Volatile Flavor Components of Dried Bonito (Katsuobushi) II. From Neutral Fraction

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The aqueous extract of dried bonito (Katsuobushi) was distilled under reduced pressure. The resulting distillate with diethyl ether and the extract was separated into acidic, phenolic, basic and neutral fractions. The neutral fraction was further fractionated into ten sub-fractions by silica gel column chromatography. All these sub-fractions were analyzed by gas chromatography and gas chromatography-mass spectrometry.

One hundred and sixty-five compounds were identified and 12 compounds were tentatively identified from the neutral fraction. Among them, 111 compounds were newly identified as flavor components of Katsuobushi.

In a previous paper,¹⁾ we have reported on 74 flavor components being identified from the acidic, phenolic and basic fractions obtained from the aqueous extract of dried bonito (Katsuobushi). The paper also showed that the characteristic smoky aroma of Katsuobushi was considered to be made up from a combination of various phenolic compounds found in the phenolic fraction.

The present study was conducted to identify the volatile flavor components in the neutral fraction.

MATERIALS AND METHODS

Materials. Katsuobushi, produced in the Makurazaki district of Kagoshima Prefecture in Japan, was used immediately after shaving.

Methods.

a) Collection of volatile flavor compounds. Twenty kilograms of Katsuobushi shaving were added to 300 liters of boiling water, the following procedures for collection of the volatiles being the same as described in the previous paper.¹⁾

From the resulting flavor concentrate which had the characteristic flavor of Katsuobushi, 200 ml were further concentrated to about 0.2 ml for gas chromatographic analysis, and the remaining 1800 ml were separated into the acidic, phenolic, basic, and neutral fractions by the

methods described in the previous paper.¹⁾

Each fraction was concentrated to about 0.1 ml, 0.5 ml, 0.1 ml, and 0.5 ml, respectively.

b) Fractionation and identification of the neutral fraction. The neutral fraction was further fractionated into ten sub-fractions by silica gel column chromatography. The neutral fraction concentrate was placed on a column (1 cm × 20 cm) packed with silica gel and successively eluted with mixtures of *n*-pentane and diethyl ether, as shown in Table I.

Each eluted sub-fraction was concentrated to about 0.1 ml. Identification of the flavor compounds was made by gas chromatography (GC) and gas chromatography-

TABLE I. SOLVENT SYSTEM USED FOR COLUMN CHROMATOGRAPHY AND PERCENTAGES OF EACH SUB-FRACTION IN THE NEUTRAL FRACTION

Sub-fraction No.	Solvents		% in neutral fraction
	ml	Ratios of <i>n</i> -pentane to ether (v/v)	
1	72	100:0	12
2	72	98:2	19
3	72	97:3	15
4	72	95:5	24
5	72	93:7	12
6	72	90:10	8
7	72	80:20	9
8	72	50:50	1
9	72	0:100	0
10	72	Methanol	0

mass spectrometry (GC-MS).

c) *Conditions of GC.* A Hewlett Packard Model 5730A gas chromatograph, equipped with an effluent splitter which was placed between the end of the GC column and three detectors, was used. The effluent was separated into three portions by the splitter at a splitting ratio of 1:1:2, the first portion being passed to a flame ionization detector (FID) circuit, the second to a flame thermionic detector (FTD) circuit and the third to a flame photometric detector (FPD) circuit. A glass SCOT column (0.5 mm i.d. $\times$ 50 m) coated with PEG 20M was used. Other operating conditions were: injection port and detector temperatures, 250°C; column temperature, programmed from 70° to 190°C at 2°C/min; carrier gas (N₂) flow, 1 ml/min.

d) *Sniffing evaluation of flavor compounds emitted at each peak on the gas chromatogram.* Sensory evaluation was accomplished by sniffing at the exit port of Hitachi Model 163 gas chromatograph fitted with an effluent splitter, while the samples were being eluted. The detailed conditions for sniffing were the same as described in the previous paper.¹⁾

e) *Conditions of GC-MS.* The GC-MS conditions were the same as described in the previous paper¹⁾ except that the column temperature was programmed from 60°C to 200°C at 2°C/min.

RESULTS AND DISCUSSION

The neutral fraction, amounting to about 46% of the whole flavor concentrate obtained

from the aqueous extract of Katsuobushi, had a somewhat spicy fish meaty aroma. Various compounds in the neutral fraction are thought to contribute to the fishy odor of Katsuobushi and a softening of the medicinal odors of the phenolic compounds.

