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Full Length Research Paper

Flavor and taste compounds analysis in Chinese solid fermented soy sauce

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The analysis of free amino acid and volatile compounds were conducted to understand the changes in taste and flavor of five samples which were solid fermented. The values of bitter, sweet and MSG-like free amino acids in these soy sauces were significantly different. The taste of soy sauce was predominated by saltiness, followed by moderate "umami" and sweet taste and slight bitterness, likely as a result of the balance and interaction among different taste components. A total of 82 kinds of volatile compounds were identified, including alcohols, acids, esters, aldehydes, ketones, phenols, heterocyclic compounds, alkynes and benzenes. The subtle aroma of the soy sauce seemed to depend not only on particular key compounds but also on a "critical balance" or a "weighted concentration ratio" of volatile compounds.

Key words: Free amino acid, volatile flavor compounds, soy sauce, HPLC, HS-SPME-GC-MS.

INTRODUCTION

Over thousands of year, Chinese have utilized micro-organisms to convert agricultural commodities into fermented food. These fermented foods have improved the qualities in the diets of Chinese people and also enriched dishes all over the world. Traditional Chinese fermented foods covers a wide range of products, such as soy sauce, sufu, vinegar, distilled spirits, rice wines, fermented vegetables and meat products (Bei-Zhong et al., 2001). Soy sauce is a kind of fermented soybean food, which is used as a condiment or seasoning sauce worldwide.

It is well known that proteolysis is the most principal and complex biochemical event occurring during the preparation of soy-fermented condiments. The degradation products, amino acids, not only have a considerable influence on the nutritional values, but also contribute directly to the taste characteristics, in some cases serving indirectly as precursors of aromatic products (Kiers et al., 2000; Han et al., 2004). It is well established that the water-soluble fraction contains the majority of taste compounds such as salt and amino acids produced during proteolysis (Kim and Lee, 2003). Consequently, quality indexes such as free amino acids of many soy-fermented foods have been reported (Omafuvbe et al., 2000; Kim and Lee, 2003; Han et al., 2004). However, little reports about Chinese solid fermented soy sauce's sensory properties such as taste have been presented.

The characteristic of flavor formation in the soy sauce depends on the manner of production process, as well as raw materials, fermentation mode and strains. The main steps of soy sauce production involved in flavor development are heat treatment of raw materials, koji culturing (mold fermentation), moromi fermentation including aging, and pasteurization (Nunomura and Sasaki, 1993). Most of the studies on the volatile flavor compounds in traditional soy sauce made in several regions such as Japan, Korea, including Indonesia have been reported (Kim et al., 1996; Kobayashi and Sugawara, 1999; Seo et al., 1996). Meanwhile, the aromatic volatile fractions of some soy-fermented foods such as Thai soy sauce, Chinese fermented soybean curd, Korean soybean paste and Korean soy sauce have been studied (Leejeerajumnean

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et al., 2001; Lertsiri et al., 2001; Wanakhachornkrai and Lertsiri, 2003; Lee et al., 2006).

In recent years, many advanced sampling technique and modern instrumental analysis have been developed rapidly including vacuum-simultaneous dissolvent extract (V-SDE), gas chromatography-olfaction (GC-O), and head-space solid-phase micro-extract gas chromatography mass spectrum (HS-SPME-GC-MS). They are used for fast volatile compound identification in food flavor research (Hyung and Hyung, 2004; Hyung and Hyung, 2005). Because of its simple operation and no solvent disturbance, HS-SPME-GC-MS has been used in volatile compound analysis especially in the Chinese traditional fermented soybean food analysis (Likang and Xiaolin, 2007). The fermentation time of Chinese solid fermented soy sauce is much shorter than the traditional process. However the study on volatile flavor compounds and the HS-SPME-GC-MS used in analysis of volatile compounds in Chinese solid fermented soy sauce has not been well conducted.

In order to optimize manufacturing of soy sauce and standardize quality aspects of Chinese solid fermented soy sauce, this study (1) investigates the formation of proteolysis taste active compounds by the assessment of free amino acid profile, and (2) identifies the complete profiles of odorants responsible for the unique flavors in solid fermented soy sauce using HS-SPME-GC-MS.

MATERIALS AND METHODS

Figure 1 shows a procedure of solid fermentation of soy sauce. Solid fermented soy sauces (A, B, C, D and E) were made by using different mold seed culture. These cultures were different in inoculate by different *Aspergillus oryzae* (A, B, C, D and E) in making mold seed which were used in different factories in China. They were all used in soy sauce fermentation respective or combination. The raw materials of these soy sauces are defatted soy bean and wheat bran.

