Fermented Vegetable (Soybean) Protein and Related Foods of Japan and China

D. FUKUSHIMA, Kikkoman Foods, Inc., PO Box 69, Walworth, WI USA

ABSTRACT

Main fermented vegetable protein foods in Japan and China are soy sauce (shoyu in Japan, chiang-yu in China), fermented soy paste (miso in Japan, chiang in China), sufu, and natto, which are all traditional foods. Chiang, which originated in China some 2,500 years ago, was introduced into Japan during the seventh century and transformed into the present Japanese shoyu and miso, which are now quite different from their Chinese counterparts. Their fermentations consist of koji fermentation by *Aspergillus* species and the subsequent brine fermentation which contains lactic acid and alcoholic fermentations. The characteristic appetizing aroma observed in Japanese style of soy sauce (shoyu) is derived through a special brine fermentation from the component of the wheat which constitutes about one-half of the materials. During the recent two decades, the fermentation technology and engineering on shoyu and miso have made great progress in Japan. Sufu (Chinese soybean cheese) is a cheese-like product originating in China in the fifth century. It is made through the fermentation by Mucor or a related mold from soybean protein curd called "tofu," which is made by coagulating soy milk. This product is widely manufactured in China on a small scale, but it is not made and consumed in Japan. On the other hand, natto is the fermented soybean protein food characteristic in Japan. It is a whole soybean product fermented by *Bacillus* species, and was originated in Northeastern Japan about 1,000 years ago. Natto is served as it is with shoyu and mustard. Besides these traditional fermented foods, a new fermented soybean product appeared on the market recently in Japan. It is a soy milk drink fermented by lactic acid bacteria. Recently, fermented soy sauce is penetrating into Western populations widely, particularly in the United States. The possibility for the universalization of traditional food flavors is discussed.

INTRODUCTION

The representative fermented vegetable protein foods of Japan and China are soy sauce, fermented soy paste, Chinese soybean cheese, and fermented whole soybeans. Recently, several reviews have been written in English on these products (1-8).

The predecessor of soy sauce (called shoyu in Japan and chiang-yu in China) and fermented soy paste (called miso in Japan and chiang in China) is "chiang," originating in China some 2,500 years ago. This chiang was introduced into Japan in the sixth century by Buddhist priests, along with the introduction of Buddhism, in which the eating of meat and fish is prohibited. Unlike China, where meat continued to be eaten, the Japanese adhered strictly to their Buddhist vegetarian diet. Therefore, this new seasoning from soybeans gradually took the place of the salted seasoning prepared from meat and fish which was used before this. This chiang, which was called "hishio" in Japan, has been transformed over the centuries into two unique distinctive foods, "shoyu" and "miso," which are now quite different from their Chinese counterparts. This is the reason why the present soy sauce and fermented soy pastes between the two countries become quite different in types.

Chinese soybean cheese called "sufu" also originated in China in ancient times. It is manufactured and consumed only in China. On the other hand, fermented whole soybeans called "natto" originated in Japan in ancient times. It is manufactured and consumed only in Japan.

During the last two decades, the fermentation technology and engineering of these traditional foods have made great progress, particularly for soy sauce and miso in Japan. In addition, new fermented protein products have been developed recently, and some of them have appeared in Japanese markets today. In the present paper, the manufacturing techniques and their recent progress on these products are described in detail.

SOY SAUCE: FERMENTED SOY SAUCE: JAPANESE AND CHINESE STYLES OF SOY SAUCE AND THEIR CHARACTERISTICS

In Japan five types of fermented soy sauce are recognized by the Japanese government at present, as shown in Table I. The total amounts of soybeans and defatted soybeans consumed for these soy sauces are shown in Table II. Table III shows the typical compositions of these five types of soy sauce.