Figure 1 shows a gas chromatogram of the neutral fraction obtained from the FID circuit. Because of the complexity of the chromatogram, the neutral fraction was further separated into ten sub-fractions by silica gel column chromatography (see Table I). All these sub-fractions were then analyzed by GC and GC-MS.

Table II lists the odor characteristics of these sub-fractions. Though the individual sub-fractions were inferior in odor to the original neutral fraction, the four sub-fractions from No. 2 to No. 5 were considered to be more important to the fish meaty and spicy aroma of Katsuobushi judging from their odors. However, the results of sniffing the effluents at the exit port of the gas chromatograph revealed the absence of an individual flavor compound having a characteristic Katsuobushi meat-like aroma in these four

TABLE II. ODOR CHARACTERISTICS OF SUB-FRACTIONS

Sub-fraction No.	Odor	Main compounds identified
No. 1	Petroleum-like	Hydrocarbons
No. 2	Fish meaty, somewhat dried small sardine-like	Alkanals, aromatic ketones, benzaldehyde, benzonitrile, safrole, isosafrole
No. 3	Spicy, vegetable-like, somewhat oily	Alkanones, alkenals, camphor, acetophenone
No. 4	Lasting spicy	Alkadienals, alkenols, dimethoxybenzenes
No. 5	Sweet fruity, somewhat meaty and green	1-Alken-3-ols, <i>cis</i> -1,5-octadien-3-ol, <i>cis,cis</i> -1,5,8-undecatrien-3-ol, trimethoxybenzenes
No. 6	Sweet, green	Alkanols, alkenols α -terpineol
No. 7	Sweet, somewhat green	Alkanols, alkenols, 2-cyclopentenones, γ -lactones
No. 8~No. 10	Oily	furfurylalcohol Not investigated

FIG. 1. Gas Chromatogram of the Neutral Fraction Detected with an FID.

FIG. 2. Gas Chromatogram of the Neutral Fraction Detected with an FTD.

TABLE III. VOLATILE FLAVOR COMPOUNDS IDENTIFIED FROM THE NEUTRAL FRACTION

Peak No.	Compounds	Peak No.	Compounds
	<i>Hydrocarbons</i>		
4*	Decane	78*	Borneol
8	Toluene	87'	<i>cis</i> -1,5-Undecadien-3-ol
12*	Undecane	95'	<i>cis,cis</i> -1,5,8-Undecatrien-3-ol
19	Dodecane	96'*	<i>p</i> -Cymen-8-ol
29*	Tridecane	97	Benzyl alcohol
42	Tetradecane	103	Phenethyl alcohol
51*	Indene	123*	β -Elemol
55	Pentadecane	126*	Cedrol
61	Methylindene ¹	141*	α -Cadinol
65	Methylindene ¹		<i>Aldehydes</i>
68	Hexadecane	4'	3-Methylbutanal
80	Heptadecane	5	Pentanal
83	Naphthalene	11'	Hexanal
87	δ -Cadinene	15'*	<i>trans</i> -2-Pentenal
94	Octadecane	17*	Heptanal
95*	Calamenene	18'*	<i>trans</i> -2-Hexenal
96	1-Methylnaphthalene	22	<i>cis</i> -4-Heptenal
100	2-Methylnaphthalene	28	Octanal
105	Nonadecane	38'*	Nonanal
112*	Diphenyl	44*	<i>trans</i> -2-Octenal
115	Eicosane	47*	<i>trans,cis</i> -2,4-Heptadienal
127	Heneicosane	49*	<i>trans,trans</i> -2,4-Heptadienal
142	Docosane	53'*	Decanal
153*	Tricosane	54	Benzaldehyde
160*	Tetracosane	57*	<i>trans</i> -2-Nonenal
170*	Pentacosane	63	2,4-Octadienal ¹
186*	Hexacosane	63	2,6-Nonadienal ¹
190*	Heptacosane	64	Octadienal ¹
192*	Octacosane	67'*	4-Methylbenzaldehyde
193*	Nonacosane	70*	Phenylacetaldehyde
	<i>Alcohols</i>	86*	<i>trans,cis</i> -2,4-Decadienal
4'	Ethanol	88*	4-Isopropylbenzaldehyde
6*	2-Butanol	92*	<i>trans,trans</i> -2,4-decadienal
11	2-Methylbutan-1-ol	113*	Cinnamaldehyde
14	1-Butanol	131*	Hexadecanal
15	1-Penten-3-ol		<i>Ketones</i>
18	3-Methylbutan-1-ol	8'	2,3-Pentanedione
23	1-Pentanol	16*	Cyclopentanone
29'*	<i>trans</i> -2-Penten-1-ol	16'	2-Heptanone
29'	Cyclopentanol	18'*	2-Methylcyclopentan-1-one
30	<i>cis</i> -2-Penten-3-ol	19'*	3-Methylcyclopentan-1-one
34	1-Hexanol	24*	3-Octanone
38*	3-Octanol	27	2-Octanone
41*	<i>trans</i> -2-Hexen-1-ol	28'*	Cyclohexanone
46	1-Octen-3-ol	34'*	5-Methyl-2-cyclopenten-1-one
46'*	1-Heptanol	35*	2,5-Dimethyl-2-cyclopenten-1-one
52*	<i>cis</i> -1,5-Octadien-3-ol	35'*	6-Methyl-5-hepten-2-one
53*	2-Hexanol	36*	4-Methyl-2-cyclopenten-1-one
58'*	Linalool	36'*	2,4-Dimethyl-2-cyclopenten-1-one
63*	2-Hydroxy- α -phenethylalcohol	37	2-Cyclopenten-1-one
66*	4-Heptanol	37'	2-Methyl-2-cyclopenten-1-one
67*	<i>trans</i> -2-Octen-1-ol	38'*	2-Nonanone
77*	α -Terpineol	43*	2-Cyclohexen-1-one

