Headspace Gas Analysis of Fish Sauce

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Volatiles from the headspace gas of fish sauce with no change in the pH (*pH as is*) were trapped in a Tenax TA column and analyzed by gas chromatography (GC) and GC-mass spectrometry (MS). Fish sauce was alkalized (*pH 11.0*) to enhance the release of nitrogenous and sulfurous compounds and was likewise analyzed. About 124 volatile compounds, including 20 nitrogen-containing compounds, 20 alcohols, 18 sulfur-containing compounds, 16 ketones, 10 aromatic hydrocarbons, 8 acids, 8 aldehydes, 8 esters, 4 furans, and 12 miscellaneous compounds, were definitely and tentatively identified. Detection of highly volatile nitrogenous and sulfurous compounds such as trimethylamine and dimethyl disulfide, which could not be easily detected by already existing methods, was enhanced after alkalization.

Keywords: *Headspace gas; volatile compounds; odor; fish sauce*

INTRODUCTION

Fish sauce, a clear brown liquid seasoning with a characteristic odor, is commonly added to traditionally prepared Southeast Asian food. It is basically produced from a mixture of fish and salt (3:1) that has been allowed to ferment for a period of 6−12 months. Production of the sauce may vary according to country of origin and species of fish used. Previous studies have indicated that fish sauce is composed of three distinctive notes; they are, ammoniacal, cheesy, and meaty (Dougan and Howard, 1975; Beddows et al., 1976). The ammoniacal note is attributable to ammonia and several amines (Saisithi et al., 1966, Dougan and Howard, 1975), and the cheesy note is associated with low molecular weight volatile fatty acids (Van Chom, 1958; Saisithi et al., 1966; Dougan and Howard, 1975; Beddows et al., 1976; Sanceda et al., 1986; Peralta et al., 1996). The possible source of the meaty note has not yet been fully clarified.

In our previous paper (Peralta et al., 1996), we reported the volatile compounds in fish sauce, identified by column concentration with Porapak Q, and related these volatiles to the three distinctive notes. We presumed that some highly volatile compounds could not be detected by this method. To our knowledge, no report on the studies of fish sauce odor by headspace gas analysis has ever been published. This study was conducted to establish a convenient method for determining highly volatile and organoleptically important compounds in fish sauce.

MATERIALS AND METHODS

Materials. Fish sauce, imported from Taiwan and contained in a 2-L bottle, was obtained from Ichiban Shokuhin Company, Ltd., Fukuoka, Japan. A Tenax TA column (60−80 mesh) was purchased from GL Sciences, Tokyo, Japan. Trimethylamine hydrochloride and methyl mercaptan sodium salt (15% in water) were from Tokyo Kasei Kogyo Company, Ltd., Tokyo, Japan.

Composition of the Standard for the Fish Sauce Sample. The composition is described in our previous paper (Peralta et al., 1996).

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Headspace Gas Analysis. A schematic diagram of the procedure is shown in Figure 1. A 100-mL sample of fish sauce, in which 5 μL of 1% cyclohexanol had been added as internal standard, was incubated for 20 min with stirring in a water bath maintained at 40 °C. The pH of this sample was not changed, and the sample is referred to as the sample with *pH as is*. Headspace gas (100 mL) was slowly suctioned for ∼2 min, with a large syringe, through a column (10 cm × 3 mm i.d.) packed with 70 mg of Tenax TA resin to adsorb the volatiles. The same procedure was used for 100 mL of fish sauce that was alkalized with 20% NaOH, to enhance the release of some nitrogenous and sulfurous compounds. We refer to this sample as an alkalized one or the sample with *pH 11.0* to distinguish it from the sample with *pH as is*. For gas chromatography (GC) and GC-mass spectrometry (MS) analyses of headspace volatiles, the flow of carrier gas was stopped, and then the Tenax TA column was inserted into the GC injection port while simultaneously cryofocusing the GC column on liquid N_2 for ∼1 min. Then, the flow rate of the carrier gas was increased to 20 mL/min. The GC injection port was maintained at a temperature of 210 °C.