The "koikuchi-shoyu" represents the largest production of Japanese soy sauce. The forerunner of this type of soy sauce had been created in the 17th century and has been improved into the present product. It is an all purpose seasoning, characterized by a strong aroma, myriad flavor, and deep red-brownish color. The second type of soy sauce is "usukuchi-shoyu," characterized by a lighter brown color and milder flavor. It is used mainly for cooking when one wishes to preserve the original flavor and color of the foodstuff itself. The other three types of soy sauce are produced and consumed only in isolated localities for special uses in Japan. On the other hand, however, the soy sauce produced in China traditionally is a "tamari-shoyu" style which forms only 2.2% of the total production of fermented soy sauce in Japan. This Chinese style of soy sauce is charac-

TABLE I

Fermented Soy Sauces (Shoyu) in Japan

Type of shoyu	Annual Pdn kiloliters	% of total
Tamari	27,000	2.2
Koikuchi	1,050,000	85.4
Usukuchi	144,000	11.1
Shiro	5,000	0.4
Saishikomi	4,000	0.3

TABLE II

Consumption of Soybeans and Defatted Soybeans in Japan, 1976

Soybean products			Whole soybean (ton)	Defatted soybean meal (ton)	Total (ton)		
For Foods	Fermented	Shoyu (soy sauce)	10,000	165,000	175,000		
		Miso (fermented soypaste)	190,500	5,000	195,500	439,500	
		Natto (fermented whole soybean)	69,000	0	69,000		1,026,000
	Nonfermented	Tofu and aburage (Soy milk curd and its derivatives)	411,500	55,000	466,500		
		Kori-tofu (Soy milk curd and its derivatives)	29,000	0	29,000	586,500	
		Others	16,000	75,000	91,000		
For feeds			30,000	1,950,000			1,980,000
Total			756,000	2,250,000			3,006,000

TABLE III

Typical Compositions of Soy Sauces Recognized by the Japanese Government

Soy sauce	Bé	NaCl (g/100ml)	Total nitrogen (g/100ml)	Formol nitrogen (g/100ml)	Reducing sugar (g/100ml)	Alcohol (Vol/100ml)	pH	Color	Annual production (%)
Koikuchi shoyu	22.5	17.6	1.55	0.88	3.8	2.2	4.7	Deep brown	85.4
Usukuchi shoyu	22.8	19.2	1.17	0.70	5.5	0.6	4.8	Light brown	11.7
Tamari shoyu	29.9	19.0	2.55	1.05	5.3	0.1	4.8	Dark brown	2.2
Saishikomi shoyu	26.9	18.6	2.39	1.11	7.5	Trace	4.8	Dark brown	0.3
Shiro shoyu	26.9	19.0	0.50	0.24	20.2	Trace	4.6	Yellow to tan	0.4

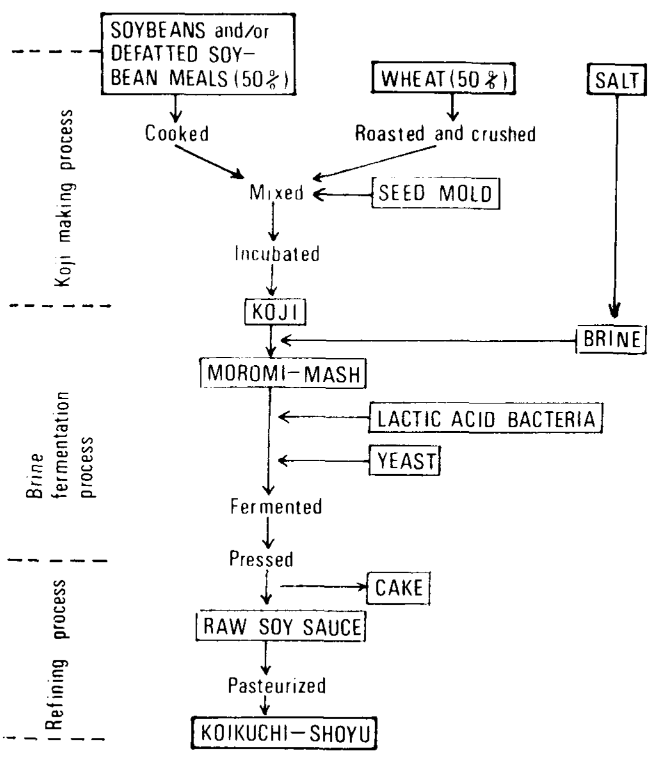

FIG. 1. Manufacturing process of "koikuchi-shoyu," the representative Japanese types of soy sauce.

terized by a deep dark color, thick taste, and poor aroma. The difference in aromas observed between Japanese and Chinese styles of soy sauce are ascribed to the different ratios of wheat and soybeans used and to the fact that Japanese style of soy sauce has a pasteurized process for raw soy sauce as one of the indispensable processes, whereas Chinese style of soy sauce does not have such a pasteurized process in principle. As seen in Figures 1 and 2, the Japanese style of soy sauce uses much more wheat, and its characteristic aroma compounds are produced mainly from the wheat constituents through the koji fermentation, alcoholic fermentation, and other special fermentations. Further, an additional, subtle, pleasant aroma is produced through the pasteurization process, as explained later.

