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Improvement of fish sauce flavor by *Eurotium herbariorum*

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Volatile compounds of various commercial fish sauces were collected by a purge-and-trap method and analyzed by gas chromatography (GC) and GC-mass spectrometry. A large amount of dimethyl disulfide (DMDS) and the branched short-chain aldehydes such as 2-methylpropanal, 2-and 3-methylbutanal, with low odor threshold values, were detected in Ika-ishiru (fish sauce made from Japanese common squid internal organs). These compounds increased with the storage of Ika-ishiru and a stinging odor developed. The effects of *Eurotium herbariorum* on DMDS and aldehyde development in Ika-ishiru were examined. The fungus apparently decomposed aldehydes and DMDS in Ika-ishiru, and changed most of authentic aldehydes into their corresponding alcohols and acids. The lower the abundance of these compounds, the more a desirable aroma developed in Ika-ishiru. Because the free amino acid compositions did not change before or after the treatment with the fungus, it was found to be desirable for the improvement of fish sauce flavor.

KEY WORDS:fish sauce, ishiru, dimethyl disulfide, volatile aldehydes, *Eurotium* sp. fungi, microorganism

INTRODUCTION

Traditionally, many kinds of fish sauces have been consumed as seasonings throughout some Asian and European countries. Free amino acids, organic acids and peptides etc. are primarily responsible for the taste of fish sauce,[1,2] which is organoleptically greater than soy sauce. Therefore, seasoning industries in Japan have shifted focus from traditional products toward tastier ones, in which fish sauce is added. It must be pointed out, however, that one of the chief difficulties in using fish sauce is its peculiar smell which is unfamiliar to the perception of most Japanese.

The volatile flavor of fermented fish sauces has been investigated,[3-12] and three distinctive notes such as ammoniacal, cheesy, and meaty have been reported to compose fish sauce flavor.[3,10] However, a few reports on the volatile flavor of fish sauces by headspace gas analysis have been published.[9,10] The details of the volatiles of Ika-ishiru, a fish sauce made from Japanese common squid internal organs and Iwashi-ishiru, a fish sauce made from sardines in the Noto district in Japan, have not been clarified. A full understanding of the volatile flavor of Japanese fish sauces is necessary to facilitate the manufacturing of new types of umami soup and related products.

Sanceda et al.[13] tried to produce fish sauce with improved aroma quality under anaerobic conditions.

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Shimoda et al.[14] reported that the unpleasant odor of fish sauce was eliminated by Amberlite resin column treatment. A company in Japan has acquired markets for fish sauce fermented with sodium chloride-(NaCl) tolerant bacteria, and this product is aimed at reducing the peculiar smell and the duration of fermentation. Its taste is, however, inferior to that of fish sauce made under the usual fermentation conditions.

Repression of fish sauce smell has never been fully investigated. Dried bonito, *Katsuobushi* in Japanese, is a fish product fermented with *Eurotium* species (once called *Aspergillus glaucus*) that smells good because this fungus greatly contributes to the formation of good flavor.

The purpose of this study was to compare the compositions of highly volatile compounds of Ishiru with those of other fish sauces by headspace gas analysis, and to determine whether *Eurotium herbariorum* plays a role in the flavor improvement of fish sauce.

MATERIALS AND METHODS

Materials

Many kinds of fish sauces were commercially available and stored at -20℃ prior to use.

