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Changes of Volatile Flavor Compounds in Low Salt-Fermented Anchovy Paste by Adding Koji

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Abstract

Volatile flavor compounds in low salt-fermented anchovy pastes by adding koji (Koji), compared with Control, were analyzed by simultaneous steam distillation-solvent extraction/gas chromatography/mass spectrometry. Volatile compounds (106) were detected in both samples during fermentation. Among these, 79 compounds were positively identified and were composed mainly of aldehydes, esters, alcohols, ketones, nitrogen-containing compounds, aromatic hydrocarbons, and furans. Aldehydes and esters were found higher amounts than other compounds in both samples. Alkylpyrazines, such as 2,6-dimethylpyrazine, 2-ethyl-6-methylpyrazine, trimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine and tetramethylpyrazine, and also 2-phenylethanol were identified only in Koji.

Key words : volatile flavor, salt-fermented anchovy, koji

INTRODUCTION

Traditionally, more than 54 of salt-fermented fish products, such as fish paste and fish sauce, have been consumed as an important protein and fat source in Korean diet[1]. Among these, 7 kinds of fermented fish products, including anchovy and shrimp paste, have been favored a large amounts by consumers nowadays in Korea[1]. However, only 2~3% of the total amounts of processed fish products were fermented products in Korea, although no accurate statistics on the amount of fermented fish products manufactured for commercial purposes were present[2].

Most of fish pastes containing only 25~30% salt are fermented for over 6 months. Therefore, much research was attempted to reduce salt content of products and to reduce fermentation time for the purpose of commercial facilitation of fermented fish products[3~5]. On the other hand, the addition of malt powder or cooked cereals to fermented fish was used to enhance flavor quality of products in a certain areas in Korea[1]. Kunimoto *et al.*[6] reported that *Aspergillus oryzae*, which is known as one of the most useful molds utilized in making koji, had a reducing effect in fishy odor. Application of koji to make sardine meal was also attempted by Kim *et al.*[7].

As a consequence of instantized and modernized diets, recent, demands have shifted from salt-fermented fish products in favor of more convenient seasoning agents in Korea. This trend has in turn initiated studies of fermented fish products to improve their composition and health impact benefits. Except for a few reports, the volatile flavor of fermented fish products has not been fully investigated[8,9]. A thorough understanding of volatile flavor in fish paste is essential if alternative products are to be successfully produced.

The objective of this study was to identify and to compare volatile flavor components in anchovy pastes by adding koji with control during fermentation.

MATERIALS AND METHODS

Materials

Anchovy, *Engraulis japonica*, were purchased from a fishery joint market in Chungmu, Korea and transported in polyethylene bags to a laboratory within 1 hr. A medium for koji production was prepared with 1 : 1 : 0.3 by weight ratio of cooked soybean, roasted barley and powdered dryed anchovy, respectively.

The mixture was inoculated with *Aspergillus oryzae (Ahlbury) cohn var.* Viride Murakami, and then incubated in an incubator (Hanyoung Co., Korea) at 25°C for 3 days until molds grew throughout the materials. The koji strain was kindly provided by Department of Food Science and Technology, National Fisheries University of Pusan. Low salt-fermented anchovy paste were prepared according to the composition shown in Table 1. Further details are described in the previous paper[10]. Samples were homogenized using a Waring blender (Waring Products Co., Winsted, CT) before analysis. Standard flavor compounds were purchased from commercial sources or were generous gifts from Aldrich Flavor and Fragrance (Milwaukee, WI).

Simultaneous steam distillation-solvent extraction (SDE)

Homogenized fish paste (500g), distilled water (1.5L) and 91.7μg of internal standard 2,4,6-trimethylpyridine (TMP) were placed in a Lickens-Nickerson[11] type SDE apparatus (Cat. No. K-523010-0000, Kontes, Vineland, NJ) to extract volatile flavor compounds into redistilled diethyl ether (100ml). The procedure has been described elsewhere[12]. Extracts were concentrated to 1.5ml under a gentle stream of nitrogen. Duplicate extractions were carried out for each sample.