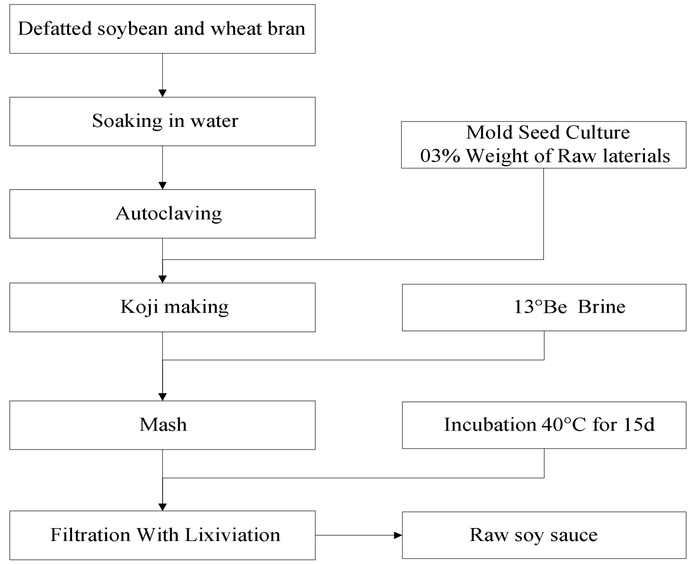

Figure 1. Procedure for manufacturing soy sauce.

Free amino acids analyses

For determination of individual free amino acids, 10 mL samples was precipitated by using 50 mL of 10 % trichloroacetic acid (TCA) for 2 h at ambient temperature to remove large peptides and then centrifuged at 10 000 rpm for 10 min. The supernatant was filtered through 0.45 µm filter. The sample solution prepared in 20 µL was analyzed by an Agilent HP1100 HPLC (Agilent, USA) equipped with an Agilent Zorbax 80A Extend-C_{18} column (150 × 4.6 mm i.d., particle size 5 µm), an o-phthalaldehyde (OPA) forward-column derivatisation autosampler, and a UV detector. The mobile phases were A, 20 mM sodium acetate (pH = 7.2) with 0.5% tetrahydrofuran; B, 20 mM sodium acetate (pH = 7.2)/ methanol/acetonitrile (1:2:2, by volume).The linear elution gradient was A:B (by volume) from 100:0 to 50:50 for 0 - 17 min, 50:50 to 0:100 for 17 - 20 min, and 100:0 for 20 - 24 min. The flow rate was 1.0 mL/min. The temperature was controlled at 40℃. The amino acids were detected at 338 nm except for proline, which was detected at 262 nm. Each amino acid was identified by comparing the samples with a standard (Sigma) analyzed under the same conditions and quantified by the calibration curve of the authentic compound.

Free amino acid grouping

According to the taste characteristics as described by Tseng et al. (2005), amino acids were grouped as MSG-like (monosodium glutamate-like) (Asp+Glu), sweet (Ala+Gly+Ser+Thr), bitter (Arg+His+Ile+Leu+Met+Phe+Trp+Try+Val), and tasteless (Cys+Lys+Pro).

Headspace Solid-Phase Micro-Extraction (HS-SPME)

Ten milliliter (10 mL) of the soy sauce sample was placed into a 15 mL headspace vial and pre-equilibrated for 15 min at 50℃ in a thermostatic bath with a vial capped using a silicon septum. Afterwards, a stainless steel needle, on which 85 µm polyacrylate (PA) (swagelok, USA) fiber was housed, was pushed through the vial septum. The fiber was pushed out of the housing and exposed to the headspace (3 cm in depth) at 50℃ for 40 min. After extraction, the fiber was pulled into the housing, and the SPME device was removed from the vial and inserted into the injection port of GC for thermal desorption of the analysis.

Gas Chromatography–Mass Spectrum (GC-MS)

GC-MS analysis was performed with Trace GC-MS (Finnigan, USA). Among which the gas chromatograph was equipped with flame ionization detector (FID). The injection was conducted in splitless mode at 250℃ for 3 min by using an inlet liner of 0.75 µm i.d. to minimize peak broadening. Chromatography separations were performed by using DB-WAX analytical capillary column 30 m × 0.25 mm × 0.25 µm (J & W Scientific, Folsom, CA) with helium as carrier gas at a constant flow rate of 0.8 mL/min. The oven temperature was programmed at an initial temperature of 40℃ for 2 min, followed by an increase of 5℃/min to 150℃ (held for 5 min), and finally at 10℃/min to 220℃ (held for 10 min). The temperature of the FID was set to 220℃. MS operating conditions (electron impact ionization mode) were an ion source temperature of 200℃, ionization voltage of 70 eV and mass scan range of m/z 33 – 450 at 2.76 scans/s.

