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Comparative Study on Volatile Flavor Compounds of Traditional Chinese-type Soy Sauces Prepared with Soybean and Defatted Soy Meal

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Abstract Volatile extracts obtained from traditional Chinese-type soy sauces prepared with soybean (SSSB) and defatted soy meal (SSDSM) by solid phase microextraction (SPME) and direct solvent extraction (DSE) were analyzed by gas chromatography-mass spectrometry (GC-MS). The volatile flavor compounds and relative contents of different chemical classes detected in SSSB and SSDSM were compared for their differences. Results showed that significant differences in both constituents of volatile flavor compounds and relative contents of different chemical classes were observed for both kinds of soy sauces. A total of 152 and 131 compounds were identified in SSSB and SSDSM, respectively, and 102 volatile flavor compounds were common in both kinds of soy sauces. Moreover, relative contents of acids, aldehydes, esters, furan(one)s, miscellaneous compounds, phenols, pyrazines, pyrrol(idinon)es, and sulfur-containing compounds in both kinds of soy sauces were all significantly different.

Keywords: soybean, defatted soy meal, volatile flavor compound, traditional Chinese-type soy sauce, gas chromatography-mass spectrometry (GC-MS)

Introduction

Soy sauce, which originated in China over 2500 years ago, is one of the indispensable fermentation condiments in China, Korea, Japan, and Southeast Asia countries. The annual production of soy sauce in China is more than 5,000,000 tons, accounting for over 55% of the world production (1,2). In China, soy sauce can be manufactured by methods of high-salt diluted fermentation and low-salt solid fermentation (National Standard: GB 18186-2000). Soy sauce manufactured by high-salt diluted fermentation method is generally regarded as traditional Chinese-type soy sauce, which mainly comprise of two kinds of soy sauces including soy sauces prepared with soybean (SSSB) and defatted soy meal (SSDSM).

Aforetime, only soybean rather than defatted soy meal was used as main material in traditional Chinese-type soy sauce fermentation, whereas defatted soy meal is used as main material in soy sauce manufacturing in some districts of China as it is available in large amounts and at relatively low cost. However, SSSB is becoming more and more popular than SSDSM in China because of its full-bodied taste, attractive aroma, and appearance. Differences in flavor profiles between SSSB and SSDSM might be caused by different levels of fat in soybean and defatted soy meal, because both materials and manufacture process employed in two kinds of soy sauces are all same except for fat content. Furthermore, previous studies showed that the composition and ratios of esters in soybean and defatted soy meal (hexane-defatted soy meal) are similar (3-5). To our knowledge, the degraded and oxidized resultants of soy fat during the long-term fermentation, such as short-chain fatty acids, monounsaturated and polyunsaturated fatty acids, alkanes, and alkenes, could be further oxidized and cyclized into aldehydes, ketones, miscellaneous compounds, lactones, alkylfurans, and so on (6-9). Therefore, the volatile flavor compounds derived from soy fat play an important role in the subtle balanced flavor of soy sauce.

Although the extraction methods of volatile flavor compounds in soy sauce have been investigated (1,2), and the aroma-impact flavor compounds in Korean-type and Japanese-type soy sauce have also been identified (10-15). The in-depth study on the differences of volatile flavor compounds and their relative contents between SSSB and SSDSM was not proceeded to date even though this research would greatly benefit to the flavor improvement of SSDSM. Therefore, one objective of this study was to investigate the differences of sensory evaluation between SSSB and SSDSM. The other objective was to try to explain the reasons caused the sensory differences between two kinds of soy sauces by comparing their differences in volatile flavor compounds and relative contents of different chemical classes.

Materials and Methods

Preparations of samples Soy sauces prepared with soybean (SSSB) and defatted soy meal (SSDSM) were directly obtained from a famous soy sauce manufacturing company (Meiweixian Flavoring Foods Co., Ltd., Zhongshan, Guangdong, China) when the moromis were brewed for 180 days. Ingredients including wheat starch (Runfon Flour Co., Ltd., Guangzhou, Guangdong, China), soybean (Guanghui Agricultural Products Co., Ltd., Linkou county,

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Heilongjiang, China), hexane-defatted soy meal (Yuwang Industrial Co., Ltd., Dezhou, Shandong, China), edible salt (Zhongshan Salt Industrial Co., Ltd., Zhongshan, Guangdong, China) and manufacture process (National Standard of Soy Sauce Industry: SB/T 10312-1999) employed in 2 kinds of soy sauces were same except for the contents of soy fat (soybean and defatted soy meal). Furthermore, the contents of total nitrogen in both moromis were kept the same during brewing according to the contents of total nitrogen in soybean and defatted soy meal measured in the present study. SSSB and SSDSM were filtered and kept in polyethylene vials, and stored in a refrigerator at –20ºC until ready for analysis.

Chemicals Dichloromethane was purchased from Merck (Darmstadt, Hessen, Germany) and redistilled prior to use. Ethyl 2-methylpropanoate, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 3-methylbutanal, phenol, and 4-ethyl-2-methoxy- were purchased from Weibo Chemicals Co., Ltd. (Guangzhou, Guangdong, China). Acetic acid, ethanol, and other chemicals were of the highest commercial grade and obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai , Shanghai, China).

Proximate analysis ***Total nitrogen, moisture, and fat analysis*****:** The contents of total nitrogen, moisture, and total fat in soybean, defatted soy meal, and lyophilized powders of SSSB, SSDSM (Alpha 1-4; Christ, Osterode, Germany) were measured according to AOAC methods (16). The contents of total nitrogen and moisture of soybean and defatted soy meal were determined to keep the same total nitrogen levels used in SSSB and SSDSM fermentation.
Formaldehyde nitrogen and total titratable acid analysis**:** Formaldehyde nitrogen and total titratable acid were measured by titration method (17). Twenty mL diluted samples were mixed with 60 mL H_2O and titrated to pH 9.6 with 0.05 M NaOH before 10 mL formalin solution (37%) was added. The consumed volume was recorded to determine total titratable acids of samples. The samples were finally titrated to pH 9.6 with 0.05 M NaOH.

Sensory evaluation Descriptive analysis (DA) was performed to determine the differences of sensory flavor characteristics between SSSB and SSDSM (15). Analysis was carried out with a panel of 9 flavorists (25 to 40 ages, 7 males and 2 females) in a professional Flavor & Fragrance Company. The flavorists selected as panelists were subjected to a ranking test with a series of 7 suprathreshold aqueous solutions (25 mL in Teflon vials) of acetic acid (sour), ethanol (alcoholic), ethyl 2-methylpropanoate (fruity), 4-hydroxy-2,5-dimethyl-3(2H)-furanone (caramel-like), 3-methylbutanal (malty), and phenol, 4-ethyl-2-methoxy- (smoky) and were asked to score the aroma intensities on a 7-point linear scale from 0 to 3. Meanwhile, the panelsits were asked to rank the aroma intensity of 0.5% acid hydrolyzed vegetable protein (HVP-B; Waychein, Baoding, Hebei, China) solution on a 7-point linear scale from 0 to 3 as well. Sensory evaluation was performed in sensory panel room at 23±2ºC at 3 different sessions. Results of sensory evaluation, the aroma intensities of chemicals, were discussed and should be obtained agreements by all the panelists eventually.

