Further Identification of Volatile Compounds in Fish Sauce

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Volatile compounds in fish sauce were isolated by a column concentration method using Porapak Q and by a simultaneous distillation and extraction method with diethyl ether under reduced pressure. Column concentration isolates were further fractionated by silica gel column chromatography and organoleptically evaluated. About 155 volatile compounds, including 14 acids, 36 carbonyls, 17 nitrogen-containing compounds, and 10 sulfur-containing compounds, were identified. Column concentration could be a suitable method for isolating volatile compounds in fish sauce. Large amounts of acids, large numbers of carbonyls and sulfur-containing compounds, such as, dimethyl sulfide, dimethyl trisulfide, and 3-(methylthio)propanal, as well as many types of nitrogen-containing compounds are thought to be major contributors to fish sauce odor.

Keywords: *Fish sauce; odor; volatile compounds*

INTRODUCTION

Fish sauce is a hydrolysis product of fish and salt (3:1−2), which is allowed to ferment from 6 to 12 months. It is an easily produced and not too costly condiment that is widely used in most countries of Southeast Asia. People from these regions have been familiar with its characteristic smell and are more concerned with the good taste it provides when blended with some local food preparations or when simply used as a sauce. However, in areas such as Japan, the United States, and Europe, people have not developed tolerance to its characteristic odor; thus, fish sauce has not become as popular as soy sauce.

Several reports have already been published on the study of fish sauce flavors. The odor of fish sauce, which had been described as a blend of three distinctive notes, ammoniacal, cheesy, and meaty (Dougan and Howard, 1975; Beddows et al., 1976), is basically derived from protein hydrolysate and lipid oxidation products brought about by autolytic and microbial fermentation (Saisithi et al., 1966; Beddows et al., 1980; McIver et al., 1982). The ammoniacal note is produced by ammonia, amines, and other basic nitrogen-containing compounds (Saisithi et al., 1966; Dougan and Howard, 1975), and the cheesy note has been attributed to low molecular weight volatile fatty acids (Van-chom, 1958; Saisithi et al., 1966; Dougan and Howard, 1975; Beddows et al., 1976; Sanceda et al., 1983). To date, no extensive report is yet available on the source of the meaty odor. Volatile compounds in fish sauce may vary due to species and origin of fish used, as well as the manner of production employed. Volatile compounds in fish sauces of various origins have been identified by several investigators by different techniques of sample preparation and methods of analysis. Saisithi et al. (1966) investigated the role of microorganisms in the development of fish sauce flavor and odor by isolating volatile compounds from Thai fish sauce, locally called nampla, and employing ion-exchange column chromatography and thin-layer chromatography. On the other hand, volatile compounds in nampla have been isolated by solvent extraction, then separated into acidic, basic, and neutral fractions and identified by gas chromatography−mass spectrometry (GC-MS) (McIver et al., 1982). Sanceda et al. (1984) separated volatile compounds in patis, a fish sauce locally produced in The Philippines, by steam distillation under reduced pressure and identified the compounds by GC-MS. Volatile compounds of shottsuru, a Japanese fish sauce, nampla, a Thai fish sauce, and noucnam, a Vietnamese fish sauce, were identified and compared by the same method (Sanceda et al., 1986).

A considerable number of studies have already been conducted on fish sauce. However, identification of volatile compounds is still incomplete and the possible contributors to the characteristic odor of fish sauce have not yet been fully elucidated. In this study, column concentration (CC) and simultaneous distillation and extraction (SDE) methods were compared. Odor fractions separated from CC concentrate were investigated. Significant results obtained from CC suggested that this method could be a more suitable technique for isolating and identifying major contributors to fish sauce odor, such as nitrogen- and sulfur-containing compounds.

