

# Analysis of volatile flavor compounds influencing Chinese-type soy sauces using GC–MS combined with HS-SPME and discrimination with electronic nose

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**Abstract** Soy sauce contains a variety of volatiles that are highly valuable to its quality with regard to sensory characteristics. This paper describes the analysis of volatile compounds influencing the flavor quality of Chinese-type soy sauces. Gas chromatography–mass spectrometry (GC–MS) combined with headspace-solid phase microextraction and electronic nose (E-nose) were applied for identifying the volatile flavor compounds as well as determining their volatile profiles of 12 soy sauces manufactured by different fermentation process. Forty one key volatile components of these 12 soy sauce products, a pure soy sauce and an acid-hydrolyzed vegetable protein sample, were compared in semi-quantitative form, and their volatile flavor profiles were analyzed by E-nose. The substantially similar results between hierarchical cluster analysis based on GC–MS data and E-nose analysis suggested that both techniques may be useful in evaluating the flavor quality of soy sauces and differentiating soy sauce products. The study also showed that there were less volatile flavor compounds in soy sauces produced through low-salt solid-state fermentation process, a traditional manufacturing technology and a widely adopted technology in Chinese soy sauce industries. In addition, the investigation suggested that the flavor quality of soy sauce varied widely in Chinese domestic market, and that the present Chinese national standards of soy sauce should be further perfected by the addition of flavor grades of soy sauce in the physical and chemical index. Meanwhile, this research provided valuable information to manufacturers and government regulators, which

have practical significance to improve quality of soy sauces.

**Keywords** Soy sauce · Volatile compounds · Flavor · HS-SPME–GC–MS · E-nose

## Introduction

As an essential traditional condiment with more than 3000 years of history in China (Zhang and Tao 2009), soy sauce plays a prominent role in Chinese food. In recent years, it has also become widely popular in Southeast Asian countries and its consumption is expanding around the world. With a production of approximate 5 million tons annually, soy sauce industry is one of the most prosperous industries in China (Feng et al. 2013a, b). Typically, soy sauce is produced from soybean and wheat by complex fermentation processes involving enzymatic catalysis by various microorganisms. Based on the differences of brewing processes, varieties of soy sauce can be classified as low-salt and high-salt (Bao 2011). Soy sauce flavor is influenced by many factors such as raw materials, yeast strain used, the pH value of the medium, fermentation temperature and climatic condition during brewing (Zhang and Liu 2000; Vander et al. 2002; Bao 2011). Each factor mentioned above has a great effect on the flavor profile of the final soy sauce and also affects its other characteristics. As a result, various brands of soy sauce products can differ in volatile flavor profile, and particularly in the overall flavor quality.

Due to their unique flavor characteristics, soy sauces produced through high-salt liquid-state fermentation processes are more popular in China and other countries in recent years (Feng et al. 2013a, b). However, low-salt

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solid-state fermentation is the commonly used traditional processing technology in soy sauce production by most Chinese soy sauce manufacturers (Zhang and Tao 2009), which has advantages such as short fermentation period, high material utilization, stable yield, low production cost and so on. Because the low-salt diet is more beneficial to human health, the low-salt fermented food research and development has become a research hotspot during the past decades. Therefore, investigation on volatile compounds influencing flavor of soy sauce is important to improve the technology of the low-salt soy sauce production and the quality of products.

Acid-hydrolyzed vegetable protein (aHVP) products, which are processed in a short time and with low cost, are foodstuffs obtained by protein hydrolysis and are used as ingredients in a wide variety of products including fermented soy sauce. Liquid aHVP, when originating from maize gluten, soybean meal or wheat gluten, typically contains 55% water, 25% organic substances, and 16% salt. Hence, there is a marked difference on flavor between aHVP and fermented soy sauce.

Flavor is one of the most important indicators of the quality of soy sauce. Volatile chemical compounds make a critical contribution to the typical flavor of soy sauce. Volatile organic compounds in various soy sauces, including Japanese-type soy sauces (Nunomura et al. 1984; Kaneko et al. 2012, 2013; Meng et al. 2012; Hayase et al. 2013; Aishima 2006; Feng et al. 2013a, b), Thai-type soy sauce (Wanakhachornkrai and Lertsiri 2003; Lertsiri et al. 2010), Korea-type soy sauces (Baek and Kim 2004; Lee et al. 2006) and Chinese-type soy sauces (Zhang and Tao 2009; Gao et al. 2010; Sun et al. 2010; Feng et al. 2015), have been investigated and some of the volatiles such as alcohols, esters, phenols, acids, and heterocyclics have been identified.

Although the technology involved all processes of soy sauce production, the possible effect of various fermentation processes on the formation of flavor compound, as well as the flavor differentiation between soy sauce and aHVP, were all important in practice, there is still very few publications involved in giving clear explain to these problems until now. This might be one of the reasons that the evaluation standard or criterion about the quality of soy sauce is too limited, particularly Chinese standard. Information about correlation between various fermentation processes and flavor profile of soy sauce, particularly the typical flavor compound of soy sauce, is still a great concern to manufacturers. Due to the sustainable growth of export and the globalization of the market, this concern has been emphasized and the need for controlling and improving soy sauce quality is getting urgent in China. Despite of all these many years study, quality control of soy sauce remains a major challenge, which needed to face

and investigate systematically. Currently, there is a pressing need for comprehensive and accurate assessment of soy sauce quality. Therefore, to address the identification of volatile compounds which influencing the flavor of soy sauce, accurate monitoring of key volatile flavor compounds in soy sauces is essential to ensure good soy sauce quality.

In this research, volatile compounds in 12 famous Chinese soy sauces including low-salt solid-state and high-salt dilute-state fermentation processes were identified by using gas chromatography-mass spectrometry (GC-MS) coupled with headspace-solid phase-microextraction (HS-SPME). 41 key volatile flavor compounds in soy sauce products, pure soy sauce sample and aHVP sample were compared in semi-quantitative term, and correlation between volatile compounds and flavor profiles were investigated. This approach may be widely applicable to the analysis and characterization of soy sauces. Electronic nose (e-nose) was also utilized for acquiring volatile profiles of soy sauces and generating the discrimination models using pattern recognition. This research may be useful in providing some valuable information on evaluating or differentiating the quality of soy sauces to manufacturers and government regulators. It also revealed that 41 volatile compounds had great effect on the flavor of Chinese-type soy sauces, and these compounds could be used to evaluate the quality of soy sauces.

## Materials and methods

### Samples

Twelve deep-colored famous brands of soy sauces, produced in various regions, were purchased from a supermarket in Beijing, China. Among these domestic products, sample 1–6 were produced from defatted soy-beans, wheat bran and wheat, and were manufactured through low-salt solid-state fermentation process, while sample 7–12 were produced from soy-beans, wheat bran and wheat, and were manufactured through high-salt dilute-state fermentation process. The pure soy sauce sample, which were produced by high-salt dilute-state fermentation process, and aHVP sample, were from Beijing Laocaichen Food Limited Company. Caramel color was from Guilin Hongxing food ingredients Co., Ltd.