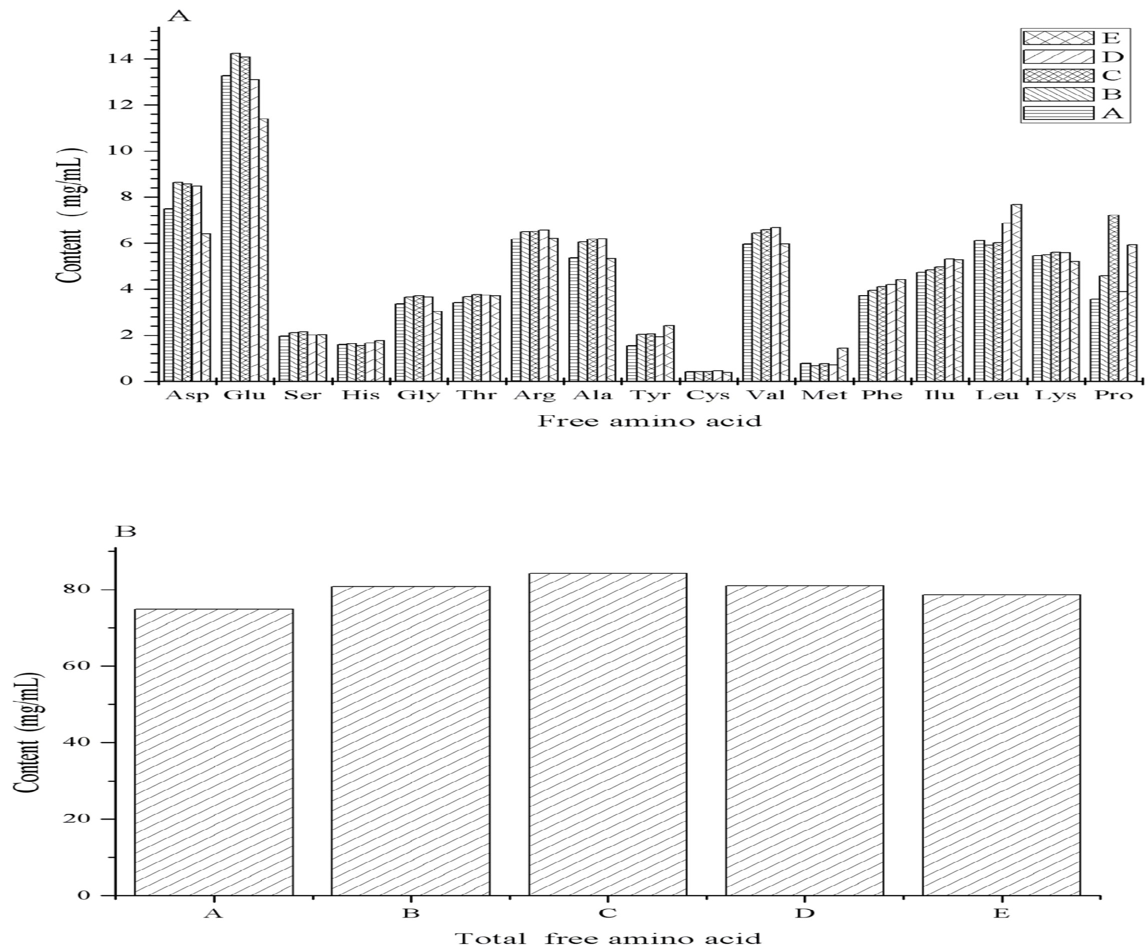

Figure 2. Content evolutions of the individual free amino acid (A), total free amino acid (B) in different type soy sauce.

Volatile compound identification and quantification

The chromatography peak identification was carried out by comparing their mass spectra with those of the bibliographic data of known compounds from the WILEY 6 library (Hewlett-Packard Co., Palo Alto, CA) and NIST 98 library (Hewlett-Packard Co. , Palo Alto, CA) mass spectral database on the basis of the criterion similarity (SI)>800 (the highest value is 1,000). According to the method (Wanakhachornkrai and Lertsiri, 2003) approximate quantification of volatile compounds was estimated by the integration of peaks on the total ion chromatogram using Xcalibur software (Vienna, VA). All analyses were performed in duplicate. Results are presented as means of the area normalized (%).