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MATERIALS AND METHODS

Materials. Fish sauce, produced in Taiwan and contained in a 2-L plastic bottle, was obtained from Ichiban Shokuhin Company, Ltd., Fukuoka, Japan. A modified Lickens-Nickerson type of SDE apparatus was used. Diethyl ether was purchased from Nakarai Tesque, Inc., Kyoto, Japan. Methanol, from Wako Pure Chemical Industries, Company, Ltd., Osaka, Japan, was further purified by passing twice through an analytical grade activated charcoal-packed column (50 × 3 cm i.d.). Porapak Q (50−80 mesh) was from Waters Associates, Milford, MA, and silica gel (100−200 mesh) was from Merck, Darmstadt, Germany.

Composition of the Standard for the Fish Sauce Sample. The fish sauce standard was composed of 25.5% NaCl (sp gr = 1.24, pH = 5.7) 1.27% amino nitrogen, 2.22% total nitrogen, and 42.6 °brix soluble solids. Specifications were obtained from Ichiban Shokuhin Company, Ltd., Fukuoka, Japan.

Simultaneous Distillation and Extraction (SDE) Method. Fish sauce (1400 mL) was distilled at ∼65−70 °C and simultaneously extracted with diethyl ether for 50 min with a modified Likens-Nickerson type of SDE apparatus under reduced pressure while a coolant was circulated at −10 °C. After extraction, cyclohexanol (1.0 ppm) was added as an

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Table 1. Volatile Compounds in Odor Concentrate Determined by SDE

peak no.	compound name	concentration (μg/L)
1	ethyl acetate	124
2	2-oxopropanol	73
3	3-methylbutanal	81
4	unknown	340
5	unknown	67
6	2-methyl-1-propanol	27
7	1-penten-3-ol	120
8	3-methyl-1-butanol	75
9	2-ethyl-6-methylpyrazine	22
10	cyclohexanol (i.s.)	1000
11	acetic acid	790
12	propanoic acid	1000
13	2-methylpropanoic acid	470
14	butanoic acid	3240
15	3-methylbutanoic acid	8910
16	pentanoic acid	72
17	2-propenylhydrazone-2-propane	39
18	4-methylpentanoic acid	400
19	hexanoix acid	51
20	phenol	120

Table 2. Odor Characteristics of Each Fraction of Volatile Concentrate

fraction no.	sensory note
1	no distinctive color
2	moderately strong, stimulating
3	strong fish sauce-like (fishy)
4	weakened odor of fraction 3
5	fermented, yeast-like
6	salty, soy sauce-like

internal standard. The ether extract was dried over anhydrous sodium sulfate and concentrated to ~200 μL for capillary GC and GC-MS analyses by evaporating ethyl ether with glass beads (to prevent bumping) in a water bath at 40 °C.

Column Concentration (CC) Method. A glass column (10 × 2 cm i.d.) was packed with Porapak Q (15 mL), washed with deionized water (70 mL) followed by purified methanol (2 × 60 mL), then washed several times with excessive amounts of diethyl ether, and then washed with purified methanol and deionized water prior to use. The fish sauce sample (1000 mL) was passed through the Porapak Q column. Water-soluble compounds were removed by washing the column with deionized water, prior to elution of adsorbed volatiles with diethyl ether (80 mL). The ether extract was dried over anhydrous sodium sulfate and concentrated to ~200 μL for capillary GC and GC-MS analyses, in the same manner as described for SDE.

Fractionation of Odor Concentrate. The odor concentrate (from CC) was passed through a silica gel-packed column (20 × 10 mm i.d.). Adsorbed volatiles were eluted with diethyl ether/*n*-pentane solution (1:1) and separated into six 10-mL fractions. The odor of each fraction was organoleptically evaluated by three co-workers. The fractions that exhibited strong fish sauce-like odor were concentrated to ~50 μL for capillary GC and GC-MS analyses in the same manner as described for SDE.