Culture of a mold

The species of *Eurotium herbariorum* (kindly supplied by Ninben Co., Kawaguchi, Japan) was grown in surface culture at 24℃ on 200 mL of medium containing 2% malt extract (Oriental Yeast Co., Tokyo, Japan), 3% glucose, 0.3% polypepton (Wako Pure Chemicals Co., Tokyo, Japan), and NaCl. After 2 weeks, the mycelial mats were harvested. One gram of the wet mycelial mats was added to 20 mL of Ika-ishiru which was then incubated on a rotary shaker in darkness at 24℃

Collection of volatiles

For capillary gas chromatography (GC), a purge-and-trap technique described in our previous paper[15] was used. Twenty-mL sample of fish sauce was shaken at 24℃ for 1 day. Five mL of the sample and 50μL of cyclohexanol (100μg/mL) as an internal standard were placed in a headspace sampling vessel, (40 mm×50 mm i.d.) held at 30℃ for 10 min, and then purged with helium gas (40 mL/min), which was passed through a column packed with the molecular sieve 5A and Tenax TA. The gas swept the headspace into a Tenax TA trap (170 mm×3 mm i.d.; 60/80 mesh; 200 mg) for 10 min. To remove moisture, the trap was further swept with helium gas for 10 min.

For headspace gas analyses, twenty-mL sample was placed in fifty-mL Erlenmeyer flask with a screw cap and shaken continuously at 30℃ for 5 min. Two-mL sample of gas was taken for analysis.

Gas chromatography (GC)

Separation of volatiles using capillary GC was achieved as follows: the Tenax TA trap was directly connected with an injection port and heated at 200℃ for 10 min. The method of concentrating volatiles from the trap included the use of cryofocusing by immersing the first column loop in liquid nitrogen. A Hewlett Packard HP-5890 II model gas chromatograph with a flame ionization detector (FID; 220℃) was used on a DB-WAX fused silica capillary column (60 m×0.25 mm i.d.; film thickness 0.25μm; J&W Scientific, Folsom, CA). Column temperature was programmed from 35℃ to 220℃ at 4℃/min with an initial hold time of 10 min. The carrier gas was helium (1.1 mL/min) with a split ratio of 1:10. A Chromatocorder 21 (System Instruments, Tokyo, Japan) was used for relative quantitative calculation.

Headspace gas analyses were achieved on a G-950 column (coated with polystyrene porous polymer; 40 m ×1.2 mm i.d.; Chemicals Inspection & Testing Inst., Tokyo, Japan) attached to a Shimadzu GC 8A model gas chromatograph equipped with an FID. The carrier gas was nitrogen, and the column temperature was 160℃. The injection and detector temperature was at 220℃.

Analysis of aqueous samples was performed on a column packed with Gaskuropack 54 (2 m×2.6 mm i. d.; GL Science, Tokyo, Japan). The instrument was a

Shimadzu GC 8A model gas chromatograph equipped with an FID. The carrier gas was nitrogen, and the column temperature was 200℃.

Gas chromatography/mass spectrometry (GC/MS)

The GC/MS system consisted of a Finnigan MAT Magnum model mass detector (ion- trap type) attached to a Varian GC-3400 gas chromatograph. GC conditions were the same as described above. The electron impact ionization voltage was 70 eV. The mass full scan mode was 26-250 a.m.u./sec, the electron multiplier voltage was 1300V, and the ion-trap manifold temperature was 220℃.

Identification of compounds

Identifications were based on standard mass library data (Magnum library search system; NIST mass spectra database 62235 compounds and private database 730 compounds), and on Retention indices. [16] Tentative identifications in the case of G-950 column analyses were based on comparison of retention times of the unknowns with those of authentic compounds.

Aldehyde decomposition by a mold

Twenty mL of 2.5 mM aqueous solutions of 2-methylpropanal, 2-methylbutanal, 3-methylbutanal and 2-butanone was kept in a 50 mL Erlenmeyer flask and 1 g of wet mycelial mats of the fungus was added which was cultured in the presence of 5% NaCl. After rotary shaking, the sample solution was filtered with a Sartorius Minisart SRP25, (Sartorius, Gottingen, Germany) and 1 μL of the filtrate was subjected to GC analysis.

Amino acid components

The free amino acid compositions were analyzed on a JEOL JLC-300 system amino acid analyzer using the ninhydrin method.

