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Identification of Distinctive Volatile Compounds in Fish Sauce

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To make clear the distinctive odorants of fish sauce, addition tests were carried out with respect to deodorized fish sauce samples. Volatiles from the headspace gas of nontreated fish sauce and deodorized fish sauce were characterized by gas chromatography−olfactometry and gas chromatography−mass spectrometry. Four compounds contributing to the distinctive odor of fish sauce were 2-methylpropanal, 2-methylbutanal, 2-ethylpyridine, and dimethyl trisulfide. Addition tests (quantitative descriptive analysis) with respect to the four odorants showed that contributors to the fishy aroma were 2-ethylpyridine and dimethyl trisulfide and that all four odorants contributed to the sweaty aroma. Furthermore, the fecal note of fish sauce was essential with 2-ethylpyridine and dimethyl trisulfide, and the rancid note was essential with all four odorants.

KEYWORDS: Fish sauce; volatile compounds; FD factors; sensory evaluation

INTRODUCTION

Fish sauce is a brown, liquid seasoning commonly used in most parts of Southeast Asia. It is called by different names such as patis in the Philippines, shotturu in Japan, budu in Malaysia, nampla in Thailand, noucnam in Vietnam, ketjap-ikan or bakasang in Indonesia, yuilu in China, and ngapi in Burma. It imparts good taste to local food preparation and supplements protein in the diet. Recently, it has been commonly used in several prepared foods and sauces due to its characteristic taste in the Japanese and European markets. However, due to its distinctive odor, there is a limit to its usage for not only home-cooked foods but also prepared foods.

Fish sauce contains ∼20 g/dL nitrogen, 16 g of which is in the form of amino acids and oligopeptides; thus, it is considered to be a good source of protein (*1*). The production of fish sauce is simple and cheap, but the process varies according to producers or species of fish used (*2*). It is basically produced from a mixture of fish and salt (3:1) that has been allowed to ferment for a period of >6 months at 30−35 °C. The resulting product has a distinctive odor and flavor, which develops progressively as the fermentation progresses.

A considerable number of studies have been done on fish sauce over the past decades (*3*−*13*). Dougan and Haward (*7*) reported that three distinctive notes contributed to the odor of fish sauce, those being ammoniacal, cheesy, and meaty notes. They are derived from protein hydrolysate and lipid oxidation products brought about by either autolytic or microbial activity or fish enzymes (*6*, *9*, *14*).

The ammoniacal note is attributed to ammonia, amines, and other basic nitrogen-containing compounds (*5*−*7*, *15*, *16*). The cheesy note is mainly due to low molecular weight volatile fatty acids (*4*−*7*, *11*, *14*, *18*, *19*) and methyl ketone (*20*). Shimoda and others (*12*) pointed out 2-methylpropanoic acid as the major contributor to cheesy and stinging notes. The meaty note is much more complicated, but it was believed that it could be produced by atmospheric oxidation of precursors that were still present in mature fish sauces (*7*).

Although a number of studies have already been conducted on fish sauce, work on the volatile compounds is still incomplete and possible contributors to its distinctive odor have not been clarified.

Our objective is to make clear some possible contributors to the distinctive odors of fish sauce by an addition test using the quantitative descriptive analysis (QDA) method.

MATERIALS AND METHODS

Materials. Fish sauce was imported from the Thai Fish Sauce Factory Co., Ltd. Because the quality of the fish sauce satisfied the standard among fish sauces, it was used as a fish sauce sample. A Tenax TA column (20−35 mesh) was purchased from GL Sciences, Tokyo, Japan. 2-Methylpropanal, 2-methylbutanal, 2-pentanone, 2-ethylpyrdine, 3-methylbutanoic acid, and 3-(methylthio)propanal were from Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan.

Dimethyl trisulfide was synthesized by Shiratori Pharmaceutical Co., Ltd., Chiba, Japan.

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Isolation of Volatiles (Headspace Gas Analysis). Fifty grams of fish sauce, to which 3 μL of 1% cyclohexanol had been added as an internal standard, was purged for 16 min with helium at 50 mL/min in a water bath maintained at 40 °C. The volatile compounds were concentrated onto a Tenax column, which was kept at 40 °C. The column consisted of a glass tube (15.8 cm × 3.0 mm i.d.) (GL Sciences) packed with Tenax TA (70 mg, 20−35 mesh).