Capillary Gas Chromatography (GC). Analysis was performed on a Shimadzu GC-14 A model gas chromatograph equipped with a flame ionization detector (FID) and connected to a Shimadzu Chromatopac C-R3A integrator. Separation was achieved on a fused silica capillary column (60 m × 0.25 mm i.d.) coated with crosslinked polyethylene glycol (20 M), with a film thickness of 0.25 μm (DB-Wax; J&W Scientific, Folsom, CA). The oven temperature was programmed from 50 to 230 °C at 2 °C/min. The injection port and detector temperatures were 220 and 250 °C, respectively. The helium carrier gas flow rate was 22 cm/s, with a split ratio of 1:25.

Capillary Gas Chromatography−Mass Spectrometry (GC-MS). Volatile compounds were identified with a JEOL Automass 50 model mass spectrometer attached to a Hewlett Packard 5890 Series II gas chromatograph. The column and chromatographic conditions were the same as described for GC. The mass spectra was obtained by electron-impact ionization at 70 eV. Mass spectral data were compared with authentic data (NIST database; *Eight Peak Index of Mass Spectra*, 1983; *Wiley/NBS Registry of Mass Spectral Data*, 1989).

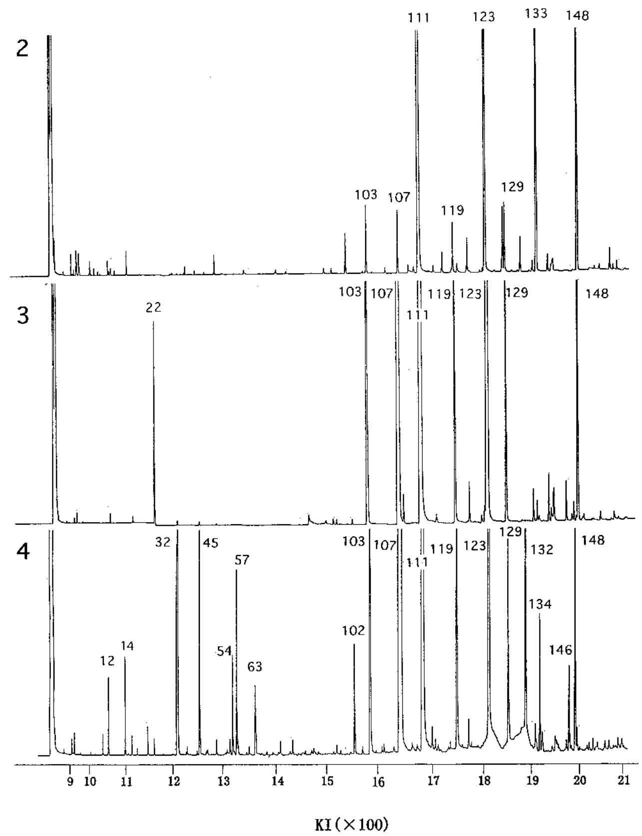

Figure 1. Gas chromatograms of fractions 2, 3, and 4 separated by silica gel column chromatography.

RESULTS AND DISCUSSION

SDE Results. The odor concentrate obtained by SDE had a strong stimulating, cheesy odor but had weak, meaty, and sulfurous odors. The identification and quantification of ~20 volatile compounds are listed in Table 1. The compositions of volatile acids were similar to the results reported by Van-chom (1958), McIver et al. (1982), and Sanceda et al. (1986).

CC Results. The odor concentrate obtained by CC, which smelled typical fish sauce-like, was further separated into six fractions with a silica gel column. The odor of each fraction was organoleptically evaluated. Fractions 2, 3, and 4 had characteristic odors, as described in Table 2. Gas chromatograms of these three fractions are shown in Figure 1. The list of the volatile compounds tentatively and definitely identified is shown in Table 3. About 155 volatile compounds were identified, including 36 carbonyls, 29 hydrocarbons, 33 alcohols, 17 nitrogen-containing compounds, 14 acids, 10 sulfur-containing compounds, 8 esters, 5 phenolic compounds, and 3 furans.