MANUFACTURING PROCESS

The manufacturing process of fermented soy sauce consists of three major processes: the koji-making process; brine fermentation process; and refining process as shown in Figure 2 and 3. The koji-making process is a unique process for manufacturing of fermented foods. This process is a technology characteristic of the Orient which has been used since olden times for the production of soy sauce, miso, sake (a rice wine), shochu (spirits), and yonezu (a rice vinegar), etc. Koji is a source of enzymes for converting the carbohydrates and proteins, etc., contained in the materials into sugars, peptides, and amino acids, etc. The nutrients which were produced through the action of these enzymes on the materials become available for the yeasts and bacteria which grow in the moromi mash during the subsequent brine fermentation process. Actual koji manufacturing of koikuchi type of soy sauce is carried out as follows. Soybeans, or more commonly defatted soybean flakes, are moistened and cooked under pressure. The cooking was done in a batch type cooker previously, but recently it is done in a continuous cooker which allows a high pressure, short cooking time for soybeans. On the other hand, the wheat is roasted by continuous roasting and then cracked into four to five pieces. The equal amounts of the resultant soybeans or defatted soybeans and wheat are mixed and then inoculated with a pure culture of *Aspergillus oryzae* or *sojae* which is called "seed mold." In the traditional way of making koji, the resultant inoculated mixture was put into small wooden trays and put into a koji room. During the development of the koji, the temperature, moisture, and aeration were controlled by manual

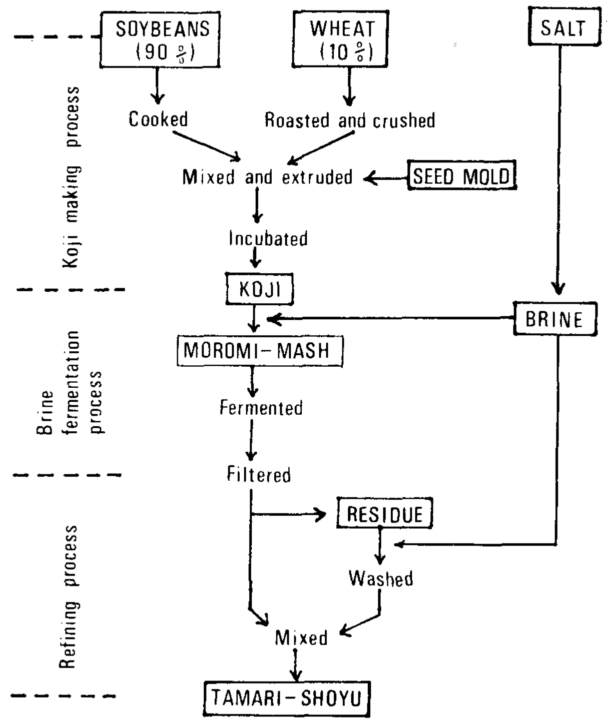

FIG. 2. Manufacturing process of "tamari-shoyu."

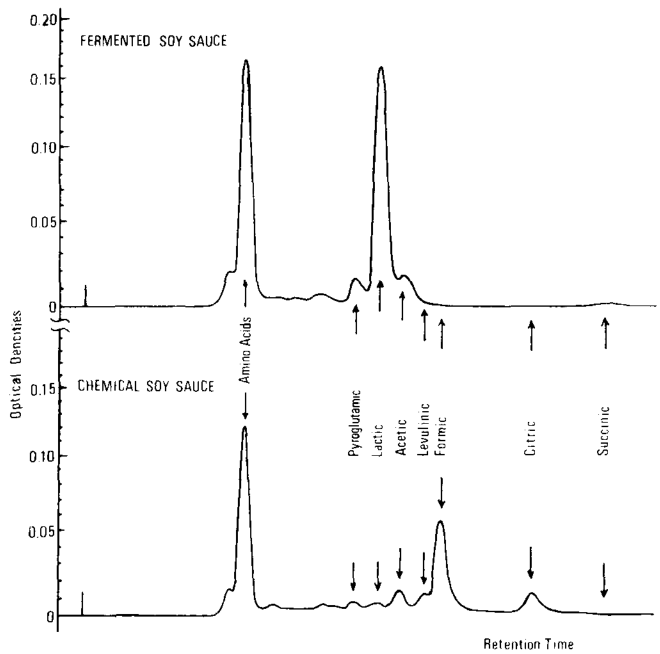

FIG. 3. Chromatograms of organic acids on fermented and chemical soy sauce. These two samples analyzed are the representative soy sauce manufactured in the U.S.A.