Sensory evaluation

Five hundred mL of each Ishiru was statically incubated with 12 g of mycelial mats of Eurotium herbariorum at 25℃ for 3 days, and then filtrated. Fungus-treated and untreated Ishiru samples were evaluated for sensory quality by 24 untrained Japanese panelists. The paired comparison tests were done to determine whether the Ishiru samples, treated with and without the fungus, could be distinguished from each other based on an unpleasant fishy flavor. The judges evaluated each sample twice.

RESULTS AND DISCUSSION

Various fish sauces commercially available were analyzed using the purge-and-trap method. A total of 41 volatile compounds were positively identified (Table 1). Typical capillary gas chromatograms of Ika-ishiru and Iwashi-ishiru are shown in Fig. 1.

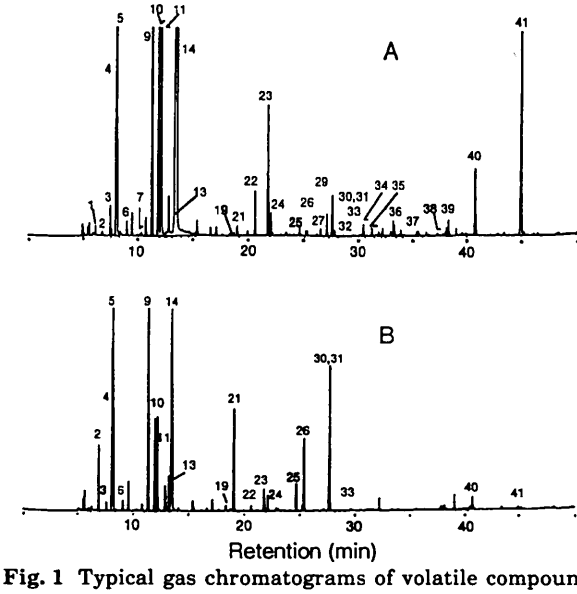

Fig. 1 Typical gas chromatograms of volatile compounds from Ika-ishiru (A) and Iwashi-ishiru (B). Peak numbers correspond to those listed in Table 1.

As shown in Table 1, amount of 2-propanone (5) and 2-butanone (9) was high in all fish sauces. The amounts of 2-methylpropanal (4), 2- and 3-methylbutanal (10,11) were substantially high, and 2-furanmethanol (41) seemed to be a specific compound largely formed in Ika-ishiru. All samples were found to have individual characteristics. The product of M company showed a specifically large amount of ethanol (14). Aldehydes such as benzaldehyde (40) and (*E*)-2-methyl-2-butenal (23)

