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Comparison of determination method for volatile compounds in Thai soy sauce

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Abstract

Dynamic headspace (DHS) sampling, direct solvent extraction (DSE) and vacuum simultaneous steam distillation–solvent extraction (V–SDE) were used for sample preparation in volatile compound analysis in Thai soy sauce. The extracts obtained from two brands were then analyzed by gas chromatography-mass spectrometry (GC–MS). A comparative study of volatile compounds obtained from these preparation techniques was performed. Some similarities were observed among different characteristic volatile profiles obtained from each preparation technique. Highly volatile compounds were detected only by DHS whereas DSE and V–SDE gave a wide spectrum of chemical classes of compounds detected. Moreover, differences of volatile compounds detected from both soy sauces were noted. This might be due to the differences of production process employed and strains of microorganism used.

Keywords: Thai soy sauce; Volatile compounds; Dynamic headspace; Direct solvent extraction; Simultaneous steam distillation–solvent extraction

1. Introduction

Soy sauce is a fermented soybean food, which is used as a condiment or seasoning sauce worldwide. Its main raw materials are soybeans, wheat, and brine. Soy sauce could be classified into Japanese-type and Chinese-type, base on the amount of wheat used (Nunomura & Sasaki, 1993). In Japanese-type soy sauce, soybeans and wheat are used with the ratio 1:1, whereas less wheat is used in Chinese-type. Thai soy sauce, as one kind of Chinese-type soy sauce, has long history in development of manufacture and is consumed widely in the Southeast Asia (Mongkolwai, Assavanig, Amnajsongsiri, Flegel, & Bhumiratana, 1997; Valyasevi & Rolle, 2002). The characteristic flavor–aroma formation in the soy sauce depends on the manner of production employed, as well as raw materials and strains of microorganism used. The main steps of soy sauce production involved in flavor development are heat treatment of raw materials, koji culturing (mold fermentation), moromi fermentation (lactic acid bacteria and yeast fermentation) including aging, and pasteurization (Nunomura & Sasaki, 1993). From this point, variation and complexity of soy sauce flavor characteristic from various origins is pronounced.

Most of the studies on the volatile flavor compounds in traditional soy sauce made in several regions such as Japan, Korean, including Indonesia have been reported (Apriyantono, Husain, Lie, Jodoamidjojo, & Puspitasari-Nienaber, 1999; Kim, Lee, Shin, Ji, Choi, & Kim, 1996; Kobayashi & Sugawara, 1999; Nunomura, Sasaki, Asao, & Yokosuka, 1976a, 1976b, 1978; Nunomura, Sasaki, & Yokosuka, 1980; Seo et al., 1996). However, the study on volatile flavor compounds in Thai soy sauce has not been well conducted, and there are no such data reported. To cope with the optimization of manufacture and standardization of quality aspects, volatile flavor compounds in Thai soy sauce should be identified.

To determine the volatile compounds in soy sauce, sample preparation is necessary prior to analysis by gas chromatography–mass spectrometry (GC–MS). The main reason for sample preparation is to obtain a concentrated analytical sample without interfering substances and to improve sensitivity for target analytes (Parliment, 1997).

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Several techniques for sample preparation of volatile compounds in soy sauce have been developed (Apriyantono et al., 1999; Kim et al., 1996; Kobayashi & Sugawara, 1999; Nunomura et al., 1976a). In those studies, sample preparation techniques were mostly based on solvent extraction and distillation. These techniques are suitable for analysis of the compounds with low volatility. However, food samples possess various volatiles with volatility ranging from high to low volatility (Parliment, 1997). Thus, one single sample preparation technique is not able to cope with such wide range of volatility. Various sample preparation should be employed for profiling the volatile compound of the food matrix. To achieve the goal, comparison of the sample preparation methods for determination of volatile flavor compounds in food sample is necessary.

In fact, each sample preparation procedure is subjected to its particular drawbacks, although it offers specific advantages under certain circumstances. Direct solvent extraction (DSE) and simultaneous steam distillation–solvent extraction (SDE) are commonly used for the determination of soy sauce volatiles (Apriyantono et al., 1999; Kim et al., 1996; Nunomura et al., 1976a, 1976b, 1978; Nunomura et al., 1980; Seo et al., 1996). The extract prepared by these techniques usually contains a wide spectrum of volatile components. On the other, dynamic headspace sampling (DHS) could be a good option for recovering highly volatile compounds lost at concentration step of DSE (Wampler, 1997). The objective of this study was to compare the sample preparation, i.e., DSE, SDE, and DHS, used for the determination of the volatile flavor compounds in Thai soy sauce.

2. Materials and methods

2.1. Samples

Two brands of Thai soy sauce were purchased from local markets. Samples were stored in the dark at room temperature.

2.2. Chemicals

Dichloromethane was purchased from Merck (Darmstadt, Germany) and was redistilled prior to use. 2-Ethyl butyric acid, 2-methyl-3-heptanone, and 2,4,6-trimethyl pyridine were obtained from Aldrich Chemical (St. Louis, MO). Other chemicals were of the best grade available supplied from Merck (Darmstadt, Germany).