Gas chromatography / mass spectrometry (GC / MS)

A 4μl aliquot from each SDE extract was injected into an HP 5790GC/5970B mass selective detector (MSD) (Hewlett Packard Co., Palo Alto, CA) by splitless mode (155°C injector temperature ; 30s valve delay). Separation of volatile components was achieved on a fused silica capillary column (Supelcowax 10 ; 60m long × 0.25mm ID × 0.25μm film thickness ; Supelco, Inc, Bellefonte, PA). The linear velocity of the helium carrier was 25.7cm/s. Oven temperature was programmed from 40°C to 175°C at a rate of 2°C/min with initial and final hold times at 5 and 30min, respectively; it was further increased to 195°C at a rate of 5°C and maintained at 195°C for 25min. Electron ionization energy was set at 70eV. Mass range was 33~300 a.m.u., electron multiplier voltage was 2000V, and scan rate was 1.6/s. Other details of GC/MSD procedure have been described by Cha *et al.*[13]. Duplicate analyses were performed on each SDE extract.

Table 1. Formulas of ingredients for salt-fermented anchovy (%)[a]

	Salt	Sorbitol	Lactic acid	Alcohol	Glucose	Koji
Control	15	6	0.5	5		
Koji	15	6	0.5	5	5	10

[a] Ratio (w/w%) to the raw anchovy, except v/w% in case of lactic acid and alcohol

Compound identification

Volatile compounds were identified by matching retention indices (RI), calculated according to Van den Dool and Kratz[14], and mass spectra of samples with those of authentic standards. Tentative identifications were based on the standard MS library data[15].

Relative abundance and peak areas of co-eluting compounds

The relative abundance of each compound was expressed by the ratio of its total ion peak area to that of the internal standard. The peak area of a co-eluted compounds was calculated as described by Hites and Biemann[16] to minimize chromatographic interference.

RESULTS AND DISCUSSION

Two types of low salt-fermented anchovy pastes, such as those made with koji (Koji) and without koji (Control) were examined for volatile flavor components during fermentation to assess the commercial feasibility of Koji. Total ion chromatograms of the volatile components in Control and Koji after 30 days of fermentation are shown in Fig. 1 and 2, respectively. One hundred and six of volatile compounds were isolated (Table 2), including 30 aldehydes, 7 ketones, 18 alcohols, 7 nitrogen-containing compounds, 23 esters, 5 aromatic hydrocarbons, 5 furans, and 11 miscellaneous compounds during fermentation. Among these, 79 compounds were positively identified. As shown in Table 2, however, a total of 87 compounds