TABLE III (continued)

Peak No.	Compounds	Peak No.	Compounds
47'	Dimethyl-2-cyclopenten-1-one ^t		<i>Esters</i>
48*	2-Methyl-2-cyclohexen-1-one	2*	Ethyl formate
48'*	3,4-Dimethyl-2-cyclopenten-1-one	3	Ethyl acetate
49'*	2-Decanone	67'*	Methyl benzoate
51'	3-Methyl-2-cyclopenten-1-one	79*	Methyl <i>o</i> -toluate
53'*	Camphor	80'*	Methyl <i>p</i> -toluate
54'	2,3-Dimethyl-2-cyclopenten-1-one		<i>Lactones</i>
58*	2,3,4-Trimethyl-2-cyclopenten-1-one	88'*	2,3-Dimethyl-2-buten-4-olide
59*	3-Methyl-2-cyclohexen-1-one	90'*	2,3-Dimethyl-2-penten-4-olide
65'	2-Undecanone	100'*	2,3-Dimethyl-2-hexen-4-olide
71*	Acetophenone	107'*	3-Methyl-4-butanolide
74*	2-Methylacetophenone	111*	2,3-Dimethyl-2-hepten-4-olide
80'*	Propiophenone	115'*	4-Decanolide
87'*	4-Methylacetophenone	132*	2,3-Dimethyl-2-nonen-4-olide
89	2-Hydroxy-3-methyl-2-cyclopenten-1-one		<i>Furans</i>
92'*	2,4-Dimethylacetophenone	23'*	2-Methyltetrahydrofuran-3-one
93*	4-Methylpropiophenone	46'	Furfural
96'*	Geranyl acetone	53'*	2-Acetylfuran
104*	4-Isopropylacetophenone	53'*	Benzofuran
107	Methylindanone ^{t1}	54'*	Furfuryl acetate
112'*	1-Indanone	61'*	Methyl furoate
117*	2-Pentadecanone	64'*	2-Methylbenzofuran
128*	2-Hexadecanone	66	4 (or 5, or 6)-Methylbenzofuran ^{t1}
	<i>Ethers</i>	67'*	2-Acetyl-5-methylfuran
19'*	1,8-Cineol	73	Furfuryl alcohol
47	1-Methoxy-3-methylbenzene	75*	Dimethylbenzofuran ^{t1}
80'	1,2-Dimethoxybenzene	77'*	2-Ethylbenzofuran
90	1,2-Dimethoxy-4-methylbenzene	78	Dimethylbenzofuran ^{t1}
92'*	1-Methoxy-4-propylbenzene	78	Dimethylbenzofuran ^{t1}
98*	Safrole	79	Dimethylbenzofuran ^{t1}
99	1,2-Dimethoxy-4-ethylbenzene		<i>Nitrogen Compounds</i>
108	1,2-Dimethoxy-4-propylbenzene	13*	4-Methylpentanenitrile
109	1,2,3-Trimethoxybenzene	54'*	Pyrrole
117*	Isosafrole	66'*	Benzonitrile
119*	1,2-Dimethoxy-4-vinylbenzene	105'*	Phenylacetoneitrile
120*	1,2,3-Trimethoxy-5-methylbenzene	110	2-Acetylpyrrole
125*	1,2,3-Trimethoxy-5-ethylbenzene	163*	Indole
133*	1,2,3-Trimethoxy-5-propylbenzene	165*	Skatole
135*	2-Methoxynaphthalene		
142'*	1,2,3-Trimethoxy-5-(1-propenyl)benzene		
145*	1,2,3-Trimethoxy-5-vinylbenzene		