Table 1 Quantitative values of volatile compounds in various fish sauces

Peak no.	Compound name by class	Area ratio[a] Ika-ishiru	Iwashi-ishiru	Japan M Co.	Japan K Co.	Vietnam	Korea
	Aldehyde						
3	Propanal	0.23	0.06	0.07	0.20	0.14	0.08
4	2-Methylpropanal	7.67	1.37	1.64	2.80	1.48	nd
6	2-Propenal	0.20	0.09	0.03	nd	0.06	0.04
7	Butanal	0.01	0.02	0.01	0.02	tr	0.02
10	2-Methylbutanal	6.57	0.64	2.33	3.88	1.42	0.07
11	3-Methylbutanal	5.96	0.37	0.94	1.46	0.58	0.07
23	(*E*)-2-methyl-2-butenal	1.30	0.19	0.07	0.06	0.06	0.06
27	Heptanal	0.10	0.01	0.01	nd	0.01	0.02
29	3-Methyl-2-butenal	0.26	0.02	0.14	nd	0.01	0.02
39	Furfural	0.29	0.09	0.02	0.02	0.02	0.01
40	Benzaldehyde	0.77	0.13	0.06	0.09	0.36	0.07
	Alcohols						
13	2-Propanol	0.14	0.43	0.15	nd	0.75	154.72
14	Ethanol	12.55	8.56	2667.34	101.56	19.05	177.31
19	2-Butanol	0.01	0.07	0.31	0.60	0.19	0.99
21	1-Propanol	0.15	1.14	0.58	0.38	1.56	8.91
24	2-Methyl-1-propanol	0.27	0.15	0.12	nd	0.01	1.01
25	1-Butanol	0.26	0.22	0.08	nd	0.10	3.59
26	1-Penten-3-ol	0.04	0.72	0.98	0.08	0.21	4.47
30	2-Methyl-1-butanol	0.69[b]	1.08[b]	0.53[b]	nd	0.36[b]	3.80[b]
31	3-Methyl-1-butanol						
33	1-Pentanol	0.01	0.02	0.05	nd	0.04	0.37
41	2-Furanmethanol	7.92	0.40	0.10	0.18	0.07	0.02
	Ketones						
5	2-Propanone	24.85	12.22	6.69	26.06	33.34	290.07
9	2-Butanone	8.60	2.26	2.85	5.09	6.04	3.50
12	3-Methyl-2-butanone	0.49	0.23	0.29	0.91	0.37	0.16
15	2-Pentanone	0.01	0.09	0.13	0.21	0.03	0.96
16	2,3-Butanedione	0.11	0.04	nd	nd	0.06	nd
18	4-Methyl-2-pentanone	nd	nd	0.01	0.06	0.01	0.01
35	3-Hydroxy-2-butanone	0.15	0.03	0.02	nd	0.06	0.06
	Miscellaneous compounds						
1	Pentane	0.07	0.04	0.39	nd	0.04	0.12
2	Dimethyl sulfide	0.06	0.32	0.08	0.54	0.11	0.11
8	Ethyl acetate	0.24	0.09	2.41	2.21	0.21	0.25
17	Acetonitrile	0.09	0.03	0.04	nd	0.10	0.22
20	Methylbenzene	0.03	0.04	0.01	0.03	0.04	0.04
22	Dimethyl disulfide	0.44	0.02	0.05	0.11	0.32	0.15
28	L-Limonene	nd	nd	0.02	nd	0.01	0.02
32	Pyrazine	0.04	nd	nd	nd	0.02	tr
34	2-Methylpyrazine	0.02	0.01	0.01	0.02	0.08	0.02
36	2,6-Dimethylpyrazine	0.04	nd	0.01	nd	0.11	0.04
37	N,N-Dimethylformamide	0.18	nd	nd	nd	nd	nd
38	Unknown	tr	0.01	0.02	nd	nd	0.04

[a] Area ratio defined as the peak area of fish sauce volatile standardized using internal standard peak area.
[b] Area ratio of 2-methyl-1-butanol plus 3-methyl-1-butanol.
tr (trace) represents concentrations of less than 0.01.
nd (not detected) represents compounds not detected.

were detected in fish sauce and fish paste.[17]

The branched short-chain aldehydes are considered to cause unpleasant oxidation flavor in foods.[18] These aldehydes with unpleasant odors could have certainly contributed to the overall odor of fish sauce due to their low threshold values.[10] 2-Methylpropanal (4) and 2- and 3-methylbutanal (10,11) had odor threshold values ranging from 2.24 to 40.7 ppb in air[19] and contributed to the formation of malty odor defects.[20] 2-Methylpropanal (4) and 3-methylbutanal (11) were responsible for the most intense odorants of ripened anchovies,[21] and 3-methylbutanal (11) was typical of salt-fermented anchovies.[22]

The amounts of 2-methylpropanal (4), 2- and 3-methylbutanal (10,11) in Ika-ishiru samples were analyzed simply by repeated headspace gas analyses reusing a G-950 column. Figure 2-B shows a typical gas chromatogram obtained for Ika-ishiru which was shaken at 24℃. Especially, levels of aldehydes and dimethyl disulfide (DMDS) (2) were notably increased. As time passed, Ika-ishiru released unpleasant and irritant odors. In the case of Iwashi-ishiru, such a remarkable change in GC profile was not observed (data unpublished). The addition of *Eurotium herbariorum* to shaken Ika-ishiru produced a good result in flavor. The fungus was quite tolerant of dryness and higher NaCl concentrations but could not grow in a medium containing more than 15%. Because Ika-ishiru contained 26.8% salt, the effect of its concentration in cultivation of the fungus on the ability to improve the flavor of Ika-ishiru was investigated in more detail.

