Volatile Flavor Components of Dried Bonito (Katsuobushi).

I. On Basic, Acidic and Weak Acidic Fractions

Izumi YAJIMA, Mikio NAKAMURA, Hidemasa SAKAKIBARA,
Tetsuya YANAI and Kazuo HAYASHI

Kawasaki Research Laboratories, T. Hasegawa Co.,
335-Kariyado, Nakahara-ku, Kawasaki 211, Japan

Received February 24, 1981

The aqueous extract of dried bonito (Katsuobushi) was distilled by using a thin film evaporator. The resulting distillate was extracted with diethyl ether, and the extract was separated into basic, acidic, weak acidic, and neutral fractions.

The basic, acidic, and weak acidic fractions were analyzed by gas chromatography and gas chromatography-mass spectrometry.

Seventy-four compounds, including 24 acids, 24 phenols, 8 pyridines, 12 pyrazines, 3 thiazoles, and 3 other compounds were identified. Thirty-six of these compounds were newly identified as volatile flavor compounds of Katsuobushi.

Katsuobushi, a traditionally used flavor enhancer of Japanese foods, is a complex of flavor compounds induced by a variety of preparation processes including boiling, sun drying, smoking and mold culturing.

Nishibori *et al.*^{1~7)} pointed out that phenolic volatiles derived from wood smoke were important to Katsuobushi flavor; 2,6-dimethoxyphenolic derivatives being especially important. Yamanishi *et al.*^{8,9)} reported that about 200 compounds were detected by gas-liquid chromatography from Katsuobushi extract and suggested that the most important flavor contributors were 1,2-dimethoxy-4-methylbenzene, *cis,cis*-1,5,8-undecatrien-3-ol and cyclotene. In a most recent paper, Hirayama *et al.*¹⁰⁾ reported that *cis*-4-heptenal contributed to the characteristic flavor of Katsuobushi.

In this manner, several valuable compounds have been identified through many studies on the volatile flavor compounds of Katsuobushi.

However, the mixing of these compounds has not been found sufficient to reproduce the true flavor and aroma of Katsuobushi, because Katsuobushi has some very delicate and complex aroma characteristics.

This investigation was undertaken to iden-

tify the additional volatile flavor components of Katsuobushi.

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.* Ten kilograms of Katsuobushi shavings were added to 150 kg of boiling water and the temperature was maintained for three minutes. After rapidly cooling and removing the residual Katsuobushi by filtering, the resulting solution was distilled under reduced pressure (28°C/10 mmHg) by using a thin film evaporator connected to a receiver and three cold traps, the first trap being cooled by dry ice-acetone and the other two by liquid nitrogen. Fourty kilograms of distillate containing the volatile flavor compounds were obtained. The distillate was then saturated with sodium chloride and extracted three times with 4 liters of redistilled diethyl ether. The ether solution was dried over anhydrous sodium sulfate and concentrated to one liter by using a Vigreux column. From this flavor concentrate, having the characteristic flavor of Katsuobushi, 100 ml were further concentrated to approximately 100 mg for gas chromatographic analysis and the remaining 900 ml were fractionated by the following method.

b) *Fractionation of flavor concentrate.* As shown in Fig. 1, the flavor concentrate was separated into basic,

FIG. 1. Fractionation of the Flavor Concentrate of Katsuobushi.

acidic, weak acidic, and neutral fractions, which were concentrated to approximately 20 mg, 15 mg, 300 mg, and 300 mg, respectively. Among them, basic, acidic, and weak acidic fractions were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS), with the exception of the acidic fraction which was analyzed after methylation.