2.3. Dynamic headspace (DHS) analysis

Soy sauce samples (100 g) was added with 10 μl methanolic solution of 2-methyl-3-heptanone as an internal standard at final concentration of 152.6 ng/g. Prior to analysis, each sample was saturated with sodium chloride, and 5 ml of the sample was drained in a sampling tube (15.2×1.6 cm i.d.). The sampling tube was then connected to a Tekmar Dohrmann 3100 purge and trap concentrator (Tekmar, Cincinnati, OH). The sample was preheated for 2 min at 40 °C, then it was purged with ultra high-purity helium (50 ml/min) for 20 min to a Tenax TA trap. After sample purging process, the trap was dry-purged for 7 min to remove moisture, then the volatiles were desorbed from the trap at temperature of 220 °C for 2 min. The desorbed volatiles were directly introduced onto GC–MS via electric pressure control–volatiles interface with split ratio of 10:1. Transfer line was maintained at temperature of 220 °C with the trap pressure of 4 psi. The Tenax TA trap of the purge and trap system was subsequently cleaned up by being baked at temperature of 225 °C for 10 min. Separations of the volatile compounds were done on HP-FFAP capillary column (polyethylene glycol modified with nitroterephthalic acid as stationary phase; 25 m×0.32 mm i.d.×0.50 μm film thickness; Agilent Co.). The GC oven temperature was held at 45 °C for 5 min, then programmed from 45 to 180 °C at a rate of 10 °C / min, following with 180 °C to 240 °C at a rate of 20 °C/ min. DHS analysis of each sample was performed in duplicate.

2.4. Direct solvent extraction (DSE)

Soy sauce samples (25 g) were added with methanolic solutions of 2-ethyl butyric acid (0.446 μg/μl), 2-methyl-3-heptanone (1.424 μg/μl), and 2,4,6-trimethyl pyridine (0.450 μg/μl), as internal standards (10 μl) for acid, neutral, and basic fractionation, respectively. The sample was saturated with sodium chloride and adjusted to pH 3.0 with hydrochloric acid. Then, the sample was extracted with dichloromethane (three times×20 ml). To separate solvent layer, the sample was centrifuged at 3000 rpm at room temperature for 15 min. After the solvent layer was recovered and pooled, the sample was re-adjusted to pH 12.0 with sodium hydroxide solution and was extracted as described above. The combined solvent layer was subjected to vacuum distillation with liquid nitrogen cold trap for 4 h. After that, resulting distillate was washed with 5% hydrochloric acid (three times×40 ml). The pooled aqueous phase was alkalized with sodium hydroxide solution to pH 12.0. Then the basic volatiles were extracted from the aqueous phase with dichloromethane (three times×40 ml), and washed with saturated sodium chloride solution (40 ml) to obtain basic fraction (DSE-B). The organic phase containing the acid/neutral volatiles was washed with 5% sodium hydroxide solution (three times×40 ml). The resulting organic layer (neutral volatiles) was washed with saturated sodium chloride solution (40 ml), to

obtain neutral fraction (DSE-N). The remaining aqueous phase was acidified with hydrochloric acid to pH 3.0 and the acid volatiles were extracted with dichloromethane (three times×40 ml) to obtain acid fraction (DSE-A). Each fraction was frozen at −20 °C overnight for water removal. The volume of each fraction was reduced to 5 ml under gentle nitrogen stream, and was dried over anhydrous sodium sulfate. The volume was further reduced to 50 μl prior to analysis. Two DSE extracts were prepared.

2.5. Vacuum simultaneous steam distillation–solvent extraction (V-SDE)

Soy sauce samples (100 g) plus deodorized water (350 ml) were added with 100 μg of 2-methyl-3-heptanone as an internal standard. The sample was extracted for 2 h with dichloromethane (450 ml) under reduced pressured (250 mbar) in an SDE apparatus (catalog no. 523010-0000, Kontes, Vineland, NJ). The modifications of the system were as follows: (1) an additional condenser was installed between SDE apparatus and vacuum generator, and the temperature of the condenser was maintained at −15 °C; and (2) a 1000 ml, three-neck-round-bottomed sample flask was connected to the apparatus, a vacuum gauge, and a thermometer. During extraction, the sample was maintained at temperature of 65–70 °C. The V-SDE extract was kept at −20 °C overnight to facilitate water removal as ice crystal, and was concentrated to 5 ml. After drying over anhydrous sodium sulfate, it was further concentrated to 1 ml under gentle nitrogen stream. V-SDE extracts were prepared in duplicate.

2.6. Gas chromatography–mass spectrometry

GC–MS analysis was conducted using the Agilent 6890 Plus GC/HP 5973 MSD, equipped with an HP-FFAP column (25 m×0.32 mm i.d.×0.50 μm film thickness) or HP-5MS (5%-phenyl-methylpolysiloxane as stationary phase; 30 m×0.25 mm i.d.×25 μm film thickness; Hewlett-Packard Co.). Each V-SDE, DSE-N fraction, and DSE-A fraction was analyzed by HP-FFAP column, whereas DSE-B fraction was analyzed by HP-5MS column. For direct injection, one microlitre of each extract was injected onto GC–MS by splitless mode with injection temperature of 230 °C. The GC oven temperature was programmed from 45 to 220 °C for HP-FFAP, or 45 to 280 °C for HP-5MS at the rate of 15 °C/min. The initial and final hold times were 2 and 11.40 min, respectively. The carrier gas was ultra high purity helium at a constant flow of 1.5 ml/min for HP-FFAP or 1.0 ml/min for HP-5MS. Mass spectrometer conditions were as follows: MSD capillary direct-interface temperature was 280 °C. Ionization energy was 70 eV. Mass range was 35–450 a.m.u. for direct injection or 20–350 a.m.u. for DHS analysis. Electron multiplier (EM) voltage was obtained from autotune, and scan rate was 3.50 scan/s for direct injection or 4.33 scan/s for DHS analysis.

