Studies on Lipids of Natto

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The lipid contents and compositions of three kinds of natto, Itohiki-, Yukiwari-, and Hama-natto, were investigated. The lipid contents of the finished products of Itohiki-, Yukiwari-, and Hama-matto were 2.8, 10.9, and 6.4%, respectively. The lipid composition was determined by high-speed liquid chromatographic analysis. The pattern of the lipids of Itohiki-natto was found to be similar to that of soybean. Yukiwari-natto contained 5 to 18% free fatty acids and Hama-natto contained 78% free fatty acids in total lipids. Gas chromatographic patterns of fatty acid composition of Itohiki- and Hama-natto were similar to those of raw soybean, and that of Yukiwari-natto was observed to contain lauric and myristic acids in addition.

Natto is a typical and popular soybean food in Japanese diets. Holding its original soybean shape, it is mainly eaten along with boiled rice and seldom used as an ingredient of soup unlike miso, which is another typical and traditional soybean fermented food. To make natto, soybean is cooked and on its surface is grown *Aspergillus oryzae* or *Bacillus natto*, different tastes and flavors being produced depending on which microorganisms are used.

Traditionally, three kinds of natto are produced in Japan and each has its own manufacturing method. Itohiki-natto is a soybean food produced with *Bacillus natto*, a strain of *Bacillus subtilis*. When fermented by *Bacillus natto*, the surface of soybean is covered with characteristic viscous substances consisting of a polymer of glutamic acids. Yukiwari-natto is made by mixing Itohiki-natto with rice koji and salt, aged at 25–30°C for 15 days. Hama-natto is made by using soybean koji. Soybean is cooked and on its surface is grown koji mold (*Aspergillus oryzae*), which is used as an enzymatic source for fermentation.

According to Kon (1969) many free amino acids were observed in Hama-natto and the pattern was similar to that of soybean miso which was known to have a tasteful amino acid flavor. Asano and Saito (1954) isolated two strains of *A. oryzae* from Hama-natto with strong activities hydrolyzing protein and starch. According to Kon and Itoh (1974), bacteria such as *Micrococcus* sp., *Streptococcus* sp., and *Pediococcus* sp. are widely distributed on the surface and on the inner part of Hama-natto. Itoh et al. (1976) have recently detected amines in Hama-natto, which was also employed in this study. It was concluded from these data that various kinds of flavors were produced by koji enzymes as well as microorganisms during fermentation of Hama-natto.

Characteristic to Hama-natto is its very unique harsh taste, which seems to be correlated to free fatty acids (Matsumoto, 1965). Thus, lipid hydrolysis has been assumed to play an important role in the production of the distinct taste and flavor of Hama-natto.

On the other hand, miso, another very popular Japanese soybean food, is in a paste form. It is generally classified into three major groups, depending on raw materials out of which miso is made: rice-miso made out of rice, soybean, and salt; barley-miso, out of barley, soybean, and salt; and soybean-miso, out of only soybean and salt. Miso is mostly used as an ingredient for making miso-soup and is sometimes used as the base of a sweet pasty miso by adding sugar, which is then used to dress meat, fish, shellfish, vegetables, and fruits. Soybean-miso, in particular, has some similarities in terms of raw materials and manufacturing method with that of Hama-natto. For soybean-miso, also, the harsh taste is existent which is regarded as tasteful and essential.

Kiuchi et al. (1975a, 1976) had already investigated the lipid composition of rice-miso and soybean-miso and found that these miso varieties contained free fatty acids that differed in composition. They also found that during the fermentation of soybean-miso the hydrolysis of lipids began at the stage of making koji with cooked and molded soybeans.

Hardly any substantial studies have been made of the lipids of natto to obtain conclusive evidence. The authors investigated the analytical conditions of the whole lipids in soybean and soybean food by high-speed liquid chromatography (HSLC) (Kiuchi et al., 1975b) discussed in the previous paper.

The present paper describes the results of an analytical survey of the three kinds of natto by applying the above-mentioned analytical conditions by HSLC (Kiuchi et al., 1975b).

MATERIALS AND METHODS

Natto. Itohiki-natto was manufactured from the following varieties of soybeans: Tokachi-nagaha, Hokkaido; Yukiwari-natto, obtained from a market in the Tohoku area; and Hama-natto, collected twice in Hamana Co. Ltd., Hamamatsu, Shizuoka prefecture.

Chemical Analyses. The dry matter and ash contents were analyzed according to the analytical methods for miso (Institute of Miso Technologists, 1968).

