Shoyu (Soy Sauce) Volatile Flavor Components: Basic Fraction†

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Received June 29, 1978

The volatile basic fraction concentrates were prepared from heated, and raw *shoyu*, separately. Each concentrate was successively analyzed by combined gas chromatography-mass spectrometry. In the above two concentrates, 35 components were identified, 27 of which have not been reported previously for their presence in *shoyu*. The identified compounds were 24 pyrazines, 5 pyridines, 2 oxazoles and 4 other compounds. Furthermore, the basic compounds of both heated and raw *shoyu* were quantitatively analyzed by GC, and the concentrations and odor units of major pyrazines which contained simple alkyl groups as the side chain were also determined. Consequently, the quantity of the basic compounds was found to increase during pasteurization; especially the total quantity of the above major pyrazines increased remarkably. Therefore, it is concluded that these confirmed pyrazines which have very low odor threshold values play an important role in the so-called "heated" flavor which is brought about by pasteurization of raw *shoyu* in the course of production. In addition, organoleptically, the basic compounds are considered to be indispensable to the *shoyu* flavor.

High-quality Japanese fermented *shoyu* is mostly made from almost equal amounts of soybeans and wheat. The manufacturing process roughly consists of cooking of raw materials, koji making, mash fermentation and pasteurization of liquid part of mash. It is well known that many flavor ingredients of *shoyu* are produced during the process.

In previous studies,^{1,2)} a total flavor concentrate of Japanese *shoyu* was analyzed by combined gas chromatography-mass spectrometry and 51 compounds were identified. A tautomeric compound that contributes greatly to *shoyu* flavor has been isolated from *shoyu* and identified as 4-hydroxy-2-ethyl-5-methyl-3(2H)-furanone or 4-hydroxy-5-ethyl-2-methyl-3(2H)-furanone.

During the last few years, a great number of basic compounds such as pyrazines have been isolated and identified as flavor components of various heated foods, and these compounds have been found to contribute greatly to the quality of cooked or roasted foods. Since *shoyu* is also made by several kinds of heating process such as cooking, roasting and pasteurization, a large number of basic compounds might be produced and contribute to a part of *shoyu* flavor. However, as heterocyclic nitrogen-containing compounds only 2-acetylpyrrole,³⁾ tetramethylpyrazine⁴⁾ and five other pyrazines¹⁾ have been found in *shoyu* flavor. On the other hand, a considerable number of pyrazines and other heterocyclic nitrogenous compounds have been proved to occur in coffee aroma.^{5~13)}

In this experiment, the basic fraction of *shoyu* flavor was separated from total volatiles and successively subjected to combined gas chromatography-mass spectrometry. Effects of pasteurization on major pyrazines were also examined.

† Studies on Flavor Components in *Shoyu*. Part IV. See references, 1, 2) and *Nippon Nôgeikagaku Kaishi*, 51, 709 (1977).

The main part of this work was presented at the Annual Meeting of the Agricultural Chemical Society of Japan, Kyoto, April 4, 1976.

MATERIALS AND METHODS

Shoyu. Commercially available regular heated *shoyu* (pH 4.8) and fermented raw *shoyu* (pH 4.8) were used for the following experiments.

Preparation of volatile basic fraction concentrates. Concentrate A was obtained from 9 liters of heated *shoyu* by the method shown in Fig. 1. A glass flash-vacuum-evaporator was utilized for distillation under reduced pressure and the distillate was condensed in an ice-NaCl trap and a dry ice-ethanol one. Another concentrate (B) was similarly prepared from 24 liters of raw *shoyu*. The all operations were carried out at a sample temperature below 40°C. Both concentrates had a fragrant and burnt odor.

FIG. 1. Scheme of Preparation of Volatile Basic Fraction Concentrate.

Combined gas chromatography-mass spectrometry (GC-MS). Combined GC-MS analyses were made on a Hitachi Model 063-RMU-7M gas chromatograph-mass spectrometer. An all-glass jet separator was utilized for the GC-MS interface which was heated at 250°C. Chromatographic separations were performed with a 0.28 mm *i.d.* $\times$ 30 m glass capillary SCOT column coated with PEG 20M. The other conditions were presented in Fig. 2. The basic fraction concentrates (A and B) were analyzed and the gas chromatogram of concentrate A obtained from heated *shoyu* is shown in Fig. 2.

Quantitative analyses of pyrazines. Each 500 ml of heated and raw *shoyu* was treated by the same method shown in Fig. 1. Distillations of *shoyu* in the amount of 50 ml were carried out 10 times for one sample by

FIG. 2. Gas Chromatogram (Capillary Column) of Basic Fraction Concentrate (A) Obtained from Heated *Shoyu*.

Instrument: Hitachi Model 063-RMU-7M (GC-MS) Column: PEG 20M, 0.28 mm *i.d.* $\times$ 30 m, glass, SCOT. Oven temp.: 50~160°C (2°C/min). Carrier gas: He, 0.1 kg/cm². Ionization electron energy: 70 eV. Ion source temp.: 240°C. Detector: total ion monitor, 10%.