Capillary Gas Chromatography (GC). Separation of volatile compounds was done on a Hitachi G-3900 model gas chromatograph equipped with a flame ionization detector (FID). The GC was connected to a thermal desorption cold trap injector CP4020 TCT (GL Sciences). Volatile compounds, which were thermally discharged from the Tenax trap at 200 °C and concentrated in a cold trap, were introduced into a capillary column. Separation was achieved on the fused silica capillary column (60 m × 0.25 mm i.d.) coated with cross-linked polyethylene glycol (20M) at a film thickness of 0.25 μm (TC-Wax; GL Sciences). 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 flow rate of helium as a carrier gas was 0.7 mL/min. For sniffing GC effluents, an outlet splitter system (OSS-2; GL Sciences) was used.

Capillary Gas Chromatography—Mass Spectrometry (GC-MS). A JMS-DX303 mass spectrometer connected to GC-06 gas chromatograph (Japan Electron Optics Laboratory, Tokyo, Japan) was used for mass spectrometry identification. Concentrated volatile compounds on the Tenax trap were thermally desorbed at 220 °C for 30 s and introduced into a capillary column, using an injection and focusing instrument (thermal desorption unit; Supelco, Bellefonte, PA). Separation was done on the same capillary column as the GC analysis. The oven temperature was programmed from 50 to 230 °C at a rate of 2 °C/min. The flow rate of helium was 0.7 mL/min. The mass spectra were obtained by electron impact ionization at 70 eV. Retention indices were calculated by using a modified Kovats index. Seven authentic compounds in material and mass spectral databases (EPA/NIH Mass Spectral Database, 1978, National Bureau of Standards, Washington, DC) were identified using a computer DA-5000 (Japan Electron Optics Laboratory). Therefore, seven compounds were completely identified, and others were tentatively identified.

GC Sniffing (Aroma Extract Dilution Analysis). According to the principle of AEDA (*21*), the headspace gas of fish sauce was absorbed by each volume of purged helium gas: 1600 mL, 800 mL (1/2), 400 mL (1/4), 200 mL (1/8), 100 mL (1/16), 50 mL (1/32), 25 mL (1/64), and 12.5 mL (1/128).

Effluents from the splitter system were sniffed by three assessors with respect to each odor concentrate on the Tenax trap. Odor acceptance was decided by two of three assessors.

Preparation of Deodorized Fish Sauce. Shimoda at el. (*12*) reported that the alkalization of fish sauce enhanced the concentration of nitrogenous and sulfurous volatile compounds in the headspace gas. For preparing deodorized fish sauce, fish sauce was alkalized at pH 9.5 by 20% NaOH and evaporated under reduced pressure at 30 °C for 4 h. After the evaporation, pH, total nitrogen, moisture, and NaCl content were adjusted to the same as they were before alkalization.

Quantification of Principal Odorants. For the quantification of principal odorants, a standard addition method was employed. Various amounts of 2-methylpropanal, 2-methylbutanal, 2-ethylpyridine, and dimethyl trisulfide dissolved in ethanol were added to nontreated fish sauce for quantification by the standard addition method (*12*).

Sensory Methods. The major contributing factors to fish sauce odor are described as ammoniacal, cheesy, and meaty notes (*7*). However, these three sensory attributes were subdivided into eight attributes as the descriptors for QDA (*22*). They were burnt, fishy, sweaty, fecal, rancid, cheesy, meaty, and ammoniacal notes.

Panelists were selected from employees of Japan Tobacco Inc. on the basis of interest, time available, and a personal acceptance of the taste of fish sauce; 4 were female and 10 were male, and ages ranged from 29 to 52 years. As different people might have different ideas on the odor descriptors of fish sauce, the odor attributes of each descriptors were explained in detail, and then typical fish sauce odors were evaluated; repeated sensory evaluations of fish sauce were made. The odor descriptors were identified by an odor profiling method with fish sauce as a sample (*23*). During the training, panelists were asked to identity and define the odor descriptors of fish sauce. Panelists were presented with a nontreated fish sauce sample, a deodorized fish sauce, and a deodorized fish sauce with some of four odorants added: 2-methylpropanal, 2-methylbutanal, 2-ethylpyridine, and dimethyl trisulfide (at 370.7, 38.5, 1.4, and 7.5 ng/mL, respectively). A blank consisted of the same volume of ethanol added to the deodorized fish sauce. All of the samples were kept at 30 °C before the presentation. Sensory evaluation was carried out in individual booths with incandescent light. For the eight descriptors, deodorized and addition samples compared to nontreated sample were scored on −2 to +2 scales: −2, very weak; −1, weak; 0, same; +1, strong; +2, very strong. The significances in the quantitative descriptive analysis were assessed by Student's *t* test.