The intensities of the 7 aroma attributes of SSSB and SSDSM were evaluated using a 7-point linear scale from 0 (no similarity) to 3 (high similarity). Meanwhile, the overall aroma evaluation of SSSB and SSDSM were also ranked on the 7-point liner scale from 0 (very bad) to 3 (very good). The results were plotted in a spider web diagram.

Collection of volatile compounds using solid phase microextraction (SPME) A SPME fiber coated with 75 μm carboxen/polydimethylsiloxane (Supelco, Bellefonte, PA, USA) was selected to collect the high volatile compounds for its high sensitivity and good selectivity to polar and non-polar compounds (2). Before sampling, the fiber was preconditioned for 1 hr and 30 min at 275 and 250ºC, respectively, in the GC injector port to eliminate the possible residues on the coated fiber. Ten mL of soy sauce saturated with NaCl were sealed in a dedicated bottle and preheated at 45ºC, stirred by a magnetic stirring bar with a speed of 200 rpm. The adsorption time was 40 min, and the concentrates were desorbed at 230ºC in the injection port of gas chromatograph (Trace 2000; Finnigan, Austin, TA, USA) by holding in the splitless mode for 3 min. The SPME fiber was cleaned by keeping it in the GC injection port for additional 5 min.

Extraction of volatile compounds using direct solvent extraction (DSE) The acidic (DSE-A), neutral (DSE-N), and basic (DSE-B) fractions of SSSB and SSDSM were extracted with redistilled dichloromethane according to the method of Wanakhachornkrai and Lertsiri (1) except for the deleted procedure that combined solvent layer was subjected to vacuum distillation with liquid nitrogen cold trap. Each fraction was sealed in a brown glass bottle and kept at –20ºC overnight for water removal, and was further dehydrated by anhydrous sodium sulfate. Then each fraction was further concentrated to approximate 5 mL with a rotary evaporator (RE-52AA; Yarong Instrument Co., Ltd., Shanghai, Shanghai, China) at 38-40ºC under reduced pressure (200-250 mbar). Prior to analysis, it was concentrated to 0.5 mL by gentle N_2 stream.

Analysis by gas chromatography-mass spectrometry (GC-MS) GC-MS analysis was carried out using the Finnigan TRACE GC-2000 GS-MSTM (Finnigan), equipped with a DB-5MS column (30 m length×0.25 mm i.d×0.25 μm film thickness, J&W Scientific, Folsom, CA, USA). Extracts of SPME and DSE-A, DSE-N, DSE-B fractions were analyzed by DB-5MS column. For direct injection, 0.5 μL sample of each extract was injected into GC-MS by splitless mode with injection temperature of 230ºC. In the case of SPME samples, volatile compounds adsorbed on the fibers were transferred into the GC injector with a splitless mode with an injection purge-off time of 3 min and injection temperature of 230ºC. The initial temperature of GC oven was held for 2 min at 50ºC, then raised up to 80ºC (held for 5 min) at a speed of 5ºC/min, finally, increased to 230ºC (held for 10 min) at a speed of 7ºC/min. Ultra high purity helium (Senyin Gas Co., Ltd., Guangzhou, Guangdong, China) was used as carrier gas at a constant speed of 1.0 mL/min for DB-5MS. Mass spectrometer conditions were as follows: MSD capillary direct-interface

Table 1. Proximate analysis of total nitrogen (TN), formaldehyde nitrogen (FN), total titratable acid (TTA), total fat (TF), and moisture contents (MC) (%)

	Soybean	Defatted soy meal	SSSB	SSDSM
TN	5.55±0.10[a1)]	7.09±0.12[a]	1.49±0.08[a]	1.59±0.06[a]
FN	NC	NC	0.86±0.08[a]	0.91±0.04[a]
TTA	NC	NC	2.03±0.07[a]	1.53±0.04[b]
TF	20.52±0.40[a]	1.09±0.06[b]	0.36±0.07[a]	0.19±0.09[b]
MC	11.86±0.08[a]	10.95±0.09[a]	NC	NC

[1)]Values are mean±SD (n=3); Different letters in the same row for each index are significantly (p<0.05) different; NC, not conducted. ANOVAs were only conducted between values of soybean and defatted soy meal or SSSB and SSDSM.

temperature was 250°C. Ionization energy was 70 eV. Mass range was 35-335 a.m.u for direct injection or SPME desorbing. Electron-multiplier voltage was 1,800V.

Identification and quantification of volatile compounds Each compound was tentatively identified by comparing its mass spectral data with those of a NIST library (including Wiley and Mainlib) and published literatures (1,11). Compounds were reported on the basis of their similarity (>750). Quantitative analysis was based on the ratios between the peak area of a particular compound and the total peak area of all compounds in the sample (18).

Statistical analysis All tests were conducted in triplicate. Data are reported as mean±standard deviation (SD). Analysis of variance (ANOVA) and significant differences among means including those of the 8 sensory attributes between SSSB and SSDSM were tested by one-way ANOVA using SPSS software (version 15.0 for Windows, SPSS Inc., Chicago, IL, USA).

Results and Discussion

Results of proximate analysis As seen from Table 1, there were significant differences in contents of total fat and total titratable acid between SSSB and SSDSM (p<0.05). Reasons for this phenomenon might be due to higher content of soy fat used in SSSB fermentation than that in SSDSM fermentation (p<0.05), because soy fat could be degraded into different kinds of unsaturated fatty acids and short-chain fatty acids, which could further generate esters by esterification with alcohols during the long-term fermentation (6-9,11). Although the levels of total nitrogen and formaldehyde nitrogen in SSDSM were found to be higher than those in SSSB, the differences were not statistically significant (p<0.05).

Sensory evaluation The sensory flavor profiles of samples based on DA test by 9 panelists are shown in Fig. 1. ANOVA showed that SSSB and SSDSM exhibited significantly different attributes of over-all, sour, fruity, and HVP-like, whereas there was no significant difference in the attributes of alcoholic, smoky, malty, and caramel-like between both kinds of soy sauces (p<0.05). The DA results indicated that the intensities of fruity and sour of SSSB were obviously stronger than those of SSDSM (p<0.05), which might be due to the higher contents of fat and total titratable acid in SSSB (Table 1). Moreover, the different sensory attributes between SSSB and SSDSM suggested that some of the volatile flavor compounds and/or their relative contents in both kinds of soy sauces might be different.

