liquors detected by the DPPH assays were 148 ± 6.3 mg/L, 161 ± 2.1 mg/L, 118 ± 2.2 mg/L, and 79.6 ± 2.4 mg/L for L1, L2, L3, and L4, respectively. The ABTS assays were 96.8 ± 0.6 mg/L (L1), 102 ± 1.4 mg/L (L2), 75.5 ± 0.3 mg/L (L3), and 55.8 ± 0.7 mg/L (L4). All of them were much higher than the typical Chinese white liquors listed in Table 2. To find the reason, it was mainly because of the raw material of these liquors. For example, the main raw material of Kinmen-Kaoliang was sorghum which contained a large amount of polyphenols. Jin contained some herb extracts which contained a lot of polyphenols, and polyphenols of highland barley might originate from barley. A lot of polyphenols had been determined in bamboo leaves which were the main raw material of Zhuyeqing. To some extent, different types and origins of raw materials and brewing procedures might affect the volatiles and antioxidant activities of wines.

4 Conclusions

In conclusion, volatile compounds of four Chinese functional liquors were extracted by HS-SPME and identified using GC-MS. OAV and ROC were successfully used to evaluate the contributions of aroma compounds to the whole flavor. The results revealed that esters and carbonyl compounds are the major aroma compounds, of which ethyl hexanoate, ethyl acetate, and ethyl octanoate being the most powerful odorants. Other volatile compounds affected the aroma of four Chinese functional liquors to different extent. In addition, the total phenolic contents of four functional liquors vary and are significantly higher ($P < 0.05$) than those of the typical Chinese white liquors, and similar trend was found for their antioxidant activities. The volatile compound profile built in this study will provide valuable information toward the sensory and health benefits of Chinese functional liquors from the chemical aspects.

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