### Sample extraction condition

The fused silica fibers coated with polyacrylate (PA) 85  $\mu\text{m}$  thickness, polydimethylsiloxane (PDMS) 100  $\mu\text{m}$  thickness, polydimethylsiloxane/divinylbenzene (PDMS/DVB) 65  $\mu\text{m}$  thickness divinylbenzene/carboxen/polydimethylsiloxane

(DVB/CAR/PDMS) 50/30  $\mu\text{m}$  thickness, were obtained from Supelco (Bellefonte, PA, USA). The fibers were conditioned prior to use according to supplier's prescriptions. Volatiles were extracted by HS-SPME after major parameters such as fiber type, time and temperature of adsorption, and ionic strength, which had large influence on extraction process, was optimized. Optimal conditions of extraction were obtained using the following procedure: 2 mL of soy sauce sample were transferred to a 20 mL vial, and the ionic strength was adjusted to 25% with NaCl. The vial was sealed and equilibrated at 45 °C for 10 min, and then the enrichment of volatile compounds on a fiber was performed for 30 min at 45 °C with a 85  $\mu\text{m}$  PA fiber when the sample were keeping under continuous agitating. The fiber desorption time in the GC injector was 3 min. Each analysis was repeated triplicate. 3-Octanol from ANPEL Laboratory Technologies (Shanghai, China) Inc. was used as reference standard compound. The stock solution of 3-octanol was prepared using methanol, and 1  $\mu\text{g}/\text{mL}$  3-octanol in sample was used for calculating the relative concentration of volatile compound.

### GC–MS conditions

The extracts of sample were analyzed by an Agilent 5975C/7890A gas chromatography tandem mass spectrometry (GC–MS) equipped with an Agilent GC sampler 80 multi mode sampler. Chromatographic column was Agilent HP-INNOWAX (J&W) fused silica capillary column (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu\text{m}$ ). The carrier gas was helium, and the flow rate was 0.8 mL  $\text{min}^{-1}$ . GC–MS determination was carried out in splitless injection mode. The initial oven temperature was set to 40 °C for 4 min. The temperature increased in two steps: 40–160 °C, at 6 °C  $\text{min}^{-1}$ , 160–220 °C at 10 °C  $\text{min}^{-1}$ , and then hold 10 min at 220 °C. The temperature of injector and transfer line were all set at 250 °C. Mass spectra electronic impact (EI) ionization was 70 eV. The mass scan range was 35–400 amu. The temperature of ion source and quadrupole were set at 230 and 150 °C respectively. The Kovats retention indices of volatiles were determined based on a series of *n*-alkanes (C8–C20) under the same GC–MS conditions.

### Compound identification and semi-quantification

RI values were calculated using *n*-alkanes as standard references. The RI of each compound was determined by running *n*-alkanes under the same chromatographic conditions and calculated according to the report (Van den Dool and Kratz 1963). Volatile compounds were positively identified by comparing retention indices (RI), and the MS fragmentation patterns with National Institute of Standards and Technology (NIST) spectral database data, and

previously reported retention indices. The peak area of volatile compound based on the total ion chromatograms peak area normalization method was used to quantify the volatiles. The relative concentration of volatile compound in sample was determined by comparison with the concentration of 1  $\mu\text{g}/\text{mL}$  3-octanol in a 2 mL sample.

### Electronic nose measurement

A PEN3 e-nose device, provided by Win Muster Airsense (WMA) Analytics Inc. (Schwerin, Germany), was used. The PEN3 electronic nose has an array of 10 different metal oxide sensors. It consists of a sampling apparatus, a detector unit containing the array of sensors, and pattern recognition software (Win Muster) for data recording. Table 1 lists all sensors and their major applications.

Soy sauce sample (2 g) was placed into a 10 mL airtight glass bottle, and sealed. The temperature of the vials was set at 45 °C by a thermostatic bath and after a headspace generation time of 15 min under agitation with the constant speed, the samples were injected in order. The measurement time was 90 s, which could make the stable response signs available. The flushing time was 250 s, which was enough to desorb volatiles from sensors and enable signals to return to the baseline. The response points at 65 s of electronic nose, which were the maximum response signal for each of the 10 sensors, were used for analysis. Each analysis was repeated 7 times. The last 5 measurements of each sample were used for further analysis. Total signals of 10 sensors during measurement of a sample are a pattern. The pattern data were analyzed by principal component analysis (PCA), linear discriminant analysis (LDA) and loading analysis using WinMuster software.

## Results and discussion

### Identification of volatile compounds by HS-SPME/GC–MS

Volatile compounds were extracted by HS-SPME using PA fiber and determined by GC–MS. Some volatile compounds, including alcohols, phenols, esters, aldehydes, ketones, heterocyclics, acids, sulphur-containing compounds, etc. were identified. Among these compounds, dozens of components were shared commonly with different amount in soy sauce products. According to the previous report (Nunomura et al. 1984; Kaneko et al. 2012, 2013; Feng et al. 2015), some typical or key volatile flavor compounds, which might be the contributors to the flavor of soy sauce, were summarized in Table 2.

Lower alcohols including ethanol, propanol and 2, 3-butanediol, are common components of brewing foodstuffs

**Table 1** Sensors used and their main applications in PEN 3

Number in array	Sensor name	General description	References
R (1)	W1C	Aromatic compounds	Toluene, 10 mg/kg
R (2)	W5S	Very sensitive, broad range sensitivity, react on nitrogen oxides, very sensitive with negative signal	NO <sub>2</sub> , 1 mg/kg
R (3)	W3C	Ammonia, used as sensor for aromatic compounds	Benzene, 10 mg/kg
R (4)	W6S	Mainly hydrogen, selectively, (breath gases)	H <sub>2</sub> , 0.1 mg/kg
R (5)	W5C	Alkanes, aromatic compounds, less polar compounds	Propane 1 mg/kg
R (6)	W1S	Sensitive to methane (environment) ca. 10 mg/kg. Broad range, similar to No. 8	CH <sub>3</sub> , 100 mg/kg
R (7)	W1W	Reacts on sulfur compounds, H <sub>2</sub> S 0.1 mg/kg. Otherwise sensitive to many terpenes and sulfur organic compounds, which are important for smell, limonene, pyrazine	H <sub>2</sub> S, 1 mg/kg
R (8)	W2S	Detects alcohol's, partially aromatic compounds, broad range	CO, 100 mg/kg
R (9)	W2W	Aromatics compounds, sulfur organic compounds	H <sub>2</sub> S, 1 mg/kg
R (10)	W3S	Reacts on high concentrations >100 mg/kg, sometime very selective (methane)	CH <sub>3</sub> , 10 CH <sub>3</sub> , 100 mg/kg

(Bao 2011). Due to their low concentration or interference from other compounds, 1-pentanol, 1-hexanol, and 1, 4-butanediol, were even not detected in a few soy sauce samples. Besides lower alcohols, soy sauce had other higher alcohols such as phenylethyl alcohol, which was related to a special taste and aroma (Bao 2011) and was one of the dominant alcohols (Sun et al. 2010). The amount of phenylethyl alcohol in pure soy sauce sample, sample 11 and sample 12 was similar. 1-Octen-3-ol, which is also known as mushroom alcohol, is a contributor to the flavor of Japanese-type soy sauce (Kaneko et al. 2012, 2013). The concentration of this compound in pure soy sauce sample was very low. Other alkyl fatty alcohols including propanol, 2-methyl-propanol, and butanol, existed only in high-salt soy sauce products. 2, 3-butanediol, which had close relation to the flavor of soy sauce, presented only in several samples with the exception of pure soy sauce sample and aHVP. It has been proved that 2, 3-butanediol itself had offensive order, but it had subtle blending function with other components (Bao 2011). As shown in Table 2, the most abundant compound in pure soy sauce was ethanol, which accounted for approximately 60% of the total volatiles. In addition, as illustrated in Table 3, total amount of alcohols presented in pure soy sauce sample were far more than that in most soy sauce products and aHVP sample. Pure soy sauce sample possessed 61% ethanol, 1.6% butanol, and 8.6% phenylethyl alcohol, while the amount of ethanol in aHVP sample was only at 0.76%. Another important alcohols, 3-(methylthio)-propanol, which contain sulfur and possess a strong roast meat-like flavor at low concentration, was identified in pure soy sauce, six high-salt dilute-state fermentation soy sauces, and three low-salt solid-state fermentation soy sauces. Ethanol concentration of sample 6 and aHVP sample was