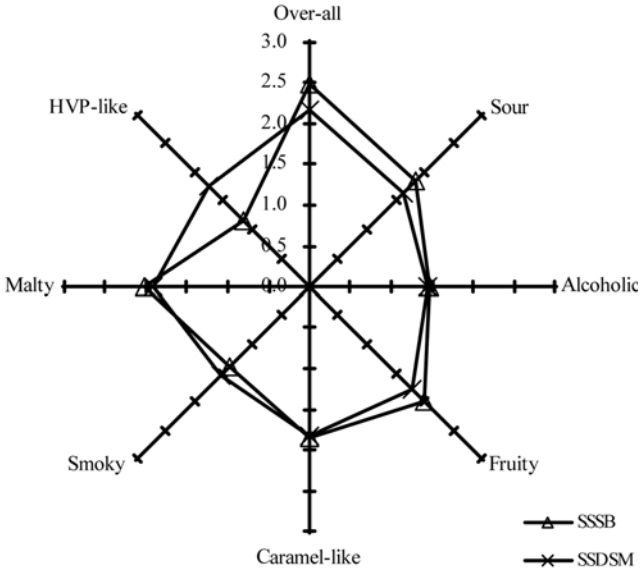

Fig. 1. Spider web diagram of sensory evaluation of soy sauces prepared with soybean (SSSB) and defatted soy meal (SSDSM).

Volatile compounds in SSSB and SSDSM identified by GC-MS To clarify the reason for the different sensory attributes in SSSB and SSDSM, volatile compounds in both kinds of soy sauces were identified and results were summarized in Table 2 and 3. Totally, 152 and 131 volatile flavor compounds were identified in SSSB and SSDSM, respectively. Alkanes and alkenes were not taken into consideration in the present study because of their neglectable aroma (19). In detail, 19 and 16 acids, 18 and 19 alcohols, 16 and 17 aldehydes, 35 and 24 esters, 12 and 11 furan(one)s, 13 and 8 ketones, 12 and 14 miscellaneous compounds, 8 and 6 phenols, 6 and 6 pyran(one)s, 4 and 4 pyrazines, 3 and 2 pyrrol(idinon)es, 6 and 4 sulfur-containing compounds were identified in SSSB and SSDSM, respectively. Although most of volatile compounds identified in both kinds of soy sauces were common, there were still 50 and 29 compounds (Table 2 and 3) belonging to SSSB and SSDSM alone.

As seen from Table 4, the relative contents of acids detected in SSSB were significantly higher than those in SSDSM (p<0.05). The result was in line with that the total titratable acid content in SSSB was higher than that in SSDSM (Table 1). Meanwhile, it further elucidated the reason for pronounced difference of sour attribute between

Table 2. Volatile flavor compounds identified in SSSB by GC-MS

RT[1)]	Compound	Percentage (%)[2)]			
		SPME	DSE		
			DSE-A	DSE-N	DSE-B
	Acids				
3.11	Acetic acid[3,4)]	1.81±0.72	ND	ND	ND
3.83	Propanoic acid[3,4)]	0.26±0.10	ND	ND	ND
4.80	Butanoic acid[3,4)]	0.07±0.06	ND	ND	ND
5.12	2-Propenoic acid	0.08±0.05	ND	ND	ND
6.53	Butanoic acid, 3-methyl-[3,4)]	0.19±0.12	ND	ND	ND
6.84	2-Butenoic acid, 2-methyl-	ND	0.35±0.14	ND	ND
7.62	Pentanoic acid, 4-methyl-[4)]	ND	0.08±0.0 4	ND	ND
8.55	Hexanoic acid[3,4)]	0.07±0.04	0.33±0.08	ND	ND
13.30	Hexanoic acid, 2-ethyl-[3)]	ND	0.17±0.08	ND	ND
14.28	Octanoic acid	ND	0.39±0.10	ND	ND
14.40	Benzoic acid[3)]	1.14±0.17	6.36±1.95	0.44±0.13	ND
15.77	Benzeneacetic acid[3)]	1.87±0.95	11.77±3.21	ND	ND
16.60	Nonanoic acid	0.13 ±0.08	0.54±0.28	ND	ND
17.60	Decanoic acid	0.52±0.16	2.44±1.61	ND	ND
20.26	Dodecanoic acid[4)]	0.13±0.08	0.40±0.32	ND	ND
22.59	Tetradecanoic acid	0.19±0.07	1.19±0.19	ND	ND
23.67	Pentadecanoic acid	ND	0.83±0.44	ND	ND
24.70	Hexadecanoic acid	ND	4.22±1.03	ND	ND
26.71	Oleic acid	ND	0.48±0.11	ND	ND
	Alcohols				
1.92	Ethanol[3,4)]	1.93±0.37	ND	ND	ND
2.60	Propanol[3,4)]	0.32±0.10	ND	ND	ND
2.96	Propanol, 2-methyl-[3,4)]	1.61±0.41	ND	ND	ND
3.25	Butanol[3,4)]	0.11±0.06	ND	ND	ND
3.99	Butanol, 3-methyl-[3,4)]	1.34±0.61	ND	ND	ND
4.08	Butanol, 2-methyl-[4)]	0.32±0.11	ND	ND	ND
4.61	2,3-Butanediol[3,4)]	1.08±0.29	ND	ND	ND
8.85	1-Octen-3-ol[4)]	3.60±0.78	ND	ND	ND
10.72	Benzyl alcohol[3,4)]	0.08±0.03	0.32±0.10	0.38±0.13	ND
11.91	1-Octenol	0.42±0.10	ND	ND	ND
12.31	Benzenemethanol, dimethyl-	ND	2.14±1.01	ND	ND
12.80	2-Nonen-1-ol, (E)-	ND	ND	0.34±0.18	ND
13.03	Phenylethyl alcohol[3,4)]	4.80±1.32	6.22±2.21	2.01±0.52	ND
16.75	1,2-Ethanediol, 1-phenyl-	ND	ND	0.18±0.12	ND
19.21	1-Undecanol[4)]	ND	0.51±0.16	0.50±0.20	ND
19.76	2-Hexyl-1-octanol	ND	ND	0.24±0.09	ND
21.12	8BetaH-cedran-8-ol	0.25±0.15	ND	ND	ND
23.40	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	ND	0.95±0.37	0.35±0.08	0.84±0.32
	Aldehydes				
3.19	Butanal, 2-methyl-[3,4)]	0.17±0.06	ND	ND	ND
8.31	Benzaldehyde[3,4)]	2.16±0.41	ND	0.35±0.14	ND
9.69	Octanal[4)]	0.30±0.13	ND	ND	ND
11.11	Benzeneacetaldehyde[3,4)]	6.11±2.09	0.14±0.06	1.45±0.54	ND
12.84	Nonanal[4)]	2.39±0.52	ND	ND	ND
15.04	Decanal[4)]	0.97±0.36	ND	ND	ND
16.06	2-Decenal, (Z)-	0.52±0.20	ND	ND	ND
16.20	Indane-4-carboxaldehyde	0.85±0.40	ND	ND	ND
16.81	Undecanal	0.52±0.14	ND	ND	ND
17.76	2-Undecenal	0.52±0.20	ND	ND	ND
18.33	Tetradecanal	0.13±0.06	ND	0.58±0.27	ND

