Fractionation and Identification of Volatile Compounds in Patis, a Philippine Fish Sauce

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Received May 16, 1984

Commercial Philippine fish sauce (Patis) was steam-distilled and the distillate was fractionated into four fractions, neutral, basic, acidic and phenolic and each fraction was analyzed using gas chromatography and gas chromatography-mass spectrometry.

As a result of this study, a total of 66 compounds were identified in Patis, 14 of which were only a tentative identification. Out of these 66 identified compounds, 40 identified compounds have not been reported in previous studies on fish sauces. These identified compounds include 19 acids, 14 alcohols, 12 nitrogen containing compounds, 5 esters, 3 sulfur containing compounds, 1 phenol, 3 carbonyls, 7 hydrocarbons and 2 others. In the acidic fraction, 5 acids were considered major constituents which accounted for about 98% of the total acids. *n*-Butanoic acid was found to be the most abundant accounting for about 50% of the total acids.

Fish sauce plays a major role in the utilization of fishes in Southeast Asia. It is a clear brown liquid produced from a mixture of 3 parts fish to 1~2 parts salt and allowed to ferment from six to twelve months. It is a commonly used food condiment in the diet of many of the Southeast Asian people.

It has been reported that the aroma of fish sauce is composed of a blend of ammoniacal, cheesy and meaty notes.¹⁾ A variety of amines, ammonia and other nitrogen containing compounds have been attributed to the ammoniacal note as described by Dougan and Howard and Saisithi *et al.*²⁾ Truong Van Chom³⁾ considered volatile fatty acids such as formic, acetic and *n*-butanoic acids as contributory factors in the aroma of fish sauce. Investigation of Noucmam, a Vietnamese fish sauce revealed that it contains a large amount of methyl ketones which probably accounts for the cheesy odor.⁴⁾ The meaty note is complicated but it was believed that it could be produced by atmospheric oxidation of precursors that were still present in mature sauces.¹⁾

In our previous paper,⁵⁾ it was reported that acids are partly responsible for the cheesy and

rancid odor of Philippine fish sauce (Patis). *n*-Butanoic acid was found to be the most abundant acid in fish sauce^{2,5,6)} whereas McIver *et al.*⁷⁾ reported that acetic acid is the major acid in Nampla, a Thai fish sauce.

The present study was carried out to identify additional volatile aroma compounds in fish sauce by using gas chromatography and gas chromatography-mass spectrometry.

MATERIALS AND METHODS

Commercial fish sauce (Patis) in glass bottles obtained in the Philippines in 1982 was used.

Collection of volatile aroma compounds by steam distillation. Samples were steam-distilled under reduced pressure at a temperature maintained at 45°C for about four hours. The whole steam volatile distillate obtained was extracted with ethyl ether. The extract was then dried by adding anhydrous sodium sulfate and allowed to stand overnight to ensure complete removal of water. It was concentrated to about 0.5 ml at 30°C under atmospheric pressure to give a whole steam volatile concentrate ready for analysis. For the execution and interpretation of subsequent analyses, the distillate was fractionated into four fractions, neutral, basic, acidic and phenolic fractions using the procedure explained in Fig. 1. Each of the four fractions was concentrated using the same procedure explained above.

FIG. 1. Fractionation of Whole Steam Volatile Distillate of Patis.

Gas chromatography (GC). For the separation of flavor compounds, the concentrate of each fraction was analyzed with a Hitachi 663-30 model gas chromatograph equipped with a flame ionization detector (FID). A fused silica capillary column (0.25 mm i.d. $\times$ 25 m) coated with PEG 20 M was used. The column temperature was programmed from 60°C to 170°C at a rate of 4°C/min and the injection port and detector were kept at 180°C and 230°C respectively. Nitrogen was used as carrier gas with a flow rate of about 1.0 ml/min. Split ratio was 1:40.