FIG. 3. Apparatus for Distillation under Reduced Pressure of *Shoyu*.

using a test tube concentrator, TAIYO Model TC-8 manufactured by Taiyo Kagaku Kogyo Co., Ltd. The apparatus is shown in Fig. 3. The obtained basic concentrates were quantitatively analyzed by the gas chromatographic method (Fig. 4). Rough concentrations of major pyrazines were calculated on the basis of the calibration curve (a straight line) of 2,5-dimethylpyrazine by the internal standard method.

RESULTS AND DISCUSSION

Identification

Thirty-five components were identified in the basic concentrates (A and B), 27 of which have not been reported previously as constituents of *shoyu*. All the components were identified by the comparison of their mass

spectra and GC retention times with those of authentic samples. The confirmed compounds are listed in Table I. Most of the identified compounds are pyrazine derivatives. Pyridines and cyclopentapyrazines were found as

new groups of compound in *shoyu*.

The number of the compounds found in heated *shoyu* is greater than that in raw *shoyu*. This is probably because their contents in raw *shoyu* were too small to be detected.

TABLE I. IDENTIFIED COMPONENTS IN BASIC FRACTION

Compounds	Samples ^a		Methods for identification	Ref. No.
	Heated	Raw		
1,1-Diethoxyethane(acetal)*	+		MS, GC	
Pyridine*, ^b	+	+	MS, GC	
3-Methylpyridine*		+	MS, GC	
2-Ethylpyridine*	+		MS, GC	
2,6-Dimethylpyridine*	+	+	MS, GC	
3-Methoxypyridine(tent.) ^c *	+		MS	(19)
Pyrazine*	+	+	MS, GC	
2-Methylpyrazine	+	+	MS, GC	
2,5-Dimethylpyrazine*	+	+	MS, GC	
2,6-Dimethylpyrazine	+	+	MS, GC	
2-Ethylpyrazine*	+		MS, GC	
2,3-Dimethylpyrazine	+	+	MS, GC	
2-Ethyl-6-methylpyrazine	+	+	MS, GC	
2-Ethyl-5-methylpyrazine*	+	+	MS, GC	
2,3,5-Trimethylpyrazine*	+	+	MS, GC	
2,3-Diethylpyrazine*	+		MS	(8)
3-Ethyl-2,5-dimethylpyrazine	+	+	MS, GC	
2,6-Diethylpyrazine*	+	+	MS	(5)
Tetramethylpyrazine	+	+	MS, GC	
2,6-Diethyl-3-methylpyrazine*	+	+	MS	(5)
2-Ethyl-3,5,6-trimethylpyrazine(tent.)*	+		MS	(8)
6,7-Dihydro-5H-cyclopenta[b]pyrazine*	+	+	MS	(17)
2-Methyl-6,7-dihydro-5H-cyclopenta[b]pyrazine*	+		MS	(17)
3-Methylthio-1-propanol(methionol)	+	+	MS, GC	
Benzoxazole(tent.)*	+		MS, GC	
2-Methylbenzoxazole(tent.)*	+		MS, GC	
2-Ethyl-6,7-dihydro-5H-cyclopenta[b]pyrazine*	+		MS	(17)
2,3,5-Trimethyl-6,7-dihydro-5H-cyclopenta[b]pyrazine*	+		MS	(17)
Ethyl nicotinate*	+	+	MS, GC	
2-Acetylpyrrole	+	+	MS, GC	
5H-cyclopenta[b]pyrazine(tent.)*	+		MS	(18)
2(or 3)-Methyl-5H-cyclopenta[b]pyrazine(tent.)*	+		MS	(18)
6-Methyl-5H-cyclopenta[b]pyrazine*	+		MS	(18)
7-Methyl-5H-cyclopenta[b]pyrazine*	+		MS	(18)
2(or 3),6(or 7)-Dimethyl-5H-cyclopenta[b]pyrazine(tent.)*	+		MS	(18)

^a +: present.

^b The compounds marked with asterisks were newly found in *shoyu*.

^c (tent.) means tentative identification.

Concentration of major pyrazines

Major pyrazines in heated and raw *shoyu* were semiquantitatively analyzed. Their concentrations and odor units are shown in Table II.

FIG. 4. Gas Chromatograms of Basic Fraction Concentrates Obtained from Heated and Raw *Shoyu*.

Instrument: Shimadzu 4BM-PF (FID).

Column: polyester FF 10%+terephthalic acid 3%, 3 mm i.d. $\times$ 2 m, glass. Oven temp.: 50~180°C (3°C/min). Injector and detector temp.: 240°C. Carrier gas: N₂, 30 ml/min. Internal standard: *m*-tert.-butylphenol (*m*-TBP).

In raw *shoyu* the concentration of ethyl-methylpyrazine was the highest; in heated one dimethylpyrazine and ethyl-methylpyrazine existed at significantly high concentrations.