As shown (Table 2 and Fig. 2), Ika-ishiru without the mycelial mats had a considerably higher amount of aldehydes, such as 2-methylpropanal (4) and 2- and 3-methylbutanal (10,11), and DMDS (2) which were

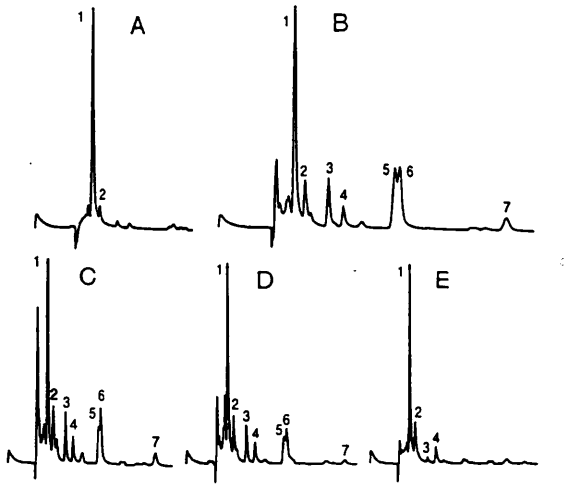

Fig. 2 Headspace gas analysis of Ika-ishiru treated with *Eurotium herbariorum*. Samples are as follows: A (At 0 time); B (After 3 days of shaking); C, D and E (After 3 days incubation with the fungus cultured in the presence of 0, 5, and 10% NaCl, respectively). Peak numbers are: 1 (Ethanol); 2 (2-Propanone); 3 (2-Methylpropanal); 4 (2-Butanone); 5 (3-Methylbutanal); 6 (2-Methylbutanal); 7 (Dimethyl disulfide).

supposed to be responsible for the unpleasant odor. Shimoda et al.[10] reported that DMDS could be one of the most potent contributors to fish sauce odor. Ika-ishiru treated with the mycelial mats, however, produced good results. The higher the concentrations of NaCl for cultivation , the lower the abundance of aldehydes and DMDS and the more a desirable aroma developed organoleptically.

The branched short-chain aldehydes might have been derived from enzymatic transamination followed by oxidative decarboxylation.[23] In the case of *in vivo* formation of 2-methylbutanal, leucine is first transaminated to the corresponding α-keto acid, which may then be oxidatively decarboxylated to the aldehyde. As for the mechanism of *in vitro* formation of these aldehydes, they are most likely formed from

Table 2 Changes in peak area (relative to an internal standard) of volatile compounds in Ika-ishiru samples treated and untreated with *Eurotium herbariorum*