RESULTS AND DISCUSSION

Changes of free amino acid

The content of individual free amino acid and total free amino acid in different fermented soy sauces are shown in Figure 2A and B. As shown in Figure 2, free amino acids were significant different in five samples. Basically coinciding with the evolution patterns of most free amino acid, the highest content of total free amino acid was 80.17 mg/mL in soy sauce C, and the lowest content of total free amino acid was 65.52 mg/mL in soy sauce E. The profiles of five major free amino acid (Glu, Asp, Arg, Val and Ala) are similar to the patterns of the change of total free amino acid content in five different soy sauces.

The free amino acids groupings, based on their taste characteristics as described by Tseng et al. (2005), are shown in Figure 3. The highest content of two free amino acid classes, MSG-like and sweet free amino acid, was 28.32 and 19.15% in soy sauce B. But for tasteless free amino acid class, the peak content level 15.72% appeared at the soy sauce C, and the peak content level of bitter free amino acid 44.74% appeared at the soy sauce E.

Saltiness and umami taste were observeded as the predominant tastes, while bitterness was slight. However, bitter free amino acid was found much higher than MSG-

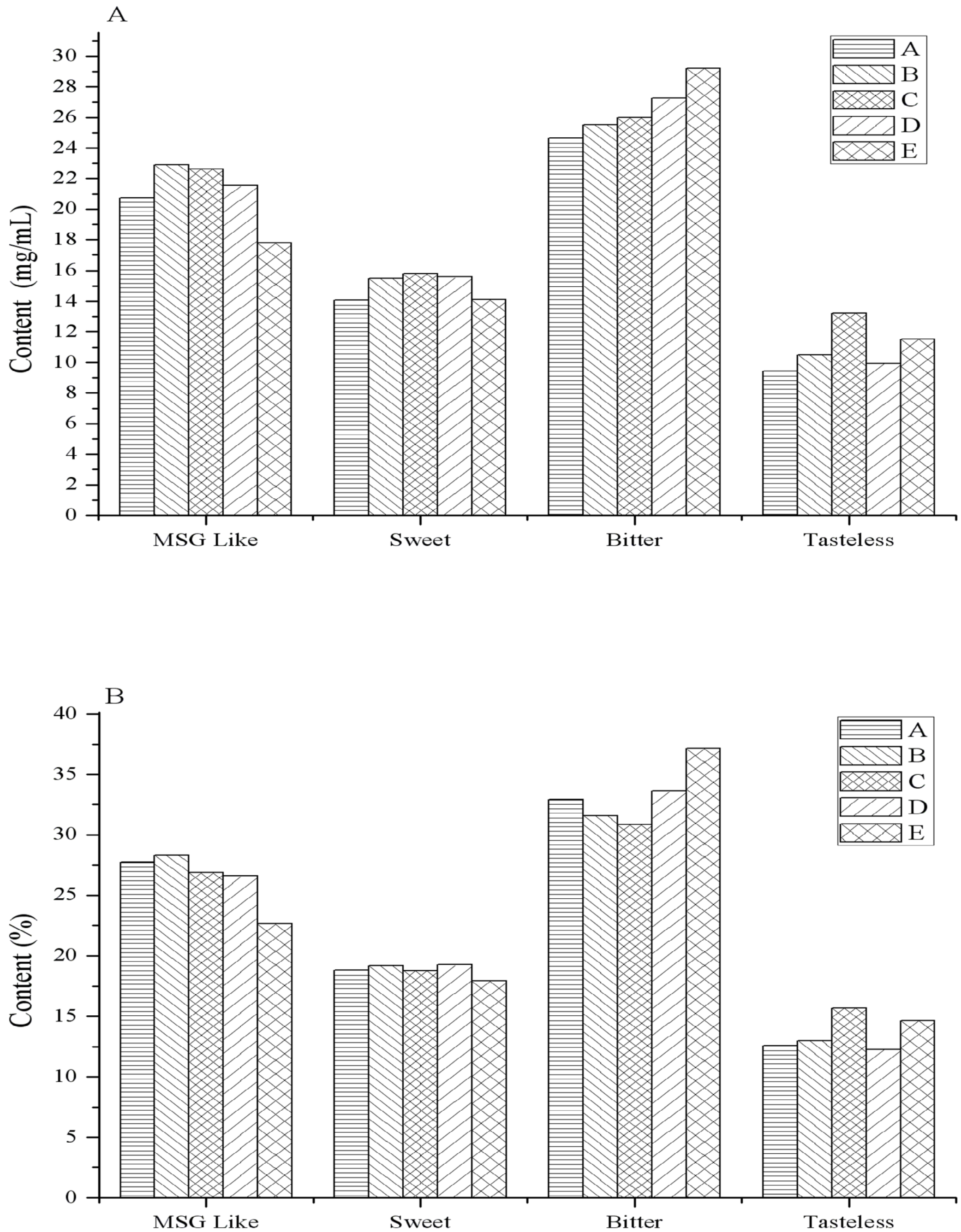

Figure 3. Content evolutions of the individual free amino acid classes (A: content in mg/mL; B: content in percent) imparted the different taste in different type soy sauce.

like and sweet free amino acids in our products. The phenomenon may be explained by the fact that the final characteristic taste of soy sauce was definitively determined by the balance and interaction between different taste components. The predominant taste saltness was directly related to the NaCl at much higher concentrations (12 – 14% dry weight). More intensive umami taste than expected was possibly due to the higher content of aspartic acid and glutamic acid (Tseng et al., 2005). The changes of unpleasant bitterness, derived from bitter free amino acids, may be attributed to the diminishing or masking effect of saltiness, umami taste, sourness and sweetness (Kim and Lee, 2003).

In these five kinds of soy sauces, the species of the free amino acid are same, while the quantities of each free amino acid change a lot, which cause the tastes of these five kinds of soy sauces different. Therefore, further study is needed for the interaction of taste components to eliminate or suppress the bitter taste and to enhance the umami and sweet taste in soy sauce.

Formation of volatile compounds