The lipid content was obtained as follows (Kiuchi et al., 1975a). The extracts of high-speed chromatographic analyses were concentrated and the solvent was removed at 48°C under reduced pressure. Then the sample was dried in a desiccator in vacuo until the weight became constant.

HSLC. Crushed soybean (1 g) or 2 g of other samples was treated according to the previously described method and washed in pure water to a final dilution of exactly 10 ml.

HSLC was carried out according to the method described previously (Kiuchi et al., 1975b). The ratio of the contents of the lipids was estimated by cutting and weighing the piece of paper corresponding to the area of peaks on the Xerox charts.

Gas chromatography employed in the study was described in the previous paper (Kiuchi et al., 1975a).

RESULTS

Figure 1 shows an outline of the flow sheet of manufacturing methods of three kinds of natto. Itohiki-natto was fermented by inoculating *Bacillus natto*. Yukiwari-natto was made by mixing Itohiki-natto with rice

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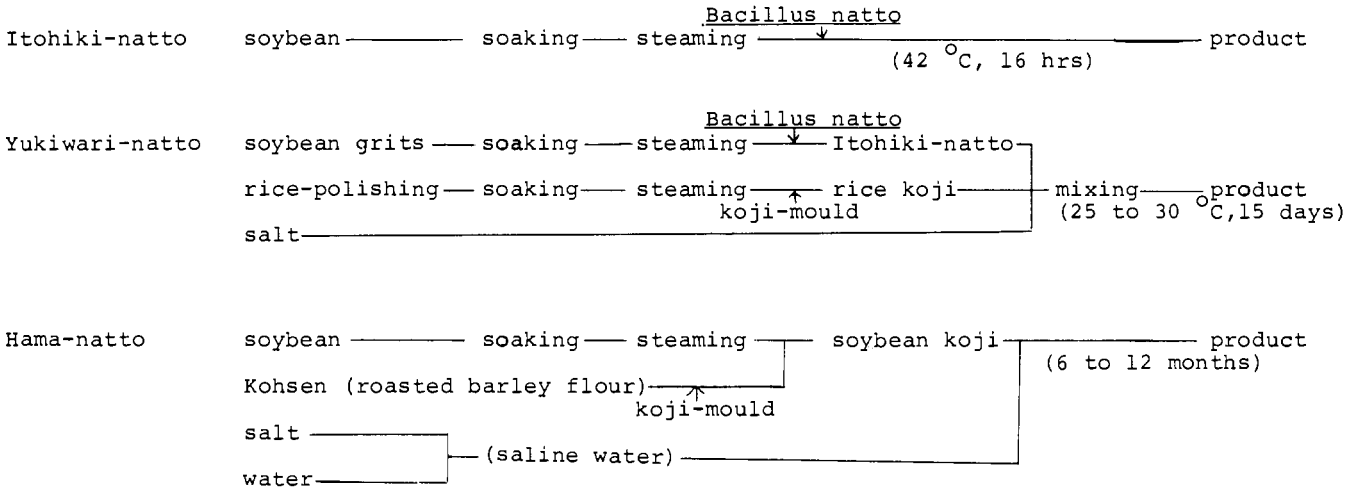

Figure 1. Manufacturing methods of natto.

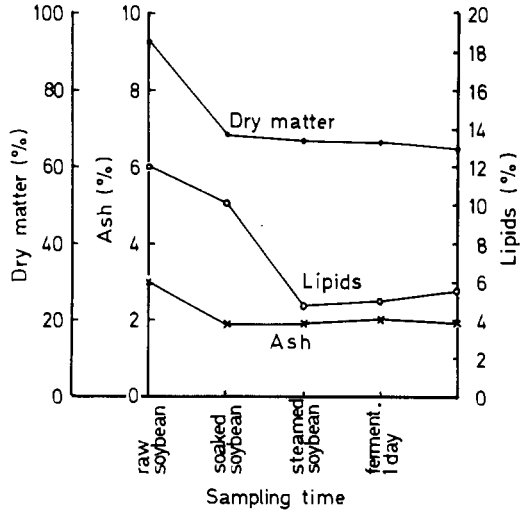

Figure 2. Contents of dry matter, lipids, and ash of Itohiki-natto.

koji (or cooked rice, on the surface of which is grown koji-mold, *Aspergillus oryzae*, which is used as an enzymatic source of fermentation) and salt. Hama-natto was manufactured using koji-mold. In the process of koji making, soybean-koji was mixed by hand (conditioning by hand: "Te-ire" in Japanese (Asai et al. (1960)), three times to keep conditions even). Then the finished soybean koji was soaked in saline water. Fermentation periods of each natto were as follows: 16 hr at 42°C for Itohiki-natto and 6 to 12 months in the case of Hama-natto. Many kinds of microorganisms were counted in Hama-natto during fermentation (Itoh et al., 1976).