operations consisting of stirring the mixture several times. In recent years, however, automatic koji-making processes have been developed to replace this traditional way in Japan. The new equipment includes a continuous cooker, automatic inoculator, automatic mixer, large perforated shallow vats in closed chambers equipped with forced air devices, temperature controls, and mechanical devices for turning the substrates during incubation. The inoculated mixture is put into this shallow perforated vat, and the forced air is circulated through the mass. After two or three days, koji is harvested a green-yellow as a result of the sporulation of *Aspergillus*.

The second step in preparation of fermented soy sauce is brine fermentation. This technique is also a unique one which makes foods through the special fermentations by osmophilic yeasts and bacteria in the presence of more than 18% salt water which effectively excludes undesirable microorganisms from the fermentation as described later. The harvested koji is transferred to deep fermentation vessels with a salt solution of 22-23% by a snake pump. The resultant mixture is called moromi or moromi mash. The moromi mash is held for six to eight months under an appropriate temperature control with occasional brief aeration to mix the contents and stimulate microbial growths. During the fermentation period, the enzymes from koji hydrolyze most of the proteins of the materials to amino acids and low molecular weight peptides. Much of the starch is converted to simple sugars which are fermented primarily to lactic acid, alcohol, and carbon dioxide. The pH drops from an initial value of 6.5-7.0 down to 4.7-4.8. The high salt concentration around 18% effectively limits growth to a few desirable osmophilic types of microorganisms. Namely, at the first stage of moromi mash, *Pediococcus halophilus* is grown, and it produces lactic acid to drop the pH. At the second stage, *Saccharomyces rouxii* is grown, and as a result, a vigorous alcoholic fermentation occurs in "koikuchi" type of soy sauce. In the Chinese style soy sauce, which is considered as a "tamari" type of soy sauce, however, very little alcoholic fermentation occurs because of its shortage of sugars with the resultant soy sauce lacking the aroma originated in ester type compounds. This is the most marked difference observed between these two brine fermentations. At the last stage of moromi fermentation, *torulopsis* strains, which are a group of salt resistant yeasts, are grown. These strains produce phenolic compounds which are important as aroma compounds of soy sauce. Recently, the pure cultured *Pediococcus halophilus* and *Saccharomyces rouxii* are often added to the moromi mash. This enables constant production of a good quality soy sauce. Usually, the fermentation of moromi is done in indoor fermentation vessels. Recently, however, closed type outdoor moromi fermenters are available commercially in Japan.

The final process of soy sauce making is refining, which includes filtering and pasteurizing. In the "koikuchi" type of soy sauce, the aged moromi is put into a cloth after the fermentation is completed and the liquid part of the mash is separated from the residue with a hydraulic press until the water content of the residue becomes around 25%. Recently new automatic pressing equipment of a vertical type or a horizontal filter press type have been available commercially in Japan. After pressing, the resulting raw soy sauce is heated to 70-80 C by a plate heater. This heating is necessary to develop the color and aroma and to inactivate most of the enzymes in "koikuchi" type soy sauce. After clarifying the resultant soy sauce by sedimentation, the clear supernatant is bottled and packed. In "tamari" type of soy sauce, on the other hand, the separation of soy sauce from the residue is done with natural gravity filtration, followed by leaching of the residue with a salt solution. A preservative, such as sodium benzoate, is added to the resultant filtered and/or leached soy sauce solution and then bottled. In "tamari" type of soy sauce, pasteurization is not usually done. In general, preservatives have been used so far in "koikuchi" types as well as in "tamari" or other types of soy sauce. However, the recent tendency for "koikuchi" type of soy sauce in Japan has been an asceptical bottling which does not need a preservative. More than 50% of soy sauce on the market in Japan does not contain preservatives.