Volatile compounds were also analyzed to understand the unique flavor of soy sauce. As seen in Table 1, a total of 55, 45, 40, 44 and 48 kinds of components were identified in these five soy sauces samples, respectively, which accounted for 84.48, 89.27, 88.27, 84.48 and 87.70% of each volatile profile. In total, 82 kinds of volatile compounds were identified, including alcohols (12), acids (16), esters (5), aldehydes (10), ketones (10), phenols (5), heterocyclic compounds (17), alkanes (6) and benzenes (1). There were significant differences in the compositions of volatile components in different soy sauce.

The above compounds were identified by comparison of retention time (RT) and mass spectral with those of NIST 98 (75 K compounds) and WILEY 6 (107 K compounds) databases and listed in order of their elution sequences. Results are presented as means of the area normalized (%) in duplicated.

A total of 16 kinds of free fatty acid were isolated from the five samples and their relative ratio to the total volatiles in the order of 52.38, 48.43, 50.66, 51.31 and 33.09% respectively. Acetic acid, propanoic acid, 2-methyl-propanoic, 3-methyl-pentanoic acid and 4-methyl-pentanoic acid were found in all five samples. Acetic acid is the main compound in these acidic compounds which contribute to the flavor. And pentanoic acid also was the important compound in quantity but not detected in soy sauce E.

A total of 20 kinds of carbonyl compounds were detected, including 10 ketones and 10 aldehydes, as2-methylbutanal,1-hydroxy-2-propanone,2,6-di -butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one, phenyl acetaldehyde, at low concentration (4.0 to 5.6%) in the five samples. The evolution of carbonyl compounds may be explained by the fact that ketones and aldehydes are intermediate unstable compounds being easily reduced to alcohols (Estrella et al., 2004).

A total of 5 kinds of ester compounds were found to constitute in 7.85, 1.59, 8.67, 3.17 and 5.57% of the total volatile fraction in the five samples. The importance of these esters which contribute to food aroma is undisputed with the fact that esters with low carbon atoms are highly volatile at ambient temperatures and the perception thresholds are 10 times lower than their alcohol precursors (Izco and Torre, 2000; Nogueira et al., 2005). In addition to imparting a fruity floral character, esters can diminish or mask the sharpness of unpleasant free amino acid-derived notes. These esters are formed by esterification between the short-chain acids and the alcohols.

Alcohols constituted approximately from 8.77 to 25.61% of the total volatiles detected in five different soy sauces. Among which 3-methyl-1-butanol, 2-(2-ethoxyethoxy)-ethanol, 3-methylthio-1-propanol and phenyl ethyl alcohol were detected in all five samples and 3-methylthio-propanal might attribute to a powerful meaty and soy sauce-like odor and flavor at high dilution (Chung, 1999). But ethanol was detected only in the soy sauce E.

