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Characterization of Flavor Properties of Sufu by HS-SPME/GC-MS, Electronic Nose and Electronic Tongue Combined with Multivariate Statistical Analysis

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Abstract: Flavor is the main quality characteristic of sufu. The flavor of different types of sufu varies greatly, which will affect the preference and selectivity of consumer for sufu. To explore the flavor characteristics and distinguishing methods in different types of sufu, the volatile flavor components and free amino acids of 5 kinds of commercial sufu were determined by HS-SPME/GC-MS and amino acid automatic analyzer, respectively. Moreover, the electronic nose and electronic tongue were used to distinguish the sufu samples. The results showed that a total of 71 volatile aroma compounds were identified, in which esters (28.43-79.17%) were the main flavor substances. The total free amino acid contents in different types of sufu were at a range of 24.8-33.89 mg/g. Bitter amino acids were dominant in all sufu (except GS). PCA of electronic nose and electronic tongue showed that the electronic nose can only distinguish the grey sufu, while the electronic tongue can effectively distinguish among types of sufu. PLS-DA based on aroma and taste substances showed that sufu can be distinguished. According to VIP, fifteen volatile flavor components and five taste amino acids could be used as the main different compounds of sufu. The research results pretended that HS-SPME/GC-MS, electronic tongue technology combined with multivariate statistical analysis method can effectively distinguish sufu. These findings will provide guiding significance to identify the types of sufu and consumers' purchase products and promote the development of sufu industry to a certain extent.

Keywords: Sufu, Volatile Flavor Components, Free Amino Acids, Electronic Nose, Electronic Tongue

Introduction

Sufu, a traditional Chinese fermented soybean product, is also known as Chinese cheese because its shape, fermentation and processing mechanism as well as texture are similar to cheese (Xie *et al.*, 2018). Attributing to the delicious taste and unique flavor of sufu, it has been widely consumed in China as an appetizer for thousands of years. Its production usually involves four steps: (1) preparation of tofu; (2) preparation of pehtze with tofu cubes inoculated microorganisms; (3) brining of pehtze and (4) ripening. According to different color and flavor, sufu can be divided into four different types, i.e., red sufu, white sufu, grey sufu and other sufu (Han *et al.*, 2004). During the

fermentation process, protein, lipid, starch and other substances of raw materials can produce amino acids, esters, alcohols, aldehydes, acids and other flavor compounds via a series of biochemical pathways (Han *et al.*, 2001, Xie *et al.*, 2019a). In addition, amino acids can participate in the esterification and reduction reactions after deamination and decarboxylation and can also produce some flavor substances (Zhao *et al.*, 2016). These flavor components (including aroma and taste) cooperate with each other and work together to give sufu unique flavor characteristics.

In recent years, with the continuous improvement of human health awareness, the development of new fermented food has become a trend. Subsequently, the types of sufu on the market are becoming more diverse.

The flavor is important characteristic to evaluate food quality, which could affect the satisfaction of consumers (Liu *et al.*, 2019). Therefore, it is necessary to explore the flavor characteristics and distinguishing methods of sufu in different types. At present, the research about the flavor of sufu has been reported. Liu *et al.* (2012) successfully applied Solid Phase Micro-Extraction (SPME) combined with Gas Chromatography-Mass Spectrometry (GC-MS) to identified 39 volatile flavors components of grey sufu, of which indole was the most important flavor substance. After, SPME/GC-MS was also employed to analyze the volatile components of Kedong sufu during ripening. The results indicated that 75 compounds were identified, among which esters were the most abundant (Fan *et al.*, 2019). Some studies have shown that free amino acids hydrolyzed by protein play an important role in taste characteristics of sufu. Moreover, statistical analysis methods have been widely used in various studies (Sunday, 2020, Umana *et al.*, 2020). At the same time, researchers found that the combination of detection technology and statistical analysis method can achieve the effect of discrimination. Dong *et al.* (2019) successfully applied electronic nose, electronic tongue and HS-SPME/GC-MS data in combination with a Principal Component Analysis (PCA) to effectively distinguish roasted coffee beans with different drying methods. Electronic nose and electronic tongue are bionic detection methods simulating human olfactory and taste, which were widely used in the quality control and origin identification of some foods (Loutfi *et al.*, 2015, Souayah *et al.*, 2017). However, there are few reports on their application in the classification of sufu types.

The purpose of this study was to: (1) Evaluate the flavor properties of different types of sufu; (2) discriminate the type of sufu using electronic nose and electronic tongue; (3) Screen differential compounds by Partial Least Squares Discrimination Analysis (PLS-DA) based on the volatile components and free amino acids. These analyses are helpful to understand the flavor characteristics of sufu and provide basis for consumers to choose relevant products.

Materials and Methods

Materials

Five kinds of commercial sufu samples, including Red Sufu (RS), White Sufu (WS), Grey Sufu (GS), Low-Salt sufu (LS) and Oil Sufu (OS), were purchased from the local supermarket in Baoding, Hebei Province, China.

Determination of Volatile Compounds

Volatile compounds of sufu samples were analysed by HS-SPME/GC-MS according to the method of with some modifications (Liang *et al.*, 2019). Briefly, 7.5 g uniform paste of each sufu sample was placed in a 20 mL headspace vial and mixed with 25 μ L of 3-octanol (300

μ g/mL in ethanol, internal standard). The vial was quickly sealed and then equilibrated for 10 min at 50 °C in a water bath. Then, the divinylbenzene/carboxen/polydimethylsiloxane fibre (length: 1 cm; df: 50/30 μ M; Supelco Ltd., Bellefonte, PA, USA) was exposed in the upper space of the headspace vial for 30 min at 50°C. After that, the fiber was immediately injected into the GC injector to desorb the extractions at 250°C for 7 min. The extractions were separated in the capillary column (HP-INNOWAX; 60 m \times 0.25 mm \times 0.25 μ M; J and W Science, Folsom, CA, USA). The oven temperature program was maintained at 35°C for 5 min, then increased to 130°C at the speed of 4°C/min and further increased to 200°C at the speed of 10°C/min. The carrier gas was purified helium (99.999%) with a flow rate of 1 mL/min. The ionization energy was 70 eV and the mass scan ranged from 40 to 350 m/z. The temperature of ion source and transmission line was 230 and 250°C respectively. The volatile components were identified by NIST 14 database and semi-quantified by internal standard.