Figure 2 and Figure 3 show the dry matter, ash, and lipid contents of Itohiki- and Hama-natto, respectively. The dry matter and lipid contents of Itohiki-natto decreased in the process of soaking and steaming and increased during fermentation (Figure 2). As shown in Figure 3, in Hama-natto's case, the dry matter and lipid content decreased after soaking and increased during koji making. Ash content increased during koji making and the fermentation process in saline water.

Table I shows the analytical data for Yukiwari-natto. Figure 4 shows a standard chromatogram of authentic substances by HSLC. The total lipids were eluted in the order triglycerides, diglycerides, cholesterol or β-sitosterol, fatty acids, and monoglycerides.

Figure 5 shows the chromatogram of the lipids of Itohiki-natto. A large peak of triglycerides and a small

Table I. Chemical Composition of Yukiwari-natto[a]

	%
Moisture	47.9
Dry matter	52.1
Crude protein	14.3
Crude fat	6.4
Ash	10.7
(Salt	9.3)
Sugar	20.6
(Reduced sugar	15.0)

[a] For this chemical analysis sample 2 in Table III was used.

Table II. Lipid Compositions of Itohiki-natto

	Lipid						
Sample	TG	DG	Un-1	Un-2	St	FFA	MG
Raw soybean	94.8	0.4	0.4	1.1	1.9	1.5	0.0
Soaked soybean	92.6	Tr	0.3	1.9	1.6	3.5	0.0
Steamed soybean	97.6	0.3	0.0	0.3	Tr	1.7	0.0
Fermentation 1 day	97.4	0.7	0.7	0.7	Tr	0.7	0.0
Fermentation 3 day	95.6	0.6	0.6	0.6	0.6	1.9	0.0
Fermentation 7 day	96.0	0.1	2.2	2.2	0.3	0.3	0.0
Fermentation 30 day	99.2	0.3	Tr	Tr	0.3	Tr	0.0

Table III. Lipid Compositions of Yukiwari-natto

Sample	TG	DG	St	FFA	MG	PL
1	78.6	2.0	0.0	17.8	0.5	1.1
2	96.3	0.0	0.0	5.7	0.0	0.0

peak of diglycerides were observed. The peak of fatty acids was scarcely observed. The pattern of the chromatogram was not changed after 7 days of storage at 30°C. From this evidence it was concluded that the fermentation had no effect on soybean lipids. As indicated in Table II, the content of free fatty acids was less than 3.5% of total lipids. Figure 6 shows the chromatogram of the lipids of Yukiwari-natto. A small peak of free fatty acid was observed in addition to the peak of triglycerides as shown in Table III. Free fatty acid occupied 5–18% of total lipids.

Figure 7 shows the changes in lipid composition during the fermentation of Hama-natto. The lipid of the original soybean consisted mainly of triglycerides and the peak of fatty acids was scarcely observable. The peak of the fatty acids was observed after inoculation of koji-mold and

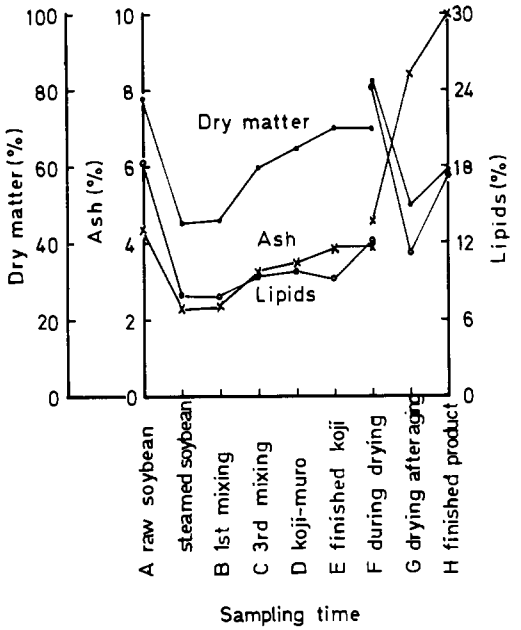

Figure 3. Contents of dry matter, ash, and lipids of Hama-natto. The letters A to H relate to the GC graphs in Figure 7.