Headspace Gas Analysis of Model Solution. Known amounts of trimethylamine hydrochloride and methyl mercaptan were added to phosphate buffer solution. Cyclohexanol was used as the internal standard. Each buffer solution was adjusted to pH values of 4.5−11.5, and was subjected to headspace gas analysis as just described.

Standard Addition Method. Various amounts of 0.1% trimethylamine and 0.1% methyl mercaptan aqueous solutions, ranging from 2 to 4 μL, were added to 100 mL of alkalized *(pH 11.0)* fish sauce. Each added sample was subjected to headspace gas analysis as already described.

Capillary Gas Chromatography (GC). Separation of volatile compounds was done on a Shimadzu GC 14A 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) at 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 3 °C/min. The injector and detector temperatures were set at 200 and 250 °C, respectively. The helium gas flow rate was 22 cm/s.

Capillary Gas Chromatography−Mass Spectrometry (GC-MS). Volatile compounds were identified with a JEOL AUTO MASS 50 model mass spectrometer attached to a Hewlett Packard 5890 model gas chromatograph, programmed under the same conditions as already mentioned. The mass spectra were obtained by electron-impact ionization at 70 eV. Retention indices were calculated by modified Kovats Index

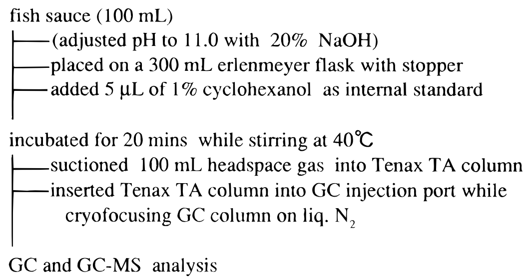

Figure 1. Procedure for headspace gas analysis.

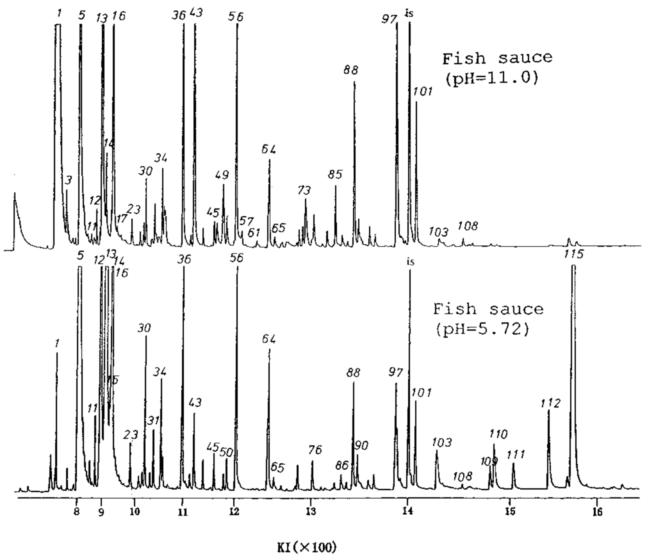

Figure 2. Gas chromatograms of headspace volatiles of fish sauce and alkalized fish sauce [is: internal standard (cyclohexanol)].

(Van den Dool et al., 1963). 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).

RESULTS AND DISCUSSION

The gas chromatograms shown in Figure 2 illustrate the volatile profiles of *pH as is* and *pH 11.0* fish sauces. The peaks of acidic compounds were reduced in the *pH 11.0* sample. On the other hand, nitrogenous and sulfurous compounds, particularly trimethylamine, dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide, were remarkably enhanced.

Identified volatile compounds with their peak area ratios to internal standard are listed in Table 1. Large amounts of acids, aldehydes, and ketones, ~15, 55, and 21%, respectively, of the total peak area of gas chromatogram were detected in fish sauce *pH as is.* Volatile fatty acids were dominated by acetic acid, propanoic acid, 2-methylpropanoic acid, butanoic acid, and 3-methylbutanoic acid, with odor threshold values of 145, 35.5, 19.5, 3.89, and 2.45 ppb in vapor phase, respectively (Devos et al., 1995). These compounds were associated with the cheesy note in fish sauce odor (Van-Chom, 1958; Saisithi et al., 1966; Dougan and Howard, 1975; Beddows et al., 1976; Sanceda et al., 1984; 1986; Peralta et al., 1996). We conclude that the volatile fatty acids, especially 2-methylpropanoic acid, could be major contributors to the cheesy and stinging odors of fish sauce, judging from their quantitative values and odor threshold values.