Table 2. Continued

RT[1)]	Compound	Percentage (%)[2)]			
		SPME	DSE		
			DSE-A	DSE-N	DSE-B
19.45	5-Methyl-2-phenyl-2-hexenal[4)]	0.26±0.08	ND	ND	ND
21.02	Tridecanedial	0.09±0.05	ND	ND	ND
22.68	3.5-Di-*tert*-butyl-4-hydroxybenzaldehyde	0.11±0.07	0.55±0.11	ND	ND
23.25	Pentadecanal	0.10±0.06	ND	ND	ND
23.47	4,8,12-Tetradecatrienal,5,9,13-trimethyl-	0.06±0.05	ND	ND	ND
	Esters				
2.83	Acetic acid, ethyl ester[3,4)]	0.75±0.23	ND	ND	ND
5.66	Butanoic acid, 3-methyl-, ethyl ester	0.06±0.03	ND	ND	ND
9.26	Hexanoic acid, ethyl ester[4)]	0.03±0.02	ND	ND	ND
10.97	l-Valine, ethyl ester[3)]	ND	ND	ND	1.56±0.32
10.03	Pentanoic acid, 2-methyl, anhydride	ND	ND	0.60±0.32	ND
12.56	Benzoic acid, methyl ester	2.32±0.68	ND	0.14±0.08	ND
12.94	DL-Isoleucine, ethyl ester[3)]	ND	ND	ND	10.72±3.59
14.34	Ethyl benzoate[4)]	4.22±1.46	6.43±2.72	3.80±0.45	ND
14.85	Octanoic acid, ethyl ester[4)]	0.37±0.11	ND	ND	ND
14.98	Benzeneacetic acid, methoxy, methyl ester	ND	ND	0.85±0.36	ND
15.72	Benzeneacetic acid, ethyl ester[4)]	1.31±0.40	ND	ND	ND
15.90	Acetic acid, 2-phenylethyl ester	4.59±1.74	ND	ND	ND
16.67	Nonanoic acid, ethyl ester	1.11±0.31	ND	ND	ND
17.48	2,2,4-Trimethyl-1,3-pentanediol, diisobutyrate	2.05±0.67	ND	ND	ND
17.82	Propanoic acid, 2-methyl-, 3-hydroxy-, 2,4,4-trimethylpentyl ester	0.73±0.24	ND	ND	ND
18.10	Decanoic acid, ethyl ester	1.36±0.40	ND	ND	ND
18.78	1-Butanol, 3-methyl, benzoate	4.29±1.54	ND	ND	ND
18.88	Dimethyl phthalate	1.66±0.49	ND	1.47±0.49	ND
19.38	2-Amino-3-phenylpropionic acid, ethyl ester	ND	ND	ND	1.82±0.42
19.54	Hexanoic acid, 2-phenylethyl ester	0.11±0.07	ND	ND	ND
20.67	Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester	5.98±2.14	ND	ND	ND
20.72	Dodecanoic acid, ethyl ester[4)]	0.21±0.12	ND	ND	ND
21.69	Acetic acid, 3,7,11,15-tetramethyl-, hexadecyl ester	ND	ND	0.13±0.09	ND
22.99	Tetradecanoic acid, ethyl ester[4)]	0.64±0.39	ND	ND	ND
23.31	Isopropyl myristate	0.29±0.14	ND	ND	ND
23.73	1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	1.95±0.65	2.18±0.83	ND	3.40±0.71
24.75	Dibutyl phthalate	0.31±0.11	ND	ND	1.29±0.38
24.87	Ethyl, 9-hexadecenoate	0.19±0.12	ND	ND	ND
24.93	Bis (2-ethylhexyl) phthalate	ND	ND	ND	25.21±5.15
25.06	Pentadecanoic acid, ethyl ester[4)]	2.36±0.97	3.59±1.48	1.35±0.56	1.65±0.35
25.30	Isopropyl palmitate	0.02±0	ND	ND	ND
27.04	9,12-Octadecadienoic acid, ethyl ester	0.70±0.44	1.02±0.42	1.92±0.73	ND
27.13	Ethyl, 9-octadecenoate, (E)-[4)]	0.46±0.16	ND	ND	1.62±0.45
27.55	Hexadecanoic acid, ethyl ester[4)]	0.08±0.07	ND	ND	ND
28.10	Phthalic acid, diisooctyl ester	1.00±0.40	11.06±5.10	17.68±4.24	ND
	Furan(one)s				
5.84	3-Furanmethanol	0.30±0.09	ND	ND	ND
6.70	2(5H)-Furanone[3)]	ND	ND	ND	0.06±0.05
6.80	2(3H)-Furanone, dihydro-[3)]	ND	ND	4.34±0.89	ND
7.91	2(3H)-Furanone, dihydro-5-methyl-[3,4)]	ND	ND	0.51±0.24	ND
8.50	2(5H)-Furanone, 3-methyl-[3)]	ND	ND	0.07±0.10	ND