For reference, their odor threshold values in water and calculated odor units are additionally presented. Ethyl-methylpyrazine can be said to affect extremely *shoyu* flavor among the identified pyrazine compounds in view of their odor units.

Effects of pasteurization

The ratio of "heated" to "raw" in Table II indicates an increase in the concentration of each pyrazine during pasteurization. It was found that the quantities of monomethyl- and dimethylpyrazines increased markedly.

Then, the total quantity of volatile basic compounds in heated *shoyu* was 1.5 times as large as that in "raw" on the basis of the quantitative GC analyses. The amount of the four major pyrazines in total in "heated" was 2.5 times as large as that in "raw"; the total quantity of the other basic compounds in "heated" was 1.3 times larger than that in "raw".

From the above results, it is recognized that not only the four major pyrazines but also the other basic compounds increase during pasteurization. The increase in the amount of these pyrazines is larger than that of the other basic compounds.

Additionally, in raw *shoyu* the total quantity of the four major pyrazines accounted for 17.0% of the volatile basic fraction concentrate; in "heated" that accounted for 28.9%.

TABLE II. CONCENTRATIONS AND ODOR UNITS OF MAJOR PYRAZINES

	Peak No. ^a	Concentrations (mg/liter)		Odor threshold ^c (ppm, in water)	Odor units		Ratio of concentrations Heated/Raw
2-Methylpyrazine	1	0.075	0.024 ^b	60 ¹⁸⁾ , ^d	0.0012	0.0004	3.1
Dimethylpyrazine	2	0.746	0.184	1.0 ²⁰⁾	0.746	0.184	4.1
Ethyl-methylpyrazine	3	0.746	0.388	0.1 ¹⁸⁾	7.46	3.88	1.9
Trimethylpyrazine	4	0.050	0.040	9 ²¹⁾	0.0056	0.0045	1.3

^a Peak No. in Fig. 4.

^b Italics mean the concentrations or odor units in raw *shoyu*.

^c Published data. The data of 2,5-dimethylpyrazine and 2-ethyl-5-methylpyrazine represented those of dimethyl- and ethyl-methylpyrazines respectively.

^d ¹⁸, ²⁰, ²¹ denote ref. Nos.

Formation of pyrazines in shoyu

Many experiments on thermal reactions regarding amino acids, proteins and sugars have been reported, and it is known that pyrazines are formed in the Maillard reaction and the Strecker degradation, although the latter is not always necessary.¹⁴⁾ In *shoyu*, pyrazine derivatives are considered to occur during the three kinds of process; heat treatment of raw materials, aging of mash, or "moromi," and pasteurization of the liquid part of mash. In the processing of raw materials, soybeans are autoclaved and wheat is roasted. The derivatives are also formed chemically and biochemically during the long period of the aging of mash. Pasteurization of raw *shoyu* is generally equivalent to heating at 80°C for 5 hr. During the pasteurization, the quantity of pyrazines increased as described above.

The formation of the alkylated five-membered alicyclic pyrazines in coffee aroma was explained as the result of the interaction between cyclic 1,2-diketones, glyoxals, and amino acids by Vitzthum and Werkhoff.¹³⁾ The proposal suggests that cyclic 1,2-diketones may exist in *shoyu* as the precursors in the formation of cyclopentapyrazines, but those diketones have not been found in *shoyu* yet.

The contribution of basic compounds to shoyu flavor

Raw *shoyu* is generally heated at 70 to 80°C in order to prevent a large number of microbial and enzymatic reactions. The pasteurization brings about the so-called "heated" flavor. Yokotsuka and Takimoto¹⁵⁾ reported that acidic compounds including organic acids and phenols constituted an important part of the "heated" flavor, and that the other "heated" flavor components were aldehydes, mercaptans, and their interreaction compounds.

Considering the facts that the concentration of pyrazines increases during pasteurization and that the pyrazine compounds have very low odor threshold values, the authors recognize that the confirmed pyrazines also play an important role in the "heated" flavor.

The study on pyrazines in potato chip aroma by Guadagni *et al.*¹⁶⁾ points out the synergistic effect of pyrazines since the best product picked out by experienced panellists had the greatest number of pyrazine compounds, but not the largest pyrazine concentration. Considering that 24 pyrazines were found in *shoyu*, the number of them as well as the concentration probably has a significant effect on *shoyu* flavor.

Organoleptically, the flavor concentrate from which the basic compounds are removed has a heavy note to some extent. From this fact, it may be concluded that the basic compounds play a role of reducing a heavy note of *shoyu* aroma rather than participating in the essential qualities of *shoyu* flavor.

The basic compounds including pyrazines are recently considered seriously as the essential components in the aroma of heated foods such as coffee,^{6~13)} cocoa,²²⁾ black tea.²³⁾ From the above organoleptical quality and the fact that many pyrazines have been isolated here from heated *shoyu*, the basic compounds in *shoyu* can be judged to be indispensable to the *shoyu* flavor.

Acknowledgement. The authors wish to thank Miss K. Harigaya for her technical assistance.

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