There were two marked advances in fermented soy sauce technology during the last two decades in Japan. One is the improvement of the treating method of soybeans and the other is mechanizations of the processes, particularly the mechanization of koji-making, which prevents koji

TABLE IV

Annual Production of Soy Sauce Marked by Japanese Agricultural Standard (JAS) in 1976

Grades	Amounts (Kl)	Amounts (Percent)
Special[a]	655,549	53.4
Upper	318,526	26.0
Standard	158,122	12.9
Non-JAS mark	94,351	7.7
Total	1,226,548	100.0

[a]Special grade is granted only for fermented soy sauce.

from contamination by undersirable microorganisms. Thus, the yield of fermented soy sauce production increased from 65% to almost 90% over the past 25 years on the basis of total nitrogen, and further, the qualities of soy sauce are also very much improved.

COMPARISON OF FERMENTED SOY SAUCE WITH CHEMICAL SOY SAUCE

In fermented soy sauce, the proteins and carbohydrates of the materials are hydrolyzed by the enzymes of *Aspergillus* species, whereas in chemical soy sauce, they are hydrolyzed with hydrochloric acid. Chemical soy sauce can be manufactured in only 8 to 10 hours, and therefore its manufacturing cost is cheaper. During the chemical hydrolysis of the materials, however, the carbohydrates maybe converted into undesirable compounds such as dark humins, levulinic acid, and formic acid, which are not present in fermented soy sauce, as shown in Figure 3. Some of the amino acids and sugars produced are also destroyed through this chemical hydrolysis process, and as a result, the undesirable compounds responsible for bad odors appear. Dimethyl sulfide, hydrogen sulfide, and furfurol, which have strong bad odors in themselves, are derived from methionine, sulfur-containing amino acids, and pentose, respectively; while tryptophane, one of the nutritionally important amino acids, is destroyed almost completely. In order to improve the odors of chemical soy sauce, semichemical soy sauce which is made by hydrolyzing the raw soybeans with 7-8% hydrochloric acid as the first step, was devised, followed by fermenting the resultant hydrolyzate with yeasts and bacteria in the presence of wheat koji. Semichemical soy sauce has a kind of fermented soy sauce flavor, but it is essentially a chemical soy sauce which has the undesirable compounds described above. Chemical soy sauce or semichemical soy sauce is not used as soy sauce in itself, but it is used as an extender of fermented soy sauce in Japan. In 1977 in Japan, 63% of the bottled soy sauce was composed of fermented soy sauce, about 30% composed of a blending mixture of semichemical and fermented soy sauce, and the remaining 7% was composed of a blending mixture of chemical and fermented soy sauce. The Japanese government recognizes three grades of soy sauce, that is, Special, Upper and Standard, which are differentiated by organoleptic evaluation, contents of total nitrogen, soluble solids other than sodium chloride, and color. Special grade is assigned to only high quality soy sauce made by fermentation. The soy sauce mixed with semichemical or chemical soy sauce cannot be Special grade. The annual production of JAS (Japanese Agricultural Standard) marked soy sauce in 1976 is shown in Table IV.

FERMENTED SOY PASTE

Fermented soy pastes are also a fermentation product of soybeans and cereals in the presence of salt. They are widely used throughout East Asia. The progenitor of these fermented soy pastes is referred to as "chiang" developed in China long before the Christian era, and transformed into "jang" in Korea, "miso" and "shoyu" in Japan, "tao-tjo" in Indonesia and Thailand, and "tao-si" in the Philippines.

There are many kinds of fermented soy pastes in China which are collectively called "chiang." Actually, the term "chiang" includes a very wide range of foods. Most of "chiang" in China are prepared at home, just as the Western people make their own jams and pickles. On the other hand, the Japanese fermented soy paste "miso" is now manufactured commercially in a modernized factory on a large scale. There are large differences in the way fermented soybean pastes are consumed in Japan and China. In China, "chiang" is used as the base for sauces served with meat, seafood, poultry, or vegetable dishes. In Japan, however, "miso" is mainly used as the base for soups. While the average annual consumption of miso is 7.2 kg per person in Japan, 80 to 85% of this consumption is consumed in the preparation of miso soup, and the balance is used as seasonings for various type of foods.