the lowest, and ethanol was the only alcohols detected in aHVP sample. Additionally, compared with low-salt soy sauces, soy sauces fermented through high-salt dilute-state process generally had higher amount of ethanol and other alcohols. Like pure soy sauce sample, sample 10, sample 11 and sample 12 had relatively high percent concentration of alcohols. The semi-quantitative data of alcohols also suggested that ethanol might play an important role in flavor of soy sauces, since it was believed that ethanol could be helpful for improving the solubility of other fragrant substance and could be helpful for them to produce aroma. Although alcohols are not the specific components of soy sauce, the free alcohols, like organic acids and esters which existed in soy sauce, are major flavor compounds (Sun et al. 2010; Feng et al. 2013a, b).

The main ester in soy sauce was ethanol esters (Bao 2011), including various fatty acid ethyl ester (ethyl acetate, butanoic acid ethyl ester, hexanoic acid ethyl ester, heptanoic acid ethyl ester, octanoic acid ethyl ester, nonanoic acid ethyl ester, dodecanoic acid ethyl ester, linoleic acid ethyl ester), and aromatic acid ethyl ester (benzoic acid ethyl ester, benzeneacetic acid ethyl ester, benzene-propanoic acid ethyl ester etc.). Compared with other samples, pure soy sauce sample had more esters. Fewer esters were found in 12 soy sauce products, while little esters were detected in aHVP sample. Table 2 listed three important ethanol esters. Obviously, ethyl acetate and pentanoic acid, 2-hydroxy-4-methyl-ethyl ester were only identified in pure soy sauce sample and several high-salt soy sauce products. They were not detected in aHVP sample and six low-salt soy sauce products. Benzeneacetic acid ethyl ester, a high boiling point ester, did not present in aHVP sample. This compound, being one of the major contributors to soy sauce flavor (Kaneko et al. 2012, 2013),

**Table 2** Volatile compounds identified in soy sauce products, pure soy sauce, and aHVP

Volatile compounds	Samples															
	P	aHVP	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12		
<i>Alcohols</i>																
Ethanol	RI: 924	a	92.88	0.16	16.18	19.31	0.034	2.00	1.41	0.014	32.96	1.42	2.30	56.38	82.19	18.47
		b	60.7%	0.76%	15.6%	16.9%	0.36%	2.2%	0.95%	0.12%	29.2%	1.81%	2.16%	36.9%	55.1%	10.1%
Propanol	RI:1042	a	0.023	-	-	-	-	-	-	-	0.0058	-	-	-	0.0027	0.018
		b	0.17%	-	-	-	-	-	-	-	0.052%	-	-	-	0.010%	0.099%
Propanol, 2-methyl	RI:1107	a	1.44	-	-	-	-	-	-	-	0.66	-	-	0.37	1.90	0.79
		b	1.07%	-	-	-	-	-	-	-	0.59%	-	-	0.24%	1.20%	0.43%
Butanol	RI:1161	a	2.38	-	-	-	-	-	-	-	0.24	-	-	-	-	-
		b	1.6%	-	-	-	-	-	-	-	0.22%	-	-	-	-	-
1-Propanol, 3-(methylthio)-	RI:1732	a	0.71	-	0.36	-	-	0.40	0.019	-	0.19	0.032	0.0076	0.59	0.57	0.71
		b	0.57%	-	0.34%	-	-	0.44%	0.13%	-	0.17%	0.32%	0.061%	0.44%	0.37%	0.40%
1-Octen-3-ol	RI:1458	a	0.0062	-	0.24	0.26	0.011	0.0099	0.0088	-	0.025	-	-	0.20	0.54	0.17
		b	0.045%	-	0.23%	0.23%	0.12%	0.12%	0.058%	-	0.23%	-	-	0.12%	0.51%	0.92%
2-Furamethanol	RI:1677	a	1.14	-	1.52	1.89	0.93	0.91	1.39	2.84	1.94	1.45	2.62	0.33	3.67	3.23
		b	0.84%	-	1.46%	1.66%	1.0%	1.0%	0.93%	2.19%	1.72%	1.58%	2.04%	2.20%	2.35%	1.77%
Phenylethyl alcohol	RI:1933	a	11.69	-	2.55	2.88	1.43	4.32	2.10	0.021	3.34	2.54	2.37	5.58	14.74	11.63
		b	8.60%	-	2.45%	2.53%	1.51%	4.71%	1.40%	0.18%	2.96%	2.55%	2.0%	3.65%	8.83%	6.25%
Benzyl alcohol	RI:1900	a	0.013	-	0.29	0.29	0.012	0.47	0.24	-	-	0.023	0.52	0.28	-	0.031
		b	0.097%	-	0.28%	0.26%	0.13%	0.52%	0.16%	-	-	0.23%	0.43%	0.15%	-	0.17%
2,3-Butanediol	RI:1554	a	-	-	1.22	1.54	-	-	-	-	-	0.12	0.0038	-	-	0.11
		b	-	-	1.17%	1.35%	-	-	-	-	-	1.19%	0.31%	-	-	0.58%
<i>Esters</i>																
Ethyl acetate	RI: 852	a	1.31	-	-	-	-	-	-	-	1.49	-	-	0.24	2.72	0.42
		b	0.90%	-	-	-	-	-	-	-	1.32%	-	-	0.14%	1.51%	0.26%
Pentanoic acid, 2-hydroxy-4-methyl-, ethyl ester	RI:1562	a	0.0011	-	-	-	-	-	-	-	0.0035	-	-	-	0.0026	-
		b	0.008%	-	-	-	-	-	-	-	0.031%	-	-	-	0.026%	-
Benzeneacetic acid, ethyl ester	RI:1799	a	0.16	-	-	0.012	-	-	-	-	0.0043	-	-	0.37	0.38	0.032
		b	0.12%	-	-	0.13%	-	-	-	-	0.039%	-	-	0.22%	0.24%	0.17%
<i>Phenols</i>																
2-Methoxy-Phenol	RI:1836	a	0.10	0.64	0.094	0.97	1.30	0.55	2.36	0.0049	0.18	0.67	0.99	1.14	0.35	1.59
		b	0.13%	3.67%	0.89%	0.85%	1.41%	0.60%	1.57%	0.041%	0.16%	0.60%	0.74%	0.66%	0.26%	0.78%
Phenol	RI: 1975	a	-	0.27	0.36	0.42	0.015	0.40	1.39	1.07	1.36	0.014	0.032	0.31	0.20	0.44
		b	-	0.88%	0.35%	0.36%	0.15%	0.43%	0.92%	0.083%	1.12%	0.15%	0.25%	0.17%	0.11%	0.22%
4-Ethyl-2-methoxy-phenol	RI:2016	a	0.69	-	0.92	0.93	0.025	0.82	1.20	-	0.83	0.79	1.78	1.76	-	10.63
		b	0.47%	-	0.88%	0.81%	0.26%	0.89%	0.80%	-	0.73%	0.86%	1.43%	1.17%	-	5.63%
4-Ethyl-phenol	RI:2181	a	-	-	0.26	0.25	-	0.010	0.78	-	0.32	-	1.25	0.46	-	2.89
		b	-	-	0.25%	0.22%	-	0.12%	0.52%	-	0.29%	-	0.97%	0.30%	-	1.59%
2-Methoxy-4-vinylphenol	RI: 1332	a	0.34	-	0.32	0.33	-	0.16	-	-	-	-	-	-	-	-
		b	0.19%	-	0.24%	0.23%	-	0.14%	-	-	-	-	-	-	-	-