RESULTS AND DISCUSSION

Identification of the Odorants in Fish Sauce and Their Flavor Dilution (FD) Factors. For determination of potent distinctive odorants in fish sauce, the method of AEDA was adopted by combination with a purge and trap technique. The FD factors were determined from 1 to 1/128 by 1/2 stepwise.

Table 1 lists the odor characteristics and FD values of 43 odorants in the headspace gas. Twenty-three compounds with high FD factors were identified by GC-MS analysis. 2-Methylpropanal (peak 2), 2-methylbutanal (peak 5), 2-pentanone (peak 7), 2-ethylpyridine (peak 21), dimethyl trisulfide (peak 26), 3-(methylthio)propanal (peak 31), and 3-methylbutanoic acid (peak 39) had FD factors >64, and their odor characteristics were burnt, burnt, fruity, grassy, fishy, grassy, and rancid, respectively. In the present study, trimethylamine had a small FD factor. There were many types of fish sauce containing various concentrations of trimethylamine.

The linearity between the peak area of GC analysis and the purge gas volume was confirmed (**Figure 1**). There was good linearity between the purge gas volume and GC peak areas except for 2-methylpropanal and 2-methylbutanal. These compounds broke through the Tenax trap with the purge gas >200 mL. The breakthrough, however, did not give cause any problems with the AEDA, because the FD factors of these aldehydes could be determined in the range of straight relationship between GC peak areas and purge gas volumes.

Quantification of Distinctive Odorants in Fish Sauce. The gas chromatogram of nontreated fish sauce is shown in **Figure 2**. Relative concentrations of odorants in the headspaces of nontreated and deodorized sauce are listed in **Table 1**. 2-Methylpropanal, 2-methylbutanal, 2-ethylpyridine, and dimethyl trisulfide were decreased significantly by the deodorization. Only four odorants were used for the following addition tests because these compounds were decreased significantly by the deodorization treatment. On the other hand, 2-pentanone and 3-(methylthio)propanal in addition to benzaldehyde (RI 1526), *n*-octanol (RI 1568), benzonitrile (RI 1613), and acetophenone (RI 1650) increased in the deodorized fish sauce.

It was proved that the volatile compounds which decreased in deodorized fish sauce would be principal contributors to the distinctive odor of fish sauce. By a standard addition method, the concentrations of 2-methylpropanal, 2-methylbutanal, 2-ethylpyridine, and dimethyl trisulfide in fish sauce were estimated to be 370.7, 38.5, 1.4, and 7.5 ng/mL, respectively.

Sensory Evaluation of Fish Sauces. The addition of individual odorants to deodorized fish sauce could not restore the distinctive odor of fish sauce, so the method of combination addition was employed. The effects of burnt, grassy, and fishy notes on the restoration of fish sauce odor were determined by using reconstituted fish sauces, which were prepared by the

Table 1. Volataile Compounds in a Fish Sauce Characterized by AEDA and GC-MS

peak	RI	odor character	compound	FD	after de-odorization[a]
1	677	fishy	trimethylamine	128	0.38
2	817	burnt	2-methylpropanal		
3	861	phenolic			
4	910	burnt			
5	926	strong burnt	2-methylbutanal	64	0.22
6	950	fruity			
7	957	fruity	2-pentanone	64	4.72
8	968	fruity			
9	1050	fruity		8	
10	1055	fruity			
11	1066	melon-like		32	
12	1083	sweaty			
13	1146	cheesy	1-butanol		
14	1170	rancid	pyridine	16	
15	1183	fruity		4	
16	1194	burnt		16	
17	1204	burnt	3-methyl-1-butanol	32	
18	1234	sweaty	2-methylpyridine	4	
19	1250	grassy			
20	1259	sweaty	4-methyl-1-hexanol		
21	1286	grassy	2-ethylpyridine	64	0.13
22	1290	sweaty	cyclopentanol		
23	1324	fruity	2,6-dimethylpyrazine		
24	1333	sweaty	ethylpyrazine		
25	1368	fishy		4	
26	1378	fishy	dimethyl trisulfide	128	0.16
27	1383	rancid	2-ethyl-6(or 5)-methyl-pyrazine		
28	1396	sweaty	2-methyl-5-(1-methyl-ethyl)pyrazine	8	
29	1435	sweaty		8	
30	1442	rancid			
31	1451	grassy	3-(methylthio)propanal	128	1.9
32	1468	fishy	4-ethyl-6-hepten-3-one	4	
33	1482	sweaty		8	
34	1508	grassy			
35	1570	sweaty			
36	1591	rancid	benzonitrile		
37	1614	cheesy	butanoic acid		
38	1650	cheesy	acetophenone	4	
39	1665	rancid	3-methylbutanoic acid	64	
40	1705	rancid		4	
41	1711	metallic			
42	1788	sweaty			
43	1847	rancid	capronic acid	4	