Determination of Free Amino Acids

The free amino acids were determined using an automatic amino acid analyzer (L-8900; Hitachi, Tokyo, Japan), following the method of (Yoon *et al.*, 2017). 2 g of the freeze-dried sample was mixed with 8 mL of 5% sulfosalicylic acid and shaken for 1 h. Then, the mixture was centrifuged at 18000 rpm for 30 min at 4°C. The supernatant was successively filtered through 0.45 μ m and 0.22 μ m organic phase filters for analysis.

Electronic Tongue Measurement

The appropriate amount of sufu sample was placed in a 40 mL headspace bottle and the sample was tested after standing for 30 min. The detection parameters are as follows: Carrier gas, clean air; gas flow rate, 1.0 L/min; cleaning flow, 6 L/min; test time, 60 s; waiting time, 10 s; detection temperature, 25°C; injection gas volume, 10 mL. Each sample was measured in five times.

Electronic Nose Measurement

Appropriate amount of sufu samples were weighed and extracted with ultrapure water and the same amount of sufu aqueous extract was taken for detection. The detection parameters are as follows: Maximum voltage, 10 V; minimum voltage, -10 V; pulse interval, 100 s; sensitivity, 10^{-4} . Each sample was measured four times.

Statistical Analysis

Statistical analysis was performed using SPSS 22.0 (SPSS Inc., Chicago, IL, USA). Analysis of Variance (ANOVA) with Duncan's test was used to assess the significance ($P < 0.05$). PCA and PLS-DA were undertaken using SIMCA 16.0.1 (Umetrics, Umea, Sweden). All data were expressed as mean \pm standard deviation.

Results

Volatile Compounds Analysis of Commercial Sufu by HS-SPME/GC-MS

A total of 71 volatile compounds including alcohols (12), esters (26), aldehydes (5), ketones (3), phenols (4), acids (3), ethers (2), hydrocarbons (5), heterocycles (8) and other compounds (3) were identified and quantified from five kinds of commercial sufu and the detailed results were shown in Table 1. In this study, alcohols and esters were the most abundant substances in all samples (except GS).

Alcohol compounds have special flavor and can also synthesize esters with organic acids, which has an important influence on the formation of unique flavor of sufu. Alcohols accounted for 44.11, 0.08, 40.41, 35.77 and 23.30% of the total concentrations in samples RS, GS, WS, LS and OS, respectively. Phenyl ethanol was identified in five kinds of sufu and its content in LS (7.16 mg/kg) was significantly higher than that of other sufu ($P < 0.05$). The top component of alcohols in the RS, WS, LS and OS was ethanol and its content was 17.83-35.19 mg/kg. However, ethanol was not detected in GS.

Esters, the most important flavor constituents in sufu, are primarily produced by the esterification or alcoholysis and usually responsible for typical fruity and sweet aromas (Liu *et al.*, 2004). The total content of ester was 270.11 mg/kg in GS, which was significantly higher than the other sufu samples ($P < 0.05$). The higher content of esters in the RS, WS and LS were ethyl palmitate and ethyl octanoate which can give sufu dense ester, cream and brandy flavor. The highest content of esters in GS and OS was ethyl linoleate, which were 155.14 and 12.99 mg/kg, respectively. The ethyl esters components of these five kinds of sufu were in the majority and the content were high. Only three kinds of methyl esters were identified and the content in OS was abundant.

Aldehydes and ketones may be derived from β -oxidation of fatty acids or catabolism of amino acids during fermentation (Yu *et al.*, 2008). For example, benzaldehyde and phenylacetaldehyde can be produced by Phe catabolism and these two aldehydes are widely present in fermented soybean products (Li *et al.*, 2016). In this study, benzaldehyde was detected in all samples, ranging from 0.22 to 0.51 mg/kg, while phenylacetaldehyde was only detected in LS (0.44 mg/kg). Three ketones were identified, namely camphor, 3-aminoacetophenone and 2-methyl-2-cyclopentenone. Although the aromas of ketones are generally pleasant, they may contribute little to the flavor because of their low concentrations detected in sufu.

Phenolic compounds are associated with smoke and woody odors and previous research showed 2-methoxy-4-vinylphenol can give soy sauce tar and smoke flavor (Parker *et al.*, 2018). At the same time, 2-methoxy-4-

vinylphenol is also the aroma active ingredient of Shaoxing rice wine (Chen *et al.*, 2013). In this study, this substance was only detected in RS and LS. Phenol and 2-methoxy-3-(2-propenyl) phenol were higher in the identified phenolic compounds. The content of phenol in GS (8.69 mg/kg) was significantly higher than that in other sufu ($P < 0.05$).

Acids are important precursors for the synthesis of esters, aldehydes and other compounds, which can be produced by long-chain fatty acid decomposition, amino acid degradation and microbial metabolism (Peng *et al.*, 2014). The content of acids in GS (40.37 mg/kg) was the highest and butyric acid accounted for about 60.94% of the total acid compounds. Butyric acid has cheese and creamy flavor at low concentrations, but it has strong stink at high concentrations. A trace amount of propionic acid was detected in GS, WS and LS, while acetic acid was the most abundant acid in WS and OS.