c) *GC-conditions.* A Hitachi Model 163 gas chromatograph, equipped with an effluent splitter (1:2), which was placed between the end of the GC column and the two detectors, was used. The effluent was separated by the splitter at a splitting ratio 1 to 2, the first portion being passed to a flame ionization detector (FID) circuit and the second portion to a flame photometric detector (FPD) circuit, the latter having a high sensitivity to sulfur-containing compounds. This GC instrument was fitted with a 0.28 mm i.d. $\times$ 50 m glass SCOT column coated with PEG 20 M. The operating conditions were; injection port and detector temperatures, 250°C; column temperature, programmed from 60°C to 190°C at 3°C/min; carrier gas (N₂) flow, 1 ml/min.

d) *Sniffing evaluation of flavor compounds emitted at each peak on the gas chromatograms.* The organoleptic evaluation was accomplished by sniffing at the exit port of a Hitachi Model 163 gas chromatograph fitted with an effluent splitter, while samples were eluted. The effluent was proportioned by the splitter at a ratio of 1 to 10, the first portion being passed to an FID circuit and the second portion to the exit port which was heated to 180°C by a small heater to prevent condensation. The gas chromatograph was equipped with a 0.5 mm i.d. $\times$ 70 m glass SCOT column coated with PEG 20 M. Other operating conditions were the same as above.

e) *GC-MS conditions.* A Hitachi Model RMU-6MG mass spectrometer was used. A glass SCOT column (0.28

mm i.d. $\times$ 50 m) coated with PEG 20 M was used. The operating parameters were as follows; column temperature, programmed from 60°C to 180°C at 2°C/min; carrier gas, helium; ionizing voltage, 70 eV; accelerating voltage, 3200 V; ion source temperature, 200°C.

RESULTS AND DISCUSSION

Table I lists the approximate yield of each fraction of the flavor concentrate from Katsuobushi. The total amount of the flavor compounds was 320 mg, which was equivalent to 32 ppm of the raw materials used. It was found that the weak acidic and neutral fractions amounted to 95% of the total flavor compounds and the basic and acidic fractions were small, being only 5% in quantity.

TABLE I. YIELDS OF FLAVOR COMPOUNDS OBTAINED FROM "KATSUOBUSHI"

Fractions	wt%	
Total	320 mg (32 ppm)*	100
Basic		3
Acidic		2
Weak acidic		52
Neutral		43

* Yield was calculated using the following equation:
Yield (mg) = weight (mg) $\times$ [100 - (Solvent peak area % on gas chromatogram)/100].

Figure 2 shows the gas chromatogram of the flavor concentrate of Katsuobushi. The chromatogram indicates the presence of over 160 compounds. Of these, the basic, acidic, and weak acidic compounds were analyzed by GC and GC-MS. Each substance was identified by comparing and matching their mass spectra and GC retention times with those of authentic compounds.

1) Basic fraction

The basic fraction, though being very small in quantity, was found to be particularly interesting in having a strong roasted and fish-like aroma.

Figure 3-A shows a gas chromatogram of the basic fraction when using FID as a detector. Peak No. 62 is one of the larger quantity compounds in the basic fraction. This compound was found to have a fish-like aroma and provided one of the characteristic elements of Katsuobushi flavor sniffed at the exit port of the gas chromatograph. In order to identify this unknown compound corresponding to peak No. 62, it was separated from other compounds by using a gas chromatograph with a 3 mm i.d. $\times$ 4 m length glass column packed with PEG-20 M, and then analyzed by GC-MS and infrared spectrometry. The infrared spectrum was obtained by JASCO IRA-2, using an ultramicro capacity cell and a reflecting beam condenser. This unknown compound indicated the following mass spectra and infrared spectrum: MS m/z (%); 109 (M^+ 100), 94 (15), 79 (13), 66 (56), 51 (14), 50 (9), 39 (59), 38 (15). IR cm^{-1} ; 1585 m, 1580 s, 1480 s, 1460 m, 1425 s, 1283 s, 1270 s, 1190 m, 1127 w, 1100 w, 1055 m, 1022 s, 800 w, 790 w, 700 w. These spectra indicated that this compound was probably 3-methoxypyridine. This was further confirmed by comparing and matching the spectra and GC retention time with those of 3-methoxypyridine synthesized from 3-hydroxypyridine and diazomethane in our laboratory. This 3-methoxypyridine had previously been found in black tea¹¹⁾ and heated soy sauce,¹²⁾ but was found for the first time in Katsuobushi.