Gas chromatography-mass spectrometry (GC-MS). A Hewlett Packard 5790 model gas chromatograph with a fused silica capillary column (0.25 mm i.d. $\times$ 50 m) coated with carbowax 20 M was used. The column temperature was programmed from 60 to 170°C at a rate of 4°C/min, the injection port at 180°C and the detector at 230°C. The carrier gas was Helium with a flow rate of about 1.1 ml/min and a split ratio of 1:28. The mass spectrometer JEOL JMS-DX 300 model was used. Analysis was conducted using two kinds of ionization methods, the chemical ionization method (CI) and the electron ionization method (EI), the latter was done at 70 eV. Interface temperature was maintained at 200°C.

Identification of aroma compounds. Aroma compounds were tentatively identified by comparing their mass spectra with the published spectra¹⁰⁾ and further confirmed by comparing their retention times with those of the standard samples using gas chromatography.

Determination of amounts of acids. The relative amount of each acid was tentatively determined by the estimation of the peak area with an integrator Shimadzu Chromatopac C-R1a.

RESULTS AND DISCUSSION

In our previous paper,⁵⁾ sensory evaluation study showed that the whole steam volatile concentrate of Patis is composed of a blend of various aromas. In the acidic fraction, 16 volatile acids were identified and *n*-butanoic acid appeared to be the most abundant. This fraction was described as having a cheesy, rancid and disagreeable odor.⁵⁾ As a result of the quantitative analysis of acids in the acidic fraction, 5 were considered as major acids which collectively accounted for about 98% of the total acids (Table I). *n*-Butanoic acid composed about 50% of the total acids while propanoic acid accounted for 22%, isopentanoic acid 17%, and acetic and isobutanoic acids about 4 and 3% respectively. *n*-Butanoic acid is characterized to have a disagreeable odor. The minor acids, which also included the newly identified ones, benzoic acid and tentatively identified 5-hexenoic and 2,5-dihy-

TABLE I. MAJOR ACIDS IN THE ACIDIC FRACTION OF PATIS

Compounds	% of fraction ^a
Acetic	3.8
Propanoic	22.2
Isobutanoic	3.3
<i>n</i> -Butanoic	51.3
Isopentanoic	17.4

^a Percent of fraction values are computed on an area percent basis.

drobenzoic acids, each present at less than 1% of the total fraction are given in the list of all the identified compounds presented in Table II.

It was reported that the ratio of acetic acid to *n*-butanoic acid was found to be 1:1 in Noucmam, whereas for a good quality Nampla, this ratio could be as high as 20:1.⁸⁾ Truong Van Chom³⁾ also found approximately equal amounts of acetic acid and *n*-butanoic acid in Vietnamese sauce. The Chinese and Hongkong fish sauces both purchased in London, were found to have a low content of volatile fatty acids thus were less cheesy and more ammoniacal.¹⁾ Furthermore, the same author reported that the fatty acid content of the commercial Philippine fish sauce fell within the range of values obtained from the Thai sauce product. However, it is interesting to note that in this study, the ratio of acetic acid to *n*-butanoic acid was 1:17 which is in contrast to the findings of Nguyen-an Cu and Vialard-Goudou.⁸⁾ These results might suggest that there is a characteristic difference in fish sauces considering the kinds of fishes used and the methods of production.

Although *n*-butanoic acid appeared to be the most predominant volatile compound in Patis, as shown in Fig. 2, its impact on the overall aroma of fish sauce is not well pronounced. This might suggest that other volatile compounds present in the neutral, basic and phenolic fractions have a great influence on the formation of the total aroma.