* Newly identified.

^{t1} Tentatively identified. (t₁: the position of the side chain or double bond was not clear.)

' Overlapping peaks in Fig. 1.

sub-fractions.

The sub-fractions from No. 2 to No. 5 amounted in total to about 70% of the original neutral fraction and consisted mainly of aliphatic aldehydes, aliphatic and aromatic ketones and methoxybenzenes.

The original neutral fraction was also sub-

jected to gas chromatographic analysis, using the FTD and FPD circuits, in order to identify nitrogen compounds and sulfur-containing compounds, respectively. The resulting chromatogram, using the FTD, indicates the presence of many nitrogen compounds as shown in Fig. 2. Among these, the compounds cor-

responding to peak No. 13, No. 54', No. 66, No. 105', No. 110, No. 163 and No. 165 were identified as 4-methylpentanenitrile, pyrrole, benzonitrile, phenylacetone, 2-acetylpyrrole, indole and skatole, respectively.

On the other hand, some sulfur-containing compounds could be detected on the FPD chromatogram, but these compounds could not be identified because of their trace quantities.

Table III lists the compounds identified from the neutral fraction of the whole flavor concentrate. One hundred and sixty-five compounds were identified and 12 compounds were tentatively identified, of which 111 compounds were identified from Katsuobushi for the first time.

Various volatile aliphatic aldehydes and ketones have been found in heat-processed herring, oxidized salmon oil²⁻⁴⁾ and oxidized milk fat.⁵⁾ Therefore, most of the aliphatic aldehydes and aliphatic ketones in Table II are thought to have been formed by the oxidation of lipids and fatty acids and by the degradation of amino acids during the Katsuobushi manufacturing process.

As can be seen from Table III, eleven 2-cyclopenten-1-one homologues were identified, all of which have been known as wood smoke flavor components.⁶⁻⁹⁾ Therefore, most of these compounds identified from the neutral fraction are thought to have come from the wood smoke used during the Katsuobushi smoking process.

Seven γ -lactones, having faintly sweet aromatic odors, 7 alkyl phenyl ketone compounds, having harshly sweet and floral odors, and 13 methoxybenzene derivatives, having sweet vanilla-like and woody odors, were also found in the neutral fraction. These compounds seemed to be important to the warm spicy and sweet odor of the neutral fraction.

2,3-Dimethyl-2-butenolide, 2,3-dimethyl-2-penten-4-olide, acetophenone, 4-methyl-

acetophenone, 1,2-dimethoxybenzene, 1,2-dimethoxy-4-methylbenzene and 1,2-dimethoxy-4-ethylbenzene have also been known as wood smoke flavor components,^{7,9)} and therefore, some of the γ -lactones, alkyl phenyl ketones and methoxybenzenes identified from the neutral fraction are thought to have come from the wood smoke.

On the other hand, Kim *et al.*¹⁰⁾ have reported that 1,2-dimethoxy-4-methylbenzene and 1,2-dimethoxy-4-ethylbenzene were considered to have been formed by methylation of 4-methylguaiacol and 4-ethylguaiacol during the molding process of Katsuobushi, because the amounts of 4-methylguaiacol and 4-ethylguaiacol decreased and 1,2-dimethoxy-4-methylbenzene and 1,2-dimethoxy 4-ethylbenzene increased remarkably during the molding process.

Further detailed experiments are necessary in order to confirm the microbial formation of other methoxybenzenes identified in this study, although the phenolic compounds corresponding to the precursors of several identified methoxybenzenes have already been identified from the phenolic fraction in our previous study.¹⁾

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