In addition, ethers, hydrocarbons and other compounds were identified in various types of sufu. 2-(2-ethoxyethoxy) - ethanol and estragole were only identified in WS and OS and the content of estragole in WS was significantly higher than that in RS ($P < 0.05$). Hydrocarbon compounds have higher flavor threshold, so they have little effect on sufu flavor. Styrene and anethole shared in five kinds of sufu and the content of anethole in RS and WS were higher, 14.01 and 12.38 mg/kg, respectively. Dimethyl trisulfide, butyramide and high concentration of indole (18.70 mg/kg) were also identified in GS, which played an important role in the formation of sufu flavor.

Analysis of Free Amino Acids

Umami taste is described as brothy, savory or meaty taste sensation (Thammarongtham *et al.*, 2001). Glu, Asp and their corresponding salts are important sources of umami. The salt added during the preparation of sufu will form sodium ammonia with Glu, thereby enhancing the umami taste. In addition, some ions can also affect the taste (Nkansah *et al.*, 2019, Durguti *et al.*, 2020). The contents of Glu in WS, LS and OS were 3.96, 3.93 and 3.84 mg/g, respectively, with no significant difference ($P > 0.05$). However, Asp contents in these three kinds of sufu were significantly different ($P < 0.05$). The content of Asp in WS was the highest (2.45 mg/g), followed by OS (2.28 mg/g). The content of umami amino acids in GS (0.38 mg/g) was the lowest. In terms of the sweet amino acids, the content of Ala was relatively high, ranged from 1.54 to 3.05 mg/g. Moreover, Gly also induced strong sweetness and its content was the highest in LS. Ser was not detected in WS. The whole taste of sufu was also affected by the threshold of taste active compounds. Although tasteless amino acids do not have taste activity, they enhance the taste intensity of other components.

Table 1: Volatile flavor components and content from five kinds of sufu

NO	Volatile compounds	Content / (mg/kg)				
		RS	GS	WS	LS	OS
Alcohols						
1	Ethanol	35.19±4.00 ^a	ND	26.20±2.20 ^b	28.41±3.15 ^b	17.83±1.28 ^c
2	1-hexanol	ND	ND	0.21±0.02 ^a	ND	0.09±0.00 ^b
3	(2R,3R)-(-)-2,3-butanediol	0.51±0.08 ^c	ND	1.01±0.04 ^a	0.63±0.03 ^b	ND
4	Linalool	ND	ND	0.19±0.02 ^a	ND	0.22±0.02 ^a
5	Terpinen-4-ol	ND	ND	0.10±0.01 ^b	ND	0.27±0.01 ^a
6	Furfuryl alcohol	0.44±0.02 ^a	ND	0.11±0.01 ^c	0.27±0.02 ^b	0.43±0.03 ^a
7	Benzyl alcohol	0.07±0.01 ^c	ND	0.11±0.01 ^b	0.12±0.01 ^b	0.18±0.02 ^a
8	Phenyl ethanol	0.78±0.11 ^b	0.26±0.02 ^c	0.54±0.03 ^{bc}	7.16±0.50 ^a	0.37±0.06 ^{bc}
9	2,7-, -dimethyl-4,5-octanediol	ND	ND	ND	0.17±0.02	ND
10	2,3-butanediol	0.49±0.05 ^b	ND	ND	0.60±0.03 ^b	2.18±0.15 ^a
11	3-methylthiopropanol	0.08±0.00 ^b	ND	ND	0.29±0.01 ^a	ND
12	5-methyl-2-furanmethanol	0.06±0.01	ND	ND	ND	ND
	∑	37.62±2.47 ^a	0.26±0.01 ^d	28.47±2.08 ^b	37.65±2.18 ^a	21.57±0.91 ^c
Esters						
13	Ethyl hexanoate	3.62±0.19 ^a	0.82±0.03 ^d	1.32±0.11 ^c	2.21±0.19 ^b	0.91±0.06 ^d
14	Ethyl palmitate	10.59±0.84 ^{bc}	0.74±0.12 ^d	11.34±0.79 ^b	19.30±1.50 ^a	9.11±0.77 ^c
15	Ethyl linoleate	ND	155.14±14.00 ^a	1.22±0.03 ^b	6.99±0.57 ^b	12.99±0.87 ^b
16	Ethyl tetradecanoate	1.97±0.11 ^a	ND	0.75±0.08 ^c	1.22±0.10 ^b	0.69±0.07 ^c
17	2,2,4-trimethyl-1,3-pentanediol diisobutyrate	0.15±0.01 ^c	0.22±0.02 ^b	0.17±0.02 ^{bc}	0.97±0.06 ^a	ND
18	Ethyl phenylpropionate	0.25±0.02 ^b	ND	0.08±0.00 ^c	0.60±0.03 ^a	ND
19	Ethyl octanoate	14.72±1.26 ^a	1.76±0.13 ^d	3.62±0.20 ^c	10.48±0.84 ^b	1.53±0.09 ^d
20	Ethyl cis-4-octenoate	ND	ND	0.12±0.01	ND	ND
21	Ethyl caprate	2.61±0.07 ^a	0.48±0.05 ^c	0.34±0.02 ^d	0.78±0.08 ^b	ND
22	Dimethyl glutarate	1.01±0.04 ^{bc}	1.11±0.10 ^b	0.79±0.08 ^d	0.84±0.06 ^{cd}	2.18±0.15 ^a
23	Dimethyl adipate	0.29±0.01 ^c	0.44±0.02 ^b	0.22±0.02 ^d	0.32±0.01 ^c	0.67±0.06 ^a
24	Ethyl nicotinoate	0.64±0.04 ^a	ND	0.06±0.00 ^c	0.41±0.02 ^b	0.11±0.01 ^c
25	Ethyl-2-ethyl hexanoate	0.49±0.05 ^a	ND	ND	0.11±0.01 ^b	ND
26	Ethyl 3-hydroxybutyrate	0.23±0.01	ND	ND	ND	ND
27	Ethyl nonylate	0.40±0.05 ^b	ND	ND	0.63±0.04 ^a	ND
28	Diethyl succinate	0.86±0.03 ^b	ND	ND	2.24±0.15 ^a	ND
29	Ethyl phenylacetate	0.51±0.05 ^a	ND	ND	0.34±0.02 ^b	ND
30	Ethyl dodecanoate	2.18±0.15 ^a	0.15±0.06 ^c	ND	0.47±0.05 ^b	0.21±0.02 ^c
31	Ethyl pentadecanoate	0.07±0.01 ^b	ND	ND	0.16±0.01 ^a	ND
32	Ethyl linolenate	ND	ND	ND	1.27±0.06	ND
33	Dimethyl succinate	ND	0.17±0.02 ^b	ND	ND	0.43±0.03 ^a
34	Ethyl stearate	ND	13.74±0.87 ^a	ND	ND	0.69±0.04 ^b
35	Ethyl oleate	ND	53.47±5.72 ^a	ND	ND	6.23±0.42 ^b
36	Ethyl 9,12,15-octadecyltrieneoate	ND	37.16±2.93 ^a	ND	ND	2.90±0.20 ^b
37	3 - hydroxy-2,2,4 - trimethylpentyl isobutyrate	ND	ND	ND	0.14±0.01	ND
38	Ethyl (9E)-9-octadecenoate	ND	4.71±0.26	ND	ND	ND
	∑	40.59±1.70 ^c	270.11±14.05 ^a	20.03±0.79 ^c	49.48±2.20 ^{bc}	38.65±1.61 ^c
Aldehydes						
39	Phenylacetaldehyde	ND	ND	1.00±0.04 ^a	ND	0.18±0.02 ^b
40	2-phenylcrotonaldehyde	0.51±0.05 ^a	ND	0.23±0.02 ^b	0.28±0.01 ^b	ND
41	Nonanal	0.39±0.05 ^b	ND	0.45±0.02 ^a	ND	ND
42	Benzaldehyde	0.51±0.04 ^a	0.44±0.02 ^b	0.26±0.02 ^d	0.33±0.02 ^c	0.22±0.02 ^d
43	Anisaldehyde	ND	ND	ND	0.44±0.04	ND
	∑	1.41±0.08 ^b	0.44±0.01 ^d	1.94±0.06 ^a	1.05±0.04 ^c	0.40±0.02 ^d
Ketones						
44	Camphor	0.19±0.03 ^a	ND	ND	0.08±0.01 ^b	ND
45	3 - aminoacetophenone	0.18±0.02	ND	ND	ND	ND
46	2-methyl-2-cyclopentenone	ND	0.07±0.01	ND	ND	ND
	∑	0.37±0.03 ^a	0.07±0.01 ^b	0	0.08±0.01 ^b	0
Phenols						
47	2-methoxy-3 - (2-propenyl) phenol	2.06±0.15 ^b	ND	0.90±0.10 ^c	2.27±0.09 ^b	3.65±0.26 ^a
48	Methyleugenol	ND	ND	ND	ND	0.09±0.01
49	Phenol	0.50±0.05 ^d	8.69±0.73 ^a	1.47±0.07 ^c	3.90±0.42 ^b	ND
50	2-methoxy-4-vinyl phenol	0.26±0.01 ^a	ND	ND	0.16±0.03 ^b	ND
	∑	2.82±0.12 ^d	8.69±0.42 ^a	2.37±0.10 ^d	6.33±0.31 ^b	3.74±0.16 ^c
Acids						