Table 2. Continued

RT[1)]	Compound	Percentage (%)[2)]			
		SPME	DSE		
			DSE-A	DSE-N	DSE-B
9.61	2-Furanmethanol, tetrahydro-, acetate	ND	ND	ND	0.58±0.17
10.44	Furan, tetrahydro-2,2-dimethyl-5-(1-methylpropyl)-	ND	ND	0.89±0.33	ND
10.59	2,5-Furandione, 3,4-dimethyl-	ND	0.23±0.06	ND	ND
10.85	4-Methyl-5H-Furan-2-one	ND	ND	0.28±0.10	ND
11.29	2(3H)-Furanone, 5-ethyldihydro-	ND	ND	0.06±0.07	ND
11.33	2,5-Dimethyl-4-hydroxy-3(2H)-Furanone	0.45±0.13	1.82±0.37	ND	ND
17.64	2(3H)-Furanone, dihydro-5-pentyl-	0.54±0.10	ND	0.19±0.08	ND
	Ketones				
3.51	Butanone, 3-hydroxy-	0.29±0.07	ND	ND	ND
10.07	2,2-Dimethyl-3-octanone	0.31±0.12	ND	ND	ND
10.30	1,2-Cyclopentanedione, 3-methyl-	ND	0.35±0.08	ND	ND
11.77	Acetophenone[4)]	0.56±0.11	ND	ND	ND
13.50	3,5-Dimethyl-4-heptanone	0.26±0.12	ND	ND	ND
16.15	5,6-Decanedione	ND	0.27±0.08	ND	ND
18.67	Acetophenone, 4-hydroxy-	ND	0.08±0.09	ND	ND
19.10	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	4.71±1.04	ND	ND	ND
19.34	Ethanone,1-(4-hydroxy-3-methoxyphenyl)-	ND	1.07±0.39	ND	ND
20.54	2',4'-Dihydroxy-3'-methylpropiophenone	ND	0.63±0.19	ND	ND
20.61	Megastigmatrienone	0.07±0.03	ND	ND	ND
23.52	2-Pentadecanone, 6,10,14-trimethyl-	0.53±0.23	ND	ND	ND
23.62	Caffeine	0.04±0.03	ND	ND	ND
	Miscellaneous compounds				
7.51	Piperidine, 1-methyl-	ND	ND	ND	0.49±0.15
11.07	1-Butanamine, 3-methyl-*N*-(3-methylbuylidene)-	ND	ND	ND	1.85±0.49
11.59	Ethanone, 1-(1H-pyrrol-2-yl)-	0.35±0.13	ND	2.05±0.59	0.13±0.09
14.76	Benzenepropanenitrile[4)]	ND	ND	ND	0.06±0.06
15.55	1-Butanamine, *N*-(2-furanylmethylene)-3-methyl-	ND	ND	ND	0.28±0.07
17.44	Pyridine,3-(1-methyl-2-pyrrolidinyl)-,[s]-	ND	ND	ND	1.00±0.29
17.69	Naphthalene, 1-isocyano-	ND	ND	ND	0.99±0.19
19.41	Formamide, *N*-(2-phenylethyl)-	ND	ND	ND	1.83±0.94
20.38	3,5-Dimethyl-1-octyl pyrazole	ND	ND	ND	0.94±0.40
20.50	1-Amino-2-methylnaphthalene	ND	ND	ND	0.73±0.24
20.63	Ethanone,1-(1a,2,3,5,6a,6b-hexahydro-3,3,6a-trimethyl) oxirenol (benzofuran-5-yl)-	ND	ND	0.33±0.07	ND
25.37	1-Acetyl-9H-pyrido(3,4-B) indole	ND	ND	ND	0.82±0.22
	Phenols				
8.66	Phenol[3,4)]	ND	0.29±0.07	ND	ND
12.25	Phenol, 2-methoxy-[3,4)]	0.18±0.08	ND	ND	ND
16.86	2-methoxy-4-vinylphenol[3,4)]	0.52±0.15	0.53±0.14	ND	ND
17.39	Phenol, 2,6-dimethoxy-[3,4)]	0.23±0.09	6.16±1.80	ND	ND
17.53	Eugenol	0.15±0.06	ND	ND	ND
19.63	Butylated hydroxytoluene	0.99±0.28	2.60±0.84	1.30±0.34	2.26±0.49
24.11	Phenol, 2,6-di-t-butyl-4-nitro-	0.20±0.12	ND	ND	ND
25.83	Phenol, 2,4-bis (1-phenylethyl)-	ND	ND	ND	5.30±1.28
	Pyran(one)s				
12.89	Maltol[3,4)]	ND	6.15±1.95	ND	ND
13.78	2H-Pyran-2-carboxaldehyde,3,4-dihydro-	ND	ND	0.10±0.08	ND
13.99	2H-Pyran-2-one, 5,6-dihydro-4-methyl-	ND	ND	1.01±0.29	0.18±0.05
14.64	2-Methyl-3-methoxy-4H-pyran-4-one	0.59±0.27	0.40±0.08	15.39±4.03	6.37±2.13

Table 2. Continued

RT[1)]	Compound	Percentage (%)[2)]			
		SPME	DSE		
			DSE-A	DSE-N	DSE-B
15.27	4H-Pyran-4-one,2-methoxy-6-methyl-	0.14±0.05	0.26±0.12	ND	ND
19.49	2H-Pyran-2-one, tetrahydro-6-phenyl-	ND	0.39±0.13	ND	ND
	Pyrazines				
6.90	Pyrazine, 2,6-dimethyl-[3,4)]	ND	ND	ND	0.36±0.17
17.13	4-Methyl, pyrrolo (1,2a) pyrazine[3)]	ND	ND	ND	1.28±0.51
24.44	Pyrrolo(1,2-a)Pyrrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	ND	3.26±0.80	13.06±4.69	4.18±1.50
24.61	5,10-Diethoxy-2,3,7,8-tetrahydro-1H, 6H-dipyrrolo(1,2a1'2'-d) pyrazine	ND	3.54±0.99	14.27±3.96	13.19±3.89
	Pyrrol(idinon)es				
9.82	1H-Pyrrole-2-carboxaldehyde	ND	ND	0.07±0.07	ND
10.77	2-Pyrrolidinone[3)]	ND	ND	ND	0.12±0.06
15.96	2,5-Pyrrolidinedione, 3-ethyl-4-methyl-	ND	0.38±0.08	ND	ND
	Sulfur-containing compounds				
6.75	Propanal, 3-(methylthiol)-[3,4)]	1.08±0.36	ND	2.23±0.48	ND
8.13	1-Propanamine, 3-(methylthiol)-	ND	ND	ND	2.93±0.86
8.72	1-Propanol, 3-(methylthio)-[3,4)]	1.27±0.31	1.24±0.35	2.82±0.93	1.22±0.41
15.16	Thiourea, tetramethyl-	ND	0.49±0.16	ND	ND
15.44	1,2-Benzisothiazole	ND	0.36±0.09	0.24±0.07	0.12±0.07
16.25	5-Thiazoleethanol, 4-methyl-	ND	ND	ND	0.27±0.12

[1)]Retention time.
[2)]Area of each compound/total area of all compounds×100; Values are mean±SD (n=3); ND, not detected.
[3,4)]Identified compounds in soy sauces were reported by references of 1 and 11, respectively.

SSSB and SSDSM. It should be noted that the fatty acids (i.e., tetradecanoic acid, hexadecanoic acid, oleic acid, etc) were the degraded products of soybean fat and contributed substantially to the flavor of soy sauce, because active lipase systems in most microorganisms present in moromi could degrade triglycerides to free fatty acids and glycerol, monoglycerides, and diglycerides (11).

There was no significant difference in the relative contents of alcohols detected by SPME and DSE for both kinds of soy sauces (Table 4), which was consistent with the results of DA evaluation. Among the alcoholics detected in both kinds of soy sauces, ethanol (alcoholic), butanol (medicinal/metallic), 1-octen-3-ol (mushroom), and phenylethyl alcohol (flowery/sweet) were reported as aroma-impact compounds in soy sauce (11,15). 1-Octen-3-ol, one of the major components in Japanese-type soy sauce, was considered as the degraded products of unsaturated fatty acid, such as linoleic and linolenic acids (11,14,20). Furthermore, alcohols can arise via the primary metabolic pathway of microorganisms or by the reduction of a carbonyl (11,21).

As seen from Table 4, the relative contents of aldehydes in SSSB detected by SPME and DSE were significantly lower than those in SSDSM, while the relative contents of ketones in both kinds of soy sauces were not significantly different even though 5 more ketones were detected in SSSB (p<0.05). Amino acid degradation and microorganism transformation are 2 formation pathways of some low-boiling aldehydes (i.e., 2-methylbutanal) detected in soy sauce (11). As for the long-chain aldehydes (i.e., nonanal, decanal, etc), the origin of them might arise from the oxidation of lipid (8). Similar to the origin of long-chain aldehydes, 2-methylketones (C_5-C_{15}) might originate from the degradation of lipid and were formed by β-oxidation and decarboxylation of fatty acids (22). Among the aldehydes, benzaldehyde, and benzeneacetaldehyde are considered as aroma-impact compounds and identified with burnt sugar, sweet, and honey-like note (15), whereas other aldehydes and ketones, particularly hexanal and heptanal, exhibited the unpopular flavor of 'beany' (23).