There are many varieties of miso in Japan as well as of chiang in China based on the ratio of substrates, salt concentration, the length of fermentation and aging. Most of miso in Japan is a paste which resembles peanut butter in consistency and is smooth in texture. Its color varies from a creamy yellowish white to a very dark brown. Generally speaking, the darker the color, the stronger the flavor. The product is typically salty and has a distinctive pleasant aroma.

As shown in Table V, miso can be classified into three major types on the basis of the raw materials, that is, rice miso, barley miso, and soybean miso. Rice miso is made from rice, soybeans, and salt; barley miso is made from barley, soybeans, and salt; and soybean miso is made from soybeans and salt. These types are further classified by the taste into three groups, that is, sweet miso, semisweet miso, and salty miso. Each group is further divided by color into white-yellow miso and red-brown miso groups. Among these miso, rice miso is the most popular one, forming 81% of the total miso consumption.

The manufactring methods for miso differ by type of miso, but the basic process is really all the same, as shown in Figure 4, for rice miso, which is the most popular miso. There are two basic differences between the miso and shoyu manufacturing,though both are very much alike. One is in koji-making. The koji of shoyu is made by using all the raw materials, that is, the mixture of soybeans and wheat, whereas the koji of miso is made by using only carbohydrate materials, that is, rice or barley. The soybeans are used in miso-making without the inoculation of koji mold, except on soybean miso. The other difference is that miso is a solid paste, and therefore the making process has no filtration step, which has a very large influence on the cost in shoyu-making. The fungus and yeast used in miso manufacturing are very similar and sometimes the same as in shoyu manufacturing.

Recently, a revolution has occurred in the manufacture of miso, which has taken the form of automated equipment and continuous processing. Particularly, the use of a rotary fermenter is used in preparation of rice or barley koji. Cooked and mold-inoculated rice is put into a large trommel of this rotary fermenter in which the temperature and moisture-controlled air is circulated. The trommel is rotated several times to prevent the rice from agglomerating during fermentation. After completion of fermentation, the resulting koji is mixed with salt, cooked whole soybeans, pure cultured yeasts, lactic acid bacteria, and water, and then kept for an appropriate period for the second fermentation. The resulting aged mixture is mashed and packaged as miso.

CHINESE SOYBEAN CHEESE (SUFU)

Sufu is a soft cheese type product made from soy milk curd by the action of microorganisms. Sufu originated in

TABLE V

Chemical Compositions of Major Types of Miso in Japan

Material	Classification		Aging time	Chemical composition					Annual production	
	Taste	Color		Protein (%)	Fat (%)	Carbohydrate (%)	Ash (%)	Moisture (%)	(Ton)	(%)
	Sweet miso	Yellowish white	5-20 days	11.1	4.0	35.9	7.0	42.0		
		Reddish brown	5.20 days	12.7	5.1	31.7	7.5	43.0		
Rice miso	Semisweet miso	Bright light yellow	5.20 days	13.0	5.4	29.1	8.5	44.0	467,000	81
		Reddish brown	3-6 months	11.2	4.4	27.9	14.5	42.0		
	Salty miso	Bright light yellow	2-6 months	13.5	5.9	19.6	14.0	47.0		
		Reddish Brown	3-12 months	13.5	5.9	19.1	14.5	47.0		
Barley miso	Semisweet miso	Yellowish to reddish brown	1-3 months	11.1	4.1	29.8	13.0	42.0	63,000	11
	Salty miso	Reddish brown	3-12 months	12.8	5.2	21.1	15.1	46.0		
Soybean miso	Salty miso	Dark reddish brown	5-20 months	19.4	9.4	13.2	13.0	45.0	46,000	8