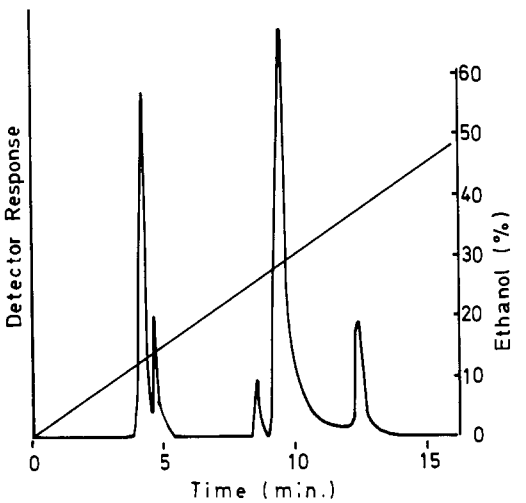

Figure 4. High-speed separation of lipids. Gradient slope shows concentration of ethanol in hexane-chloroform (2:1): (1) tristearin; (2) distearin; (3) cholesterol; (4) stearic acid; (5) monopalmitin.

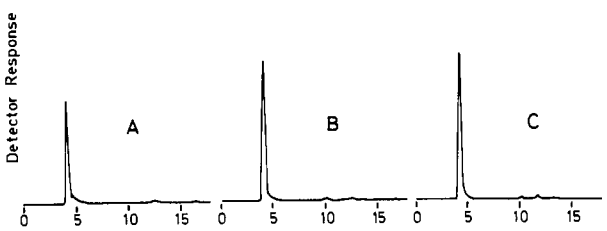

Figure 5. High-speed separation of lipids of Itohiki-natto: (A) soybean; (B) Itohiki-natto (1st day); (C) Itohiki-natto (7th day).

increased markedly between the first conditioning and the third conditioning of koji.

Table IV shows the ratio of lipid composition during the fermentation. Though some deviations were found among the samples, the fatty acid fraction was 13.9% of total lipids after the first mixing and increased up to 47 to 60% after the third conditioning. Hydrolysis of lipids was scarcely made during the fermentation and the content of

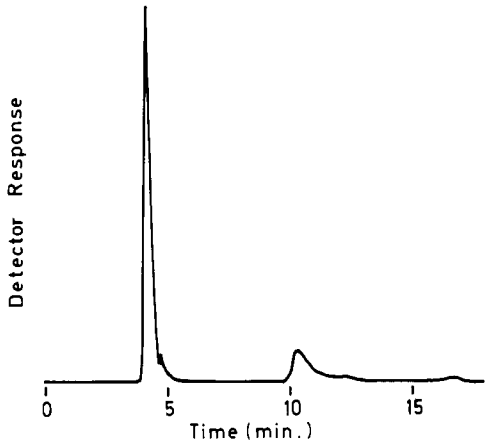

Figure 6. High-speed separation of lipids of Yukiwari-natto.

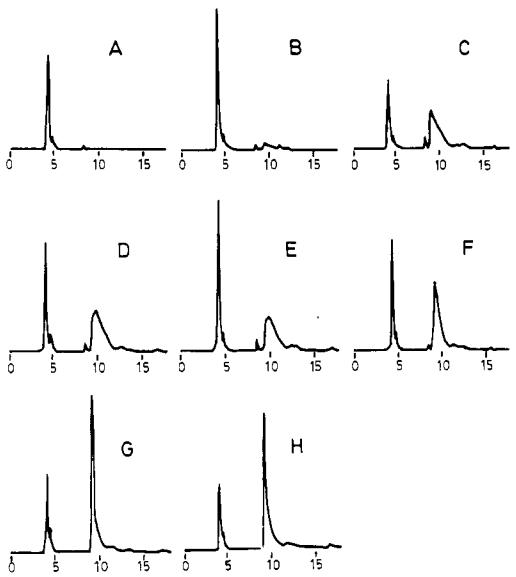

Figure 7. High-speed separation of lipids of Hama-natto. The abscissas show time in minutes and the ordinates show detector response at 1.28×10^{-10} A, full scale division: (A) raw soybean; (B) 1st mixing; (C) 3rd mixing; (D) koji-muro; (E) finished koji; (F) during drying; (G) drying after aging; (H) finished product.