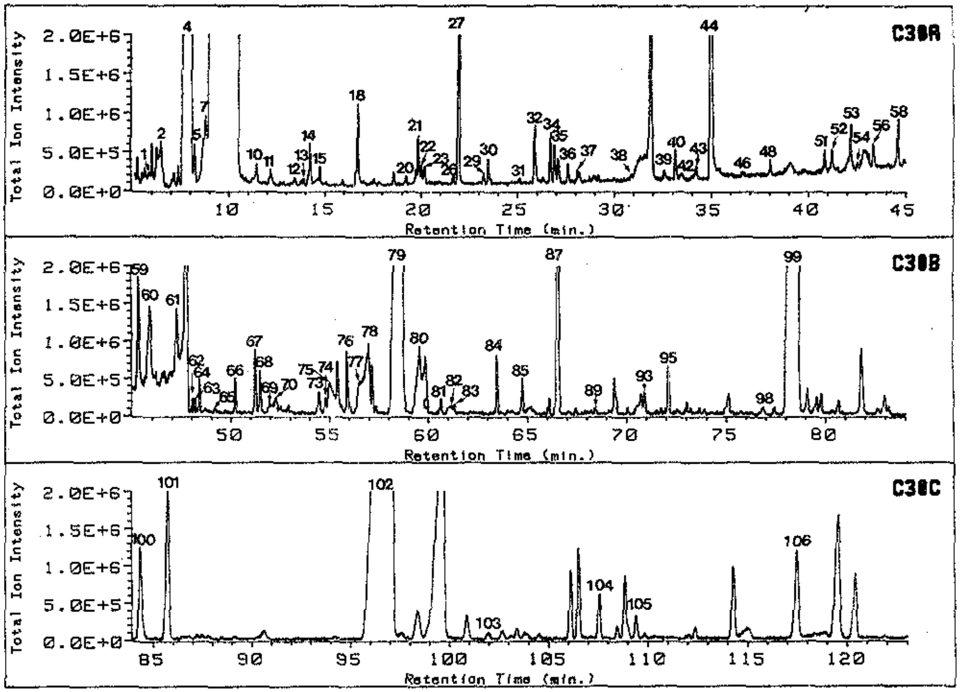

Fig. 1. Total ion chromatogram of volatile components in low salted anchovy paste (Control) after 30days of fermentation.
Peak numbers correspond to those listed in Table 2.

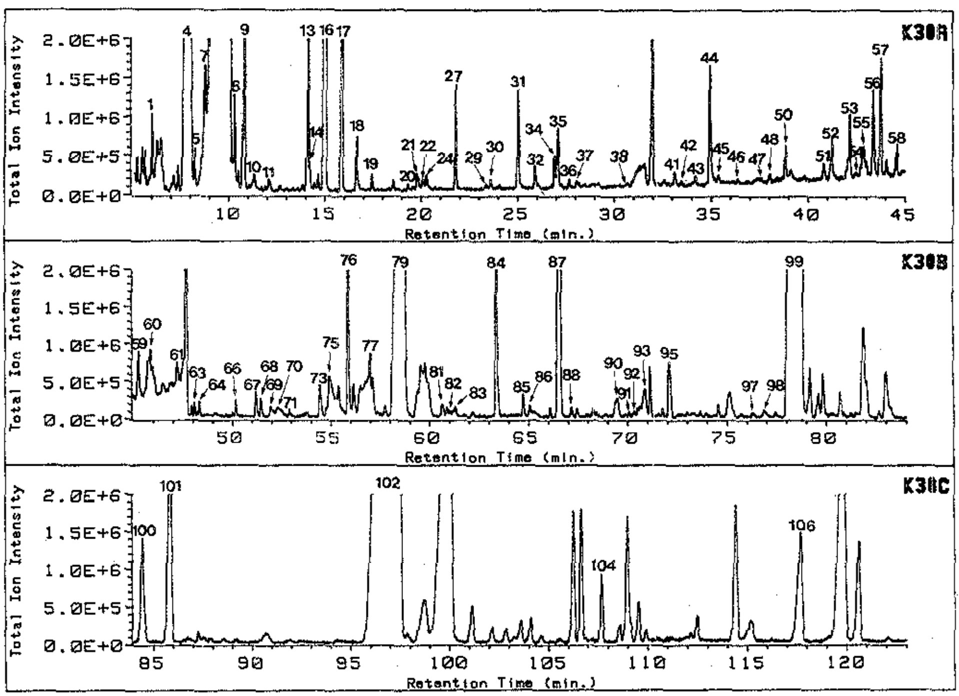

Fig. 2. Total ion chromatogram of volatile components in low salted anchovy paste by adding koji(Koji) after 30days of fermentation.
Peak numbers correspond to those listed in Table 2.

Table 2. Changes of volatile flavor components in low salted anchovy paste by adding koji during fermentation