FIG. 2. Gas Chromatogram of the Flavor Concentrate of Katsuobushi.

FIG. 3-A. Gas Chromatogram of the Basic Fraction of Katsuobushi when FID is Used as a Detector.

FIG. 3-B. Gas Chromatogram of the Basic Fraction of Katsuobushi when FPD is Used as a Detector.

On the presumption that the sulfur-containing compounds in the basic fraction were important, we conducted a gas chromatographic analysis, using FPD as the detector, to isolate the existence of these compounds.

The resulting chromatogram is shown in Fig. 3-B. It indicates the presence of more than 30 compounds, of which three sulfur containing substances, namely, thiazole (peak No. 22'), 2,4-dimethylthiazole (peak No. 27'), and 2,4,5-trimethylthiazole (peak No. 38'), were identified. The thiazole, which has a nutty aroma, has been known to exist in roasted peanuts,¹³⁾ cooked beef¹⁴⁾ etc., the 2,4-dimethylthiazole, which has a green nutty aroma, has been known to exist in coffee,¹⁵⁾ black tea,¹¹⁾ cooked beef,¹⁴⁾ etc. and 2,4,5-trimethylthiazole, which also has a nutty aro-

ma, has been known to exist in coffee,¹⁵⁾ black tea,¹¹⁾ and cooked beef¹⁴⁾ etc. These substances were found for the first time in Katsuobushi. The compounds corresponding to peak No. 43' and peak No. 57' were tentatively identified by GC retention times as 2,5-dimethyl-4-ethylthiazole and 2,5-diethyl-4-methylthiazole, respectively. Likewise, peak No. 45'' (M_w 141) was assumed to be a thiazole derivative but could not be positively identified.

Table II shows the compounds identified from the basic fraction. Eight of these compounds were identified as pyrazine compounds, of which seven were found in Katsuobushi for the first time. Likewise, 12 compounds were identified as pyrazine compounds. Among them, seven were newly found in Katsuobushi,

TABLE II. VOLATILE COMPOUNDS IDENTIFIED FROM THE BASIC FRACTION

Peak No.	Compounds	Amounts
<i>Pyridines</i>		
17 ^b	Pyridine	+
21*	2-Methylpyridine	+
28*	2-Ethylpyridine	+
29*	3-Methylpyridine	+
30*	4-Methylpyridine	+
38*	3-Ethylpyridine	+
47*	3-Vinylpyridine	+
62*	3-Methoxypyridine	+++
<i>Pyrazines</i>		
23*	Pyrazine	+
24'	2-Methylpyrazine	+
34	2,5-Dimethylpyrazine	+
35	2,6-Dimethylpyrazine	++
35'	2-Ethylpyrazine	+
36'	2,3-Dimethylpyrazine	+
40	2-Ethyl-6-methylpyrazine	++
41	2-Ethyl-5-methylpyrazine	+
42	2,3,5-Trimethylpyrazine	+++
45'	2,5-Dimethyl-3-ethylpyrazine	+
47'	2,6-Dimethyl-3-ethylpyrazine	++
50'	Tetramethylpyrazine	+++
<i>Thiazoles</i>		
22'	Thiazole	+
27'	2,4-Dimethylthiazole	+
38'	2,4,5-Trimethylthiazole	+
43*	2,5-Dimethyl-4-ethylthiazole ^a	+
57'	2,5-Diethyl-4-methylthiazole ^a	+
<i>Others</i>		
106'	Quinoline	+
111'	Isoquinoline	+

* Newly identified.