2.7. Compound identification

Positive identification of a component was performed by comparison of its retention index (RI) and mass spectrum. Tentatively identified compounds were uniquely identified on the basis of the mass spectra from the Wiley 275.L mass spectral database (Hewlett-Packard Co.). The integration of peaks was done on HP chemstation software (Hewlett-Packard Co.). The minimum peak area for detection was 10,000 counts for DHS analysis, and 100,000 count for DSE and V–SDE.

3. Results and discussions

The chromatograms shown in Figs. 1 and 2, illustrate the volatile profiles of Thai soy sauce brand A and B, obtained from DHS, DSE, and V-SDE. Identified volatile compounds with their relative peak area are summarized in Tables 1–3, for DHS, DSE, and V–SDE, respectively. Totally, ninety-three compounds were detected from both samples. They included 10 acids, 15 alcohols, 3 aldehydes, 10 esters, 7 furans, 11 furanones, 11 ketones, 5 phenols, 7 pyrazines, 3 pyrones, 5 sulfur-containing compounds, and 6 miscellaneous compounds. Fifteen of these compounds, i.e., 2-methyl-1-propanol, 3-methyl-1-butanol, benzeneethanol, benzaldehyde, furfural, 2-furanmethanol, dihydro-2(3H)-furanone, 3-hydroxy-2-butanone, 2-methoxy-phenol, phenol, 4-ethyl-2-methoxy-phenol, methyl pyrazine, S-butyl thiohexanoate, methionol, and 2-pyrrolyl methyl ketone, were found in all extracts. Only some compounds were detected by both DHS and DSE. Those were 1-butanol, benzenemethanol, dihydro-2-methyl-3(2H)-furanone, and 2,5-dimethyl pyrazine. DHS and V-SDE profiles showed similarity in volatile compounds detected, i.e., acetic acid, 2-methylpropanoic acid, butanoic acid, benzeneacetaldehyde, ethyl lactate, dihydro-3-methyl-2(3H)-furanone, 2(5H)-furanone, 1-hydroxy-2-propanone, 1-hydroxy-2-butanone, and 2,6-dimethyl pyrazine. In addition, 2-ethylhexanoic acid, 2-ethyl-hexanol, 4-hydroxy-2-ethyl-5-methyl-3(2H)-furanone (HEMF), 4-ethyl-phenol, maltol, ethyl maltol, and S-propyl thiopentanoate were detected only by DSE and V-SDE. Possessing low volatility, these compounds, particularly hydroxy furanone compound (i.e., HEMF) and hydroxy pyrone compound (i.e., maltol and ethyl maltol), could not be detected by DHS analysis.

In this study, acetone, ethyl acetate, 2-butanone, 2-methyl-butanal, ethanol, 4-methyl-2-pentanone, 2-butanol, ethyl butanoate, and 1-propanol were detected in

Thai soy sauce only by DHS. Generally, DHS exploits the difference in volatility among analyte and sample matrices. With this technique, headspace over the sample is swept by an inert gas for a period of time in DHS sampling device (purge and trap concentrator). The analytes partitioning in the headspace are subsequently trapped and concentrated in porous polymer trapping unit, prior to GC–MS analysis. Hence, DHS analysis is capable for the detection of highly volatile analytes with low molecular weight. Such analytes could not be detected by the other techniques. DHS offers the various advantages, including analyzing the sample without