Peak No.	Compound name by class	Retention index	Control						Koji					
			Fermentation time						Fermentation time					
			0day		13day		30day		0day		13day		30day	
			Mean area*** ratio	S.D.	Mean area*** ratio	S.D.	Mean area*** ratio	S.D.	Mean area*** ratio	S.D.	Mean area*** ratio	S.D.	Mean area*** ratio	S.D.
	Aldehydes (30)													
3	Butanal	858			6.26	3.46								
6	2-Methylbutanal	897	3.13	2.25	12.70	8.06								
7	3-Methylbutanal	900	11.63	8.73	71.03	33.94	33.06	19.14	14.46	19.08	34.54	38.51	96.00	45.14
10	Pentanal	971	4.13	1.89	12.82	4.44	4.00	2.04	2.05	1.02	4.50	4.04	3.75	0.50
15	(E)-2-Butenal	1043			3.65	2.96	1.06	0.31						
18	Hexanal	1078	18.00	8.55	82.86	28.10	12.44	6.85	10.90	13.10	9.25	5.85	15.00	4.76
19	2-Methyl-(E)-2-butenal	1095			4.76	1.83					1.08	0.17	3.25	0.50
21	(E)-2-Pentenal	1125	2.75	1.04	16.13	5.07	4.00	1.83	1.70	1.36	3.67	3.62	4.50	0.58
26	2-Methyl-(E)-2-pentenal	1155					0.88	0.25						
30	Heptanal	1181	4.25	2.02	20.66	4.26	2.31	1.28	2.58	1.65	1.96	0.75	2.75	0.96
32	(E)-2-Hexenal	1214	6.25	3.18	21.65	7.10	6.50	1.73	3.51	2.13	3.83	1.45	4.50	1.00
36	(Z)-4-Heptenal	1240	4.13	1.93	6.96	2.63	2.06	1.36	1.73	0.79	1.33	0.47	2.25	0.50
38	Octanal	1287			7.65	3.46	1.44	1.05	0.46	0.39	1.21	0.63	1.25	0.50
42	(E)-2-Heptenal	1323	0.88	0.25	4.15	2.75	1.94	2.05	0.84	0.32	1.04	0.67	1.00	0.00
49	Nonanal	1392			9.90	4.37								
51	(E)-2-Octenal	1429	2.38	1.49	14.84	5.30	2.13	1.44	1.87	0.34	2.92	2.17	3.25	0.96
59	(E,E)-2,4-Heptadienal	1493	16.50	10.78	17.78	12.12	14.56	5.32	4.63	1.38	7.54	4.77	13.00	4.83
61	Benzaldehyde	1523			21.11	7.10	20.25	6.45	3.38	1.28	15.04	9.64	12.75	9.03
64	(E)-2-Nonenal	1540	2.00	1.15	18.18	6.31	3.06	0.97			2.63	1.11	3.50	1.00
67	(E,Z)-2,6-Nonadienal	1582	6.00	2.71	17.93	7.31	7.69	2.44	2.05	1.41	3.96	2.06	6.00	0.00