Among the aldehydes, 2-methylpropanal, 2-methylbutanal, and 3-methylbutanal, with odor threshold values in vapor phase ranging from 2.24 to 40.7 ppb (Devos et al., 1995), were predominantly present. Aldehydes have been derived from lipid oxidation during fermentation (Karahadian and Lindsay, 1989), or these branched, short-chain aldehydes might have resulted from deamination of amino acids. These aldehydes were considered to cause unpleasant oxidation flavors in foods (Heath and Reineccius, 1986). They were reported to cause off flavor in canned meat (Belitz and Grosch, 1986). They could have certainly contributed to the overall odor due to their low odor threshold values. The decrease in the volatilities of these compounds by alkalization simplified their identification.

On the other hand, the concentrations of 2-butanone, 3-methyl-2-butanone, 2-pentanone, and 3-methyl-2-pentanone, were particularly high among the ketones. These compounds were thought to be responsible for the cheesy note in fish sauce odor, but could not have an impact on fish sauce odor because of their high odor threshold values in the vapor phase that ranges from 7.76 to 1.55 ppm (Devos et al., 1995).

Trace amounts of nitrogen-containing compounds, such as, pyrazines, pyridines, pyrimidines, amines, and nitrile, were detected in fish sauce *pH as is.* These compounds, with burned and amine-like complicated odor, together with aldehyde compounds might be responsible for the meaty note. The volatilities of the nitrogen-containing compounds were enhanced by increasing the sample pH. The peak area ratio to internal standard of trimethylamine increased to 65 from 0.33 after alkalization. Because of its low odor threshold value of 2.4 ppb in the vapor phase (Devos et al., 1995) and its characteristic ammonia-like and fish smell (Dravnieks, 1985), trimethylamine was presumed to be the source of fishy, ammoniacal odor and somewhat meaty note in fish sauce *pH as is.*

The volatilities of sulfur-containing compounds [i.e., dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide, with threshold values of 2.24, 0.43, and 1.66 ppb in vapor phase (Devos et al., 1995), respectively] were quite low in fish sauce *pH as is.* Judging from their odor threshold values and their concentrations in headspace vapor, dimethyl disulfide and dimethyl trisulfide, together with trimethylamine, seemed to be the most potent contributors to a hedonic fish sauce odor and they could play important roles. The volatilities of these sulfide-containing compounds were enhanced and the compounds were readily detected in alkalized fish sauce *(pH 11.0).*

Toluene constituted the largest amount among the aromatic hydrocarbons, but did not seem to have contributory importance considering its high odor threshold value of 1.55 ppm in vapor phase (Devos et al., 1995).

A large number of normal and branched alcohols, which could have insignificant contributions to fish sauce odor due to their relatively high odor threshold values (Devos et al., 1995), were detected in small concentrations.

A number of esters were detected, but only *tert*-butyl propanoate seemed to have contributory importance. Esters have been found to be present in most fermented seafoods (Sanceda et al., 1984 and 1986; Josephson et al., 1987; Cha and Cadwallader, 1995). These compounds might be products of esterification of alcohols with carboxylic acids that are formed by microbial and enzymatic decomposition of lipids.

Furans could not have contributed to fish sauce odor because of their very low concentrations and quite high odor threshold values.

