Studies on the Volatile Fatty Acids and Volatile Bases in "Shiokara"—I

Volatile Fatty Acids and Volatile Bases in Shiokara from Commercial Source.

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Shiokara is a marine food-stuff, made by allowing cuttle fish muscle and viscera to ferment in the presence of a considerable amount of common salt. It tastes salty and slightly bitter, involving favorable flavors. In any food-stuff good taste and flavor are very important factors. From this view point, the majority of the present work has been accelerated on the clarification of the taste of Shiokara. It was found that the increase of free-amino acid was responsible for the good taste, and the tasty components were produced in the early stages of ripening before the product became palatable.¹⁻³⁾ Hence, it may be that the ripening of Shiokara is caused not only by the increase of amino acid, but also by changes of some other chemical and physical characters in the raw material.

This study was planned to clarify the nature of the acidic and basic fraction of flavor in Shiokara from commercial sources.

Experimental

Materials: Ika (squid)- and Katsuo (skipjack)-Shiokara were obtained from commercial sources. The chemical composition of both Shiokara used here was shown in Table 1. Authentic samples: Various volatile fatty acids from C₁ to C₆ and the volatile bases such as, trimethylamine, ammonia, dimethylamine, isobutylamine and *n*-butylamine were obtained from Wako Co., Japan.

Determination of the volatile fatty acids. *Preparation of sample:* Three hundred grams of Shiokara was subjected to steam distillation and 1 liter of distillate was caught in 0.1N-sodium hydroxide solution. To this was added dil. hydrochloric acid in order to bring its pH to 7.0, it was then evaporated to dryness

Table 1. Chemical composition of Ika- and Katsuo-Shiokara. (%)

Composition	Ika-Shiokara	Katsuo-Shiokara
Moisture	63.0	62.8
Crude protein	12.3	12.9
Crude fat	5.1	3.2
Crude ash	19.0	20.7
Crude fiber	0.0	0.0
Nitrogen free extract	0.7	0.3

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at 50°C under the reduced pressure by rotary evaporator.

To the residue, a small amount of 1N-hydrochloric acid was added, fatty acids being extracted with ether. The slight moisture in the ether-extract was eliminated with a small amount of dried sodium sulfate. The ether-solution was submitted to gas-chromatography directly.

Gas-chromatography for the volatile fatty acids: Many reports have been presented on gas-chromatographic analyses of volatile fatty acids from various sources.⁴⁻⁸⁾ Analyzing the fatty acids without esterification has been common recently, so such a procedure was adopted in this study. A liquid phase consisting of DEGS-Phosphate mixture was applied for the direct analysis. A Shimadzu Model GC-IB gas-chromatographic unit, with a thermal conductivity detector installed, was used. The detector cell was operated at 200°C. The U-shaped column of 4 mm inside-diameter and 225 cm long was packed with DEGS containing 5 parts of phosphate. The temperature of column and preheater were maintained at 130°C and 250°C, respectively. The flow rate of carrier-gas (helium) was adjusted to 4 ml per minute. The sample mentioned above was applied directly, and the relative retention time was compared with that of authentic samples.

Determination of the volatile bases. *Preparation of the sample:* Three hundred grams of Shiokara was subjected to steam distillation and 1 liter of distillate was caught with 0.1N-hydrochloric acid solution. To this was added dil. sodium hydroxide in order to bring its pH to 7.0, it was evaporated to dryness at 50°C under reduced pressure. To the residue, a small amount of 1N-sodium hydroxide was added, and the volatile bases were extracted with ether. The slight moisture in the ether-extract was eliminated with a small amount of dried sodium sulfate. The ether-solution was submitted to gas-chromatography directly.

Gas-chromatography for the volatile bases: The above mentioned gas-chromatographic unit was used. The U-shaped column of 4 mm inside-diameter and 225 cm long was packed with triethanolamine (25 parts) on Shimalite (75 parts). The temperatures of the column and preheater were maintained at 90°C and 220°C, respectively.