[a] Fish sauce was deodorized by alkalization at pH 9.5 by 20% NaOH and evaporated under reduced pressure at 30 °C for 4 h. The value is the concentrate ratio of deodorized fish sauce and nontreated fish sauce.

addition of four distinctive odorants in various combinations. The combinations of odorants added were as follows: (A) all four volatiles (2-methylpropanal, 2-methylbutanal, 2-ethylpyridine, and dimethyl trisulfide), (B) three volatiles (2-methylpropanal, 2-methylbutanal, and dimethyl trisulfide), (C) three volatiles (2-methylpropanal, 2-methylbutanal, and 2-ethylpyridine), (D) two volatiles (2-ethylpyridine and dimethyl trisulfide), and (E) two volatiles (2-methylpropanal and 2-methylbutanal).

QDA was performed to examine the contributors to the distinctive odor of fish sauce (**Figure 3**). Sample A showed that four volatiles cooperatively contributed to the fishy, sweaty, rancid, cheesy, and ammoniacal notes. In samples A−D, the fishy note was well restored but it was decreased in sample E. Therefore, 2-ethylpyridine and dimethyl trisulfide could contribute to the fishy note. Although 2-ethylpyridine was described as grassy by GC-sniffing (**Table 1**), this compound was found to contribute to the fishy odor of fish sauce. The sweaty note was restored in every reconstituted sample, which indicated that every odorant is involved in the sweaty note. From the sensory evaluations of samples A and D, 2-ethylpyridine and dimethyl

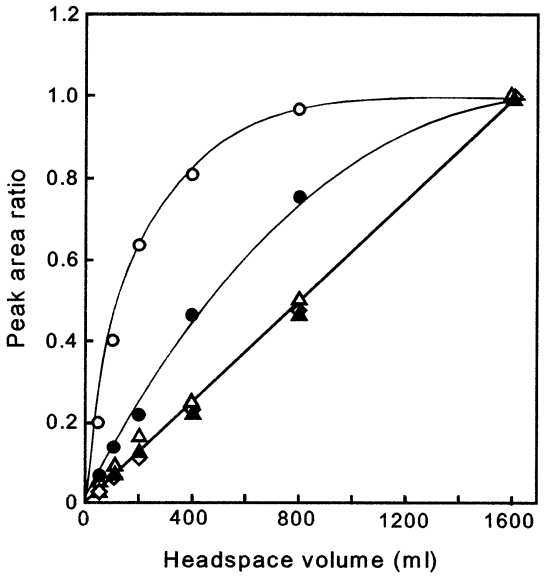

Figure 1. Relationships between peak area ratios (I.S. = 1.0) and purge gas volumes: (○) 2-methylpropanal (peak 2); (●) 2-methylbutanal (peak 5); (▲) 2-pentanone (peak 7); (△) dimethyl trisulfide (peak 26); (◇) 3-(methylthio)propanal (peak 31). Peak numbers correspond to **Table 1**.