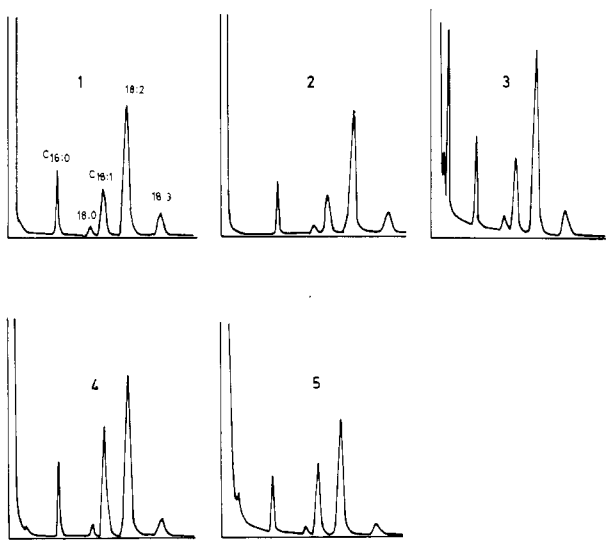

Figure 8. Gas chromatograms of lipids of three kinds of natto: (1) Itohiki-natto; (2) Itohiki-natto, 7 day; (3) Yukiwari-natto product; (4) Hama-natto raw soybean; (5) Hama-natto product.

Table IV. Lipid Compositions of Hama-natto[a]

Sample		Lipid						
		TG	DG	St	FFA	MG	Un	PL
Expt 1								
Raw soybean	A	96.4	2.0	1.0	Tr	Tr	0.7	Tr
Steamed soybean		97.5	0.4	0.2	Tr	Tr	1.8	Tr
1st mixing	B	81.1	0.6	1.8	14.1	1.2	0.0	0.8
3rd mixing	C	30.2	0.7	4.1	60.9	0.3	2.3	1.4
Koji-muro	D	32.0	1.1	1.1	62.1	0.9	1.7	1.1
Finished koji	E	32.6	1.7	2.3	60.6	0.8	1.4	0.8
During drying	F	45.7	1.7	2.6	47.1	1.0	1.2	0.6
Expt 2								
Raw soybean		99.0	0.3	Tr	Tr	Tr	0.8	0.3
During drying		16.5	1.8	0.4	77.5	Tr	2.0	1.8
Drying after aging	G	27.5	1.7	Tr	69.2	1.2	0.4	0.9
Finished product	H	25.1	0.7	Tr	70.8	1.8	0.5	1.1

[a] The letters A to H indicate the samples shown in Figure 7.

fatty acid remained 69 to 78% of total lipids.

Figure 8 shows the results of GLC of the three kinds of natto. No differences were observed between the pattern of fatty acids of soybean and products of Itohiki-natto and Hama-natto. The gas chromatographic peaks of lauric and myristic acids were observed in Yukiwari-natto. Perhaps they originated from rice-koji (Yasumatsu and Moritaka, 1964; Fujino and Mano, 1972).

DISCUSSION

Muto (1929) found out qualitatively that *Bacillus natto* produced lipase. However, as shown in Figure 5 and Table II, the change of soybean lipids of Itohiki-natto was scarcely observed during fermentation. Taira et al. (1964) reported that amino acids of soybean were liberated during fermentation. Shibasaki et al. (1968) observed that the protein body in natto and miso was hard to fix dye for protein and suggested that the protein was hydrolyzed during fermentation. Furthermore, according to the electron microscopic observation of Saio and Watanabe (1968), protein in a protein body of soaked soybean cotyledon was hardened as it was, and was not diffused, but the protein was dotted in the cotyledon cell of natto after fermentation for 20 hr. These facts seemed to prove that some enzymes invaded the cell to disintegrate the protein bodies. However, if lipase was produced by *B. natto*, oil in the soybean cells would be hydrolyzed and the content of free fatty acids would be increased. From the data in Figure 5 and in Table II, it can be seen that the oil did not change, indicating that *B. natto* did not produce lipase.

Since Yukiwari-natto and Hama-natto were produced with koji-mold, the lipase must result from koji-mold. Since the aging period of Yukiwari-natto was shorter than that of Hama-natto (Figure 1), the ratio of free fatty acids in total was much less than that of Hama-natto. In Hama-natto the hyphae of koji-mold or its lipase would invade into the cotyledon of a soybean.

Calculated from the data of Table IV and Figure 3, the free fatty acid content of Hama-natto amounted to 12% of the wet weight of Hama-natto. Therefore, the harsh taste caused by the free fatty acids should have been very strong. However, by the sweet taste from sugars which not only existed originally in soybean but also appeared from the degradation of the cell walls and the hulls of soybean by koji enzymes during the aging process, by amino acids produced by degradation of the protein, and so on, the harsh taste would have been made mild.

Because the harsh taste in soybean miso caused by free fatty acids has been appreciated as tasteful, free fatty acids liberated from soybean in Hama-natto and Yukiwari-natto also would play an important role in the flavor. This is so to a greater extent in the case of Hama-natto.

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