68	(E,E)-2,4-Octadienal*	1586	3.00	1.78	7.34	2.53	4.31	1.21	1.39	0.71	2.42	1.26	3.75	1.26
74	(E)-2-Decenal	1639	2.50	1.00	17.65	5.39	1.88	0.63						
75	Phenylacetaldehyde	1642			7.34	2.01	6.25	3.23	2.09	0.81	7.00	1.83	8.50	0.58
83	2-Undecenal*	1746	1.63	1.11	12.13	4.25	0.94	0.13	1.14	0.50	2.00	0.82	1.50	0.58
85	(E,E)-2,4-Decadienal	1806	4.25	2.60	11.91	4.06	4.69	1.38	2.55	1.77	3.96	2.06	6.50	1.29
86	2-Chlorobenzaldehyde*	1813							0.64	0.49	1.58	0.50	1.50	0.58
93	Tetradecanal*	1918	31.75	31.84	11.53	8.53	4.94	2.07	6.03	3.00	12.29	2.58	11.75	3.30
94	alpha-Ethylidene-phenylacetaldehyde*	1928							1.08	0.94				
98	Pentadecanal*	2021	7.00	3.83	3.38	1.61	1.00	0.00					1.50	0.58
100	Hexadecanal*	2128	145.75	40.15	86.87	73.16	24.94	14.14	14.19	5.22	38.96	15.37	45.25	26.00
	Ketones(7)													
28	2-Heptanone	1177							0.87	0.46				
48	2-Nonaone	1389	2.38	0.95	6.53	2.99	1.50	0.58	1.52	0.42	1.33	0.47	1.75	0.50
62	1-(2-Furanyl)-ethanone*	1534					6.13	10.59						
66	(E,E)-3-5-Octadien-2-one*	1567	3.88	1.93	6.98	2.52	3.31	0.85	1.74	0.98	1.33	0.47	3.00	0.82
69	2-Undecanone	1593	2.25	0.29	5.62	0.87	1.44	1.05	1.34	0.55	2.25	1.26	2.00	0.82
88	Geranylacetone	1850							0.71	0.20	2.13	1.31	2.50	1.29
97	Pentadecanone*	2012	3.13	3.97					0.51	0.16	2.13	1.31	1.00	0.00
	Alcohols(18)													
25	Butanol	1139							0.49	0.16				
27	1-Penten-3-ol	1156	27.00	12.94	76.74	16.49	31.25	10.50	14.54	10.97	15.00	6.22	21.50	1.73
31	3-Methyl-1-butanol	1204					0.88	0.25	6.43	4.46	14.54	4.86	23.00	6.27
37	Pentanol	1246	1.38	0.48	4.83	3.54	1.19	0.55	0.81	0.36	1.25	0.50	2.25	1.89
39	(Z)-2-Penten-1-ol	1310	2.00	0.82	1.28	0.53	1.69	1.03						
40	(E)-2-Penten-1-ol	1318	5.75	3.69	6.21	1.81	2.44	1.48	2.54	1.18				
45	Hexanol	1351							1.30	0.57	1.21	0.25	1.50	0.58