Table 1: Continue

51	Propionic acid	ND	2.81±0.09 ^a	0.21±0.02 ^c	0.50±0.05 ^b	ND
52	Butyric acid	ND	24.60±2.20 ^a	1.70±0.08 ^b	ND	0.38±0.03 ^b
53	Acetic acid	ND	12.96±0.85 ^a	ND	7.88±0.52 ^b	4.41±0.37 ^c
	∑	0	40.37±1.81 ^a	1.91±0.06 ^c	8.38±0.33 ^b	4.79±0.23 ^c
	Ethers					
54	2-(2-ethoxyethoxy) - ethanol	ND	ND	0.09±0.01 ^a	ND	0.08±0.01 ^a
55	Estragole	ND	ND	0.98±0.10 ^a	ND	0.55±0.10 ^b
	∑	0	0	1.07±0.06 ^a	0	0.63±0.06 ^b
	Hydrocarbons					
56	Styrene	0.54±0.05 ^a	0.19±0.01 ^d	0.27±0.02 ^c	0.40±0.06 ^b	0.35±0.04 ^b
57	Tetradecane	ND	ND	ND	0.08±0.00	ND
58	Anethole	0.19±0.03 ^e	0.64±0.04 ^c	12.38±1.11 ^b	0.39±0.04 ^d	14.01±0.80 ^a
59	1,3,5-trimethylbenzene	ND	0.10±0.01	ND	ND	ND
60	(Z)-1-methoxy-4-(1-propenyl) - benzene	ND	ND	ND	ND	4.65±0.40
	∑	0.73±0.05 ^c	0.93±0.03 ^c	12.65±0.65 ^b	0.87±0.06 ^c	19.01±0.72 ^a
	Heterocycles					
61	2-pentylfuran	1.50±0.20 ^a	0.35±0.04 ^e	0.66±0.04 ^d	1.03±0.04 ^b	0.84±0.06 ^c
62	Indole	ND	18.70±1.26 ^a	0.73±0.08 ^b	ND	1.86±0.11 ^b
63	Naphthalene	ND	0.18±0.02 ^a	0.20±0.02 ^a	0.17±0.03 ^a	ND
64	2-methylnaphthalene	ND	ND	0.07±0.01	ND	ND
65	1,6-dimethylnaphthalene	0.13±0.00	ND	ND	ND	ND
66	2,6-dimethylpyrazine	ND	ND	0.35±0.03	ND	ND
67	2,3,5-trimethylpyrazine	ND	ND	ND	0.23±0.02	ND
68	2-acetylpyrrole	ND	ND	ND	ND	0.97±0.08
	∑	1.63±0.12 ^c	19.23±0.76 ^a	2.01±0.10 ^c	1.43±0.09 ^c	3.67±0.14 ^b
	Others					
69	Azulene	0.11±0.02 ^a	ND	ND	ND	0.12±0.01 ^a
70	Dimethyl trisulfide	ND	0.20±0.02	ND	ND	ND
71	Butyramide	ND	0.86±0.05	ND	ND	ND
	∑	0.11±0.01 ^b	1.06±0.04 ^a	0	0	0.12±0.01 ^b
	Total	85.28±4.57 ^c	341.16±17.15 ^a	70.45±3.90 ^c	105.27±5.17 ^{bc}	92.58±3.86 ^c

a, b, c, d, e Different letters in the same row indicate statistical differences at the 0.05 level according to Duncan test. ND, not detect