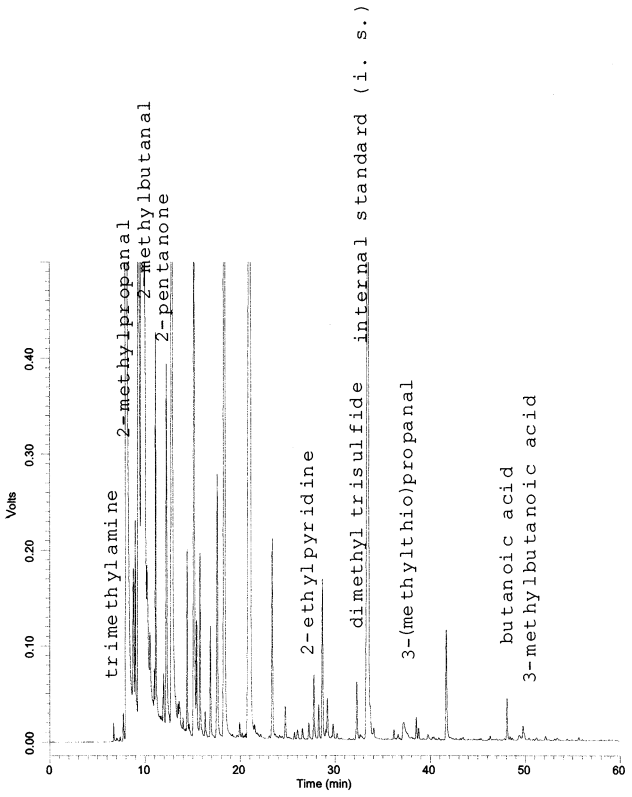

Figure 2. Gas chromatogram of headspace volatiles of fish sauce.

trisulfide were found to be essential to the fecal note, because the fecal note was significantly decreased by the omissions of 2-ethylpyridine and dimethyl trisulfide. These compounds had grassy and fishy odors, respectively, but they contributed to the fecal note in the fish sauce as a result of olfactory interaction. A rancid note was fully restored only in sample A. This indicated that the four volatiles were essential to the development of the rancid note. The cheesy note was restored in samples A and C, and in sample D it was partially restored, with an average score in cheesy note, but it was completely diminished in sample B. 2-Pentanone and volatile fatty acids had been