Table 2. Continued

Peak No.	Compound name by class	Retention index	Control						Koji					
			Fermentation time						Fermentation time					
			0day		13day		30day		0day		13day		30day	
			Mean area*** ratio	S.D.	Mean area*** ratio	S.D.	Mean area*** ratio	S.D.	Mean area*** ratio	S.D.	Mean area*** ratio	S.D.	Mean area*** ratio	S.D.
53	1-Octen-3-ol	1449	5.75	2.72	11.44	3.44	5.88	2.32	5.15	0.73	9.50	3.70	14.25	3.86
54	Heptanol	1454			2.79	0.85	3.50	2.89	1.75	0.50	2.08	1.07	4.25	3.86
58	2-Ethyl-1-hexanol	1484	8.25	5.68	7.93	2.45	4.63	2.50	2.83	0.67	5.67	3.68	6.75	2.06
65	Octanol	1552					0.88	0.25						
71	(E)-2-Octen-1-ol	1608											1.00	0.00
82	Decanol	1744	4.25	3.84	1.28	0.53	1.13	0.25	0.52	0.23	1.58	0.96	1.75	0.50
89	Benzylalcohol	1874	1.00	0.41	1.70	1.01	1.69	1.55	0.48	0.43				
92	2-Phenylethanol	1907							0.39	0.09	1.33	0.47	1.25	0.50
96	Dedecanol	1971	2.50	1.08										
103	Pentadecanol	2299					2.50	3.00						
105	Hexadecanol	2376					5.00	2.97						
	N-Containing compounds (7)													
33	2,4-Dimethylpyridine*	1216			5.70	3.16								
41	2,6-Dimethylpyrazine	1320									2.00	0.82	3.00	0.82
46	2,4,6-Trimethylpyridine (I.S.)**	1363	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00	1.00	0.00
47	2-Ethyl-6-methyl-pyrazine	1384									1.13	0.25	1.00	0.00
50	Trimethylpyrazine	1400							2.53	0.80	5.50	1.73	6.50	1.73
55	2-Ethyl-3,5-dimethyl-pyrazine	1461									6.96	3.33	8.50	4.80
57	Tetramethylpyrazine	1471							6.73	6.19	25.42	8.63	36.00	4.97
	Esters(23)													
4	Ethyl acetate	867	358.75	287.26	59.93	47.85	219.56	140.22	125.51	108.38	302.88	172.30	313.00	121.58
9	Ethyl-2-methylpropanoate	956							3.48	2.18	30.79	20.99	39.50	5.69
13	Ethyl butanoate	1031			4.78	2.34	2.19	2.54	2.82	1.50	15.38	3.82	40.50	11.56
16	Ethyl-2-methylbu-tanoate*	1047							3.80	2.58	74.50	26.29	195.00	41.99
17	Ethyl-3-methylbu-tanoate*	1062							9.75	7.51	43.33	21.75	101.00	19.34
22	Ethyl pentanoate	1130					1.44	0.72	1.65	1.20	2.88	1.55	2.25	0.50
35	Ethyl hexanoate	1230	1.63	0.75	10.02	3.32	4.50	3.54	1.59	0.47	4.46	3.52	11.25	7.27
43	Ethyl heptanoate	1333	1.00	0.41	1.84	1.09	1.31	0.47	1.03	0.53	1.46	0.42	2.25	0.50
44	Ethyl-2-hydrox-ypropanoate*	1344	102.00	50.73	22.73	7.35	48.94	36.96	29.73	19.22	33.38	23.61	28.25	4.57
52	Ethyl octanoate	1435	1.63	0.75	4.68	2.55	2.31	1.60	3.66	0.46	7.00	2.71	11.25	0.96
63	Ethyl nonanoate	1535	2.00	1.58			4.56	6.97	1.18	0.56	1.58	0.50	2.75	0.96
73	Ethyl decanoate	1633	3.75	1.19	3.14	1.76	3.69	0.99	3.03	1.92	8.42	5.10	12.25	2.06
78	Ethyl-3-(2-furyl)-propanoate*	1674					11.19	2.64						
81	Ethyl undecanoate*	1736	37.88	65.12	3.21	1.31	1.31	0.47	1.28	0.71	5.88	3.12	4.50	0.58
84	Ethyl phenylacetate*	1784	9.00	4.53	24.87	8.88	6.06	1.88	6.70	4.09	38.46	18.39	70.75	11.70
87	Ethyl dodecanoate*	1840	75.63	54.26	101.78	32.21	69.50	33.76	23.54	6.76	121.58	49.48	146.00	31.84
90	Methyl-2,8-dimethylun-decanoate*	1892							0.88	0.65	4.96	2.08	4.25	3.20
95	Ethyl tridecanoate*	1939	10.00	7.93	11.73	5.72	7.75	2.87	3.54	1.46	15.83	6.69	12.75	9.46
99	Ethyl tetradecanoate*	2049	252.75	282.57	1360.67	411.40	653.63	360.60	221.38	75.72	643.71	274.33	1194.25	363.60
101	Ethyl pentadecanoate*	2143	48.75	16.46	115.58	79.22	32.13	9.75	14.13	5.80	58.04	12.49	85.50	53.31
102	Ethyl hexadecanoate*	2255	104.63	72.35	1432.64	700.63	326.88	271.93	219.30	77.94	469.42	172.43	215.75	60.29
104	Ethyl heptadecanoate*	2357	19.13	11.01	16.70	10.68	11.69	9.13	2.24	0.62	16.38	9.91	21.50	3.70