Results and Discussion

Volatile fatty acids. The results of gas-chromatography of Ika- and Katsuo-Shiokara were shown in Fig. 1, Fig. 2 and Table 2. Six peaks were detected by gas-chromatography in Ika-Shiokara, which were identified as formic acid, acetic acid, propionic acid, iso-butyric acid, *n*-butyric acid and *n*-caproic acid, respectively. In Katsuo-Shiokara, ten peaks were detected by gas-chromatography and the peaks 1, 2, 3, 6, 7 and 8 were identical with the above mentioned six components of Ika-Shiokara in the relative retention time, respectively. Peak 5 was fitted for iso-valeric acid, but peak 4, 9 and 10 were left un-identified.

It was reported that the tasty components of Shiokara were usually produced by

Table 2. Value of tR and the ratio of volatile fatty acids in Shiokara.

Katsuo-Shiokara			Ika-Shiokara			Authentic fatty acids	
Peak No.	tR	Ratio (%)	Peak No.	tR	Ratio (%)	Name	tR
1	0.81	2	1	0.81	1	Formic	0.83
2	1.00	30	2	1.00	64	Acetic	1.00
3	1.28	8	3	1.28	9	Propionic	1.20
4	1.40	6		—		Unknown	
5	1.60	6		—		iso-Valeric	1.63
6	1.77	24	4	1.77	18	iso-Butyric	1.76
7	2.20	6	5	2.20	3	<i>n</i> -Butyric	2.17
8	3.30	5	6	3.30	4	<i>n</i> -Caproic	3.34
9	5.03	6		—			
10	6.24	7		—

Fig. 1. Gas-chromatogram of the volatile fatty acids in Ika-Shiokara. **Fig. 2.** Gas-chromatogram of the volatile fatty acids in Ika-Shiokara.

enzymic reactions in the early stages of ripening,⁹⁻¹¹⁾ whereas the production of flavor was referred to bacterial activity at the later stages. These facts seem to reveal that the volatile fatty acids are derived from amino acids by the following reaction.

Although these volatile acids might be attributed to the carbohydrate added in the process of the production of Shiokara, from the result of general analyses of both Shiokara as shown in Table 1, it may not be conceivable that these acids originated from the constituents of the muscle or viscera itself.

Among these acids, the main were such acids as acetic acid, propionic acid, iso-

butyric acid; the sum of them amounted to 92 and 62 per cent of total volatile acids in Ika- and Katsuo-Shiokara, respectively. The other acids could be found only in small amounts. It is noteworthy that the chief ingredient is acetic acid in the both Shiokara. When both Shiokara were compared, no difference could be found in the acids, except the differences in the ratio of acids between them. That is, in Katsuo-Shiokara, the content of acetic acid was nearly equal to that of iso-butyric acid. The differences of ratio among the acids were less than those of Ika-Shiokara.

Volatile bases. The results of gas-chromatography of Ika- and Katsuo-Shiokara were shown in Fig. 3, Fig. 4 and Table 3.

Table 3. Value of tR and the ratio of volatile bases in Shiokara

Katsuo-Shiokara			Ika-Shiokara			Authentic volatile bases	
Peak No.	tR	Ratio(%)	Peak No.	tR	Ratio(%)	Name	tR
1	1.00	4	1	1.00	3	Trimethylamine	1.00
2	1.30	58	2	1.30	55	Ammonia	1.30
3	1.75	3	3	1.75	2	Dimethylamine	1.79
4	2.00	3	4	2.00	2	Unknown	
5	2.43	1	5	2.43	4	Monomethylamine	2.42
6	3.16	1	6	3.16	13	Unknown	
7	4.24	16	7	4.24	15	iso-Butylamine	4.24
8	4.90	9	8	4.90	2	Unknown	
9	6.75	1	9	6.75	2	<i>n</i> -Butylamine	6.75
10	7.67	2	10	7.67	9	Unknown

Fig. 3. Gas-chromatogram of the volatile bases in Ika-Shiokara.

Fig. 4. Gas-chromatogram of the volatile bases in Katsuo-Shiokara.