**Table 2** continued

		Samples													
Volatile compounds		P	aHVP	SI	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
<i>Aldehydes and ketones</i>															
Butanal, 3-methyl-	RI: 910	a	0.011	0.078	1.77	2.16	0.015	0.0039	0.43	-	0.57	-	0.020	0.015	0.28
		b	0.078%	0.26%	1.70%	1.90%	0.17%	0.046%	0.28%	-	0.51%	-	0.13%	0.098%	0.090%
Pentanal	RI: < 910	a	0.16	0.11	-	-	-	-	-	-	0.0091	0.013	0.012	-	-
		b	0.13%	0.35%	-	-	-	-	-	-	0.093%	0.10%	0.078%	-	-
Benzaldehyde	RI:1536	a	0.015	0.39	0.43	0.56	0.021	0.34	0.85	0.0028	0.42	0.59	0.019	0.26	0.78
		b	0.11%	1.28%	0.41%	0.49%	0.18%	0.37%	0.56%	0.023%	0.38%	0.64%	0.15%	0.16%	0.33%
Benzeneacetaldehyde	RI:1657	a	0.40	0.21	0.76	0.84	0.046	0.45	0.84	0.0016	0.54	0.037	0.012	0.34	0.049
		b	0.28%	0.69%	0.73%	0.74%	0.48%	0.49%	0.54%	0.013%	0.48%	0.37%	0.094%	0.22%	0.26%
Benzeneacetaldehyde, $\alpha$ -(2-methylpropylidene)-	RI:1959	a	0.0053	-	0.59	0.62	-	-	0.029	-	0.022	-	0.45	-	-
		b	0.038%	-	0.56%	0.55%	-	-	0.19%	-	0.20%	-	0.29%	-	-
Benzeneacetaldehyde, $\alpha$ -ethylidene-	RI:1952	a	0.0029	-	0.46	0.51	0.025	-	0.62	-	0.44	-	0.27	0.34	-
		b	0.022%	-	0.45%	0.44%	0.26%	-	0.41%	-	0.39%	-	0.14%	0.23%	-
2-Butanone, 3-hydroxy-	RI:1297	a	0.0068	-	0.012	0.012	0.0054	0.020	0.0060	-	0.0033	0.0094	0.0074	0.0048	0.019
		b	0.049%	-	0.13%	0.12%	0.056%	0.23%	0.040%	-	0.030%	0.096%	0.060%	0.028%	0.10%
2-Propanone, 1-hydroxy-	RI:1316	a	0.012	-	0.27	0.28	0.024	0.29	0.020	-	0.0088	0.019	0.076	0.37	0.0091
		b	0.085%	-	0.26%	0.24%	0.25%	0.32%	0.13%	-	0.080%	0.19%	0.61%	0.24%	0.049%
Maltol	RI:1987	a	0.0059	-	-	-	0.036	-	0.019	-	0.011	0.020	0.76	0.020	0.019
		b	0.043%	-	-	-	0.34%	-	0.12%	-	0.10%	0.21%	0.59%	0.12%	0.10%
Furfural	RI:1478	a	-	0.063	0.098	0.053	-	-	-	-	-	-	-	-	-
		b	-	4.12%	1.04%	0.55%	-	-	-	-	-	-	-	-	-
Propanal, 3-(methylthio)-	RI: 1467	a	0.0088	0.012	0.011	0.011	0.0028	0.0089	-	-	0.0088	0.0067	-	0.0065	-
		b	0.064%	0.76%	0.12%	0.11%	0.030%	0.10%	-	-	0.080%	0.068%	-	0.059%	-
<i>Acids</i>															
Acetic acid	RI: 1471	a	5.72	0.70	7.50	8.75	9.68	8.2	20.06	4.71	10.43	9.97	19.51	9.92	13.88
		b	4.14%	2.31%	7.22%	7.67%	10.5%	8.95%	13.4%	3.64%	9.23%	10.19%	15.7%	5.90%	7.52%
Propanoic acid	RI:1555	a	0.011	1.51	0.011	0.17	0.026	0.014	0.46	0.019	0.0068	0.012	0.035	0.27	0.0088
		b	0.083%	1.01%	0.12%	0.15%	0.28%	0.16	0.31%	0.16%	0.062%	0.12%	0.28%	0.17%	0.053%
Octanoic acid	RI: 2060	a	-	-	0.46	0.51	0.70	0.32	0.31	0.014	0.29	0.73	0.36	0.014	0.75
		b	-	-	0.44%	0.44%	0.76%	0.35%	0.21%	0.11%	0.25%	0.73%	0.26%	0.17%	0.40%
Nonanoic acid	RI: 2168	a	0.35	-	1.23	1.49	2.73	0.92	1.62	1.13	1.36	3.23	1.62	0.65	0.45
		b	0.24%	-	1.18%	1.26%	2.90%	1.00%	1.1%	0.87%	1.21%	2.84%	1.23%	0.78%	2.14%
<i>Heterocycles</i>															
Pyrazine, trimethyl-	RI:1408	a	0.0017	0.0021	0.0028	0.0029	0.0076	-	0.0044	-	0.0014	-	0.0020	0.0052	0.0054
		b	0.013%	0.14%	0.030%	0.030%	0.029%	-	0.029%	-	0.012%	-	0.013%	0.031%	0.029%
Pyrazine, 2-ethyl-6-methyl-	RI:1389	a	-	0.035	0.0036	-	0.013	-	0.0066	-	0.0012	0.0044	0.0029	0.0037	0.0027
		b	-	0.12%	0.038%	-	0.14%	-	0.044%	-	0.011%	0.045%	0.023%	0.024%	0.014%
3-Phenyl-furan	RI:1835	a	0.26	0.23	1.00	1.44	-	-	-	-	0.11	-	0.041	0.37	-
		b	0.15%	0.62%	0.75%	0.99%	-	-	-	-	0.082%	-	0.17%	0.19%	-