Peak no.	Compound name by class	Area ratio[a]						
		Before incubation	After incubation[b]					
			Fresh[c]			Deteriorated[d]		
		Fresh[e]	Untreated	Treated		Untreated	Treated	
				A[e]	B[f]		A[e]	B[f]
	Aldehydes							
3	Propanal	0.07	0.18	0.04	0.01	0.63	0.07	0.02
4	2-Methylpropanal	1.65	7.09	0.76	0.32	11.06	0.75	0.10
6	2-Propenal	0.08	0.32	0.08	0.04	0.28	0.08	0.02
7	Butanal	0.01	0.02	0.02	nd	0.03	0.04	tr
10	2-Methylbutanal	1.45	5.41	0.55	0.22	8.05	0.38	0.11
11	3-Methylbutanal	0.40	3.92	0.45	0.19	10.26	0.72	0.18
23	(*E*)-2-methyl-2-butenal	tr	0.15	0.02	nd	2.68	0.36	0.03
27	Heptanal	0.01	0.01	0.02	0.03	0.02	0.02	0.04
29	3-Methyl-2-butenal	nd	0.02	0.01	nd	0.36	0.24	0.10
39	Furfural	0.22	0.24	0.31	0.27	0.28	0.24	0.23
40	Benzaldehyde	0.04	0.31	0.13	0.03	7.36	0.07	0.20
	Alcohols							
13	2-Propanol	0.01	0.03	0.06	0.09	0.12	0.11	0.20
14	Ethanol	1.34	1.30	0.45	0.73	1.27	1.68	1.06
19	2-Butanol	tr	tr	0.03	0.05	0.05	0.10	0.20
21	1-Propanol	0.01	0.01	0.02	0.01	0.01	0.03	0.03
24	2-Methyl-1-propanol	0.04	0.03	0.10	0.11	0.11	0.77	0.26
25	1-Butanol	0.04	0.01	0.05	0.01	0.03	0.10	0.02
26	1-Penten-3-ol	0.08	0.05	0.08	0.06	0.05	0.06	0.06
30	2-Methyl-1-butanol	0.10[g]	0.14[g]	0.54[g]	1.01[g]	0.11[g]	2.61[g]	1.48[g]
31	3-Methyl-1-butanol							
33	1-Pentanol	0.01	0.01	0.48	0.82	0.04	0.26	1.00
41	2-Furanmethanol	6.00	7.35	6.53	6.06	5.84	4.93	5.84
	Ketones							
5	2-Propanone	4.89	10.26	9.57	7.56	36.84	30.68	34.62
9	2-Butanone	0.96	2.09	1.65	1.12	14.30	11.21	11.80
12	3-Methyl-2-butanone	0.06	0.12	0.09	0.05	0.25	0.18	0.17
15	2-Pentanone	0.02	0.02	0.01	0.01	0.03	0.03	0.03
16	2,3-Butanedione	0.03	0.12	0.01	nd	0.26	0.06	nd
18	4-Methyl-2-pentanone	tr	nd	nd	nd	0.02	0.01	tr
35	3-Hydroxy-2-butanone	0.06	0.08	0.04	0.01	0.07	0.03	0.02
	Miscellaneous compounds							
2	Dimethyl sulfide	0.61	0.29	0.22	0.19	0.02	tr	0.01
8	Ethyl acetate	0.02	0.02	0.01	nd	0.01	0.02	0.01
17	Acetonitrile	0.04	0.02	0.03	0.01	0.06	0.05	0.05
20	Methylbenzene	0.05	0.02	0.06	0.01	0.09	0.07	0.04
22	Dimethyl disulfide	0.07	0.10	0.02	0.01	0.79	0.23	0.28
28	L-Limonene	tr	0.01	0.03	0.01	0.02	0.01	0.05
32	Pyrazine	0.01	0.01	0.01	0.01	0.15	0.12	0.13
34	2-Methylpyrazine	0.01	0.02	0.01	0.02	0.11	0.09	0.10
36	2,6-Dimethylpyrazine	0.01	0.03	0.02	0.02	0.04	0.02	0.04
37	N,N-Dimethylformamide	0.01	0.04	0.08	0.04	0.09	0.02	0.07
38	Unknown	tr	tr	0.34	0.20	0.01	2.00	2.81

[a] Area ratio defined as the peak area of fish sauce volatile standardized using internal standard peak area.
[b] Sample were shaken at 24℃ for 1 day. [c] Fresh sample opened just prior to analysis.
[d] Sample reciprocally shaken at 24℃ for 1 wk. [e,f] Samples treated with mycelial mats of Eurotium herbariorum cultured in the presence of 5 and 10% NaCl, respectively. [g] Area ratio of 2-methyl-1-butanol plus 3-methyl-1-butanol.
tr (trace) represents concentrations of less than 0.01. nd (not detected) represents compounds not detected.