Phenols constituted 2.08, 2.56, 2.18, 2.92 and 2.26% of the total volatiles in the five samples. Four important savory compounds, phenol, 2-methoxy-phenol (guaiacol) and 2-methoxy- 4-vinyl phenol were detected in all these five soy sauces. Phenol and 4-ethylphenol were believed to generate from the degradation of lignin glycoside during the fermentation (Kobayashi and Sugawara 1999), while 2-methoxy-4-vinylphenol characterizing the cooked soybean, together with 2-methoxy-phenol (guaiacol) were thought to be the thermal degradation products of lignin-related phenolic carboxylic acids (Chung, 1999).

Heterocyclic compounds are powerful aromatic compounds with very low odor threshold values in most heat-treated foods. 17 kinds of heterocyclic compounds were detected: five pyrazines (methyl pyrazine, 2,5-dimethyl pyrazine, 2,6-dimethyl pyrazine, 2-ethyl-6-methylpyrazine, trimethylpyrazine), five furans (tetrahydro-3-methyl-5-oxo-2-furancarboxylic, tetrahydro-2,2-dimethyl-5-(1-methylethy-l)-furan, 2-furanmethanol, 2(5H)-furanone, 2,5-dimethyl-4-hydroxy-3(2H)-furanone (HDMF)), three pyrans (4- benzoyloxy-2H-pyran-3-one, 3-hydroxy-2,6-dimethyl-4H-pyran-4-one, 3,5-dihydroxy-6-methyl-4H-pyran-4-one), two pyrrols (1-(1H-pyrrol-2yl)-ethanone, 2-carboxaldehyde-1H-pyrrole) and one pyridine(3-phenyl-pyridine). Among which 2,6-dimethylpyrazine, 2,5- dimethyl-4-hydr oxy-3(2H) furanone (HDMF) and 2-furanmethanol were detected in all five samples. Alkyl pyrazines have a nutty aroma and could be generated naturally during the aging process by the condensation of amino ketones formed through the Maillard reaction and Strecker degradation (Sarkar et al., 2002). HDMF detected in all the five soy sauces also has been reported as the character impact compound of Japanese soy sauce. Pyrone and pyridine could be the products of the Maillard reaction. Low concentration in the soy sauce may be attributed to its floral note (Chung, 1999).

Conclusion

Flavor is a key parameter that determines the overall acceptability of food quality. Each type of soy-fermented food has its characteristic profile of free amino acids that results from the balance of the degradation of the peptides to free amino acids and the inter-conversion of the

Table 1. Volatile compounds in five different soy sauce (A, B, C, D and E) by headspace solid-phase microextraction and chromatography-mass-spectrometry (SPME-GC-MS).