Table 2 continued

		Samples													
		P	aHVP	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
Volatile compounds															
3-Acetyl-1H-pyrroline	a	0.37	-	2.98	3.36	5.47	1.37	4.85	-	2.36	2.36	2.73	4.85	3.60	0.37
	b	0.25%	-	2.85%	2.97%	5.95%	1.52%	3.09%	-	2.13%	2.55%	2.01%	3.19%	2.31%	0.25%
2-Ethyl-4-hydroxy-5-methyl-3(2H)-furanone	a	0.028	-	-	-	-	-	-	-	-	-	-	-	-	-
	b	0.21%	-	-	-	-	-	-	-	-	-	-	-	-	-
3(2H)-furanone, dihydro-4,4-dimethyl-	a	0.0010	-	-	-	-	-	-	-	-	-	-	-	-	-
	b	0.007%	-	-	-	-	-	-	-	-	-	-	-	-	-
Ethanone, 1-(2-furanyl)-	a	-	0.5	0.32	0.40	1.17	0.43	0.47	1.99	0.42	0.019	0.0086	0.32	0.022	0.36
	b	-	0.58%	0.31%	0.34%	1.27%	0.48%	0.32%	1.52%	0.38%	0.19%	0.069%	0.21%	0.13%	0.20%
<i>Sulfides</i>															
Dimethyl trisulfide	a	-	-	0.0075	0.0096	0.0011	0.0048	0.0071	-	0.0068	0.0088	0.0045	0.0012	0.0088	0.011
	b	-	-	0.079%	0.1%	0.01%	0.056%	0.047%	-	0.061%	0.090%	0.036%	0.079%	0.081%	0.058%

## RI retention indices

a Relative concentration of compound calculated, with the reference standard compound, 1 µg/mL 3-octanol

b Peak area percent concentration of compound, calculated with peak area normalization method

- The compound could not be detected

P Represent pure soy sauce sample produced by high-salt dilute state

was found at low concentration in pure soy sauce sample and several soy sauce products. It is noteworthy that the amount of fatty acid ester in soy sauce depends on varieties of soybean which were used in the brewing material. Compared to defatted soybean, soybean during fermentation might generate much fatty acid ester (Gao et al. 2009). This certainly should be one of the important factors which had influence on the flavor of soy sauces.

Aldehydes and ketones were important volatile compounds in soy sauce. For instance, 2-methyl-butanal, and pentanal were identified in pure soy sauce sample, aHVP sample, and several soy sauce products while benzaldehyde and benzeneacetaldehyde were identified in all samples. A-(2-methylpropylidene)-benzeneacetaldehyde, and  $\alpha$ -ethylidene-benzeneacetaldehyde were not found in aHVP sample. Furfural was only detected in aHVP sample and two low-salt soy sauce products. Aliphatic aldehyde, 3-(methylthio)-propanal, which contain sulfur and possess a strong onion and roast meat-like flavor, was also identified in pure soy sauce, aHVP, and three soy sauce products. It was the key aroma compound and major contributor of fragrance in soy sauces (Kaneko et al. 2012, 2013). Like benzeneacetaldehyde, 3-(methylthio)-propanal was also found to be positively related to 'musty' and 'soy sauce-like' odors and exhibited the greatest aroma intensities (Feng et al. 2013a, b, 2015). As far as ketone is concerned, 3-hydroxy-2-butanone, 1-hydroxy-2-propanone, and maltol were identified in pure soy sauce sample and 12 soy sauce products except for aHVP sample. Maltol, 3-hydroxy-2-methyl-4-pyrone, which is likely to be derived from roasted wheat and is used primarily as a flavor enhancer, was detected in pure soy sauce and six high-salt soy sauce products as well as two low-salt soy sauce products, but not in aHVP sample. It has a strong aroma of caramel malt. As it listed in Tables 2 and 3, the total amount of aldehydes and ketones in aHVP was much higher than that in pure soy sauce sample, and most soy sauce products. Moreover, furfural was the main aldehydes in aHVP sample.

Phenolic compounds including 4-ethyl-2-methoxy-phenol (4-EG), 4-ethyl-phenol (4-EP), 2-methoxy-phenol, 2-methoxy-4-vinylphenol and phenol, represent an important aroma class in soy sauce. Phenols which were found in pure soy sauce sample at relatively higher levels were 4-EG. It was also identified in most soy sauce products with the exception of aHVP sample, sample 6 and sample 11. 4-EG, which was formed from lignin degradation by asperillus during fermentation (Bao 2011; Nunomura et al. 1984; Gao et al. 2010), was an important contributor to soy sauce aroma with 1 ppm could be sensory (Bao 2011). In contrast to other phenols, the amount of 4-EG in soy sauce was not significantly higher. Relatively speaking, compared with other samples, sample 12 had quite higher amount of 4-EG. Other phenolic derivatives, like 2, 4-bis

**Table 3** Relative amount of volatile compounds identified in soy sauce products, pure soy sauce, and aHVP

Volatile compounds	Samples														
	P	aHVP	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	
Alcohols	a	6.07	0.16	22.4	26.2	2.42	8.11	5.17	2.88	39.4	5.59	7.82	63.7	103.6	35.2
	b	73.7%	0.76%	21.5%	22.9%	3.12%	8.99%	3.63%	2.49%	35.1%	7.68%	7.00%	43.7%	68.4%	20.7%
Esters	a	1.47	–	–	0.012	–	–	–	–	1.49	–	–	0.61	3.10	0.45
	b	1.03%	–	–	0.13%	–	–	–	–	1.39%	–	–	0.36%	1.78%	0.43%
Phenols	a	1.13	0.91	1.95	2.90	1.34	1.94	5.73	1.07	2.69	1.47	4.05	3.67	0.55	15.5
	b	0.79%	4.55%	2.61%	2.47%	1.82%	2.18%	3.81%	0.12%	2.30%	1.61%	3.39%	2.30%	0.37%	8.22%
Aldehydes and Ketones	a	0.63	0.86	4.40	5.05	0.17	1.11	2.81	0.0044	2.03	0.69	0.89	2.94	1.36	1.16
	b	0.90%	7.46%	5.40%	5.14%	1.77%	1.56%	2.27%	0.036%	2.25%	1.67%	1.60%	2.16%	1.16%	0.93%
Acids	a	0.039	2.21	9.20	10.9	13.1	9.45	22.5	5.86	12.1	13.9	21.5	15.6	10.6	15.6
	b	4.46%	3.32%	8.96%	9.52%	14.5%	10.5%	15.0%	4.78%	10.8%	13.8%	17.5%	10.4%	6.45%	10.3%
Heterocycles	a	0.66	0.62	4.31	5.20	6.66	1.80	5.33	1.99	2.89	2.38	2.74	5.22	4.00	0.74
	b	0.63%	1.26%	3.98%	4.33%	7.39%	2.00%	3.48%	1.52%	2.61%	2.79%	2.10%	3.57%	2.69%	0.49%
Dimethyl trisulfide	a	–	–	0.0075	0.0096	0.0011	0.0048	0.0071	–	0.0068	0.0088	0.0045	0.012	0.0088	0.011
	b	–	–	0.079%	0.10%	0.010%	0.056%	0.047%	–	0.061%	0.090%	0.04%	0.079%	0.081%	0.058%

P Pure soy sauce produced by high-salt dilute state

a Total relative concentration calculated, with the reference standard compound, 1 µg/mL 3-octanol

b Total peak area percent concentration of compound

– The compound could not be detected



(1, 1'-dimethylethyl)-phenols, were not only found in pure soy sauce sample but also in aHVP sample. Nevertheless, the typical compound of soy sauce, 4-EG, and 4-EP, were not found in aHVP sample. 2-methoxy-phenol, typically described as smoky, spicy, and medicine-like (Nunomura et al. 1984; Kaneko et al. 2012, 2013), was detected in all samples, while phenol was not found in pure soy sauce sample. By calculation, it was clear that the total amount of phenols in pure soy sauce sample was not high and phenols accounted for a little part of volatile flavor compounds. Nevertheless, although pure soy sauce sample and aHVP sample had almost identical concentration of phenols, phenols in aHVP sample took proportionately more of its total volatiles, especially 2-methoxy-phenol. Therefore, it was believed that phenols might only be a small influencing factor on flavor differentiation of soy sauce.