free amino acids by Strecker degradation.[21,24]

If squid enzymes and microorganisms contributed to the formation of these aldehydes, it is impossible that these could be newly produced in thermally treated fish sauce. Triqui and Reineccius[25] concluded that microorganisms play a minor role in the development of the characteristic flavor of ripened anchovies. The addition of mycelial mats to Ika-ishiru would decompose the aldehydes, so they should not be present in it.

Commercially available Ika-ishiru was supposed to be thermally treated. When the fish sauce treated at 100℃ for 15 min was shaken at 24℃ for 1 day, the amounts of 2-methylpropanal and 2- and 3-methylbutanal were increased (Fig. 3-B). These results suggest that the aldehydes were not formed enzymatically but through another formation mechanism. The addition of living mats of *Eurotium herbariorum* could repress the formation of aldehydes, but the autoclaved mats did not show any depression effect (Fig. 3-C and 3-D). These facts indicate that the living fungus itself plays an important role in repression of the production of unpleasant odor and its decomposition, and some metabolites from *Eurotium* sp., such as flavoglaucin and its derivatives,[26] did not contribute to the improvement of flavor.

Purge-and-trap data (Table 2) show that the alcohols, 2-methylpropanol and 2- and 3-methylbutanol, with respective to odor threshold values of 832, 229, and 44.7 ppb,[19] were in high abundance in samples treated with the fungus. The amount of aldehydes, on the contrary, apparently decreased. These data suggest that the fungus could change aldehydes to the corresponding alcohols and acids. In spite of the greater amounts, the alcohols might not have an impact on fish sauce flavor because of their high thresholds and low volatilities. Irrespective of fresh and deteriorated samples, the fungus yielded desirable results in terms of a pleasant flavor. In the deteriorated sample, a large amount of the unknown compound (peak no. 38) was formed by incubation with the fungus. It must be clarified whether this unknown has an immediate connection with the flavor improvement.

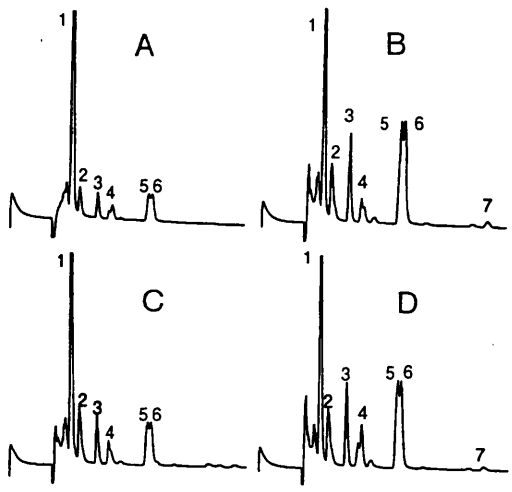

Fig. 3 Headspace gas analysis of Ika-ishiru heated and treated with *Eurotium herbariorum*. Samples are as follows: A (Immediately after heating); B (After 1 day of shaking); C (After 1 day of incubation with the living fungus); D (After 1 day of incubation with the autoclaved fungus). Peak numbers correspond to those shown in Fig. 2.

Table 3 Changes in the amounts of volatile component in Ika-ishiru samples treated with *Eurotium herbariorum*

Volatiles	Original	days treated 1day	2days
2-Methylpropanal	100[a]	5.6	-[b]
2-Methylpropanol	0	12.4	-
2-Methylpropionic acid	0	75.1	-
2-Methylbutanal	100	8.1	-
2-Methylbutanol	0	23.9	-
2-Methylbutyric acid	0	63.4	-
3-Methylbutanal	100	9.4	-
3-Methylbutanol	0	57.6	-
3-Methylpropionic acid	0	31.8	-
2-Butanone	100	96	95.4

[a] Abundance shown in %.
[b] Not measured.