^a Tentatively identified.^b In the cases of two peaks overlapping in Fig. 2 and Fig. 3-A (or Fig. 3-B), peak numbers are marked with superscript '.

+, trace component; ++, minor component; +++, major component.

though they had previously been identified in roasted food products¹⁶⁾ such as coffer, beef, sesame seeds, *etc.* All these pyridines and pyrazines are thought to be formed through Maillard reactions during the process of Katsuobushi manufacturing, especially during

FIG. 4. Gas Chromatogram of the Phenolic Fraction of Katsuobushi.

TABLE III. VOLATILE COMPOUNDS IDENTIFIED FROM THE PHENOLIC FRACTION

Peak No.	Compounds	Amounts
89	Cyclotene	+
96	Guaiacol	+++
103	2,6-Dimethylphenol	+
108	4-Methylguaiacol	+++
112	Phenol	+++
112'	<i>o</i> -Cresol	+++
115	4-Ethylguaiacol	++
121*	2,5-Dimethylphenol	+
122	<i>p</i> -Cresol	+++
123	<i>m</i> -Cresol	++
125'	4-Propylguaiacol	+
125'' ^a	Methyl isoeugenol(<i>cis</i>)	+
129*	2,3-Dimethylphenol	+
132'	Eugenol	+
132	3,5-Dimethylphenol	+
133'	2-Ethylphenol	++
134	Methyl isoeugenol(<i>trans</i>)	+
138	3,4-Dimethylphenol	++
142	Isoeugenol(<i>cis</i>)	+
145	2,6-Dimethoxyphenol	++
154'	Isoeugenol(<i>trans</i>)	+
155	2,6-Dimethoxy-4-methylphenol	++
155'*	4-Vinylphenol	+
161	2,6-Dimethoxy-4-ethylphenol	+
165	2,6-Dimethoxy-4-propylphenol	+

* Newly identified.

^a In the cases of three peaks overlapping in Fig. 2 and Fig. 4, peak numbers are marked with superscript ''.

+, trace component; ++, minor component; +++, major component.

the boiling and smoking processes.

The results of exit port sniffing revealed that most of the peaks on the chromatogram possessed a fish-like or roasted odor.

2) Weak acidic fraction

The weak acidic fraction was large in quantity and had the characteristic smoky aroma of Katsuobushi. Figure 4 shows a gas chromatogram of this fraction. In the same manner as with the analysis of the basic fraction, FPD was used to confirm the existence of sulfur-containing compounds in this fraction, but such peaks did not appear.

Table III shows the flavor compounds identified from the weak acidic fraction. Of these compounds, 2,5-dimethylphenol, 2,3-dimethylphenol and 4-vinylphenol were found in

TABLE IV. VOLATILE COMPOUNDS IDENTIFIED FROM THE ACIDIC FRACTION

Peak No.	Compounds	Amounts
M 1	Propionic acid	++
M 2	<i>iso</i> -Butyric acid	++
M 3	<i>n</i> -Butyric acid	+++
M 5	<i>iso</i> -Valeric acid	++
M 6	<i>n</i> -Valeric acid	++
M 7*	<i>iso</i> -Hexanoic acid	++
M 8*	Tiglic acid	+
M 10*	<i>n</i> -Hexanoic acid	+++
M 12*	4-Hexenoic acid	+
M 14*	<i>n</i> -Heptanoic acid	++
M 15*	Lactic acid	+
M 19	<i>n</i> -Octanoic acid	+
M 23*	Furoic acid	+
M 24*	Succinic acid	+
M 26*	Benzoic acid	+
M 30*	5-Methyl furoic acid	+
M 32*	<i>o</i> -Toluic acid	+
M 33*	<i>p</i> -Toluic acid	+
M 34*	<i>m</i> -Toluic acid	+
M 35*	Phenylacetic acid	+
M 37*	Phenylpropionic acid	+
M 39	Myristic acid	+
M 43	Palmitic acid	+
M 44*	9-Hexadecenoic acid	+

* Newly identified.