Analysis of Electronic Nose

PCA was used as a method to reduce the dimensionality of the data in multivariate analysis, which could explain the maximum variance with the least principal component. Generally, the greater cumulative contribution rate of principal component, the more effective it was to reflect the information of original data (Zheng *et al.*, 2013). PCA was applied on the data set obtained by the electronic nose and the results were shown in Fig. 1. The cumulative contribution rate of the principal components PC1 (65.9%) and PC2 (16.9%) was 85.6%, which can basically represent the information of samples. The overlap and crossover among RS, WS, LS and OS samples were observed in Fig. 1, indicating similar odor characteristics of these sufu samples. Meanwhile, it also showed that PCA combined with electronic nose cannot distinguish these four types of sufu. The GS sample was divided into one area separately without overlap with other sufu, indicating that the odor components of GS sample were quite different from other samples. In short, the PCA combined with electronic nose was more suitable to identify the types of GS.

Analysis of Electronic Tongue

The data collected by the electronic tongue were also used to perform PCA and the results were shown in Fig. 2. The accumulated variance contribution rate of the first two principal components was 95.96% (PC1

and PC2 represented 92.10 and 3.86% of the total variance, respectively), indicating that the principal components PC1 and PC2 could explain most of the information of samples. GS were located in the second quadrant, which were far from other types of sufu, suggesting that there was a remarkable difference between GS and other sufu samples. It can be observed that the samples were divided into five groups without overlapping. Therefore, the electronic tongue can generally distinguish different kinds of sufu samples.

Analysis of Volatile Flavor Components and Free Amino Acids in Sufu Using PLS-DA

PLS-DA, a multivariate statistical method widely used for classification, can better distinguish differences between groups. In addition, the potentially differential compounds can be screened from Variables Important in the Projection (VIP) values. Generally, the compounds with VIP values > 1 were essential for distinguishing between different kinds of sufu samples.

PLS-DA based on volatile flavor components was conducted and results were shown in Fig. 3 and 4. The PLS-DA score plot showed clear discrimination among different kinds of sufu samples. The RS and LS samples were located to the lower right of the X-axis, while the WS and OS samples were located to the upper left of the X-axis. For electronic nose analysis, the samples of RS, LS, OS and WS all clustered in the

lower right side of PCA diagram, which were not obvious distinction between each other. However, five kinds of sufu could be effectively distinguished by PLS-DA. This is mainly because the former relies on the response of the sensor to a certain category of flavor components, while the latter mainly depends on the specific components in sufu.

Based on the standard of VIP >1, it can be found that nonanal, phenyl ethanol, 2,2,4-trimethyl-1,3-pentanediol diisobutyrate and anisaldehyde had a great contribution to the difference of the sufu samples. Therefore, these substances were used as differential components in various sufu. In addition, ethyl palmitate, furfuryl alcohol, 3-methylthiopropanol, ethyl caprate, ethyl dodecanoate, diethyl succinate, dimethyl adipate, dimethyl glutarate, estragole, 2-methoxy-3 - (2-propenyl) phenol and azulene also affected the classification of sufu.

PLS-DA based on free amino acids was performed and results were shown in Fig. 5 and 6. According to Fig. 5, the five kinds of sufu were all distributed in the 95% confidence interval and different sufus can be well distinguished. The GS and WS samples were located to the second quadrant and the fourth quadrant, respectively. As shown in Table 2, the content of umami amino acids in WS was the highest and that in GS were the lowest. Moreover, there was no significant difference in the total content of sweet amino acids between WS and GS, so as the content of bitter amino acids ($P < 0.05$). In addition, the difference of tasteless amino acids in these two sufus was mainly reflected in the high content of Lys in WS samples. Therefore, the distribution characteristics of WS and GS on the score plot may be related to Asp, Glu and Lys. As shown in Fig. 6, the VIP values of Met, Phe, Ser, Tyr and Lys were all greater than 1, which showed that these amino acids could be used as differential compounds in various sufu.