Table 2. Continued

Peak No.	Compound name by class	Retention index	Control						Koji					
			Fermentation time						Fermentation time					
			0day		13day		30day		0day		13day		30day	
			Mean area*** ratio	S.D.	Mean area*** ratio	S.D.	Mean area*** ratio	S.D.	Mean area*** ratio	S.D.	Mean area*** ratio	S.D.	Mean area*** ratio	S.D.
106	Ethyl octadecanoate*	2456	29.00	13.34	35.83	35.06	22.56	6.36	4.69	2.46	25.54	12.85	62.00	41.86
	Aromatic hydrocarbons(5)													
14	Toluene	1037			13.31	4.80	3.81	2.19					3.00	1.83
20	Ethylbenzene	1119			2.20	1.05	1.19	0.55			1.33	0.47	1.00	0.00
23	*p*-Xylene	1132			2.61	1.34	1.50	0.71						
24	*m*-Xylene	1134			4.58	2.73			0.53	0.15	2.25	1.26	2.50	1.29
29	*o*-Xylene	1178			2.77	1.70	1.69	1.55			2.38	1.11	1.25	0.50
	Furans(5)													
8	2-Ethylfuran	940			130.75	51.17			2.30	1.29			50.00	43.42
12	2-Propylfuran	1024			9.69	3.30	1.06	0.13						
34	2-Pentylfuran	1227	2.25	1.19	26.83	9.29	5.69	2.17	4.02	3.18	6.25	5.19	6.25	1.50
56	Furfural	1466	1.88	0.63	4.68	2.55	2.50	1.78	2.24	0.52	12.58	4.40	18.50	3.00
76	Furfurylalcohol	1656	11.75	5.27	1.80	0.40	6.81	2.41	2.24	1.92	29.46	13.48	49.50	6.19
	Miscellaneous compounds (11)													
1	Octane	812			48.79	23.68	4.00	2.42			5.13	2.59	17.00	4.97
2	1,2-Dimethylcyclo-hexane*	827			4.49	2.48	9.44	10.56						
5	Nonane	891			15.83	14.53	9.06	6.04			10.88	6.84	25.00	21.40
11	Decane	1002			5.43	2.03	3.19	1.97					3.50	1.73
60	Pentadecane	1501	40.38	27.90	3.32	1.49	16.50	9.98	7.09	2.26	12.42	5.76	15.25	5.38
70	Hexadecane	1598	2.50	1.47	7.54	2.57	2.44	1.78	0.99	0.47	3.96	5.37	1.75	0.50
72	3,5-Dimethyl-1,2,4-trithiolane*	1618							0.50	0.36				
77	2,6,10,14-tetramethylpentadecane	1667	9.38	5.56	2.84	1.58	4.31	4.03	2.67	1.36	10.71	6.30	16.75	11.27
79	Heptadecane	1698	84.38	73.00	538.23	166.50	294.75	164.65	59.13	18.86	282.08	146.53	415.25	80.02
80	Heptadecene	1717					13.00	7.97						
91	Nonadecane	1899							0.46	0.25	1.83	1.45	1.00	0.00

*Compound tentatively identified by MS data only, ** I.S. : Internal standard
***Mean are ratio : Compound peak area/I.S. peak area from the average of 2 SDE extractions, and 2 injections of each extract
S.D. : Standard deviation, Control and Koji correspond to those expressed in Table 1

were detected in Control and 88 compounds in Koji. Twenty-nine aldehydes were identified in the Control and 24 in the Koji (Table 2). The amounts of the aldehydes increased with a fermentation period in Koji, while the amounts of aldehydes in Control increased the most in 13 days. Some of aldehydes, such as 3-methylbutanal, pentanal, hexanal, (E)-2-pentenal, heptanal and (E)-2-hexenal, were a large amounts in both samples during fermentation. However, certain other aldehydes, such as butanal, 2-methylbutanal, 2-methyl-(E)-2-butenal, nonanal and (E)-2-decenal, were identified only in Control. These straight-chain alkanals and alkenals might have been due to oxidation of polyunsaturated fatty acids[17,18]. Two branched aldehydes, particularly, 2-methylbutanal and 3-methylbutanal, were known to be originated from Strecker or microbiological degradation of amino acids[19]. Cha *et al.*[20] reported that anchovy paste contains a high proportion of ω-3 fatty acids (18 : 3, 20 : 5, 22 : 5, 22 : 6) and ω-6 fatty acids (18 : 2, 20 : 4) which are susceptible to lipid oxidation. In particular, C_8 and C_9 series of short-chain carbonyls and alcohols have been known to derive from ω-3 and ω-6 series fatty acids by action of lipoxygenase in fish[21~23]. Ho *et al.*[24] reported that some aldehydes which do not contribute to good flavor could act as important flavor precursors of the good aroma, such as heterocyclic compounds. Two aldehydes, benzaldehyde and phenylacetaldehyde, were identified in all of Koji, while those in Control were identified after 13 days. These compounds were known to have sweet, almond-like odor and penetrating aroma in many cooked foods[25].