Another compound which was considered to be an important flavor contributor to soy sauce flavor was 2-ethyl-4-hydroxy-5-methyl-3 (2H)-furanone (HEMF). HEMF is very stable in soy sauce, and has a strong aroma like western dessert sweet with very low sensory threshold (Bao 2011; Nunomura et al. 1984; Kaneko et al. 2012, 2013). HEMF has very strong aroma activity, and it is also a dramatic anticancer compound. Our present study showed HEMF was not found in aHVP sample and 12 soy sauce products. It was only identified in pure soy sauce sample. Besides HEMF, other furans which have aroma like caramel color, such as 4-methoxy-2, 5-dimethyl-3 (2H)-furanone, dihydro-4, 4-dimethyl-3 (2H)-furanone, dihydro-2-methyl-3 (2H)-furanone, were also identified in pure soy sauce sample. Dihydro-2-methyl-3 (2H)-furanone was found in sample 10 and sample 11. In addition, the total amount of furans in aHVP sample and 12 soy sauce products were far lower in comparison with pure soy sauce sample. 2-Acetyl-1-pyrroline (Wongpornchai et al. 2003), abbreviated 2AP, with the IUPAC name 1-(3, 4-dihydro-2H-pyrrol-5-yl) ethanone, is an aroma compound, and gives white bread customary smell. 2-Acetyl-1-pyrroline can be formed by Maillard reactions during heating of food or high temperature. Both compounds have odor thresholds below 0.06 ng/L (Harrison and Dake 2005). However, 2AP was not found in aHVP sample and sample 6. Actually the heterocyclic compounds which were identified in aHVP sample were greatly different from that in pure soy sauce sample. A wide variety of heterocyclics, particularly pyrazine derivatives, were found in aHVP sample. Heterocyclics of some soy sauce products were really similar. In addition, these pyrazines mainly played an important role in providing nutty, baked and roasted notes to aHVP as well as soy sauce samples. Obviously, furans which was listed in Table 2 and was the significant flavor compounds in food, especially in soy sauce, could hardly be found in aHVP sample and most soy sauce products.

The aroma of the sulfur-containing compound in food, which has the characteristic malodorous smell, is one of the favorite specific flavors of Chinese. Besides of 3-(methylthio)-1-propanol and 3-(methylthio)-propanal, dimethyl trisulfide, which was detectable in 11 soy sauce products, should be noted because it has been confirmed as a volatile compound which contributes to the foul odor like cooked onion in food. The degradation of sulfur-containing amino acids probably produced the sulfur-containing compounds.

Among acids identified in all samples, the most abundant of which was acetic acid, followed by octanoic acid, nonanoic acid and propanoic acid. These acids were produced by diversity of microorganism during fermentation. The data showed long-chain fatty acid, both octanoic acid and nonanoic acid were not detected in aHVP sample. Acetic acid comprised about 2.3% of the total volatile compounds in aHVP sample, while it was about 4% in pure soy sauce sample, and around 3–15% in soy sauce products. Apart from formic acid, propanoic acid and other acid, acetic acid was also detected in caramel color. According to Chinese national standards (Chinese national standard GB/T 2760-2010; Chinese national standard: Fermented soy sauce GB 18186-2000), caramel color is approved for use in soy sauce, but application and use has level restrictions. It has an odor of burnt sugar and a somewhat bitter taste. Since 12 soy sauce samples were all deep-colored products, and were labeled as the caramel color addition in ingredient lists, it could be concluded that some acetic acid in soy sauce products were from caramel color.

Obviously, the overall volatile compounds of each soy sauce products were less than that of pure soy sauce sample, many of the same components were identified in pure soy sauce sample, and soy sauce products. With a few exceptions, for example alcohols and acetic acid, most of the volatile compounds in pure soy sauce sample were present at <1%. Although the manufacturing process of soy sauce varied, some common compounds generated owing to the same raw material used in production process. For instance, the brewing raw material of low-salt and high-salt soy sauce samples, all include wheat, wheat bran and soybean, the only differentiation was defatted soybean used in low-salt soy sauce products sample 1–6, and soybean used in high-salt soy sauce products sample 7–12. Nevertheless, with the exception of several compound such as 4-ethyl-2-methoxy-phenol, 2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone and other furan compounds, most compounds maybe not the typical flavor compounds of soy sauce.

It was believed that there was no single compound could be responsible for the whole flavor of soy sauce. Flavor of

soy sauce might be a result of comprehensive combination of volatile substances in soy sauce in specific proportions, and the difference of flavor might be each soy sauce had different amount of volatile substances. Consequently, their quantitative constitution is of great importance, which is the key to research of soy sauce flavor, especially the finding of typical flavor attributes and specific compounds which might be responsible for the particular flavor. Based on the overall amount of each kind of chemical compound illustrated in Table 3, it could be concluded that flavor quality of 12 soy sauces varied widely. Flavor of sample 10 and sample 11 were more closely resembled pure soy sauce sample, while flavor of sample 6 was more closely resembled aHVP sample.

### Statistical analysis: clustering of soy sauce samples

Cluster analysis was performed using SPSS software (version 20) from SPSS Inc (USA). The data matrix from Table 3 was subjected with flavor descriptions to hierarchical cluster analysis (HCA) based on dissimilarities. As it showed in Fig. 1, soy sauce samples have clustered together. Pure soy sauce sample and soy sauce sample 11 have the most similar flavor profiles, whereas aHVP sample and soy sauce sample 6 have the most similar flavor profiles. Sample 1, sample 2 have similarities with sample 12. Sample 7 groups together with sample 10. HCA on their volatile compounds of sample 3, sample 4, sample 5, sample 8, and sample 9 also obtained very good classification results according to GC–MS data.

### Electronic nose analysis of volatile compounds from soy sauce

E-nose was used to determine volatile profiles of pure soy sauce sample, aHVP sample, and 12 soy sauce products. E-nose differentiated overall volatile compounds associated with each sample.

### Classification of soy sauce using PCA and LDA

For data analysis of e-nose determination, PCA, LDA were used for discriminating and forming clusters among these samples based on the difference of volatile chemical compounds and signal intensity of the sensor array. PCA and LDA analysis were performed, and the principal component 1 (PC1) and 2 (PC2) as well as first and second linear discriminant LD1 and LD2 were shown in Fig. 2a, b respectively. The data showed a shift of different sample along the first principal component, PC1, which represented 96.81% of the total variance with value 98.85%. The second principal component (PC2) represented 2.04% of the variation. Several soy sauce products were less

separated and the measurement results were overlapped for several groups.