RT[1] (min)	Compounds	Area normalized (%)				
		A	B	C	D	E
	Alcohols (12)	**8.77 (6)**	**14.59 (7)**	**10.58 (6)**	**9.48 (5)**	**25.61 (8)**
3.76	ethanol	ND [2]	ND	ND	ND	3.47
7.27	2-methyl-1-propanol	0.17	0.09	ND	ND	0.70
9.89	3-methyl-1-butanol	1.34	1.90	1.78	1.47	5.63
16.27	2-ethyl-1-hexanol	0.22	ND	ND	ND	0.35
18.28	2,3-octanediol	ND	ND	0.28	ND	ND
18.37	5-methyl-2-(1-methylethyl)-cyclohexanol	ND	0.22	ND	ND	ND
18.64	4-methyl-3-methylidenepentane-1,2-diol	ND	ND	0.40	ND	ND
18.81	2-(2-ethoxyethoxy)-ethanol	0.85	1.80	1.14	0.98	1.02
19.16	L-(-)-menthol	ND	1.82	ND	ND	ND
20.40	3-methylthio-1-propanol	0.34	0.48	0.34	0.40	2.19
22.76	benzyl alcohol	ND	ND	ND	0.18	0.23
23.26	phenyl ethyl alcohol	5.85	8.28	6.64	6.45	12.02
	Acids(16)	**52.38 (13)**	**48.43 (7)**	**50.66 (6)**	**51.31 (9)**	**33.09 (10)**
15.29	acetic acid	11.20	17.27	19.92	16.70	20.63
15.61	4-methyl-2-oxovaleric acid	ND	ND	ND	ND	0.57
17.22	propanoic acid	0.29	0.46	0.55	0.53	0.21
17.78	2-methyl-propanoic	1.70	2.61	2.94	2.51	1.64
19.59	pentanoic acid	11.51	20.32	19.67	18.56	ND
19.61	2-methyl-butanoic acid	ND	ND	ND	ND	9.24
21.10	benzene propanoic acid	ND	0.27	ND	ND	ND
21.64	3-methyl-pentanoic acid	0.52	1.21	1.17	1.26	0.30
21.82	4-methyl-pentanoic acid	3.23	6.29	6.41	7.15	0.50
23.85	2-ethyl-hexanoic acid	0.34	ND	ND	0.87	ND
21.10	benzene propanoic acid	0.27	ND	ND	ND	ND
26.57	nonanoic acid	0.90	ND	ND	ND	ND
28.00	decanoic acid (capric acid)	11.04	ND	ND	1.00	ND
32.87	Tetradecanoic acid (myristic acid)	10.07	ND	ND	2.73	ND
33.48	Z-9-tetradecenoic acid	0.63	ND	ND	ND	ND
34.60	Pentadecanoic acid (pentadecylic acid)	0.68	ND	ND	ND	ND
	Esters (5)	**7.85 (5)**	**1.59 (2)**	**8.67 (3)**	**3.17 (2)**	**5.57 (3)**
22.67	isobutanoic acid 3-hydroxy-2,2,4-trimethylpenthyl ester	0.48	ND	0.63	ND	0.85
22.89	2,2,4-trimethyl-1,3-pentanediol diisobutyrate	0.27	ND	ND	ND	ND
28.85	diethyl phthalate	0.51	0.23	0.87	ND	0.52
30.68	isobutyl phthalate	6.46	1.36	7.17	2.80	4.20
32.63	1,2-benzenedicarboxylic acid dibutyl ester	0.13	ND	ND	0.37	ND
	Aldehydes (10)	**5.4 (8)**	**3.42 (3)**	**3.70 (4)**	**6.63 (6)**	**1.92 (4)**
3.31	2-methyl butanal	1.0	1.39	1.32	1.42	0.33
11.72	octanal	ND	0.05	ND	ND	ND
14.11	nonanal	0.11	ND	ND	ND	0.11
16.42	decanal	0.16	ND	ND	ND	ND
16.79	benzaldehyde	0.36	ND	0.56	0.56	ND
19.03	phenyl acetaldehyde	1.02	1.98	1.49	1.70	1.14

Table 1. Contd.