Volatile fatty acids were the most abundant volatile compounds in the concentrates obtained by both methods, comprising about 80% of total peak area of chromatogram. These acids could be formed from amino acids through bacterial fermentation (Saisithi et al., 1966; Beddows et al., 1980; McIver et al., 1982) or from the oxidation of unsaturated fatty acids. Major volatile fatty acids were butanoic acid, 3-methylbutanoic acid, pentanoic acid, and 4-methylpentanoic acid and they

Table 3. Volatile Compounds Identified in Fractions 2, 3, and 4

peak no.	compound name	% area in each fraction			peak no.	compound name	% area in each fraction		
		2	3	4			2	3	4
				Acids					
102	propanoic[a]	nd[b]	0.04	0.21	123	4-methylpentanoic[a]	12.70	20.98	9.01
103	2-methylpropanoic[a]	0.72	6.90	4.45	129	hexanoic[a]	0.81	5.14	1.23
107	butanoic	0.67	17.76	33.57	136	(*E*)-3-hexenoic	nd	0.06	0.06
111	3-methylbutanoic[a]	67.14	32.40	37.73	141	2-ethylhexanoic	tr	nd	nd
119	pentanoic[a]	0.57	6.99	3.76	142	heptanoic[a]	tr	0.62	nd
121	2-methylpentanoic[a]	0.39	0.64	0.04	149	ethyl-3-hydroxylhexanoic	nd	nd	0.03
122	3-methylpentanoic	0.06	0.13	tr[c]	150	octanoic	0.10	0.12	nd
				Alcohols					
4	2-propanol	nd	nd	tr	69	2,3-epoxyhexanol	nd	nd	tr
9	2-butanol	nd	0.01	0.06	75	(*Z*)-3-hexenol	nd	nd	tr
16	3-pentanol	nd	nd	tr	78	2-octanol[a]	nd	tr	nd
17	2-methyl-2-propanol	nd	0.04	0.01	82	1-heptyn-4-ol	nd	tr	nd
18	3-methyl-2-butanol	nd	0.01	0.01	83	2-ethylhexanol	nd	tr	nd
31	2-methylbutanol	nd	0.06	nd	84	2-ethyl-1-hexanol	nd	0.12	nd
32	3-methylbutanol[a]	nd	nd	2.72	98	4-heptenol	nd	nd	0.03
35	3-methyl-2-pentanol	nd	nd	tr	101	2,7-dimethyloctanol	nd	nd	tr
41	4-methyl-2,3-pentanediol	nd	tr	nd	108	furfuryl alcohol[a]	nd	nd	0.05
43	2-methyl-1-penten-3-ol	nd	tr	nd	109	(*E*)-3-nonen-2-ol	nd	nd	0.03
46	1-hepten-4-ol	nd	tr	tr	124	decanol	nd	0.05	nd
55	(*E*)-2-pentenol	nd	tr	0.31	125	2-phenyl-2-propanol	nd	nd	tr
57	(*Z*)-2-pentenol	nd	tr	0.60	132	benzenemethanol[a]	nd	0.01	1.70
60	2,3-butanediol[a]	nd	nd	0.05	135	benzeneethanol[a]	nd	nd	0.10
61	2-nitro-*tert*-butanol	nd	tr	nd	147	tetrahydro-5-methyl-2-furanmethanol	nd	nd	tr
67	1-chloro-2-butanol	nd	tr	nd	152	1,4-benzenedimethanol	nd	nd	tr
68	(*E*)-3-hexenol	nd	nd	tr					
				Carbonyls					
1	2-butanone[a]	0.13	0.10	0.13	86	1-(2-furanyl)ethanone	nd	0.12	nd
2	3-methylbutanal	0.05	0.02	nd	91	2,3-epoxy-2-methyl-4-octanone	nd	nd	tr
6	2,3-butanedione	nd	0.02	nd	93	(*E*)-2-heptanal	nd	tr	nd
8	2-pentanone	nd	nd	tr	97	decanal	nd	nd	tr
19	2,4-dimethyl-3-pentanone	nd	tr	nd	99	benzaldehyde[a]	0.45	0.01	0.01
24	2-ethylbutanal	nd	tr	nd	104	1-(2-pyridinyl)ethanone	nd	nd	tr