Some of ketones, such as 2-nonanone, (E,E)-3,5-octadien-2-one and 2-undecanone, were detected a larger amounts than other ketones in Control compared with Koji. Generally, lower aroma threshold values of volatile ketones was known greatly to contribute to overall fresh fish-like odors[21]. These ketones may be produced by thermal oxidation/degradation of polyunsaturated fatty acids[21]. In particular, 3,5-octadien-2-one, contributing a fatty-fruity odor, may play a significant role in samples.

Among 18 alcohols detected, 14 compounds were identified in Control and 13 in Koji. The amount of alcohols detected, however, was lower than those of aldehydes in both samples. Two alcohols, 3-methyl-1-butanol and 2-phenylethanol, which are known to have suppressive effect on fishy odor[26], were detected only in Koji except 3-methyl-1-butanol in Control after 30 days. Kunimoto *et al.*[6] reported that Koji made from *Aspergillus oryzae* had a reducing effect in content of trimethylamine which do mainly contribute to fishy odor. However, alcohols may not contribute to the overall flavor of anchovy pastes because of their high threshold values[27].

Five alkylpyrazines were detected only in Koji. It is suggested that these pyrazines might be produced by adding koji. Tetramethylpyrazine was in highest concentration, followed by 2-ethyl-3,5-dimethylpyrazine, trimethylpyrazine, and 2,6-dimethylpyrazine. MacLeod and Ames[28] reported that a lot of alkylpyrazines, having nutty, roasted, and toasted aroma, were identified in soybeans, which was used as one of medium ingredients for making koji in this study. Generally, heterocyclic compounds, such as pyrazines and pyridines, were reported to be formed by Maillard and pyrolysis reaction through Strecker degradations in heat processed foods[29]. Further research is needed to determine origin of pyrazines whether come from enzymic action of *A. oryzae* or from medium of koji.

A large number of esters were identified in both samples. Esters, in particular ethylacetate, ethyldodecanoate, ethyltetradecanoate, ethylpentanoate and ethylhexadecanoate, were a high amounts in both samples during fermentation. Esters may have arose from the esterification of various alcohols and carboxylic acids that were formed from microbial decomposition of lipid and protein in fermented fish pastes. Cha and Cadwallader[9] reported that over 20 ester compounds were detected in Korean fermented fish pastes. In particular, even carbon number ethyl esters from butanoate to hexadecanoate, having a sweet odor (fruity or floral), have been found in many cheeses[30,31]. However, more research is needed to determine the sensory role of esters in fermented fish pastes in future.

Among 5 aromatic hydrocarbons detected, 5 compounds were found in Control and 4 in Koji. These compounds were slightly decreased and also detected in small amounts during fermentation in both samples.

Five furans, which are known to have burnt, sweet, bitter and coconut flavor in some foods[32], were found in both samples. Among these, 2-ethylfuran and 2-pentylfuran were the most amounts in Control, while 2-ethylfuran, furfurylalcohol and furfural were in Koji.

Alkanes were the major volatile compounds among the miscellaneous compounds. However, alkanes may not contribute to the characteristics flavor of fermented anchovy pastes because of their high threshold values. Sulfur containing compound, 3,5-dimethyl-1,2,4-trithiolane, which is known to have onion-like odor in boiled Antarctic krills[32], was only detected in Koji.

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Koji를 이용한 멸치젓 숙성중 휘발성 향기성분의 변화에 관한 연구

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요 약

*Koji*를 첨가한 저식염 멸치젓의 숙성 중 휘발성 향기성분의 변화를 대조구와 분석 비교하였다. 총 106개의 휘발성 성분이 검출되었는데 이 중에서 79개의 화합물은 표준품으로 확인 동정되었다. 이들은 주로 aldehyde, ketone, alcohol, ester, aromatic hydrocarbon, furan 및 nitrogen-containing화합물로 구성되어 있었다. 숙성 중 두시료에서 aldehyde 및 ester류의 화합물의 종류와 함량이 가장 많았다. 특히 koji를 첨가한 멸치젓에서는 대조구에서는 검출되지 않은 2,6-dimethylpyrazine, 2-ethyl-6-methylpyrazine, trimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine 및 tetramethylpyrazine이 검출되었으며 2-phenylethanol도 검출되었다.