19.15	4-methylbenzaldehyde	ND	ND	0.33	ND	ND
23.51	2-phenyl-2-butenal	0.30	ND	ND	0.48	ND
23.65	methyl phenyl pentenal	0.16	ND	ND	0.20	ND
24.78	5-methyl-2-phenyl-2-hexenal	2.29	ND	ND	2.27	0.34
	Ketones (10)	**1.73 (4)**	**4.72 (7)**	**3.62 (6)**	**1.26 (3)**	**4.31 (4)**
11.76	3-hydroxy-2-butanone	ND	ND	0.09	ND	ND
11.89	1-hydroxy-2-propanone	0.21	0.11	0.33	0.13	0.44
19.31	4-(benzoyloxy)-2H-3-one	0.24	0.38	ND	ND	ND
19.21	acetophenone	ND	ND	0.31	ND	ND
21.34	2,2,6-trimethyl-1,4-cyclohexanedione	ND	0.10	ND	ND	ND
23.51	ethylidene-benzeneacetaldehyde	ND	ND	0.63	ND	ND
25.41	5-methyl-2-phenyl-2-hexenal	ND	2.35	ND	ND	ND
25.70	2,6-di(t-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one	1.16	0.93	1.91	0.63	3.10
27.00	megastigma trienoone	0.12	0.37	ND	ND	0.38
30.07	diphenyl-methanone	ND	0.48	0.35	0.50	0.39
	Phenol (5)	**2.08 (5)**	**2.56 (4)**	**2.18 (4)**	**2.92 (4)**	**2.26 (4)**
22.41	1-naphthalenol	0.28	0.37	0.26	0.52	0.25
22.50	2-methoxy-phenol	0.85	1.12	0.86	1.15	1.16
24.47	phenol	0.51	0.64	0.64	0.51	0.42
26.50	2-methoxy-4-(2-propenyl)-phenol	0.18	ND	ND	ND	ND
26.84	2-methoxy-4-vinyl-phenol	0.26	0.43	0.42	0.74	0.43
	Heterocyclic compounds (17)	**2.87 (10)**	**9.9 (13)**	**4.74 (8)**	**5.81 (12)**	**9.73 (12)**
11.19	methyl pyrazine	0.20	0.18	0.25	0.18	ND
12.52	2,5-dimethyl pyrazine	0.17	0.28	0.28	0.26	ND
12.70	2,6-dimethyl pyrazine	0.25	0.52	0.50	0.51	0.18
14.01	2-ethyl-6-methyl pyrazine	ND	0.07	ND	0.06	ND
14.42	trimethyl pyrazine	0.13	0.09	ND	0.03	0.03
18.04	tetrahydro-3-methyl-5-oxo-2-furancarboxylic	0.15	0.25	ND	ND	1.14
18.63	tetrahydro-2,2-dimethyl-5-(1-methylethyl)-furan	0.20	0.32	ND	0.33	0.20
19.31	4-benzoyloxy-2H-pyran-3-one	ND	ND	ND	ND	1.60
19.42	2-furanmethanol	0.55	1.49	1.60	1.44	0.59
20.91	2(5H)-furanone	ND	ND	ND	ND	0.25
23.96	3-hydroxy-2,6-dimethyl-4H-pyran-4-one	0.30	0.47	0.39	0.32	0.33
24.06	1-(1H-pyrrol-2yl)-ethanone	ND	3.50	ND	ND	ND
24.77	2-carboxaldehyde-1H-pyrrole	ND	0.76	0.43	0.69	0.63
24.91	2,5-dimethyl-4-hydroxy-3(2H)furanone	0.23	0.58	0.42	0.45	1.93
25.94	parabanic acid(2,4,5-trioxoimidazolidine)	ND	ND	ND	ND	2.25
27.56	3-phenyl-pyridine	0.92	1.39	0.87	1.02	ND
27.75	3,5-dihydroxy-6-methyl-4H-pyran-4-one	ND	ND	ND	0.52	0.60
	Alkanes (6)	**3.4 (4)**	**1.9 (2)**	**3.62 (2)**	**3.90 (3)**	**4.55 (2)**
21.09	1-(4-methoxyphenyl)-1-methoxypropane	0.25	ND	ND	0.37	0.28
22.89	2,2,4-trimethyl-1,3-dioldiisobutyrate-pentane	ND	ND	0.35	ND	ND
23.42	2-phenylethene	ND	0.34	ND	0.36	ND
24.06	5-bromo-3-methylidene-1-methoxycyclohexane	1.74	ND	3.27	3.17	4.27
25.89	1-methyl-2-(phenylmethyl)-benzene	0.37	ND	ND	ND	ND
26.00	1-methyl-4-(phenylmethyl)-benzene	1.04	1.56	ND	ND	ND

Table 1. Contd.

	Benzenes (1)	**0**	**0**	**0.50 (1)**	**0**	**0.66 (1)**
30.18	7-ethoxy-8-methoxy-2,2-dimethyl-2H-chromene	ND	ND	0.50	ND	0.66
	Total volatiles (82)	84.48 (55)	89.27 (45)	88.27 (40)	84.48 (44)	87.70 (48)

[1] Retention time (RT) on DB-WBXcolumn in GC-MS.
[2] ND: not detected

different free amino acids. Soy sauce, a traditional Chinese soy fermented-condiment, was analyzed in this initial study. From the results it can be concluded that free amino acids, such as Glu, Asp, Arg, Leu, and Ala, were abundant and are recognized as important contributors to taste of the soy sauce. The final taste is determined by balance and interaction among different taste components.

Among the 82 volatile compounds identified, 20 compounds including alcohols (3), acids (5), esters (1), aldehydes (1), ketones (2), phenols (4), heterocyclic compounds (4) were found to appear in different type soy sauce at different concentrations. The main components are ethanol (alcohols), acetic acid (acids), 2-methoxy-phenol (guaiacol), 2-methoxy- 4-vinyl phenol (4-EG), and 2,5-dimethyl-4-hydroxy-3(2H)-furanone (HDMF). But others also have important influence to the flavor of soy sauce. Compare with the other soy sauces, such as Japanese soy sauce and Korean soy sauce, the other main flavor component 4-hydroxy-2-ethyl-5-methyl-3-furanone (HEMF) were not found in these solid-fermented soy sauces. It should because these soy sauce were fermented by *A. oryzae* only, while HEMF is from yeast fermentation.

From the results it could be speculated that the subtle taste and aroma of soy sauce seems to be depend on not only particular key components, but also a “critical balance” or a “weighted concentration ratio” of all components present. Therefore, further study is needed for the interaction of taste components to eliminate or suppress the bitter taste and to enhance the umami and sweet taste in soy sauce.

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