27	(*E*)-2-hexenal	nd	tr	nd	105	2,3-octadione	tr	nd	nd
28	5-methyl-2-hexanone	tr	nd	nd	110	phenylethanone	0.13	nd	nd
36	4-methyl-3-penten-2-one	nd	tr	nd	117	4-ethylbenzaldehyde	0.05	nd	nd
37	3-methylcyclopentanone	nd	tr	nd	118	phenylacetone	nd	nd	tr
45	dihydro-4,5-dimethyl-2(3*H*)-furanone	nd	nd	0.83	126	1-(3,4-dimethylphenyl)ethanone	0.01	0.01	nd
50	2,2-dimethyl-1,3-dioxane-4,6-dione	nd	nd	0.02	128	2,6,6-trimethyl-1,4-cyclohexanedione	nd	nd	tr
54	cyclohexanone	nd	nd	0.01	131	1-(4-ethylphenyl)ethanone	0.03	0.02	nd
58	4-methyl-2,3-pentanedione	nd	nd	tr	134	4-methylcyclheptanone	nd	nd	0.11
63	tetrahydro-3,6-dimethyl-2*H*-pyran-2-one	nd	nd	0.52	137	α-ethylidenebenzeneacetaldehyde	0.15	0.47	tr
71	5-methoxy-2-pentanone	nd	tr	nd	143	4-(1-hydroxyethyl)benzaldehyde	nd	nd	tr
72	2-methyl-2-cyclopenten-1-one	nd	nd	tr	144	pyrrole-2-aldehyde	nd	nd	tr
77	octanal	nd	nd	tr	145	myrtenal	nd	0.48	nd
				Esters					
3	ethyl acetate[a]	nd	0.03	nd	130	methyl 3-methyl furoate	nd	nd	0.01
10	methyl 3-methyl-2-oxobutanoate	nd	tr	nd	140	ethenyl cyclopentanoate	nd	nd	tr
49	ethoxyethyl acetate	nd	tr	nd	153	methylhexadecanoate	0.03	nd	nd
120	methylbenzene acetate	tr	nd	nd	155	methyl 10-octadecenoate	tr	nd	nd
				Nitrogen-Containing Compounds					
29	tetrahydro-2-methoxy-2*H*-pyran	nd	tr	nd	89	2-ethyl-3,5-dimethylpyrazine	nd	nd	tr
53	2-(3,3-dimethylethyl)pyridine	nd	nd	0.09	92	2,5-dimethyl-3-propylpyrazine[a]	nd	nd	tr
59	2-*tert*-butoxytetrahydro-2*H*-pyran	nd	tr	nd	94	*O*-decylhydroxyamine	nd	nd	tr
65	benzeneacetonitrile	tr	tr	nd	95	2-ethenyl-6-methylpyrazine[a]	nd	nd	tr
66	propylpyrazine	nd	nd	tr	106	2-acetylpyridine	nd	nd	0.01
73	2-methyl-5-(3-methylbutyl)pyridine	nd	nd	tr	115	1-piperidine	nd	nd	0.05
81	3-hydroxypyrrolidine	nd	tr	nd	138	3-methyl-1,2-benzisoxasole	nd	nd	tr
85	2-methyl-5-(1-methylethyl)pyrazine	nd	nd	tr	139	2-acetylpyrrole[a]	nd	nd	tr
87	*O*-(3-methylbutyl)hydroxyamine	nd	nd	tr					
				Sulfur-Containing Compounds					
13	dimethyl disulfide[a]	0.19	tr	nd	100	*S*-propyl ethanethioate	nd	0.01	nd
44	bis(1-methylethyl) disulfide	nd	tr	nd	112	ethyl 1-methylethyl disulfide	nd	nd	0.02
70	dimethyl trisulfide	0.05	tr	nd	114	2-formylthiophene	nd	0.01	nd
80	3-(methylthio)propanal[a]	tr	0.50	nd	116	1-formyl-2-thiohydantoin	nd	0.03	nd
90	3-(methylthio)propanol	nd	tr	0.01	127	2-*tert*-butylthiazole	nd	nd	tr
				Furans					
26	tetrahydro-2-methylfuran	nd	nd	tr	113	3-methylfuran	nd	0.15	nd
88	2-methylfuran	nd	nd	tr					
				Phenolic Compounds					
133	2,6-*tert*-butyl-*p*-cresol	4.89	0.77	0.02	151	4-methylphenol	nd	0.03	nd
146	3,4-diethylphenol	nd	0.20	0.15	154	2,4-bis(1,1-dimethylethyl)phenol	0.18	nd	nd
148	phenol	7.27	3.94	1.50					