Table 1. Quantitative Values of Volatile Compounds Identified in Fish Sauce

peak no.	Kovats index[a]	compound name	area ratio pH as is	area ratio pH = 11.0	peak no.	Kovats index[a]	compound name	area ratio pH as is	area ratio pH = 11.0
				Acids					
103	1435	acetic[b]	0.57	0.01	115	1574	2-methylpropanoic[b]	12.77	tr
106	1445	formic[b]	tr[c]	nd[d]	120	1631	butanoic[b]	0.61	tr
110	1470	2,2-dimethylpropanoic[b]	0.10	tr	122	1652	2-methylbutanoic[b]	0.01	nd
112	1535	propanoic[b]	0.36	nd	124	1670	3-methylbutanoic[b]	0.03	nd
				Alcohols					
15	912	4-penten-2-ol	0.09	0.05	65	1255	3-methyl-1-butanol[b]	0.03	0.03
31	1041	2-butanol[b]	0.20	0.30	68	1262	5-methyl-3-hexanol	0.01	0.02
33	1049	1-propanol	tr	0.02	76	1296	2-heptanol	0.20	0.10
42	1110	2-methyl-1-propanol[b]	0.30	1.01	85	1318	2-ethyl-1-butanol	tr	nd
45	1154	2-methyl-2-propanol[b]	0.14	0.08	86	1327	(*E*)-2-penten-1-ol[b]	0.03	0.03
46	1161	3-methyl-2-butanol[b]	nd	0.06	87	1336	(*Z*)-2-penten-1-ol[b]	0.02	0.02
49	1172	3-pentanol[b]	0.04	0.07	89	1351	2-nitro-*tert*-butanol[b]	0.21	0.02
52	1188	2-pentanol[b]	tr	nd	102	1414	4-methyl-1-hexanol	tr	nd
55	1194	1-butanol[b]	nd	0.01	105	1444	2-ethyl-1-hexanol[b]	tr	nd
64	1249	2-methyl-1-butanol	0.81	0.13	123	1658	3-methyl-2-heptanol	tr	nd
				Aldehydes					
2	686	acetaldehyde[b]	0.03	0.01	14	911	3-methylbutanal[b]	4.97	0.14
4	784	propanal[b]	0.07	0.10	38	1097	hexanal[b]	tr	nd
5	800	2-methylpropanal	35.51	7.96	44	1104	2-methyl-2-butenal[b]	0.02	0.02
13	906	2-methylbutanal[b]	13.75	3.07	108	1459	benzaldehyde[b]	0.01	0.02
				Aromatic Hydrocarbons					
17	938	benzene[b]	0.04	0.06	67	1260	1-ethyl-3-methylbenzene[b]	tr	nd
34	1058	toluene[b]	0.45	0.22	74	1278	methyl(1-methylethyl)benzene	tr	0.03
48	1168	ethylbenzene[b]	tr	nd	78	1302	2-ethyl-3-methylbenzene	tr	nd
51	1184	1,2-dimethylbenzene[b]	tr	nd	95	1383	ethyl(1-methylethyl)benzene	nd	tr
54	1192	1,4-dimethylbenzene[b]	tr	nd	121	1637	naphthalene[b]	tr	nd
				Esters					
11	863	ethyl acetate[b]	0.28	0.08	56	1197	*tert*-butyl propanoate	2.29	1.15
20	961	ethyl propanoate[b]	tr	0.01	58	1205	pentyl formate	nd	tr
22	977	propyl acetate	tr	tr	93	1379	cyclohexyl acetate	nd	tr
32	1044	ethyl butanoate[b]	tr	0.01	94	1382	hexyl formate	nd	tr
				Furans					
19	957	2-ethylfuran[b]	tr	0.01	111	1522	5-methyl-2-furancarboxaldehyde[b]	tr	nd
107	1457	1-(2-furanyl)ethanone	tr	0.01	114	1565	2-Furanmethanol[b]	0.08	0.05
				Ketones					
12	888	2-butanone[b]	13.05	0.02	44	1128	2-(1-methylpropyl)cyclopentanone	0.10	0.02