The relative contents of esters detected in SSSB by SPME and DSE were significantly higher than those in SSDSM (p<0.05) (Table 4), which was in accordance with the difference of total fat contents in both kinds of soy sauces (Table 1). It was a reasonable explanation for the significant difference in fruity attribute between 2 kinds of soy sauces because most of esters, especially ethyl esters, are responsible for fruity note (p<0.05). Meanwhile, esters also could contribute a good flavor by minimizing the sharpness and bitterness imparted by amines and fatty acids (6,24). As seen by a comparison of Table 2 and 3, esters were observed to be the major volatile compounds identified in both kinds of soy sauces in the present study, and 11 more esters (i.e., 1-valine, ethyl ester, DL-isoleucine, ethyl ester, decanoic acid, ethyl, etc) were detected in SSSB. The formation of esters was related to the metabolism of lipids by yeast, which provided a large number of acids and alcohols that may undergo esterification to yield a variety of esters (11).

In the present study, the relative contents of furan(one)s

Table 3. Volatile flavor compounds identified in SSDSM by GC-MS

RT[1)]	Compound	Percentage (%)[2)]			
		SPME	DSE		
			DSE-A	DSE-N	DSE-B
	Acids				
3.11	Acetic acid[3,4)]	1.50±0.57	ND	ND	ND
3.83	Propanoic acid[3,4)]	0.16±0.10	ND	ND	ND
4.80	Butanoic acid[3,4)]	0.13±0.08	ND	ND	ND
6.52	Butanoic acid, 3-methyl-[3,4)]	0.09±0.05	ND	ND	ND
7.14	Butanoic acid, 4-hydroxy-	0.76±0.27	ND	ND	ND
14.26	Octanoic acid	0.09±0.02	0.43±0.14	ND	ND
14.40	Benzoic acid[3,4)]	0.17±0.06	2.45±0.87	1.01±0.43	ND
15.77	Benzeneacetic acid[3)]	1.01±0.42	6.74±2.41	ND	ND
17.60	Decanoic acid	ND	0.20±0.12	ND	ND
20.26	3-Hydroxy-4-methoxybenzoic acid	ND	0.33±0.17	ND	ND
22.60	Tetradecanoic acid	0.04±0.03	0.45±0.17	ND	ND
23.69	Pentadecanoic acid	ND	0.71±0.34	ND	ND
24.52	9-Hexadecenoic acid	ND	0.59±0.23	ND	ND
24.70	Hexadecanoic acid	ND	2.75±1.47	ND	ND
26.71	Oleic acid	ND	0.53±0.18	ND	ND
26.83	9-Octadecenoic acid, (E)	ND	2.81±1.07	ND	ND
	Alcohols				
1.92	Ethanol[3,4)]	1.44±0.49	ND	ND	ND
2.60	Propanol[3,4)]	0.16±0.07	ND	ND	ND
2.96	Propanol, 2-methyl-[3,4)]	1.13±0.98	ND	ND	ND
3.24	Butanol[3,4)]	0.07±0.05	ND	ND	ND
3.99	Butanol, 3-methyl-[3,4)]	0.26±0.10	ND	ND	ND
4.61	2,3-Butanediol[3,4)]	1.40±0.33	ND	ND	ND
6.20	Cyclohexanol	ND	ND	0.32±0.07	ND
8.11	Ethanol, 2,2'-oxybis-	ND	ND	0.21±0.12	ND
8.86	1-Octen-3-ol[4)]	2.43±0.97	ND	ND	ND
9.44	3-Octanol	0.65±0.24	ND	ND	ND
10.72	Benzyl alcohol[3,4)]	0.57±0.20	1.26±0.44	1.17±0.45	ND
12.79	2-Nonen-1-ol, (E)-	ND	ND	0.20±0.11	ND
13.05	Phenylethyl alcohol[3,4)]	3.25±0.99	3.68±0.94	1.82±0.61	ND
19.24	1-Undecanol[4)]	ND	0.37±0.10	0.44±0.20	ND
19.76	2-Hexyl-1-octanol	ND	ND	0.19±0.06	ND
21.13	8BetaH-cedran-8-ol	0.01±0	ND	ND	ND
21.35	3-Oxi-?ionol	ND	ND	0.50±0.18	ND
22.56	Cyclopentanol,3-methyl-2-(2-pentenyl)-	ND	2.70±0.98	ND	ND
23.47	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	ND	1.17±0.55	0.44±0.17	1.07±0.59
	Aldehydes				
3.20	Butanal, 2-methyl-[3,4)]	0.22±0.06	ND	ND	ND
8.32	Benzaldehyde[3,4)]	4.80±1.16	ND	0.31±0.15	ND
11.13	Benzeneacetaldehyde[3,4)]	5.31±2.00	0.05±0.05	0.73±0.31	ND
11.56	2-Octenal, (E)-	0.91±0.29	ND	ND	ND
12.84	Nonanal[4)]	2.43±0.66	ND	ND	ND
14.11	2-Nonenal, (E)-	0.11±0.05	ND	ND	ND
15.05	Decanal[4)]	2.07±0.99	ND	ND	ND
16.07	2-Decenal, (Z)-	1.18±0.90	ND	ND	ND
16.19	Indane-4-carboxaldehyde	0.82±0.33	ND	ND	ND
16.30	Benzaldehyde, ethenyl-	ND	1.20±0.31	ND	ND
16.82	Undecanal	1.35±0.41	ND	ND	ND
17.70	2-Undecenal	1.37±0.46	ND	ND	ND
17.79	Benzaldehyde, 4-ethoxy-	ND	0.27±0.15	ND	ND