Table 2: Composition and content of free amino acids from five kinds of sufu

Amino acids	Content (mg/g)				
	RS	GS	WS	LS	OS
umami amino acids					
Aspartic acid (Asp)	1.67±0.02 ^d	0.16±0.01 ^e	2.45±0.03 ^a	1.75±0.04 ^c	2.28±0.04 ^b
Glutamate (Glu) sweet amino acids	3.56±0.03 ^b	0.22±0.03 ^c	3.96±0.17 ^a	3.93±0.15 ^a	3.84±0.12 ^a
Threonine (Thr)	1.05±0.01 ^c	0.03±0.00 ^d	1.77±0.04 ^a	1.26±0.03 ^b	1.23±0.03 ^b
Glycine (Gly)	1.06±0.01 ^d	0.07±0.00 ^e	1.13±0.02 ^c	1.22±0.02 ^a	1.16±0.01 ^b
Alanine (Ala)	1.54±0.02 ^d	3.05±0.05 ^a	1.65±0.02 ^c	1.95±0.04 ^b	1.67±0.05 ^c
Serine (Ser) bitter amino acids	1.35±0.03 ^b	0.24±0.01 ^d	ND	0.81±0.01 ^c	1.64±0.02 ^a
Valline (Val)	2.79±0.03 ^e	5.27±0.20 ^a	3.99±0.02 ^b	3.11±0.10 ^d	3.35±0.08 ^c
Phenylalanine (Phe)	3.69±0.15 ^b	3.39±0.12 ^c	2.67±0.04 ^d	4.03±0.20 ^a	2.61±0.05 ^d
Methionine (Met)	1.46±0.05 ^a	0.91±0.02 ^e	1.27±0.03 ^b	1.00±0.04 ^d	1.20±0.03 ^c
Isoleucine (Ile)	1.80±0.10 ^c	3.18±0.12 ^a	2.07±0.02 ^b	1.82±0.05 ^c	1.79±0.04 ^c
Leucine (Leu)	3.14±0.09 ^{cd}	4.87±0.17 ^a	3.68±0.03 ^b	3.31±0.10 ^c	3.10±0.12 ^d
Histidine (His)	0.25±0.01 ^d	0.02±0.00 ^e	0.79±0.01 ^a	0.32±0.00 ^c	0.72±0.00 ^b
Tyrosine (Tyr)	1.08±0.01 ^d	1.58±0.02 ^b	2.01±0.03 ^a	1.14±0.03 ^c	0.89±0.01 ^e
Arginine (Arg) tasteless amino acids	0.03±0.00 ^b	ND	0.07±0.00 ^a	ND	ND
Proline (Pro)	2.74±0.04 ^a	ND	1.99±0.04 ^d	2.35±0.04 ^b	2.26±0.04 ^c
Lysine (Lys)	1.36±0.05 ^d	1.39±0.02 ^{cd}	4.05±0.20 ^a	1.49±0.02 ^c	2.80±0.12 ^b
Cysteine (Cys)	0.27±0.01 ^d	0.42±0.02 ^a	0.34±0.00 ^b	0.26±0.01 ^d	0.30±0.02 ^c
total free amino acid	28.84±0.66 ^c	24.80±0.79 ^d	33.89±0.52 ^a	29.75±0.78 ^c	30.84±0.80 ^{bc}

a, b, c, d, e Different letters in the same row indicate statistical differences at the 0.05 level according to Duncan test. ND, not detect