16	929	3-methyl-2-butanone[b]	7.45	1.22	53	1190	5-methyl-2-hexanone[b]	tr	nd
18	948	3-buten-2-one[b]	tr	tr	63	1226	3-methylcyclohexanone	tr	nd
23	980	2-pentanone[b]	0.17	0.08	81	1314	2-ethylcyclopentanone	tr	nd
28	1003	2-hexanone	0.02	0.01	82	1315	cyclohexanone[b]	tr	nd
30	1016	3-methyl-2-pentanone	0.52	0.07	84	1317	1-hydroxy-2-propanone	0.02	0.08
35	1080	2,3-pentanedione[b]	0.01	tr	113	1542	1-phenylethanone	tr	nd
37	1096	5-methyl-3-hexanone[b]	0.02	nd	119	1620	1-phenyl-2-propanone	tr	nd
				Nitrogen-Containing Compounds					
1	570	trimethylamine[b]	0.33	65.46	88	1348	2,5-dimethylpyrimidine	0.10	0.57
26	993	2-methylpropanenitrile	nd	tr	90	1363	4,6-dimethylpyrimidine	0.04	0.05
40	1102	*N*-methylethanamine	nd	tr	91	1371	2-ethylpyrazine[b]	tr	0.01
60	1213	hydrogen azide	tr	0.01	92	1377	2,3-dimethylpyrazine[b]	nd	0.01
66	1257	pyrazine[b]	tr	0.01	96	1385	2-isopropylpyrazine	nd	0.01
72	1266	2,6-dimethylpyridine	0.02	0.03	98	1393	2-ethyl-5-methylpyrazine[b]	0.28	0.30
73	1270	2-methylpyrazine[b]	0.10	0.13	99	1395	4,5-dimethylpyrimidine	0.02	0.02
79	1307	benzenemethanamine	tr	0.01	100	1402	trimethylpyrazine[b]	0.02	0.02
80	1311	2-ethylpyridine[b]	tr	0.03	101	1409	5-isopropyl-2-methylpyrazine	0.41	0.52
83	1316	2-propylpyridine	tr	0.01	104	1438	3-ethyl-2,5-dimethylpyrazine[b]	0.01	0.02
				Sulfur-Containing Compounds					
3	761	methanethiol[b]	0.02	0.22	61	1216	methanesulfonyl chloride	0.01	0.02
6	814	1-ethenylmethyl disulfide[b]	0.06	0.07	62	1224	trimethyloxazole	tr	0.01
7	818	dimethyl sulfide[b]	0.06	0.34	71	1265	thiazole	0.01	0.01
8	829	(methylthio)ethane	0.04	0.01	75	1282	2,4-dithiapentane	0.01	0.09
10	840	2-(methylthio)propane	0.02	0.03	97	1387	dimethyl trisulfide[b]	0.51	1.21
36	1090	*S*-methyl ethanethioate[b]	1.32	1.45	109	1462	*S*-methyl butanethioate[b]	0.14	1.21
43	1120	dimethyl disulfide[b]	0.30	1.04	116	1585	isothiocyanatocyclohexane	0.01	nd
47	1166	1-propanesulfonyl chloride	nd	tr	117	1605	methyl *N*-hexyl disulfide	tr	nd
59	1210	2-(ethylthio)butane	nd	tr	118	1615	3-(methylthio)-1-propanal[b]	tr	nd
				Miscellaneous Compounds					
9	831	hexamethylcyclotrisiloxane	0.05	0.03	39	1100	undecane	tr	nd
21	970	3-ethylheptane	0.01	nd	50	1181	2-nitropropane	0.13	0.05
24	982	2-methylheptane[b]	tr	nd	57	1203	*d*-limonene[b]	0.01	0.07
25	987	1-(ethenyloxy)2-methylpropane	tr	nd	69	1263	benzocyclobutane	tr	nd
27	1000	decane[b]	tr	nd	76	1264	1,3,5,7-cyclooctatetraene	tr	nd
29	1010	trichloromethane[b]	0.03	0.04	77	1300	tridecane[b]	tr	nd