Table 3. Continued

RT[1)]	Compound	Percentage (%)[2)]			
		SPME	DSE		
			DSE-A	DSE-N	DSE-B
18.34	Tetradecanal	0.24±0.14	ND	0.52±0.19	ND
20.55	4-Ethoxy-3-anisaldehyde	1.02±0.41	6.03±2.10	ND	ND
22.69	3,5-Di-*tert*-butyl-4-hydroxybezaldehyde	0.14±0.09	0.98±0.48	ND	ND
23.30	Pentadecanal	0.16±0.12	ND	ND	ND
	Esters				
2.83	Acetic acid, ethyl ester[3,4)]	0.47±0.12	ND	ND	ND
5.66	Butanoic acid, 3-methyl-, ethyl ester	0.09±0.04	ND	ND	ND
9.26	Hexanoic acid, ethyl ester[4)]	0.05±0.04	ND	ND	ND
14.35	Ethyl benzoate[4)]	6.47±2.52	6.10±2.61	2.20±0.63	ND
14.86	Octanoic acid, ethyl ester[4)]	0.70±0.30	ND	ND	ND
15.98	Benzeneacetic acid, methoxy, methyl ester	ND	ND	1.25±0.44	ND
15.71	Benzeneacetic acid, ethyl ester[4)]	1.61±0.41	ND	ND	ND
15.92	Acetic acid, 2-phenylethyl ester	1.49±0.53	ND	ND	ND
16.61	Nonanoic acid, ethyl ester	0.16±0.10	ND	ND	ND
16.75	Ethyl(s)-, (+)-mandelate	ND	ND	0.16±0.06	ND
17.49	2,2,4-Trimethyl-1,3-pentanediol, diisobutyrate	0.26±0.07	ND	ND	ND
17.84	Propanoic acid, 2-methyl-, 3-hydroxy- 2,4,4-trimethylpentyl ester	0.27±0.09	ND	ND	ND
18.88	Dimethyl phthalate	2.47±0.66	ND	2.33±0.82	ND
20.68	Pentanoic acid, 2,2,4-trimethyl-, 3-carboxyisopropyl, isobutyl ester	1.30±0.43	ND	ND	ND
20.72	Dodecanoic acid, ethyl ester[4)]	0.82±0.21	ND	ND	ND
22.99	Tetradecanoic acid, ethyl ester[4)]	0.65±0.28	ND	ND	ND
23.35	Isopropyl myristate	0.17±0.07	ND	ND	ND
23.73	1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	2.96±0.74	0.73±0.26	ND	4.63±1.61
24.75	Dibutyl phthalate	0.38±0.30	ND	ND	11.54 ± 4.33
24.87	Ethyl, 9-hexadecenoate	0.37±0.17	ND	ND	ND
25.05	Pentadecanoic acid, ethyl ester[4)]	3.37±0.93	1.70±0.36	1.72±0.60	1.49±0.45
27.05	9,12-Octadecadienoic acid, ethyl ester	1.33±0.43	0.70±0.28	0.26±0.15	ND
27.14	Ethyl, 9-octadecenoate, (E)-[4)]	0.74±0.34	ND	ND	2.75±0.78
28.10	Phthalic acid, diisooctyl ester	0.71±0.27	10.03±3.16	15.76±5.91	ND
	Furan(one)s				
5.39	2-Furanmethanol[3)]	0.58±0.25	ND	ND	ND
5.84	3-Furanmethanol	0.22±0.10	ND	ND	ND
6.79	2(3H)-Furanone, dihydro-[3)]	ND	ND	1.55±0.62	ND
7.90	2(3H)-Furanone, dihydro-5-methyl-[4)]	ND	ND	0.36±0.14	ND
8.52	2(5H)-Furanone, 3-methyl-[3)]	ND	ND	0.07±0.06	ND
9.70	2-Furanmethanol, tetrahydro-,acetate	ND	ND	ND	0.24±0.14
10.85	4-Methyl-5H-Furan-2-one	ND	ND	0.77±0.36	ND
11.30	2(3H)-Furanone, 5-ethyldihydro-	ND	ND	0.10±0.07	ND
11.38	2,5-Dimethyl-4-hydroxy-3(2H)-Furanone	0.18±0.07	0.51±0.27	ND	ND
17.65	2(3H)-Furanone, dihydro-5-pentyl-	1.36±0.66	ND	0.44±0.26	ND
26.36	2(3H)-Furanone, 5-dodecyldihydro	ND	0.97±0.31	ND	ND
	Ketones				
3.51	Butanone, 3-hydroxy-	0.43±0.13	ND	ND	ND
6.42	2-Cyclopenten-1-one, 2-hydroxy-	ND	ND	0.39±0.13	ND
13.47	3,5-Dimethyl-4-heptanone	0.21±0.09	0.20±0.10	ND	ND
14.31	1,4-Cyclohexanedione, 2,2,6-trimethyl-	ND	ND	1.06±0.31	ND
19.10	2,5-Cyclohexadiene-1,4-dione, 2,6-bis (1,1-dimethylethyl)-	4.47±1.78	ND	ND	ND

Table 3. Continued

RT[1)]	Compound	Percentage (%)[2)]			
		SPME	DSE		
			DSE-A	DSE-N	DSE-B
19.35	Ethanone, 1-(4-hydroxy-3-methoxyphenyl)-	ND	1.07±0.33	ND	ND
20.60	Megastigmatrienone	0.16±0.07	ND	ND	ND
23.52	2-Pentadecanone, 6.10.14-trimethyl-	0.77±0.31	ND	ND	ND
	Miscellaneous compounds				
6.69	Oxime-, methoxy-phenyl-	1.54±0.42	ND	ND	ND
7.52	Piperidine, 1-methyl-	ND	ND	ND	0.36±0.15
11.61	Ethanone, 1-(1H-pyrrol-2-yl)-	1.52±0.59	ND	3.63±1.15	0.64±0.20
15.86	Caprolactam	ND	ND	ND	0.33±0.12
16.51	Benzene, 1-methoxy-4-(1-propenyl)-	0.56±0.24	ND	ND	ND
17.44	Pyridine,3-(1-methyl-2-pyrrolidinyl)-,[s]-	ND	ND	ND	0.83±0.32
18.04	Benzeneacetamide[4)]	ND	ND	0.16±0.05	ND
19.04	1,2,3-Trimethoxy benzene	ND	1.19±0.38	ND	ND
19.40	Formamide, *N*-(2-phenylethyl)-	ND	ND	ND	1.16±0.41
19.68	Acetamide, *N*-(2-phenylethyl)-	ND	ND	1.91±0.67	ND
20.38	3,5-Dimethyl-1-octyl pyrazole	ND	ND	ND	1.58±0.45
20.63	Ethanone,1-(1a,2,3,5,6a,6b-hexahydro-3,3, 6a-trimethyl) oxirenol (benzofuran-5-yl)	ND	ND	1.27±0.37	ND
22.71	1H-Indole-3-ethanol	ND	ND	1.72±0.58	ND
25.38	1-Acetyl-9H-pyrido (3,4-B) indole	ND	ND	ND	0.73±0.29
	Phenols				
12.31	Phenol, 2-methoxy-[3,4)]	1.36±0.40	ND	ND	ND
16.25	Phenol, 4-ethyl-2-methoxy-[3,4)]	0.46±0.10	ND	ND	ND
16.86	2-Methoxy-4-vinylphenol[3,4)]	0.36±0.25	1.33±0.44	ND	ND
17.40	Phenol, 2,6-dimethoxy-[4)]	0.23±0.12	4.44±1.91	ND	ND
17.54	Eugenol	0.12±0.09	ND	ND	ND
19.62	Butylated hydroxytoluene	3.11±1.01	11.03±3.29	1.93±0.73	3.28±1.33
	Pyran(one)s				
8.06	2H-Pyran-2-one, tetrahydro-	ND	ND	0.20±0.07	ND
12.90	Maltol[3,4)]	ND	3.17±1.19	ND	ND
13.78	2H-Pyran-2-carboxaldehyde,3,4-dihydro-	ND	ND	0.46±0.19	ND
13.99	2H-Pyran-2-one, 5,6-dihydro-4-methyl-	ND	ND	0.16±0.13	ND
14.66	2-Methyl-3-methoxy-4H-pyran-4-one	0.55±0.25	0.59±0.17	12.99±4.68	6.08±2.32
15.29	4H-Pyran-4-one, 2-methoxy-6-methyl-	0.21±0.06	0.31±0.21	ND	ND
	Pyrazines				
6.85	Pyrazine, 2,6-dimethyl-[3,4)]	ND	ND	ND	2.61±1.02
17.13	4-Methyl, pyrrolo (1,2a) pyrazine[3)]	ND	ND	ND	5.61±1.91
24.45	Pyrrolo(1,2-a)Pyrrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	ND	4.11±1.66	9.89±3.97	16.59±4.99
24.61	5,10-Diethoxy-2,3,7,8-tetrahydro-1H, 6H-dipyrrolo(1,2a1'2'-d) pyrazine	ND	6.13±2.36	10.02±3.57	25.16±8.97
	Pyrrol(idinon)es				
11.77	2-Pyrrolidinone, 1-methyl-	ND	ND	1.17±0.43	ND
15.98	2,5-Pyrrolidinedione, 3-ethyl-4-methyl-	ND	0.31±0.10	ND	ND
	Sulfur-containing compounds				
6.74	Propanal,3-(methylthiol)-[3,4)]	0.64±0.36	ND	1.55±0.71	ND
8.15	1-Propanamine, 3-(methylthiol)-	ND	ND	ND	0.55±0.22
8.74	1-Propanol, 3-(methylthio)-[3,4)]	4.10±1.43	2.92±0.54	8.28±2.74	3.09±0.63
15.43	1,2-Benzisothiazole	ND	0.27±0.12	0.17±0.13	0.40±0.19