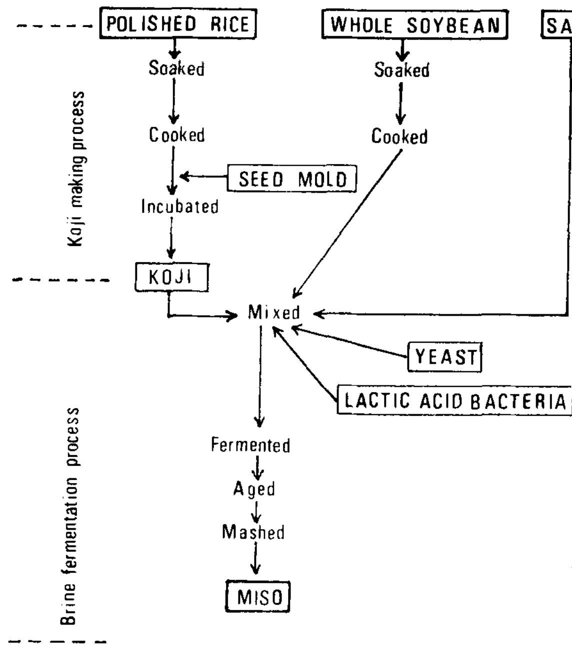

FIG. 4. Manufacturing process of "rice miso."

the fifth century in China and has been widely consumed as a relish by Chinese people. However, sufu is not consumed in Japan.

Sufu-making process consists of three major steps; they are preparation of soy milk curd, molding process, and brining process as shown in Figure 5. The first step, that is, soy milk curd-making is essentially the same process as used for making "tofu." Tofu can be consumed directly and is widely eaten throughout the Far East. In the case of making sufu, however, tofu is made so hard that its water content may be less than 70%, while the water content of directly consumed ordinary tofu is about 90%.

The second process of sufu-making is the molding process. After the hard made tofu is cut into 3 cm cubes, the cubes are heated for pasteurization and for reducing the water content of the cube surface, and then the mold is inoculated on it. The molds belonging to the genus of *Mucor* or *Actinomuccor* are usually used, but the molds belonging to the genus of *Rhizopus* are also used sometimes. For instance, *Actinomuccor elegans, Mucor hiemalis, Mucor silvaticus, Mucor praini, Mucor subtilissimus,* and *Rhizophus chinensis,* etc., are used for the inoculation. The time of mold fermentation differs depending upon the varieties of mold. It takes about 7 days at 12 C for *Rhizopus chinensis,* 3 days at 24 C for *Mucor hiemalis* and *Mucor silvaticus,* and 2 days at 25 C for *Mucor praini.*

The last process of sufu-making is brining and aging. The freshly molded cubes are placed in various types of brining solution depending on the flavor desired. The usual brining solution consists of salted fermented rice mash, soy sauce moromi mash, fermented soy paste or 5% NaCl solution containing rice wine having ca. 10% ethyl alcohol. The time of aging ranges from one to twelve months depending upon the varieties of the brining solution. Finally, the product is bottled with the brine, sterilized, and marketed as sufu.

Sufu is a creamy cheese type product which has a mild flavor, and therefore it would be suitable for Western people in using it the same way as cheese.

FERMENTED WHOLE SOYBEAN (NATTO)

Natto is a traditional fermented food originating in the northern parts of Japan 1,000 years ago. There are two major types of natto. One is itohiki-natto and the other is

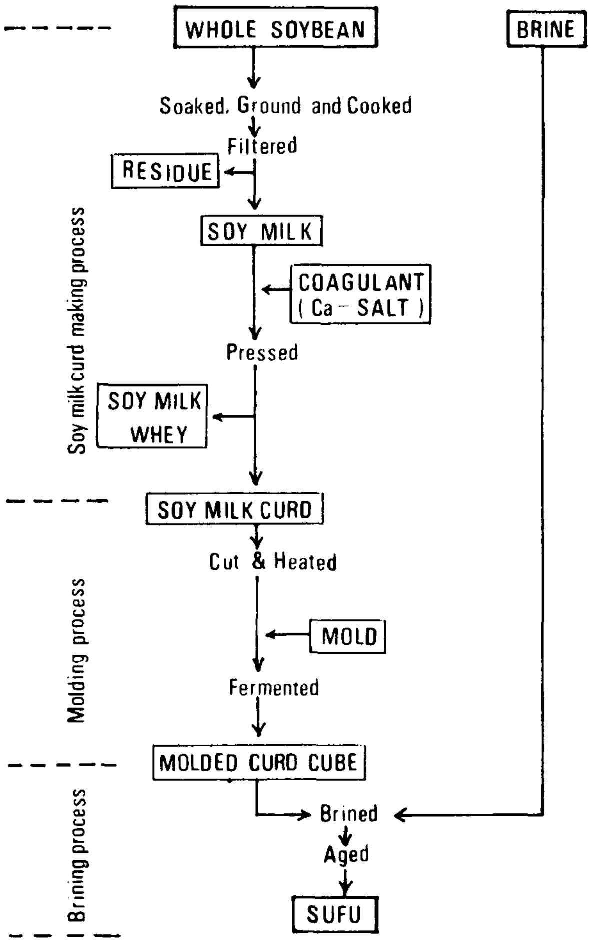

FIG. 5. Manufacturing process of "sufu."