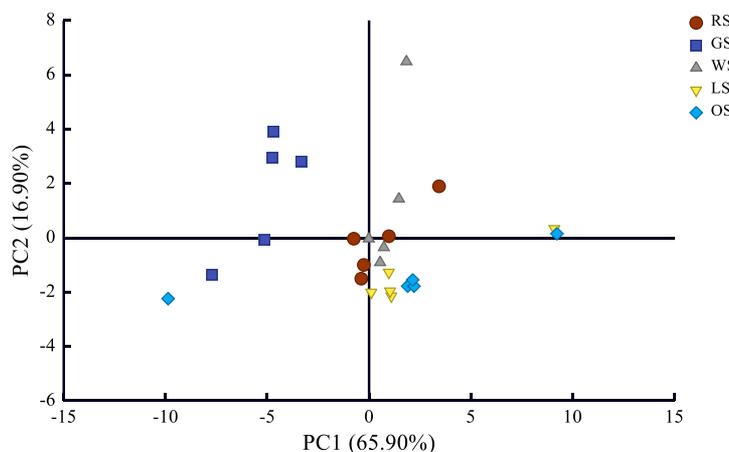


Fig. 1: Electronic nose PCA discrimination of five kinds of sufu

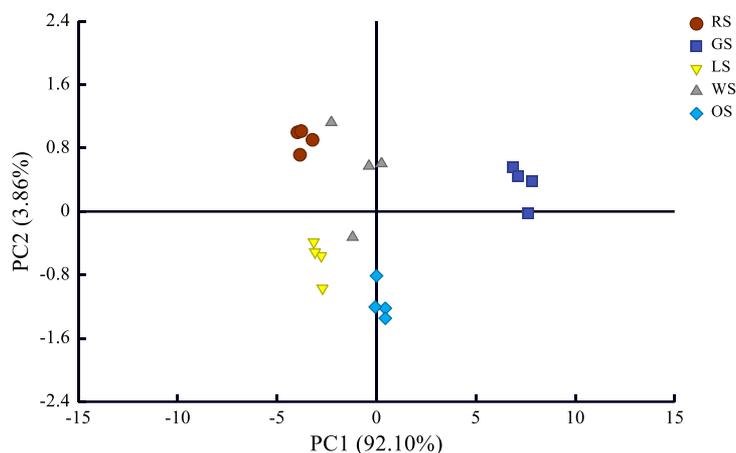


Fig. 2: Electronic tongue PCA discrimination of five kinds of sufu

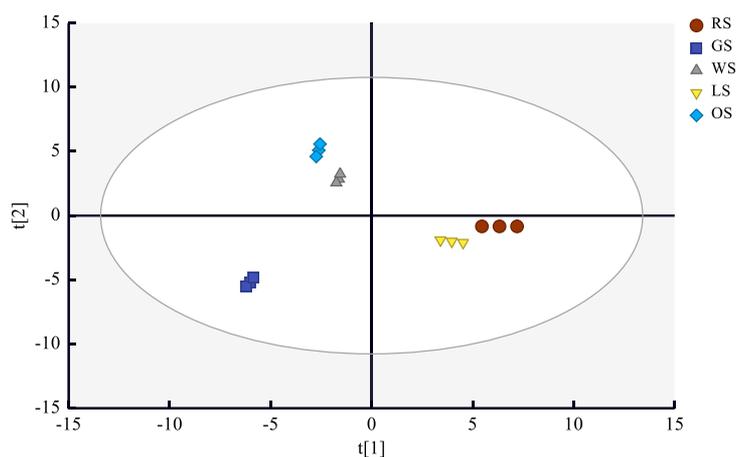


Fig. 3: The score plot of PLS-DA based on volatile flavor components

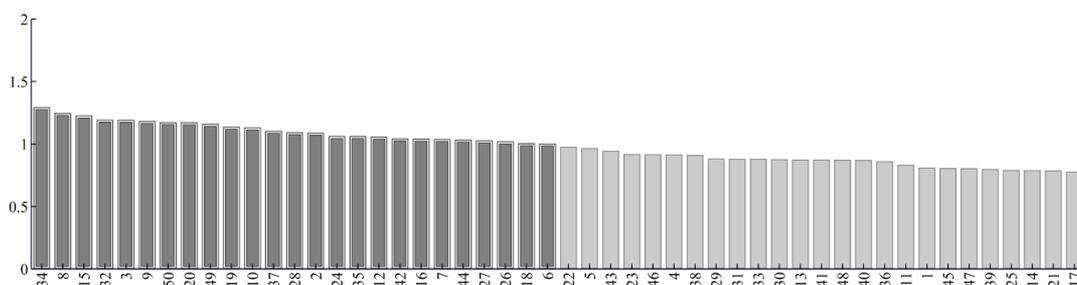


Fig. 4: Distribution of VIP forecast values of PLS-DA (1. Ethanol; 2. 1-hexanol; 3. (2R, 3R) - (-) -2,3-butanediol; 4. Linalool; 5. Terpinen-4-ol; 6. Furfuryl alcohol; 7. Benzyl alcohol; 8. Phenyl ethanol; 9. 2,3-butanediol; 10. 3-methylthiopropanol; 11. Ethyl caprate; 12. Ethyl palmitate; 13. Ethyl linoleate; 14. Ethyl tetradecanoate; 15. 2,2,4 - trimethyl-1,3 - pentanediol diisobutyrate; 16.3-Ethyl phenylpropionate; 17. Ethyl octanoate; 18. Ethyl caprate; 19. Dimethyl glutarate; 20. Dimethyl adipate; 21. Ethyl nicotinoate; 22. Ethyl-2-ethyl hexanoate; 23. Ethyl nonylate; 24. Diethyl succinate; 25. Ethyl phenylacetate; 26. Ethyl dodecanoate; 27. Ethyl pentadecanoate; 28. Dimethyl succinate; 29. Ethyl stearate; 30. Ethyl oleate; 31. Ethyl 9,12,15-octadecyltriene; 32. Anisaldehyde; 33. 2- phenylcrotonaldehyde; 34. Nonanal; 35. Benzaldehyde; 36. Camphor; 37. 2-methoxy-3- (2-propenyl) phenol; 38. Phenol; 39. 2-methoxy-4-vinylphenol; 40. Propionic acid; 41. Butyric acid; 42. Acetic acid; 43. 2-(2-ethoxyethoxy) - ethanol; 44. Estragole; 45. Styrene; 46. Anethole; 47. 2-pentylfuran; 48.Indole; 49. Naphthalene; 50. azulene. Dark gray represents VIP >1)

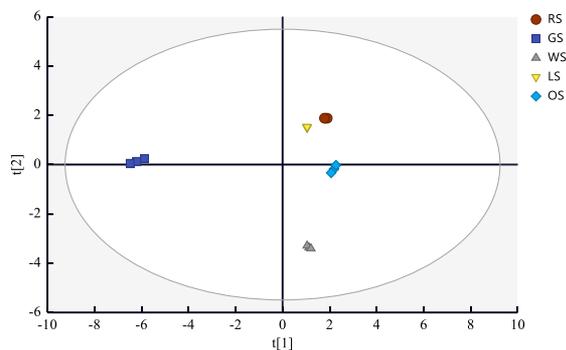


Fig. 5: The score plot of PLS-DA based on free amino acids

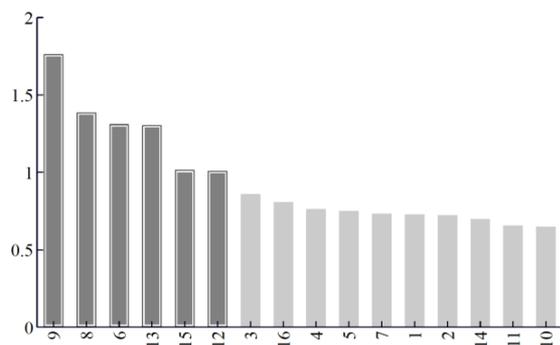


Fig. 6: Distribution of VIP forecast values of PLS-DA based on free amino acids (1. Asp; 2. Glu; 3. Thr; 4. Gly; 5. Ala; 6. Ser; 7. Val; 8. Phe; 9. Met; 10. Ile; 11. Leu; 12. His; 13. Tyr; 14. Pro; 15. Lys; 16. Cys. Dark gray represents VIP > 1)

Discussion

In this study, the types and contents of the volatile compounds in different sufu were various, but alcohols and esters were dominant in all samples (except GS). The results were similar to the report of Chung (2000). Moy *et al.* (2012) detected 52 volatile flavor components in enzyme-ripened sufu, which was lower than that detected in this study. This is mainly caused by different processing technologies. Enzyme-ripened sufu was fermented with rice-soybean koji mash, while the sufu in this study was fermented with *Mucor*. The content of ethanol in GS was significantly lower than that in other sufu, which may be mainly related to the production technology. Generally, the dressing mixtures added to RS and WS during the post-fermentation contain alcohol, while the most ingredients in seasoning mixtures of GS are yellow serofluid. Hwan and Chou (1999) explored the effect of ethanol addition during post-fermentation period on volatile components of sufu and the results indicated that only a small amount of alcohols were produced by multiple enzymes during ripening and most alcohols were added by exogenous sources. Phenyl ethanol, an alcohol shared in all sufu, can be formed by phenylalanine

catabolism, which provided rose-like sweet floral flavor for sufu (Ardo, 2006). In addition, the methyl ester compounds in OS were more abundant. Wei *et al.* (2021) also detected methyl esters in oil sufu and the main component being methyl salicylate. Sufu also contains ingredients beneficial to human health. For example, Ji (2020) in the study about volatile components in commercial sufu mentioned that ethyl linoleate can reduce cholesterol and blood lipids, with fewer side effects than linoleic acid with the same effect.