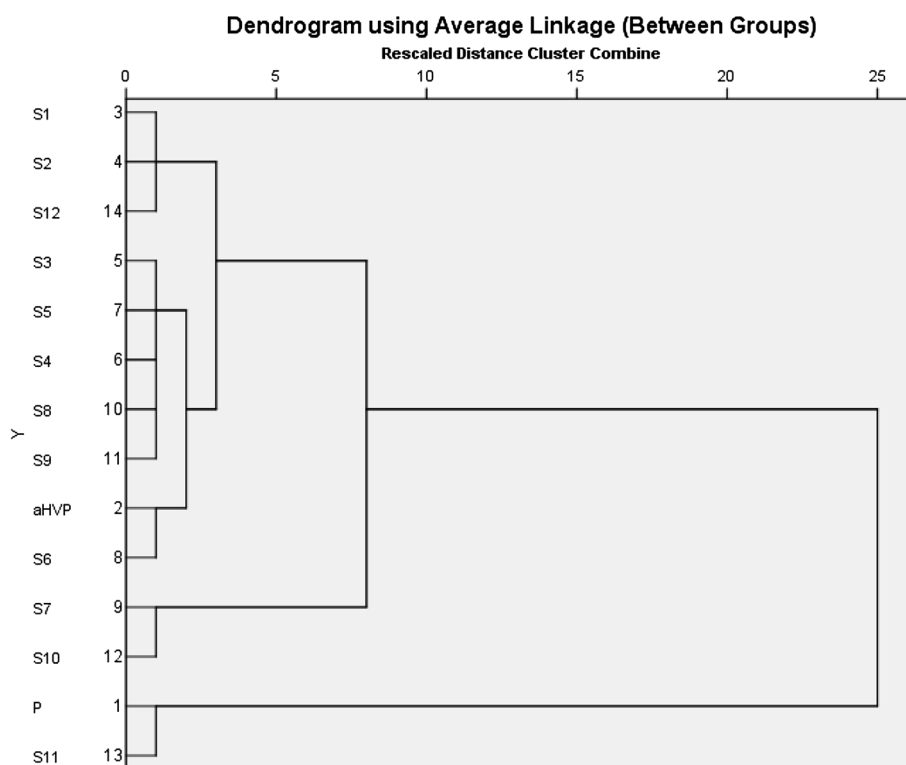
Moving left to right along the axis-x (PC1), aHVP sample were separated from other high-salt dilute-state soy sauce samples, while axis-y (PC2) could distinguish aHVP sample and other soy sauce samples with the exception of sample 6. This could be explained by the fact that most soy sauce samples had higher overall volatile intensity than aHVP sample. It was found that some soy sauce products with different fermentation types could not be discriminated. Apparently, it was hard to discriminate sample 10 from sample 11. Sample 1, sample 2, and sample 12 could hardly be differentiated from each other. There was some difficulty to make a distinction between sample 3, sample 4, sample 5, and sample 8. There still existed similarity between sample 6 and aHVP sample, and differences were not evident between sample 2 and sample 7. Nevertheless, results showed significant differences for the volatile attributes of pure soy sauce sample and aHVP sample.

For supporting the PCA results, LDA was used to discriminate these samples. After analyzing the same data set using LDA, 14 groups were more clearly distinguished than using PCA (Fig. 2b). It could be seen from Fig. 2B, about 97.84% of the total data variance was displayed. Functions 1 (LD1) and 2 (LD2) accounted for 87.69 and 10.15% of the variance, respectively. LDA analysis showed markedly differentiation among groups of pure soy sauce sample, aHVP sample, sample 3, sample 4, sample 5, sample 6, sample 7, sample 8, sample 9, sample 10, and sample 11. The sample of pure soy sauce sample, aHVP sample, sample 3, sample 4, sample 5, sample 6, sample 7, sample 8, sample 9, sample 10, and sample 11 would be completely distinguished using LDA analysis. The group of sample 1, sample 2, and sample 12 has almost wholly overlapped. This indicated sample 1, sample 2, and sample 12 would hardly be distinguished with each other using LDA analysis and their flavor were almost similar. However, sample 1, sample 2, and sample 12 could be clearly differentiated from other samples.

### Loading analysis

The important sensors, which had a major correlation to quality determination, were identified. The loading analysis will be a helpful tool to identify the important sensors which might be responsible for discrimination in the current pattern file. Sensors with high values of loading parameters for a particular principal component indicates a discriminating sensor, whereas loading parameters near to zero have a low contribution to the total response of the array of sensors. Figure 3 showed the loading analysis results and the loading factor associating to first and second principal components (PC1 and PC2) for each sensor was

**Fig. 1** Agglomerative hierarchical clustering (AHC) of 12 investigated soy sauce product samples, pure soy sauce sample, and aHVP sample



represented. Sensor groups having almost identical loading parameters might be represented by just one sensor. As could be seen, the sensors R(8), R(9), R(2) and R(7) have higher influence in the current pattern file, while the sensors R(4), R(10), R(3) and R(5) have low influence. Hence, it could be concluded that some aromatic compounds, sulfur organic compounds and alcohols must be the major contributors to flavor of soy sauces.

### Comparison of e-nose analysis and GC–MS data

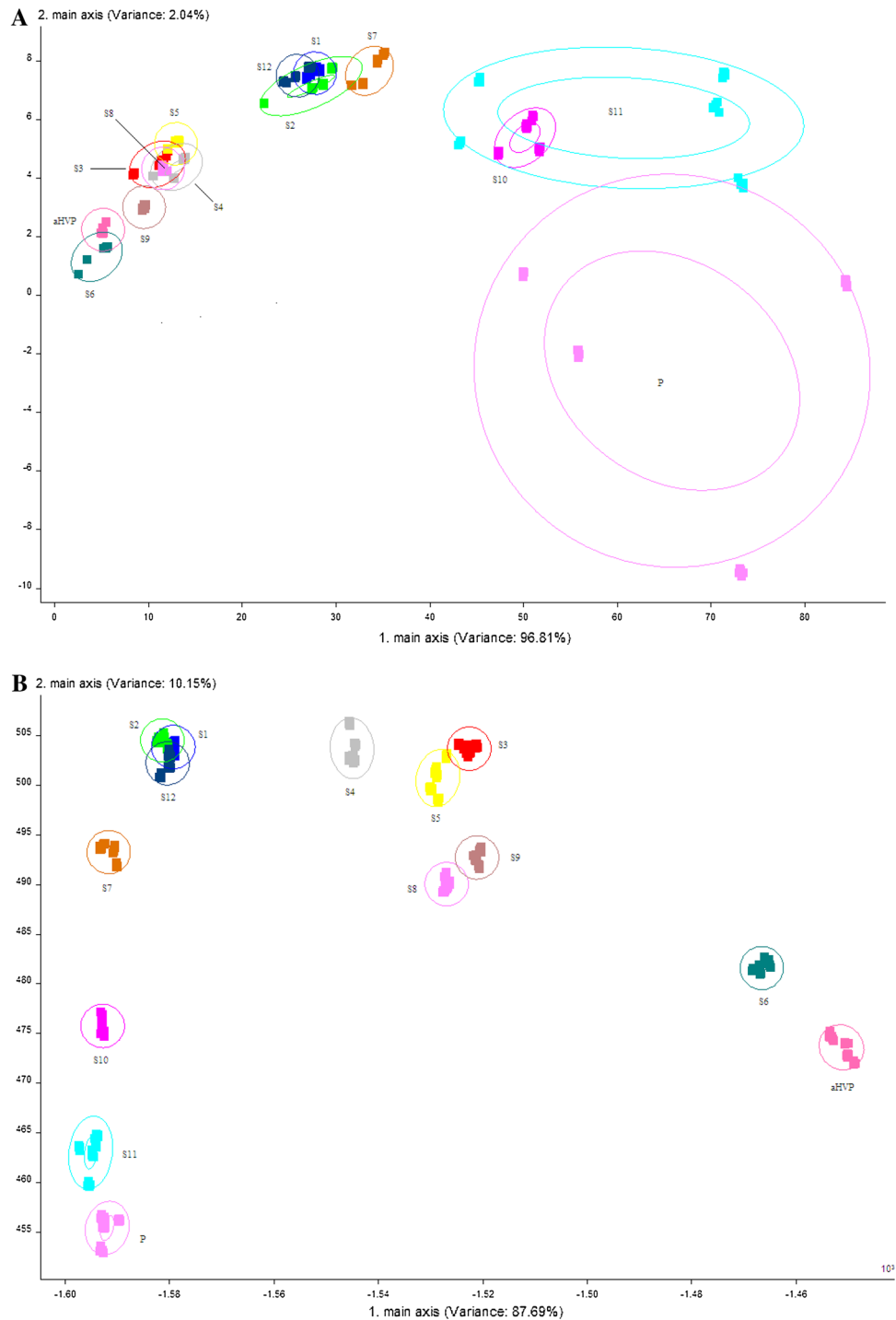
The volatile profiles and the amount of volatiles were analyzed to describe flavor profiles and qualities of soy sauce samples and aHVP sample. Based on GC–MS results shown in Tables 2, 3 and Fig. 1, all samples can be classified into several groups. As can be seen in Table 3, the amount of volatiles of pure soy sauce sample and aHVP sample had great difference, especially alcohols, while the amount of volatiles of sample 11 were much close to pure soy sauce sample. The good agreement was obtained between GC–MS statistical data and PCA analysis coming from e-nose. It indicated that flavor profile of pure soy sauce sample and aHVP sample was totally different, while flavor profile of sample 11, and pure soy sauce sample were much closer. Furthermore, flavor profile of sample 11 and sample 10 was hardly distinguished. Their subtle flavor difference probably came from the different amount of some typical compound including furans and other