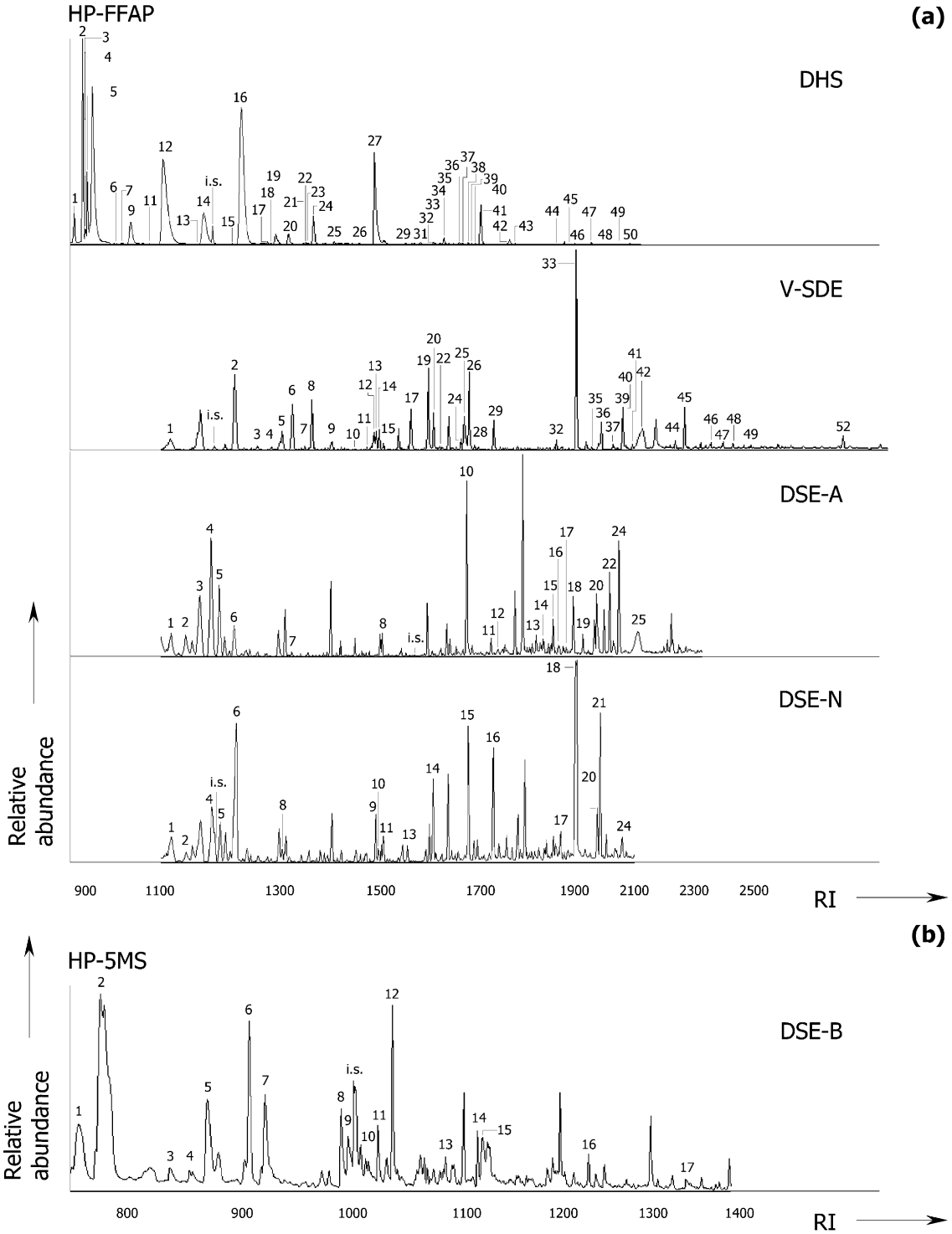

Fig. 1. Total ion count (TIC) chromatograms of the volatile components of Thai soy sauce brand A derived from (a) DHS, V-SDE, DSE-A, and DSE-N, (b) DSE-B.

the interference of solvent peak, discrimination to analyze only volatiles, less steps in sample preparation without using organic solvent, and being capable for routine work with high reproducibility. From these points of view, DHS could be used to monitor the change of volatile profile during fermentation as well as storage of Thai soy sauce. There were, however, some limitations and obstacles in this technique. Low sensitivity to the low volatile compounds is one of the limitations of DHS. As shown in Table 1 and Fig. 3, the compounds in the chemical classes of furanone (hydroxy furanone; HEMF) and particularly pyrone (hydroxy pyrones; maltol and ethyl maltol) were not detected by the mean of DHS. The direct injection of