In our previous study, it was reported that

the neutral fraction has a sweet, somewhat burnt and rice bran like smell.⁵⁾ Compounds identified in this fraction which are listed in Table II contained a number of alcohols, almost all of which appeared in low concentrations except, 2,2-dimethyl-1, 3-propanediol, benzyl alcohol and 2-phenyl ethanol, which appeared in a fairly large amount. The latter has a rose like floral aroma. Methyl ketone, considered by Van Veen to be responsible for the cheesy odor of fish sauce,⁴⁾ was found in this sauce. Phenol, also found in the phenolic fraction showed a high concentration. Other compounds identified were nitrogen containing compounds which are mostly composed of pyrazines and pyridines, compounds known to possess respectively a burnt and disagreeable odor, and they appeared at low concentrations. Ide *et al.*⁹⁾ also identified these compounds in their study on Thai sauce. Among the sulfur containing compounds identified, 1-ethenyl-methyl disulfide was found to appear at a high concentration but there might be a possibility that other compounds with the same retention time could have been contaminated in this peak. A few esters and carbonyls were among those identified as minor components. The presence of hydrocarbons were also observed although the identification was only tentative using gas chromatography-mass spectrometry. We could not identify aldehydes in this sample although other authors reported its presence in other fish sauces.^{6,7)} Dougan and Howard and Saisithi *et al.*^{1,2)} explained that aldehydes formed in the samples would have been consumed in the enzymatic browning.

The GC profile obtained in the analysis of the basic fraction indicated the presence of nitrogen containing compounds including trimethylamine which were confirmed using GC-MS. This fraction was characterized to have a burnt and acetamide like smell.⁵⁾ The phenolic fraction, which was reported to have a rice bran smell,⁵⁾ revealed the presence of phenol but no other phenolic compounds could be detected. This finding might support our previous report that the phenolic fraction has the

TABLE II. AROMA COMPOUNDS IDENTIFIED IN PATIS

Peak No.	Compounds	Fraction ^f	References ^r	Methods of identification
<i>Acids</i>				
37	Acetic	A	a,b,c,d,e,f	GC-MS, t_R
44	Propanoic	A	a,c,d,e,f,h	GC-MS, t_R
47	Isobutanoic	A	a,c,d,e,f	GC-MS, t_R
58	<i>n</i> -Butanoic	A	a,b,c,d,e,f,h	GC-MS, t_R
64	Isopentanoic	A	a,c,d,e,f,h	GC-MS, t_R
69	<i>n</i> -Pentanoic	A	a,f	GC-MS, t_R
70-a*	5-Hexenoic	A	**	GC-MS
70	Isohexanoic	A	a,f	GC-MS, t_R
76	<i>n</i> -Hexanoic	A	a,e,f	GC-MS, t_R
88	Isoheptanoic	A	a	GC-MS, t_R
90	<i>n</i> -Heptanoic	A	a	GC-MS, t_R
94	Isooctanoic	A	a	GC-MS, t_R
96	Isononanoic	A	a	GC-MS, t_R
97	<i>n</i> -Octanoic	A	a	GC-MS, t_R
99	<i>n</i> -Nonanoic	A	a	GC-MS, t_R
100	Isodecanoic	A	a	GC-MS, t_R
101	<i>n</i> -Decanoic	A	a	GC-MS, t_R
106*	2,5-Dihydrobenzoic	A	**	GC-MS
107	Benzoic	A	**	GC-MS, t_R
<i>Alcohols</i>				
2	Ethanol	N	e,f	GC-MS, t_R
3	Propanol	N	e	GC-MS, t_R
9	Butanol	N	e,f	GC-MS, t_R
18*	2-Methyl-2-Pentanol	N	**	GC-MS
21	Isopentanol	N	e,h	GC-MS, t_R
38	Heptanol	N	**	GC-MS, t_R
40	2-Octanol	N	**	GC-MS, t_R
43	1-Octanol	N	**	GC-MS, t_R
52	Nonanol	N	**	GC-MS, t_R
54	Decanol	N	**	GC-MS, t_R
59	Furfuryl alcohol	N	**	GC-MS, t_R
78	2,2-Dimethyl-1,3-propanediol	N	**	GC-MS, t_R
82	Benzyl alcohol	N	**	GC-MS, t_R
85	2-Phenyl ethanol	N	**	GC-MS, t_R
<i>Nitrogen containing</i>				
19	Pyridine	B,N	**	GC-MS, t_R
24	Pyrazole	B,N	**	GC-MS, t_R
26	2-Ethyl pyridine	B,N	**	GC-MS, t_R
27	2,5-Dimethyl pyrazine	B,N	h	GC-MS, t_R
28	2,6-Dimethyl pyrazine	B,N	f	GC-MS, t_R
31	Trimethyl pyrazine	B,N	**	GC-MS, t_R
32	Trimethylamine	B	d,e,f	GC-MS, t_R
33	2,3,5-Trimethyl pyrazine	B,N	h	GC-MS, t_R
34	2,5-Diethyl pyrazine	B,N	**	GC-MS, t_R
73*	3- <i>t</i> -Butylmercapto pyridine	B,N	**	GC-MS
104*	<i>n</i> -Formyl-5-methoxy-2-indolinol	B,N	**	GC-MS
105	Indole	B,N	f	GC-MS, t_R
<i>Esters</i>				
1	Ethyl acetate	N	**	GC-MS, t_R
4	Ethyl propionate	N	**	GC-MS, t_R
5	Isopropyl propionate	N	**	GC-MS, t_R
10	Ethyl <i>n</i> -butyrate	N	**	GC-MS, t_R
42	Octyl <i>n</i> -butyrate	N	**	GC-MS, t_R