In both Shiokara, ten peaks were detected in gas-chromatogram, and by their relative retention times six of them were found to be identical with trimethylamine, ammonia, monomethylamine, dimethylamine, iso-butylamine and *n*-butylamine, res-

pectively. However, the other four peaks (4, 6, 8 and 10) were left un-identified. Among these volatile bases, ammonia, iso-butylamine and an unknown substance (peak 8) were predominant and the others were found in relatively small amounts. In case of basic components, no marked difference could be observed between the two types of Shiokara. The sum of ammonia, iso-butylamine and an unknown (peak 8) amounted approximately to 80 per cent of the total volatile bases.

It was perceived that in the case of fish muscle only a small amount of ammonia was produced in the course of autolysis, and the ammonia was probably generated from purine bases.¹²⁾ However, it was reported that a considerable amount of ammonia was produced from the pyloric coeca through autolysis.¹³⁻¹⁴⁾ Accordingly, the ammonia in Shiokara may not attributed only to the deamination product of amino acids by microorganisms.

At all events, it seems that these components affect the quality of Shiokara considerably. Volatile fatty acids and bases were nearly equal in amount and in composition in the both Shiokara, excepting the slight difference in volatile fatty acids. Moreover, as these acidic and basic volatile substances usually generate disagreeable flavor, it is quite reasonable that the palatable flavor of Shiokara should be supposed to be due to the neutral components such as carbonyl, phenol, alcohol and terpene etc.

Summary

Some investigations of the volatile fatty acids and the volatile bases in Shiokara from commercial source were carried out by gas-chromatographic analysis.

In the acidic fraction of Ika-Shiokara, six sorts of volatile fatty acids were found. They were identical to formic acid, acetic acid, propionic acid, iso-butyric acid, *n*-butyric acid and *n*-caproic acid. Ten sorts of base were contained in the basic fraction, six of them were identified as trimethylamine, ammonia, dimethylamine, monomethylamine, iso-butylamine and *n*-butylamine, however, the other components were left un-identified.

In Katsuo-Shiokara, the same components were found except iso-valeric acid and small amount of three unknown substances. In both Shiokara the chief acidic and basic ingredients were acetic acid, iso-butyric acid, propionic acid, ammonia, iso-butylamine and an another un-identified substance.

References

- 1) S. NAGASAKI and T. YAMAMOTO: This Bull., **20**, 613 (1954).
- 2) S. NAGASAKI and T. YAMAMOTO: *ibid.*, **20**, 617 (1954).
- 3) T. MORI, Y. HASHIMOTO, Y. KOMATA and S. EGUCHI: *ibid.*, **23**, 37 (1957).
- 4) C. L. HANKINSON, W. J. HARPER and E. MIKOLAJCIK: *J. Dairy. Sci.*, **41**, 1502 (1958).
- 5) E. S. ERWIN, G. J. MARCO and E. N. EMERY: *ibid.*, **44**, 1768 (1961).
- 6) I. R. HUNTER, N. G. HAWKINS and J. W. PENCE: *J. Food. Sci.*, **26**, 578 (1961).

- 7) H. SUGISAWA, J. S. MATTEWS and D. R. MACGREGOR: *ibid.*, **27**, 435 (1962).
- 8) R. G. ACKMAN and R. G. BURGHER: *Anal. Chem.*, **35**, 647 (1963).
- 9) K. SHIMADA and R. BABA: *This Bull.*, **1**, 227 (1933).
- 10) K. NAGAO and T. KIMURA: *Bull. Fac. Fish., Hokkaido Univ.*, **1**, 81 (1950).
- 11) Z. OOSHIRO and N. KOREEDA: *Mem. Fac. Fish., Kagoshima Univ.*, **8**, 56 (1960).
- 12) T. OYA and E. SUMI: *Journ. Imp. Fisher. Institute.*, **20**, 20 (1926).
- 13) K. KASHIWADA: *This Bull.*, **21**, 496 (1955).
- 14) K. KASHIWADA: *ibid.*, **21**, 498 (1955).