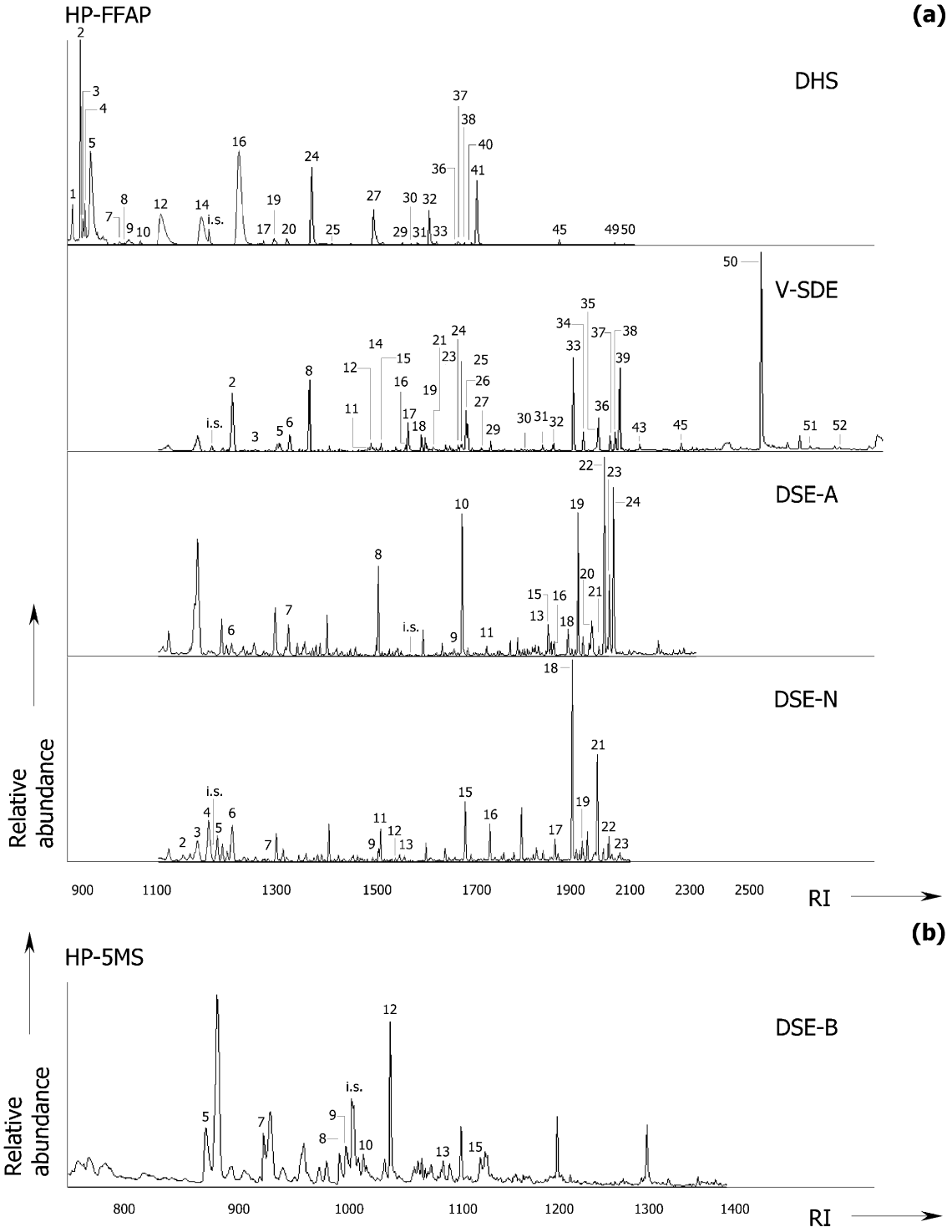

Fig. 2. TIC chromatograms of the volatile components of Thai soy sauce brand B derived from (a) DHS, V-SDE, DSE-A, and DSE-N, (b) DSE-B.

desorbed analytes via electric pressure control-volatiles interface to the GC inlet might cause broad shape of peaks (Figs. 1 and 2). Using of cryogenic focusing on the GC inlet sharpens the peak shape on the chromatogram (Wampler, 1997). DHS could also lead to the errors of analysis due to cross-contamination from sample to sample. Thus, the proper cleaning steps are needed when DHS is employed.

Solvent extraction has been performed as the general method for the analysis of flavor compounds in food samples. In this study, after DSE, vacuum distillation and pH fractionation of the resulting distillate were

Table 1
Volatile compounds of Thai soy sauce from DHS analysis

Peak no.	RI[a]	Compound name	Area normalize		Peak no.	RI[a]	Compound name	Area normalize	
			A	B				A	B
Acids									
27	1465	Acetic acid	5.62	2.92	32	>1500	2-Methylpropanoic acid	0.12	1.78
31	>1500	Propanoic acid	0.09	0.10	37	>1500	Butanoic acid	0.03	0.18
Alcohols									
5	944	Ethanol	19.01	20.30	14	1164	1-Butanol	5.87	7.86
7	1029	2-Butanol	0.07	0.26	16	1225	3-Methyl-1-butanol	25.12	25.15
9	1045	1-Propanol	2.80	0.96	46	>1500	Benzenemethanol	0.02	
12	1104	2-Methyl-1-propanol	19.90	10.74	47	>1500	Benzeneethanol	0.07	
Aldehydes									
4	920	2-Methyl-butanal	4.36	3.22	39	>1500	Benzenacetaldehyde	0.02	
30	>1500	Benzaldehyde	0.02	0.03					
Esters									
2	903	Ethyl acetate	8.41	9.19	24	1356	Ethyl lactate	1.32	5.38
8	1038	Ethyl butanoate		0.06	26	1436	Ethyl hydroxyacetate	0.03	
Furans									
28	1487	Furfural		0.12	41	>1500	2-Furanmethanol	1.54	3.88
29	>1500	2-Furyl methyl ketone	0.02	0.07	44	>1500	3-Phenyl furan	0.01	
33	>1500	2-Furyl ethyl ketone	0.04	0.11					
Furanones									
15	1207	5-Methyl-2(5H)-furanone	0.01		36	>1500	Dihydro-5-methyl-2(3H)-Furanone		0.03
17	1276	Dihydro-2-methyl-3(2H)-furanone	0.09	0.19	38	>1500	Dihydro-2(3H)-furanone	0.05	0.08
35	>1500	Dihydro-3-methyl-2(3H)-furanone	0.02		43	>1500	2(5H)-Furanone	0.01	
Ketones									
1	832	Acetone	2.39	4.09	19	1295	3-Hydroxy-2-butanone	0.69	0.61
3	914	2-Butanone	0.74	1.61	20	1315	1-Hydroxy-2-propanone	0.57	0.42
6	1013	4-Methyl-2-pentanone	0.01		25	1390	1-Hydroxy-2-butanone	0.13	0.06
10	1067	2,3-Pentanone		0.30	40	>1500	1-Phenyl ethanone	0.03	0.07
13	1154	5-Methyl-2-hexanone	0.01						
Phenols									
45	>1500	2-Methoxy-phenol	0.07	0.17	50	>1500	4-Ethyl-2-methoxy-phenol	0.03	
49	>1500	Phenol	0.01	0.05					
Pyrazines									
18	1283	Methyl pyrazine	0.03		22	1344	2,6-Dimethyl pyrazine	0.07	
21	1339	2,5-Dimethyl pyrazine	0.02		23	1348	Ethyl pyrazine	0.01	
Sulfur-containing compounds									
11	1079	Dimethyl disulfide	0.02		42	>1500	Methionol	0.22	
34	>1500	S-propyl thiohexanoate	0.26						
Miscellaneous compound									
48	>1500	2-Pyrrolyl methyl ketone	0.01						

[a] RI: Retention index, calculated according to the retention time of n-alkanes standard on HP-FFAP column.