The concentration of aldehydes in the tested samples was lower, which may be attributed to the chemical properties of aldehydes themselves. Their properties are relatively reactive and they are easily oxidized to corresponding acids or reduced to corresponding alcohols during fermentation (Smit *et al.*, 2009). 2-methoxy-4-vinylphenol is the aroma active ingredient of Shaoxing rice wine (Chen *et al.*, 2013). At the same time, rice wine can also be used as the soup component of post fermentation. So, 2-methoxy-4-vinyl phenol may be derived from the mixed soup and the thermal degradation of lignoside or ferulic acid (Wang *et al.*, 2019). Previous studies have reported that lactic acid can be converted to butyric acid by butyrate bacteria (Bourriaud *et al.*, 2005, Louis and Flint, 2009). Therefore, high concentrations of butyric acid in GS may be related to *Clostridium butyricum*. Moreover, sufu also contained propionic acid. Song *et al.* (2021) also detected propionic acid in grey sufu and indicated that excessive intake of propionic acid would lead to vomiting, nausea and other symptoms. Chinese national standard GB 2760-2014 stipulates that the content of propionic acid in bean products shall not exceed 2.5 g/kg. In this study, the content of propionic acid in sufu was low. So, eating a small amount of sufu will not cause harm to human body. The content of indole in sufu was different from that reported by Huang *et al.* (2018). In addition, Huang *et al.* (2018) also found that the synthesis of indole in sufu was related to *Weissella* and *Lactococcus*. Previous studies have shown that there were some same microorganisms in various sufu, but their abundance was obviously different (Tan *et al.*, 2020). Therefore, the content of flavor substances in sufu is also different. Sufu is a complex food matrix and its flavor will be affected by raw materials, microorganisms, processing technology and other factors.

Compared with WS, the level of total free amino acid in RS was lower, which was also the same case in the report of Tan *et al.* (2020). Xie *et al.* (2019b) showed that the content of bitter and umami amino acids in different sufu were rich and the highest detectable amount bitter amino acids in grey sufu was Leu. The results of this study were similar. The formation of these bitter amino acids may be related to the imbalance of protein and peptide hydrolysis. Some studies have pointed out that there is an interaction between bitterness and freshness. When Phe (0.5-5.0 mM) was added, the umami tastes can

significantly improved (Chen and Zhang, 2007, Kim *et al.*, 2015). Therefore, Phe in sufu will affect its umami characteristics to a certain extent. The interaction between different amino acids also affects the flavor characteristics of sufu (Lioe *et al.*, 2005).

Electronic nose and electronic tongue had different identification effects on various productions. Chen *et al.* (2018) research showed that jujubes could be classification by E-nose technology combined with multivariate statistical analysis. It shows that the electronic nose has certain applicability in the differentiation of products. Xing *et al.* (2021) distinguished the processing degree of *Polygonum multiflorum* using electronic tongue. In addition, some studies also showed that the electronic tongue technology can be applied to milk, meat and so on (Di Rosa *et al.*, 2017). In this study, the electronic tongue has an obvious effect on distinguishing all kinds of sufu, but the electronic nose can only distinguish GS. GS contained some volatile components with strong irritation, such as indole and sulfide, which were significantly different from other types of sufu. Therefore, the electronic nose was easier to distinguish grey sufu. In addition, Di Donato *et al.* (2021) showed also volatile substances (obtained by HS-SPME/GC-MS) coupled with PLS-DA was effective in the discrimination of typical Italian Pecorino cheeses. Meanwhile, fourteen components related to the discrimination of the Pecorino cheeses were screened by VIP value. This experiment also used this method to reach the aim of distinguished the types of sufu.

Conclusion

The present study revealed the volatile flavor compounds of five kinds of sufu by HS-SPME/GC-MS method. The flavor characteristics of different sufu were diversities. The total content of volatile flavor compounds in GS was the highest, followed by LS and the lowest in WS. Esters were the most abundant flavor substances in all sufu samples, which had a higher contribution to the sufu characteristic flavors. Evaluation of amino acid profiles from commercial sufu demonstrated that Glu, Val, Leu and Phe were high content taste amino acids in sufu. Compared with other sufu, GS has the defect of insufficient umami. PCA combined with electronic tongue could accurately discriminate all kinds of sufu. In contrast, the electronic nose could only distinguish grey sufu. The PLS-DA analysis based on volatile components and flavor amino acids could clearly distinguish five different types of sufu and differential compounds were screened, such as nonanal, phenyl ethanol, 2,2,4-trimethyl-1,3-pentanediol diisobutyrate, anisaldehyde, Met, Phe, Ser, Tyr and Lys. In summary, when the specific components are known, PLS-DA analysis can accurately distinguish various samples from the perspective of aroma and taste to achieve the purpose of

identification. When the specific components are unknown, electronic tongue is more suitable for distinguishing various types of sufu. In order to ensure the reliability of the experimental results, electronic tongue combined with HS-SPME/GC-MS technology can be used. These results are applicable to the quality control of sufu and the development of new products, which is beneficial to the development of sufu industry. The effect of microorganisms on the flavor of sufu was not analyzed in this study. After that, we can further study the core microbial flora of sufu and screen out the strains related to flavor, which are particularly important for standardized sufu production.

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Author's Contributions

Xiaoli Xi: Has contributed in all experiments, paper writing and publication.

Yanli Ma and Jingxuan Ke: Have contributed in the paper writing, reviewed and revised the manuscript.

Yinzhuan Wang and Xiaodong Gu: Have assisted in the experiment.

Yaqiong Liu, Jianfeng Sun and Jianlou Mu: Has organized the study, reviewed and revised the manuscript.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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