heterocyclics in soy sauce. Concentration of several volatiles including phenols, aldehydes, ketones, and acids in high-salt soy sauce product sample 7 and sample 10, were almost identical. Their concentration of other volatiles, such as alcohols, esters and heterocycles were different. However, flavor profile of sample 7 and sample 10 were still very similar. Therefore, based on volatiles concentration, quality grade of pure soy sauce sample and other soy sauce products could be discriminated. Concentration of volatiles in high-salt soy sauce sample 12 was compared with that in low-salt soy sauce product including sample 1 and sample 2. Three soy sauce samples had similar amount of alcohols and acids. Their discrimination lied in different amount of phenols, esters, aldehydes, ketones, as well as heterocyclics, which were possessed by each sample. Flavor profile of these three samples was very similar, and their quality grade was almost the same. Obviously, GC–MS chemical information have produced results essentially in agreement with figures of PCA based on e-nose determination.

The varieties and concentration of volatile compounds in sample 3, sample 4, sample 5 and sample 8 were of high similarity. Thus, flavor quality of them was hardly to discriminate.

Volatile compounds in aHVP sample and low-salt soy sauce sample 6, which presented at relatively low level, had similar flavor quality. This result could be proved by PCA analysis. Moreover, sample 11 and sample 10 had the

**Fig. 2** A PCA of headspace compounds from pure soy sauce, aHVP and 12 soy sauce samples. The x and y axes are the first and second principal components respectively and account for 96.81 and 2.04% of the variance in the data. B LDA of headspace compounds from pure soy sauce, aHVP and 12 soy sauce samples

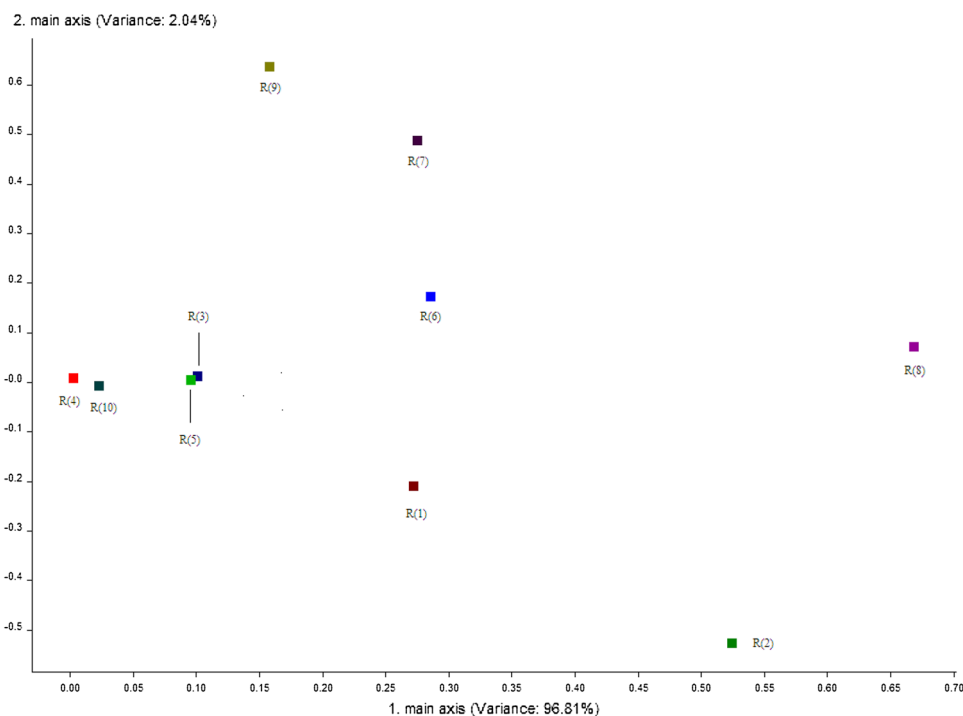


best flavor quality, and sample 6 had the worst flavor quality.

To our knowledge, the aroma activity or flavor attributes of each volatile substance in particular foodstuff not only depends on its concentration, but on its flavor threshold as well as its interaction with other compounds. Although the study of volatile compounds in

individual soy sauce provided limited information on which compounds were the key contributors to flavor quality of soy sauce, acquiring the amount of certain volatiles which commonly shared by soy sauce, such as sulfur organic compounds and alcohols, was still an effective approach for evaluation of the flavor quality of soy sauce.

**Fig. 3** Loading analysis related to PC1 and PC2 for soy sauces with total variance 98.86%



## Conclusion

Forty one key volatile compounds, which had great effect on the flavor of Chinese-type soy sauces, were identified by using GC–MS combined with HS–SPME, and compared in semi-quantitative terms. Statistical analysis method, HCA, which successfully clustered different types of soy sauces into several groups, was used to characterize flavor quality of soy sauce samples. The investigation demonstrated that volatile flavor compounds of soy sauce samples had a greater difference, and the flavor quality of soy sauces manufactured by low-salt solid-state fermentation process generally had fewer volatile organic compounds. Meanwhile, the overall volatiles profiles were also analyzed by e-nose. E-nose determination and GC–MS statistical analysis had similar results, and both methods had good potential application in evaluating flavor quality, differentiating soy sauce products, and supervising quality of soy sauces. Particularly, on the one hand, this survey revealed that soy sauce products with uneven quality distributed in the Chinese domestic market. This conclusion highlights the necessity and importance of making much effort on perfecting the relevant standard of soy sauce because physical and chemical index is too limited in present Chinese national standards of soy sauce. Assessment indexes of soy sauce quality should include flavor quality or flavor grade besides of concentration of amino acid nitrogen. On the other hand, this research provides the experimental evidence and theoretical basis for evaluating the flavor quality of soy sauces. Therefore, it is valuable

information to both manufacturers and government regulators, which have an important practical significance to formulate relevant policy or measures to improve the brewing technology and quality of low-salt soy sauce in China.

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