Table 2
Volatile compounds of Thai soy sauce from DSE extract

Peak no.	RI[a]	Compound name	Area normalize		Peak no.	RI[a]	Compound name	Area normalize	
			A	B				A	B
DSE-A									
Acid									
19	1968	2-Ethylhexanoic acid	1.15	12.60					
Alcohols									
1	1110	2-Methyl-1-propanol	4.30		7	1326	4-methyl-2-pentanol	0.48	5.27
2	1136	2-Pentanol	3.95		8	1503	2-Ethyl-hexanol	1.29	7.97
3	1162	1-Butanol	12.85		16	1908	Benzenemethanol	0.56	1.67
4	1182	2-Hexanol	18.08		18	1947	Benzeneethanol	3.51	2.95
6	1224	3-Methyl-1-butanol	4.25	2.22					
Ester									
5	1197	Butyl 2-Propenoate	7.80						
Furan									
10	1687	2-Furanmethanol	10.94	13.80					
Furanones									
9	1668	Dihydro-2(3H)-furanone		0.93	25	2111	HEMF[b]	6.64	
12	1759	3-Methyl-2(5H)-Furanone	0.42						
Phenols									
15	1893	2-Methoxy-phenol	2.21	2.81	24	2065	4-Ethyl-2-methoxy-phenol	7.51	15.16
22	2039	Phenol	4.90	18.77	26	2209	*p-Ethyl phenol*	2.42	
Pyrones									
20	2003	Maltol	3.80	3.91	23	2052	Ethyl maltol		7.71
Sulfur-containing compound									
11	1744	Methionol	1.05	0.96					
Miscellaneous compound									
13	1861	Corylone[c]	0.47	0.73	21	2006	2-Pyrrolyl methyl ketone		2.54
14	1868	3,4-DMCP[d]	1.03						
17	1926	3-Ethyl-2-hydroxy-2-cyclopenten-1-one	0.39						
DSE-N									
Acid									
19	1968	2-Ethylhexanoic acid		2.30					
Alcohols									
1	1108	2-Methyl-1-propanol	4.74		6	1224	3-Methyl-1-butanol	17.74	10.62
2	1135	2-Pentanol	0.96	1.17	11	1503	2-Ethyl-hexanol	1.44	3.99
3	1161	1-Butanol		0.98	17	1908	Benzenemethanol	1.86	1.10
4	1182	2-Hexanol	7.98	14.03	18	1947	Benzeneethanol	23.65	27.86
Aldehyde									
13	1553	Benzaldehyde	1.04	0.92					
Ester									
5	1197	Butyl 2-Propenoate	3.72	6.05					
Furans									
10	1494	Furfural	0.72		15	1687	2-Furanmethanol	8.00	8.05
12	1534	2-Acetylfuran		0.47					
Furanone									
7	1286	Dihyro-2-methyl-3(2H)-furanone		0.63					

(*continued on next page*)

Table 2 (*continued*)

Peak no.	RI[a]	Compound name	Area normalize		Peak no.	RI[a]	Compound name	Area normalize	
			A	B				A	B
Ketones									
8	1306	3-Hydroxy-2-butanone	0.74						
Phenols									
22	2039	Phenol		2.76	23	2065	4-Ethyl-2-methoxy-phenol		0.41
Pyrone									
20	2004	Maltol	3.19						
Sulfur-containing compounds									
9	1488	S-propyl thiopentanoate	2.62	0.63	16	1744	Methionol	7.02	4.40
14	1609	S-butyl thiohexanoate	4.84						
Miscellaneous compounds									
21	2008	2-Pyrrolyl methyl ketone	8.11	13.64	24	2067	2-Formylpyrrole	1.63	
DSE-B									
Alcohols									
1	<800	3-Methyl-1-butanol	12.85		12	1031	2-Ethyl-1-hexanol	9.09	28.16
2	<800	2-Hexanol	17.75		15	1120	Benzeneethanol	3.94	6.95
Esters									
6	902	Butyl 2-propenoate	13.09		14	1114	L-Isoleucine, ethyl ester	2.60	
11	1017	L-Valine, ethyl ester	3.03		16	1232	6-Methylheptyl 2-propenoate	1.40	
Furans									
4	854	2-Furanmethanamine	0.97		5	866	2-Furanmethanol	12.65	26.18
Phenol									
9	989	Phenol	3.75	10.78					
Pyrazines									
3	836	Methyl pyrazine	1.80		13	1083	3-Ethyl-2,5-dimethyl pyrazine	1.04	2.83
7	917	2,5-Dimethyl pyrazine	7.54	11.77	17	1340	4-Methyl-pyrrolo(1,2-A)pyrazine	0.67	
10	1005	Trimethyl pyrazine	0.72	4.09					
Sulfur-containing compound									
8	983	Methionol	7.12	9.24					

[a] RI: Retention index, calculated according to the retention time of n-alkanes standard on HP-FFAP column (DSE-A and DSE-N) and HP-5MS column (DSE-B).
[b] HEMF: 4-Hydroxy-2-ethyl-5-methyl-3(2H)-furanone.
[c] Corylone: 2-Hydroxy-3-methyl-2-cyclopenten-1-one.
[d] 3,4-DMCP: 3,4-Dimethyl cyclopentenolone.

applied to exclude non-volatile materials from the extract, and to facilitate chromatographic technique, respectively. Unlike DHS, this technique did not discriminate the extraction of compounds based on their volatility. However, its discrimination was based on the solubility of the analyte in the solvent (Parliment, 1997). This factor is directly related to the partitioning property of the analyte between sample matrices and solvent layer. Moreover, DSE was operated at lower temperature than the SDE conducted. Thence the thermal generation of artifacts in DSE should not occur. On the other hand, DSE also caused some difficulties for detection of highly volatile compounds and routine analysis. In this study, DSE detected a large number of semi-volatiles (i.e., furanone and pyrone; Fig. 3), but the highly volatile compounds were lost during sample preparation and concentration. Moreover, the presence of solvent peak could mask the analytes' peaks eluted at the beginning of the chromatography. The main disadvantage of DSE was that it took several steps in operations and was time-consuming in sample preparation. This also led to the sample loss and the contamination from the environment during the sample preparation.