+, trace component; ++, minor component; +++, major component.

Katsuobushi for the first time. It is clear that the major portion of these compounds in Table III came from the wood smoke used during the smoking process of Katsuobushi manufacturing.

It was found when sniffing at the exit port and matching the peaks on the gas chromatogram that none of the compounds individually contributed to the smoky aroma of Katsuobushi. It was, therefore, presumed that the characteristic smoky aroma of Katsuobushi is made up by the combination of various compounds found in the weak acidic fraction.

3) Acidic fraction

This fraction was small in quantity and had a pungent acidic aroma. Figure 5-A shows a gas chromatogram of the methylated acidic fraction using diazomethane. Figure 5-B

FIG. 5-A. Gas Chromatogram of the methylated Acidic Fraction when FID is Used as a Detector.
M1 ~ M44 indicate the peak numbers of methylated acidic compounds.

FIG. 5-B. Gas Chromatogram of the Methylated Acidic Fraction when FPD is Used as a Detector.

shows a gas chromatogram, when FPD was used as the detector. It clearly shows the existence of sulfur-containing compounds, but none were able to be identified due to their being so small in quantity.

The compounds identified in this acidic fraction are shown in Table IV. Of the 23 compounds identified, 15 compounds were found in Katsuobushi for the first time.

Summarizing the above findings, from the basic, weak acidic and acidic fraction of Katsuobushi, 74 flavor compounds were identified, amongst which 36 compounds were identified in Katsuobushi for the first time. As to the neutral fraction, further research is now in progress as to their olfactory contributions.

REFERENCES

- 1) K. Nishibori, *Nippon Suisan Gakkaishi*, **31**, 41 (1965).
- 2) K. Nishibori, *Nippon Suisan Gakkaishi*, **31**, 47 (1965).
- 3) K. Nishibori and K. Okamoto, *Nippon Suisan Gakkaishi*, **37**, 156 (1971).
- 4) K. Nishibori and K. Okamoto, *Nippon Suisan Gakkaishi*, **37**, 610 (1971).
- 5) K. Nishibori and K. Okuyama, *Nippon Suisan Gakkaishi*, **38**, 231 (1972).
- 6) K. Nishibori and K. Kasahara, *Nippon Suisan Gakkaishi*, **41**, 1027 (1975).
- 7) K. Nishibori and K. Kasahara, *Nippon Suisan Gakkaishi*, **44**, 389 (1978).
- 8) T. Yamanishi, A. Kobayashi, N. Nakayama and Y. Nakasone, *Nippon Nôgeikagaku Kaishi*, **40**, 311 (1966).
- 9) K. Kim, T. Yamanishi, Y. Nakatani and T. Matsui, *Nippon Nôgeikagaku Kaishi*, **45**, 328 (1971).
- 10) K. Hirayama and K. Ishiguro, Abstracts of Papers, Annual Meeting of the Agricultural Chemical Society of Japan, Tokyo, 1976, p. 333.
- 11) O. G. Vitzthum and P. Werkhoff, *J. Agric. Food Chem.*, **23**, 999 (1975).
- 12) N. Nunomura, M. Sasaki, Y. Asao and T. Yokotsuka, *Agric. Biol. Chem.*, **42**, 2123 (1978).
- 13) J. P. Walralt, A. O. Pittet, T. E. Kinlin, R. Muralidhara and A. Sanderson, *J. Agric. Food Chem.*, **19**, 972 (1971).
- 14) R. A. Wilson, C. J. Mussinan, I. Katz and A. Sanderson, *J. Agric. Food Chem.*, **21**, 873 (1973).
- 15) O. G. Vitzthum and P. Werkhoff, *J. Food Sci.*, **39**, 1210 (1974).
- 16) J. A. Maga and C. E. Sizer, "CRC Critical Reviews in Food Technology," 1973, p. 39.