Table 3 (Continued)

peak no.	compound name	% area in each fraction 2	3	4	peak no.	compound name	% area in each fraction 2	3	4
				Hydrocarbons					
5	2-methyl-1,3-dioxolane	nd	tr	tr	39	1-ethyl-3-methylbenzene	0.05	tr	tr
7	2,4-dimethylhexane	0.06	tr	nd	40	1,2,3-trimethylbenzene	0.18	tr	tr
11	4-ethylheptane	0.04	nd	nd	42	2,2,3-trimethylbenzene	0.06	tr	nd
12	toluene[a]	0.12	0.18	0.22	47	1-methylethylbenzene	nd	tr	nd
14	1-chloro-2-methylpropane	nd	tr	nd	48	1-ethyl-4-methylbenzene	0.04	nd	nd
15	octene	nd	tr	nd	51	tridecane	tr	tr	nd
20	ethylbenzene	nd	tr	nd	52	3-methyltridecane	tr	tr	nd
21	1,2-dimethylbenzene	nd	tr	nd	56	1,2-diethylbenzene	nd	tr	nd
22	1,3-dimethylbenzene	nd	tr	tr	62	1,2,4-trimethylbenzene	nd	tr	nd
23	dimethyldecane	0.89	tr	nd	64	diethylbenzene	tr	tr	nd
25	trimethylbenzene	nd	tr	nd	74	pentylbenzene	0.03	nd	nd
30	dodecane	tr	nd	nd	76	(*E*,*E*)-2,4-heptadiene	nd	tr	nd
33	limonene	0.04	nd	nd	79	3-methyltetradecane	tr	nd	nd
34	propylbenzene	0.08	tr	0.01	96	pentadecane	tr	nd	tr
38	1-ethyl-2-methylbenzene	tr	tr	nd					

[a] Compounds previously identified (Van-Chom, 1958; Saisithi et al., 1966; Beddows et al., 1976; McIver et al., 1982; Sanceda et al., 1983, 1984, 1986, 1990). [b] nd, not detected. [c] tr, trace concentrations of <0.01.

had threshold values of 3.89, 2.45, 4.79, and 7.10 ppb in vapor phase (Devos et al., 1995), respectively. Threshold value is defined as the lowest concentration of a compound that can still be directly recognized by its odor (Belitz and Grosch, 1986). Because the threshold values for volatile fatty acids were relatively low, they were considered to be associated with the cheesy note in the fish sauce odor. Although the percentage of volatile fatty acids was high, these compounds may not be the major contributors to the overall odor of fish sauce.