TABLE II. (continued)

Peak No.	Compounds	Fraction ^f	References ^r	Methods of identification
<i>Sulfur containing</i>				
8	Dimethyl disulfide	B,N	**	GC-MS, t_R
72	1-Ethenyl methyl disulfide	B,N	**	GC-MS, t_R
103*	2-Methyl 4- <i>t</i> -butylthiophenol	B,N	**	GC-MS
<i>Phenol</i>				
91	Phenol	P,N	**	GC-MS, t_R
<i>Carbonyls</i>				
1-b	Methyl ethyl ketone	N	g	GC-MS, t_R
20	Cyclopentanone	N	**	GC-MS, t_R
22	2,4-Dimethyl pentanone	N	**	GC-MS, t_R
<i>Hydrocarbons</i>				
17	<i>m</i> -Dimethyl benzene	N	**	GC-MS, t_R
23*	<i>cis</i> -2-Pentene	N	**	GC-MS
61*	1,1,3-Trimethyl cyclopentane	N	**	GC-MS
75*	2,6,10-Dimethyl octane	N	**	GC-MS
79*	2,6,10-Trimethyl tridecane	N	**	GC-MS
92*	2,6,10-Trimethyl hexadecane	N	**	GC-MS
93*	2,6,10-Trimethyl pentadecane	N	**	GC-MS
<i>Others</i>				
25*	Dioxane	N	**	GC-MS
60*	1,1-Bis-1-ethoxy carbonyl cyclopropane	N	**	GC-MS

* Tentative identification.

** Not previously reported.

 t_R retention time.^f A, acidic fraction; B, basic fraction; N, neutral fraction; P, phenolic fraction.^r a, Sanceda *et al.* (1983); b, Truong Van Chom (1957); c, Dougan and Howard (1975); d, Beddows *et al.* (1979); e, Nonaka *et al.* (1975); f, McIver *et al.* (1982); g, Van Veen (1941); h, Ide *et al.* (1982).

FIG. 2. Gas Chromatogram of the Whole Steam Volatile Distillate of Patis.

lowest yield among the fractions analyzed.⁵⁾

In summary, 66 compounds were identified in Patis, 14 of which were only a tentative identification. Out of these 66 compounds

identified, 40 compounds have not been reported in previous studies. These identified compounds include 19 acids, 14 alcohols, 12 nitrogen containing compounds, 5 esters, 3

sulfur containing compounds, 1 phenol, 3 carbonyls, 7 hydrocarbons and 2 others.

So far no conclusive evidence on the mechanism of the formation of volatile compounds has been reported. For further clarification, some studies on a small scale fish sauce preparation is being conducted. It is hoped that good information can be obtained from this model experiment and results will be reported in a succeeding paper.

Acknowledgment. The authors wish to thank Dr. K. Hayashi of the Kawasaki Research Laboratories, T. Hasegawa Co. for his valuable discussion on this research.

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