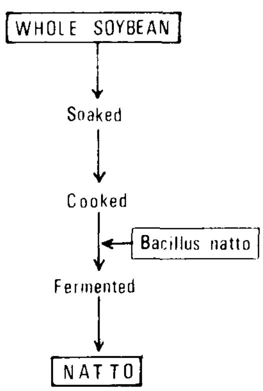

FIG. 6. Manufacturing process of "natto."

hama-natto. Hama-natto is a food fermented by *Aspergillus* and rather resembles soybean miso in taste. The production of hama-natto is very limited, as it is produced only in certain localities. On the other hand, itohiki-natto is very popular and produced in large amounts particularly around Tokyo and at the eastern part of Japan. Therefore, the word "natto" usually means "itohiki-natto." Natto is a unique soybean product fermented by *Bacillus natto.* In this product, the shape of cooked whole soybean particles is kept as it is, and the surface of the particles is covered with a very viscous substance, which consists of glutamic acid polymers produced by *Bacillus natto.*

The manufacturing of natto is very simple (Figure 6), and the time of fermentation is also short. A small quantity of the inoculated cooked whole soybeans is put into a small plastic tray with cover and packed. The resulting packed

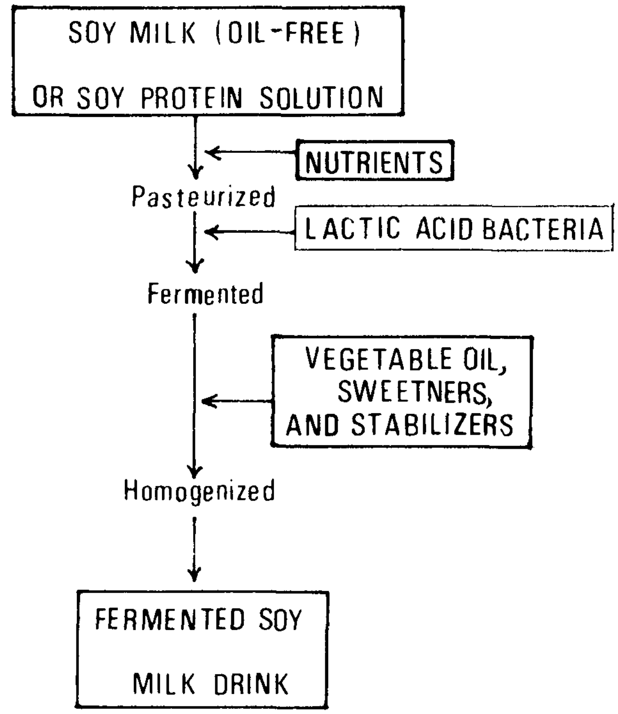

FIG. 7. Manufacturing process of fermented soy milk drink.

tray is kept at 40 C for the fermentation by *Bacillus natto.* After 14-18 hr, the packed tray is cooled to 2-7 C and then shipped to the market. Natto is a very cheap and nutritious protein food, and its annual production is about 124,000 tons. Natto is tasty and does not have much odor, but its viscous fluid is distinctive; each soybean is covered with a viscous, stringly substance produced by the bacteria. Natto is usually served as is with shoyu and mustard.

NEW FERMENTED SOYBEAN PRODUCTS

The fermented vegetable protein foods found in Japan and China are all traditional ones as described above. Recently, however, many investigations have been made in Japan regarding new fermented foods using vegetable protein foodstuffs such as cheese and yogurt type fermentation products. Most of these have not been available commercially yet, except for a fermented soy milk drink which appeared on the Japanese market last year. According to the recent studies on fermented soy milk drinks (9,10), the off-flavor found in soy milk disappears by the fermentation and the addition of oil, sugar, and stabilizers. The basic process of the fermented soy milk drink is shown in Figure 7. *Lactobacillus casei, Lactobacillus acidophillus,* and *Lactobacillus bulgaricus,* etc., are usually used as the starters.

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