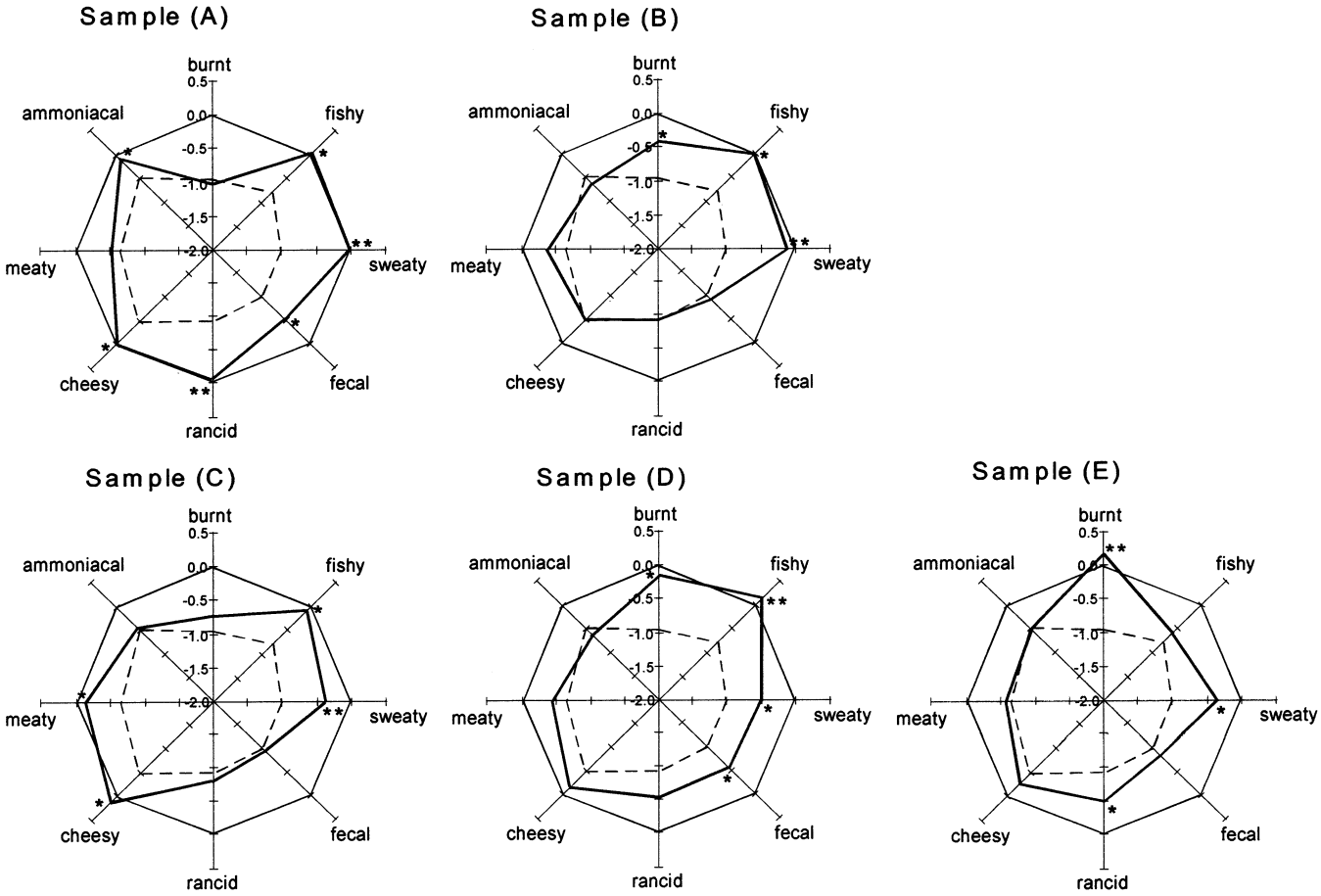

Figure 3. QDA of fish sauce odor: (—) nontreated; (•••) deodorized; (**—**) addition samples. Addition samples were prepared with deodorized fish sauce to which four odorants, 2-methylpropanal (370.7 ng/mL), 2-methylbutanal (38.5 ng/mL), 2-ethylpyridine (1.4 ng/mL), and dimethyl trisulfide (7.5 ng/mL), had been added: sample A, all four volatiles added (2-methylpropanal, 2-methylbutanal, dimethyltrisulfide and 2-ethylpyridine); sample B, three volatiles added (2-methylpropanal, 2-methylbutanal, and dimethyl trisulfide); sample C, three volatiles added (2-methylpropanal, 2-methylbutanal, and 2-ethylpyridine); sample D, two volatiles added (2-ethylpyridine and dimethyl trisulfide); sample E, two volatiles added (2-methylpropanal and 2-methylbutanal). Significance between deodorized and addition sample was assessed by Student's *t* test: *, $p < 0.05$; **, $p < 0.01$.

considered to be responsible for the cheesy note (*12*, *24*). In the present experiment, 2-pentanone increased ~4 times after the alkali treatment; on the other hand, there was no difference in volatile fatty acids contents before and after the treatment. Therefore, it was considered that 2-ethylpyridine in addition to 2-pentanone and volatile acids was an essential factor for the development of the cheesy note. Shimoda and others (*12*) reported that nitrogen-containing compounds together with aldehydes were responsible for the meaty note of fish sauce. The result of sample C indicated that 2-ethylpyridine together with 2-methylpropanal and 2-methylbutanal could be responsible for the meaty note. The ammoniacal note was restored only in sample A despite the absence of ammonia and volatile amines. The burnt note was restored in samples B, D, and E, but the note decreased in samples A and C. These results did not show any contributors to burnt odor.

In this study, the contribution of volatile fatty acids could not be investigated because there was no difference in the volatile fatty acids contents before and after the alkali treatment (data were not shown). Further study is needed to investigate contributions of volatile fatty acids.

Conclusion. 2-Methylpropanal, 2-methylbutanal, 2-pentanone, 2-ethylpyridine, dimethyl trisulfide, 3-(methylthio)-propanal, and 3-methylbutanoic acid were principal contributors to the distinctive odor of fish sauce. 2-Ethylpyridine and dimethyl trisulfide were found to contribute to the fishy note. The sweaty note could be attributed to all four volatiles. 2-Ethylpyridine and dimethyl trisulfide were essential to the fecal note. All four volatiles were essential to the development of the rancid note. 2-Ethylpyridine in addition to 2-pentanone and volatile acids was essential to the cheesy note. 2-Ethylpyridine together with 2-methylpropanal and 2-methylbutanal was responsible for the meaty note. The burnt note was developed in the presence of 2-ethylpyridine and dimethyl trisulfide and also in the presence of 2-methylpropanal and 2-methylbutanal.

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