Small concentrations of a series of normal and branched alcohols (C_3-C_{10}) could not have influence the overall odor of fish sauce because of their relatively high threshold values (Devos et al., 1995). In spite of their large concentrations in fraction 4, (*E*)-2-pentenol and (*Z*)-2-pentenol seemed to have no direct influence because the fish sauce-like odor in fraction 3 was stronger than in fraction 4.

Aliphatic aldehydes, such as 3-methylbutanal, octanal, and decanal, have threshold values of 2.24, 1.35, and 0.891 ppb in vapor phase (Devos et al., 1995), respectively, so 2-ethylbutanal, (*E*)-2-hexenal, and (*E*)-2-heptenal might have partially contributed to the overall fish sauce odor. A large quantity of benzaldehyde was detected in fraction 2. Therefore, benzaldehyde could not have contributed to the strong fish sauce-like odor characterized in fraction 3. Several kinds of ketones, which were reported as contributors to blue camembert cheese flavors (Azzara et al., 1992), were detected. These ketones as well as volatile fatty acids could be responsible for the cheesy odor of fish sauce. 1-(2-Furanyl)-ethanone, α-ethylidenebenzeneacetaldehyde and myrtenal were predominantly present in fraction 3. Their sensory characteristics should be elucidated. However, authentic chemicals of these compounds are not commercially available.

Several esters with low concentrations were detected. Their importance to fish sauce odor has not yet been fully explained.

Trace amounts of nitrogen-containing compunds, such as pyrazines, pyridines, nitrile, pyrrolidine, amine, piperidine, benzisoxasole, and pyrrole, were detected. These compounds might be the source of burned and amine-like complicated odors in fish sauce.

Among the sulfur-containing compounds, dimethyl disulfide and dimethyl trisulfide [with threshold values of 0.427 and 1.66 ppb in vapor phase (Devos et al., 1995), respectively], bis(1-methylethyl) disulfide, 3-(methylthio)propanal, and *S*-propyl ethanethioate could be the most potent sources of the unpleasant odor in fish sauce. The sensory importance of 2-formylthiophene and 1-formyl-2-thiohydantoin was not clearly elucidated.

Three methylfurans, namely, tetrahydro-2-methylfuran, 2-methylfuran, and 3-methylfuran, were detected, but their contributions to fish sauce odor may be insignificant due to their low concentrations and high threshold values.

A large amount of 2,6-*tert*-butyl-*p*-cresol was present. The source of this compound could have been diethyl ether, wherein it has been added as an antioxidant. Other phenolic compounds identified were phenol, 3,4-diethylphenol, and 4-methylphenol. These compounds impart a phenolic and somewhat medicinal odor to different kinds of aged Italian cheese (Ha and Lindsay, 1991).

Several types of alkylbenzenes and branched alkanes might have contributed to solvent-like odor in fish sauce.

To summarize, ~155 compounds were identified in the odor concentrates by the CC method, 25 of which have been previously identified (Van-chom 1958; Saisithi et al., 1966; Beddows et al., 1976; McIver et al., 1982; Sanceda et al., 1983, 1984, 1986, 1990). More significant results were obtained by employing CC compared with SDE. This result suggests that the CC method would be a suitable technique for isolating and identifying volatile compounds in fish sauce. A large amount of volatile fatty acids; a large number of carbonyls with low concentrations; sulfur-containing compounds, such as dimethyl disulfide, bis(1-methylethyl) disulfide, dimethyl trisulfide, and 3-(methylthio)propanol; and nitrogen-containing compounds, such as 3-hydroxypyrrolidine, 2-acetylpyrrole, 1-piperidine, 2-ethyl-3,5-dimethylpyrazine, 2-methyl-5-(1-methylethyl)pyrazine, and 2-(3,3-dimethylethyl)pyridine, were thought to be major contributors to fish sauce flavors. However, trimethylamine could not be detected by the present method. Most of it might have escaped while ethyl ether vaporized, and/or it could not be separated from the peak of ethyl ether in the gas chromatogram.

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