SDE exploits the differences in volatility and polarity among the analytes and the other non-volatile compo-

Table 3
Volatile compounds of Thai soy sauce from V-SDE extract

Peak no.	RI[a]	Compound name	Area normalize		Peak no.	RI[a]	Compound name	Area normalize	
			A	B				A	B
Acids									
11	1476	Acetic acid	0.46	0.58	34	1965	2-Ethylhexanoic acid	0.60	1.74
18	1586	2-Methylpropanoic acid		1.51	50	2473	Benzoic acid		26.36
23	1648	Butanoic acid		0.63	51	>2600	Benzeneacetic acid		0.52
27	1688	3-Methylbutanoic acid		2.36	52	>2600	Benzenepropinoic acid	1.43	0.43
31	1864	Hexanoic acid		0.66					
Alcohols									
1	1108	2-Methyl-1-propanol	3.33		19	1594	1,3-Buanediol	6.71	1.27
2	1224	3-Methyl-1-butanol	12.06	12.56	21	1612	Propylene glycol		0.50
15	1502	2-Ethyl-hexanol	0.47	0.79	33	1944	Benzeneethanol	14.90	8.86
17	1558	2,3-Butanediol	3.59	3.33					
Aldehydes									
16	1553	Benzaldehyde		0.77	25	1674	Benzeneacetaldehyde	4.49	1.12
Esters									
8	1362	Ethyl lactate	4.17	8.07	53	>2600	Ethyl vanillate	0.49	
28	1697	Diethyl succinate	0.30						
Furans									
14	1492	Furfural	1.55	0.34	26	1685	2-Furanmethanol	5.01	3.91
Furanones									
22	1625	Dihydro-3-methyl-2(3H)-furanone	0.23		42	2107	HEMF[b]	8.17	
24	1666	Dihydro-2(3H)-furanone	0.75	0.53	48	2378	Dihydro-5-(1-hydroxyethyl)	0.39	
30	1796	2(5H)Furanone		0.32			−2(3H)-furanone		
40	2070	Dihydro-3-hydroxy-4,4-dimethyl-2(3H)-furanone	0.22		49	2431	4-(1-hydroxyethyl).gamma. butanolactone	0.22	
Ketones									
3	1262	3-Hydroxy-3-methyl-2-butanone	0.49	0.39	9	1399	1-Hydroxy-2-butanone	1.01	
5	1307	3-Hydroxy-2-butanone	2.20	1.32	10	1469	1-Hydroxy-2-pentanone	0.27	
6	1326	1-Hydroxy-2-Propanone	6.33	3.11					
Phenols									
32	1890	2-Methoxy-phenol	0.77	0.72	44	2207	*p-Ethyl phenol*	0.59	
37	2035	Phenol	0.41	1.50	45	2234	4-Vinyl-2-methoxy-phenol	3.18	0.77
39	2061	4-Ethyl-2-methoxy-phenol	3.25	7.57					
Pyrazines									
4	1287	Methyl pyrazine	0.33		7	1349	2,6-Dimethyl pyrazine	0.29	
Pyrones									
35	1998	Maltol	0.25	0.61	46	2311	4H-Pyran-4-one, 2,3-dihydro	0.37	
38	2050	Ethyl maltol		1.71			−3,5-dihydroxy-6-methyl		
Sulfur-containing compounds									
12	1482	Methional	1.71	0.76	20	1607	S-butyl thiohexanoate	2.55	
13	1486	S-propyl thiopentanoate	1.52		29	1742	Methionol	2.16	0.74
Miscellaneous compounds									
36	2003	2-Pyrrolyl methyl ketone	1.93	3.17	47	2348	4-Methyl-5-(2-hydroxyethyl)	0.47	
41	2089	2-Pyrolidinone	0.39				thiazole		
43	2117	2,6-di(t-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadiene-1-one		0.46					

[a] RI: Retention index, calculated according to the retention time of n-alkanes standard on HP-FFAP column.
[b] HEMF: 4-Hydroxy-2-ethyl-5-methyl-3(2H)-furanone.