Table 4 Sensory evaluation of unpleasant fishy flavor in fungus-treated and untreated Ishiru samples

Sample	Unpleasant odor -1	0	+1
Ika-ishiru	5	1	18[a]
Iwashi-ishiru	4	1	19[a]

-1: Untreated sample < fungus-treated sample.
0: Untreated sample = fungus-treated sample.
+1: Untreated sample > fungus-treated sample.
[a] Significantly different at 5% level ($p < 0.05$).

Table 5 Free amino acid compositions of Ishiru before and after the treatment with *Eurotium herbariorum*

(mg/100mL)

Amino acid	Iwashi-ishiru		Ika-ishiru	
	Untreated	Treated [a]	Untreated	Treated [a]
Taurine	197	203	664	653
Aspartic acid	415	416	830	818
Threonine	285	273	516	508
Serine	279	278	483	476
Glutamic acid	772	791	2266	2233
Glycine	206	208	451	439
Alanine	408	408	703	694
Proline	140	126	486	483
Citrulline	nd	nd	nd	nd
Valine	354	352	576	563
Cystine	23	22	35	28
Methionine	173	149	225	220
Isoleucine	300	300	414	407
Leucine	539	539	551	542
Tyrosine	51	53	86	77
Phenylalanine	216	216	376	370
Ornithine	26	26	266	262
Lysine	519	527	851	836
Histidine	263	263	158	155
Arginine	339	341	291	287
Total	5505	5491	10228	10051

[a] Samples are the same as shown in Table 4.
nd (not detected) represents compounds not detected.

Aqueous solutions of authentic flavor compounds were treated with 1g of mycelial mats of *Eurotium herbariorum* (cultured in 5% NaCl) (Table 3). Aldehydes were easily converted into their corresponding alcohols and acids. 2-Methylpropanal and 2-methylbutanal tended to form large amounts of the respective acids, but the results were reversed in the case of 3-methylbutanal. The fungus could not exert any effect on 2-butanone. Because the purge-and-trap method could not detect acids, further research is required to confirm the abundance of these acids in fish sauce before and after the treatment with the fungus.

Sensory evaluation showed that a great majority of panelists could detect the differences in acceptability between the fungus-treated and untreated Ishiru (Table 4). Thus it is noted that the fungus could act as an improver for fish sauce flavor.

Free amino acid compositions in both the fungus-treated and untreated fish sauces did not change before or after analysis (Table 5). The typical taste of fish sauce is correlated with amino acids.[1,2] Therefore, *Eurotium* sp. was found to be a desirable fungus.

Clarification of the mechanism producing aldehydes responsible for the unpleasant odor and the overall odor of Ika-ishiru is under investigation.

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(和文要旨)

Eurotium herbariorum による魚醤油の臭気改善

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市販魚醤油の揮発性成分をパージ・トラップ法で捕集し，キャピラリーGC及びGC-MS分析した。主にスルメイカの内臓を原料としたイカイシルからは，匂い閾値の低い分岐短鎖アルデヒドおよびdimethyl disulfide(DMDS)が多く検出された。イシルの貯蔵に伴って，これらの成分は増加し，刺激臭が増した。そこで，イカイシルのアルデヒド及びDMDSの生成に及ぼす*Eurotium herbariorum*菌体処理の効果を調べた。処理によってアルデヒドが顕著に減少し，DMDSも減少した。そして，これらの成分の量が少ないほど，イカイシルの刺激的な不快臭は減少した。菌体は大部分のアルデヒドを相当するアルコールと酸に変換した。処理の前後でイカイシルの遊離アミノ酸組成が変わらないので，魚醤油の臭気を改善する方法として*Eurotium*菌体処理が有用な方法であることが分かった。