[a] Modified Kovats indices calculated for DB-Wax capillary column. [b] Previously identified (Van-chom, 1987; Saisithi et al., 1966; Dougan and Howard, 1975; Nonaka et al., 1975; Beddows et al., 1980; McIver et al., 1982; Sanceda et al., 1984, 1989, 1990; Peralta et al., 1996). [c] tr, trace concentrations of <0.01. [d] nd, not detected.

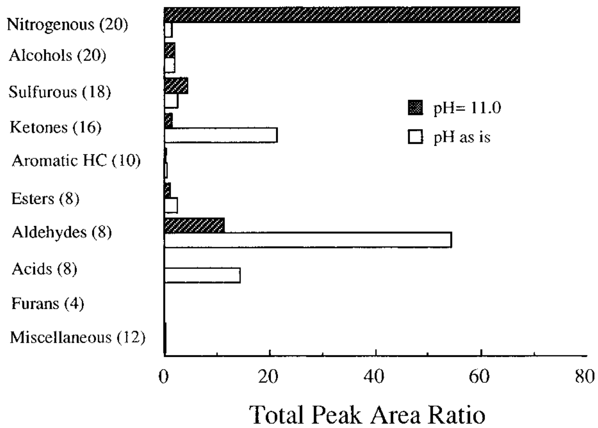

Figure 3. Effect of alkalization on the percentage distribution of chemical classes of volatile compounds in fish sauce.

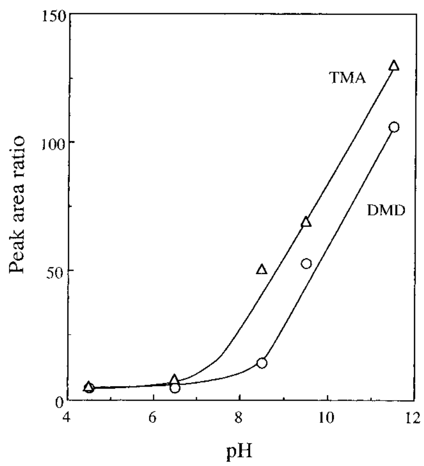

Figure 4. Effect of pH on volatilities of trimethylamine and dimethyl disulfide.

A number of alkylbenzenes and branched alkanes detected might have been the source of solvent-like odor in fish sauce.

A graphical representation of the effect of alkalization on the volatilities of compounds in fish sauce is shown in Figure 3. After alkalization, the concentrations in the headspace of highly volatile nitrogen-containing compounds increased to 65%, and concentrations of sulfur-containing compounds were enhanced by ~5%. On the other hand, concentrations of fatty acids, aldehydes, and ketones were decreased.

To measure the effect of alkalinity on the volatilities of trimethylamine and dimethyl disulfide, their phosphate buffer solutions at different pH values were applied to headspace gas analysis. The data show that the volatilities of these two compounds increased at almost the same rate as alkalinity increased (Figure 4). This result suggested that methanethiol and dimethyl disulfide were in equilibrium state.

The results obtained were highly reproducible, so we determined the concentrations of trimethylamine and dimethyl disulfide in fish sauce by the standard addition method. From the standard curve shown in Figure 5 it is evident that the concentrations of trimethylamine and dimethyl disulfide are 21 and 7.5 ppb, respectively.

To summarize, 124 volatile compounds were identified by headspace gas analysis, 68 of which have been previously identified (Van-chom, 1958; Saisithi et al., 1966; Dougan and Howard, 1975; Nonaka et al., 1975; Beddows et al., 1980; McIver, 1982; Sanceda et al., 1984, 1986, 1989, 1990; Cha and Cadwallader, 1995; Peralta et al., 1996). On the basis of the results of this study

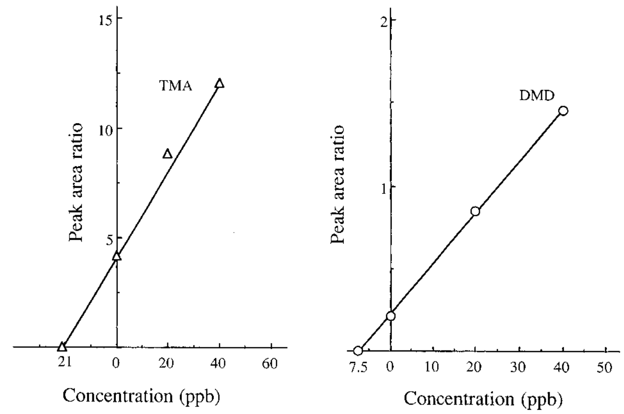

Figure 5. Determination of trimethylamine and dimethyl disulfide in fish sauce by standard addition method [trimethylamine (21.0 ppb), dimethyl disulfide (7.5 ppb)]. The pH value of fish sauce was adjusted to 11.0 by adding 20% NaOH.

we conclude that headspace gas analysis under alkalized condition could be a suitable method for realizing a sensory-attributed odor profile of fish sauce samples.

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