[1)]Retention time.
[2)]Area of each compound/total area of all compounds×100; Values are mean±SD (n=3); ND, not detected.
[3),4)]Identified compounds in soy sauces were reported by references of 1 and 11, respectively.

Table 4. Relative contents of different chemical classes in soy sauces prepared with soybean (SSSB) and defatted soy meal (SSDSM)

Compound class	Relative contents (%)[1)]			
	SPME		DSE	
	SSSB	SSDSM	SSSB	SSDSM
Acids	6.46±1.25[a]	3.95±0.45[b]	10.00±0.87[a]	6.33±0.90[b]
Alcohols	15.86±2.85	11.37±1.82	4.99±1.30	5.18±0.57
Aldehydes	15.26±1.46[b]	22.13±2.13[a]	1.02±0.22[b]	3.36±0.40[a]
Esters	39.15±2.68[a]	26.84±2.16[b]	33.16±4.22[a]	21.12±2.79[b]
Furan(one)s	1.29±0.17[b]	2.34±0.25[a]	3.01±0.42[a]	1.67±0.16[b]
Ketones	6.77±0.73	6.04±1.19	0.80±0.08	0.91±0.14
Mis.cpds[2)]	0.35±0.13[b]	3.62±0.41[a]	3.83±0.06[b]	5.17±0.34[a]
Phenols	2.27±0.21[b]	5.64±0.56[a]	6.15±0.36[b]	7.34±0.40[a]
Pyran(one)s	0.73±0.22	0.76±0.23	10.08±1.07	7.99±1.09
pyrazines	ND	ND	16.71±0.55[b]	26.71±2.00[a]
Pyrrol(idinon)es	ND	ND	0.19±0.07[b]	0.49±0.18[a]
S-cpds[3)]	2.35±0.59[b]	4.74±1.07[a]	3.97±0.19	5.74±0.76

[1)]Area of each chemical class/total area of all compounds. Values are mean±SD (n=3); ANOVAs were only conducted within SPME or DSE, means with different letters in the same row for each chemical classes are significantly (p<0.05) different; ND, not detected.
[2)]Miscellaneous compounds.
[3)]Sulfur-containing compounds.

between both kinds of soy sauces were significantly different (p<0.05). SSSB was found to contain more high volatile furan(one)s (detected by SPME), while SSDSM had more low volatile furan(one)s (detected by DSE). Among the furan(one)s detected in SSSB and SSDSM, 2(3)-furanone, 2(5)-furanone, and 2,5-dimemethy-4-hydroxy-3(2H)-furanone (caramel-like) were reported as aroma-impact compounds in soy sauce and cheese (6,25). Notably, it has been reported that 2,5-dimemethy-4-hydroxy-3(2H)-furanone with low thresholds of 160 μg/L can be produced by yeast from intermediates of Maillard reaction (15,25).

As seen from Table 4, the relative contents of miscellaneous compounds detected by SPME and DSE in SSSB were significantly lower than those in SSDSM (p<0.05), which might be contribute to the difference of over-all attribute between SSSB and SSDSM, because miscellaneous compounds comprised of abundant odorants detected in both soy sauces in the present study (Table 2 and 3). For instance, indole, which is likely to be a degradation product of tryptophan, is regarded as a main odorant (musty) of water buffalo mozzarella (6). Moreover, it should be noted that some derivatives of pyrrole, pyridine, pyrazole, and furan (Table 2 and 3) were detected in both kinds of soy sauces, all these compounds are possible aroma-impact compounds and contribute to the overall aroma of soy sauce (6,26-28).

Among the phenols, methoxyphenols, i.e., phenol, 2-methoxy-, phenol, and 2,6-dimethoxy-, were components responsible for smoke flavor and significantly contribute to the flavor of Japanese-type soy sauce (7,11). Furthermore, phenol, 4-ethyl-2-methoxy- (only detected in SSDSM), which mainly arises from wheat fraction by *Candida* (*Torulopsis*) yeasts fermentation, is a very strong contributor to the good aroma of Japanese-type soy sauce (29). Notably, the relative contents of phenols in SSSB were lower than those in SSDSM (p<0.05), which might be the main reason for stronger smoky attribute in SSDSM (Fig. 1).

As seen from Table 2-4, most of the pyran(one)s, pyrazines, and pyrrol(idon)es were detected by DSE. Moreover, the higher relative content of pyrazines detected in SSDSM might be one of the important reasons for the markedly different HVP-like attribute (Fig. 1) between SSSB and SSDSM because pyrazines were the main volatile compounds of acid-hydrolyzed soy sauce (p<0.05) (11). Pyrazines mainly originate from the reactants of Strecker aldehydes and α-aminoketones, or were produced by microbial (*Aspergillus oryzae*) metabolism (11,30). Additionally, some pyrroles and pyranones, such as pyrrole and maltol, can be produced by Maillard reaction (31).
As shown in Table 2 and 3, 6 and 4 sulfur-containing compounds were detected in SSSB and SSDSM, in which propanal, 3-(methylthiol)- 1-propanol, and 3-(methylthiol)- were identified as aroma-impact compounds in soy sauce (11,15). Hence, the difference of relative contents of sulfur-containing compounds in SSSB and SSDSM can bring on the final differences of sensory flavor profiles between these two kinds of soy sauces to a certain extent.

Results of our study suggested that the differences of sensory evaluation between SSSB and SSDSM should result from their differences of volatile flavor compounds and relative contents of different chemical classes, and the latter differences were caused mainly by different contents of soy fat in SSSB and SSDSM during brewing.

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