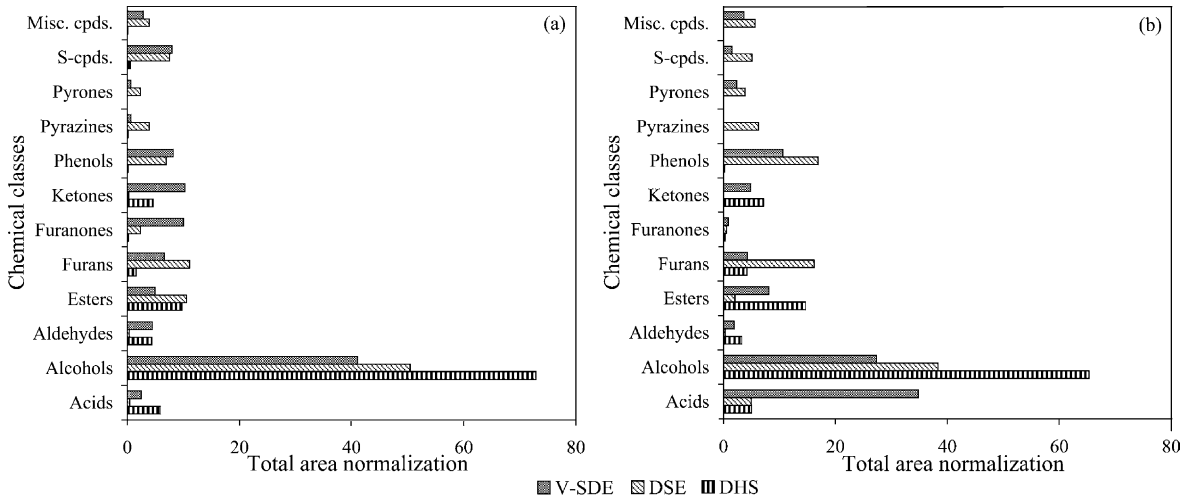

Fig. 3. Distribution of chemical classes of volatile compounds in Thai soy sauce (a) brand A and (b) brand B prepared by DHS, V–SDE, and DSE, (Misc. cpds., Miscellaneous compounds; S-cpds., Sulfur-containing compounds).

nents in food (Parliment, 1997). In this study, the SDE system was operated under vacuum. This facilitated distillation of the sample at relatively low temperature, in order to reduce thermal generation of the artifacts. This technique also gave a wide spectrum of chemical compounds detected. In addition, the fractionation of vacuum SDE (V-SDE) extract by pH (i.e., acid, neutral, basic, and phenol fractions) might be applied to facilitate further chromatographic technique (Seo et al., 1996). The major advantage of V-SDE was that it required only two single operations, i.e., extraction and concentration without sample cleaning. However, there was the possibility for the artifacts generated from heating, even the system was operated under vacuum conditions. In this study, methional, a product of Strecker degradation from methionine was an evidence for the chemical changes in the sample during V-SDE procedure (Table 3). In Thai soy sauce sample, methional was not detected by both of DHS and DSE, although it is thought to be present in the soy sauce at low concentration. Generally, methional occurs during the brewing process and can be converted into methionol by microbial biotransformation, and the redox conditions of the fermentation. This compound has more acceptable flavor (Kobayashi & Sugawara, 1999).

Both brands of Thai soy sauce showed slightly different pattern among chemical compounds detected (Tables 1–3, and Fig. 3). The number of peaks detected by each method in brand A was higher than in brand B, except in neutral fraction of DSE extract (DSE-N). The chemical compounds showing remarkable differences between two brands were acids, alcohols, furanones, and phenols (Fig. 3). The percentage of alcohols distributed in brand A was higher than in brand B. In fact, alcohols were produced by yeast fermentation during moromi process. This could be used as an index to monitor fermentation process of the Thai soy sauce. Brand A might involve in more effective yeast fermentation than brand B, since it contained higher percentage of alcohols. More acid compounds were detected in brand B. This included benzoic acid, which was not detected in brand A. Benzoic acid might be used as a preservative. During moromi fermentation, acids were produced by lactic acid bacteria. In general, alcohol formation in Thai soy sauce is limited as seen in low consumption of reducing sugar (Lertsiri, Muangma, Assavanig, & Bhumiratana, 2001).

In case of furanones, especially hydroxy furanone; HEMF, have been reported as the character impact compound of Japanese soy sauce (Nunomura et al., 1976b; Nunomura & Sasaki, 1993). HEMF is biosynthesized by yeasts (*Zygosaccharomyces rouxii* and *Candida* sp.) via pentose phosphate pathway (Nunomura & Sasaki, 1993). None of HEMF is produced in soy sauce fermentation, unless these microorganisms are presented. Thus, the fermentation of brand B might have less efficiency in HEMF formation. For phenols, the concentration in brand B was higher than in brand A. These compounds are generated from the degradation of lignin glycoside during fermentation (Kobayashi & Sugawara, 1999). Koji mold produces phenol compounds by releasing them from lignins in cereal bran. In addition, ethyl maltol, which is a synthetic chemical, was found only in brand B. This compound might be used as an artificial flavoring additive.

To determine the odor-active compounds being present in Thai soy sauce, the use of GC–Olfactometry (GC–O) technique must be employed. Application of GC–O has been used to determine the character impact compound of Indonesian soy sauce as well as Korean soy sauce (Apriyantono et al., 1999; Kim et al., 1996). The extracts prepared from SDE and V–SDE are easily analyzed by GC–O. In some cases, the sample prepared from DHS can be applied for sniffing on GC–O as well

(Lee, Suriyaphan, & Cadwallader, 2001). The techniques based on dilution analysis can be used for the determination of the most potent odorant in food sample (Blank, 1997). Those are aroma extract dilution analysis (AEDA) and Charm analysis. AEDA technique can be applied with DSE and V–SDE extracts as well as DHS preparation (Lee et al., 2001). The determination of aroma active compounds as well as character impact compounds in Thai soy sauce will be performed in the future.

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