Proteins are complex systems composed of 21 amino acids, of which all but 8 can be synthesized by the human body. These 8 are called essential amino acids because they must be obtained from the food we eat. A protein is like a chain with amino acids as the links, and the chain is not stronger than its weakest link. Therefore, proteins may be present in sufficient quantity but the contribution to nutritional value may be limited by the quantity of a particular essential amino acid in the protein. In wheat, the amino acid lysine limits the nutritional value of its protein; the limiting amino acid in soya is methionine.

Fortunately, proteins from different foods complement each other, and by combining proteins, the nutritive value of the food is dramatically improved. Such is the case of combining soya protein with wheat protein in the manufacture of bakery foods.

Hoover (9) reported that the addition of higher levels of soya flour brought about dramatic changes in the protein nutritive value of bread. The Protein Efficiency Ratio (PER) compares the nutritive value of a system with the protein nutritive value of casein at 2.5. By comparison, the PER of defatted soya flour, lightly toasted, is 2.03-2.3 (10).

The PER for white bread is ca. 0.7 and for bread with 3% soy flour added, ca. 0.83. When the soya flour is increased to the 6% level, the PER increases to 1.3, and at the 12% level, the PER is 1.95. In addition to the improvement in protein quality at the 12% soya flour level, the protein content is increased by 50%. Feeding studies with rats indicated a 3- to 4-fold increase in growth rates of rats fed diets based on the fortified bread, compared to unfortified white bread.

Tsen et al. (11) noted that high levels of soy flour produced depressed volume and poor bread characteristics. They found that the addition of sodium stearoyl-2-lactylate (SSL), or ethoxylated monoglycerides provided a system which permitted the addition of higher levels of soya flour to bread stuffs without detrimental effects on their eating qualities.

Tsen also reported (12) that excellent protein fortified cookies could be made using a blend of 88 parts bread flour, 12 parts defatted soya flour and 0.5 parts SSL.

Soya fortified flour has been used worldwide in mass feeding programs, including school lunch programs, since 1975. This commodity is available through the U.S. Food for Peace Programs (P.L. 480). Millions of pounds of this blended food are used each year in all types of bakery foods.

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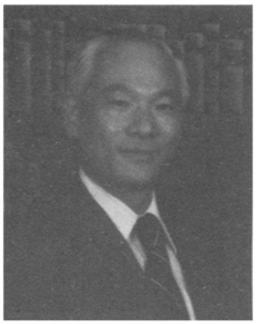

Soy Proteins for Foods Centering around Soy Sauce and Tofu

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ABSTRACT

In the Orient, soy proteins have been used for human consumption for centuries, during which various kinds of unique soy protein foods have been established. Some of these products, such as tofu, have a bland flavor, but there are also those products having a distinctive flavor and an aroma like fermented soy sauce. Both types are acceptable for worldwide populations. In earlier times, the consumption of these soy protein foods in the U.S. was mostly confined to Orientals. Recently, however, the koikuchi type of soy sauce has been consumed widely by non-Orientals through nationwide supermarkets. Annual production in the U.S. has reached more than 16,500 kl. Tofu has also become popular in the U.S. because of its bland flavor which enables it to be used in many dishes. Fermented soy sauce is completely different in the constituents of aroma and flavor from chemical soy sauce. Koikuchi shoyu is a typical Japanese type of fermented soy sauce characterized by a strong, appetizing aroma. The fermentation process consists of koji fermentation by *Aspergillus* species and the subsequent brine fermentation, which contains lactic acid and alcoholic fermentations. Miso is a paste product produced through a similar fermentation. Tofu is a soy milk curd product made through a nonfermented process from soy milk.

INTRODUCTION

There are significant differences in the use of soy proteins for human consumption in the U.S. and in the Orient. In the U.S., the use of soy protein as food is a new development. Soy proteins are used as ingredients of a wide variety of foods, e.g., hamburgers, sausages, meatloaves, dairy products, breads, pastries and cookies. In the Orient, however, soy proteins have been consumed for thousands of years, not as ingredients, but as characteristic, unique soy protein foods.

These traditional soy protein foods are divided into two groups: fermented and nonfermented. Fermented foods are shoyu, miso, natto, sufu, and tempeh; nonfermented products are soy milk, tofu and its derived products, kori-tofu, yuba, kinako and moyashi. Several reviews on these products have been published (1-23). In the U.S., the consumption of these soy protein foods was mostly confined to the Orientals in earlier times. Recently, however, some of these foods have become popular with Americans. For instance, fermented soy sauce (shoyu) has been consumed widely among the U.S. population because of its excellent aroma. Tofu has rapidly penetrated the U.S. market because of its bland flavor which enables it to be used as a nutritious dish by itself or as an ingredient in many dishes.

This paper discusses traditional Oriental soy protein foods which are growing rapidly in popularity in the U.S. among the non-Oriental population. A recent paper (24) describes an interesting conclusion on the origins of soy sauce and miso. A discussion also is made on the comprehensive mechanisms at the molecular level for the protein coagulations, which are very important for the manufacturing of nonfermented soy protein foods.

SOY SAUCE: VARIETIES

Generally speaking, soy sauce is divided into two groups: fermented soy sauce and chemical soy sauce. Fermented soy sauce has a long history as a human food, whereas chemical soy sauce has a history of only several decades. In fermented soy sauce, the proteins and carbohydrates contained in the materials are hydrolyzed very slowly under mild conditions below 30 C for over six months, whereas in chemical soy sauce they are hydrolyzed quickly by hydrochloric acid at 80 C for 8-10 hr. Chemical hydrolysis is a cheap and rapid process, but during the hydrolysis, various secondary reactions occur and produce undesirable compounds, e.g., dark humins, furfurol, dimethyl sulfide, hydrogen sulfide, levulinic acid and formic acid, which are not present in fermented soy sauce. Furfurol, dimethyl sulfide and hydrogen sulfide, which have strong, bad odors in themselves, are derived from pentose, methionine and sulfur-containing amino acids, respectively. Furthermore, tryptophane, one of the nutritionally important amino acids, is destroyed almost completely. As shown in Figure 1, the main organic acid of fermented soy sauce is lactic acid, whereas the main organic acid of chemical soy sauce is formic acid. Levulinic acid, present in chemical soy sauce, does not exist naturally. Therefore, we can determine the quantities of the chemical soy sauce mixed with fermented soy sauce by measuring the levulinic acid content. To improve the odors of chemical soy sauce, semichemical soy sauce was devised. It is made by hydrolyzing raw soybeans with a lower concentration of hydrochloric acid (7-8%) as the first step, followed by fermenting the hydrolysate with osmophilic yeasts in the presence of wheat koji. The odor and flavor of the resultant semichemical soy sauce are improved partly by the fermentation procedure, but it is essentially a chemical soy sauce which has the undesirable compounds previously described. Chemical soy sauce is not used as soy sauce in itself, but it

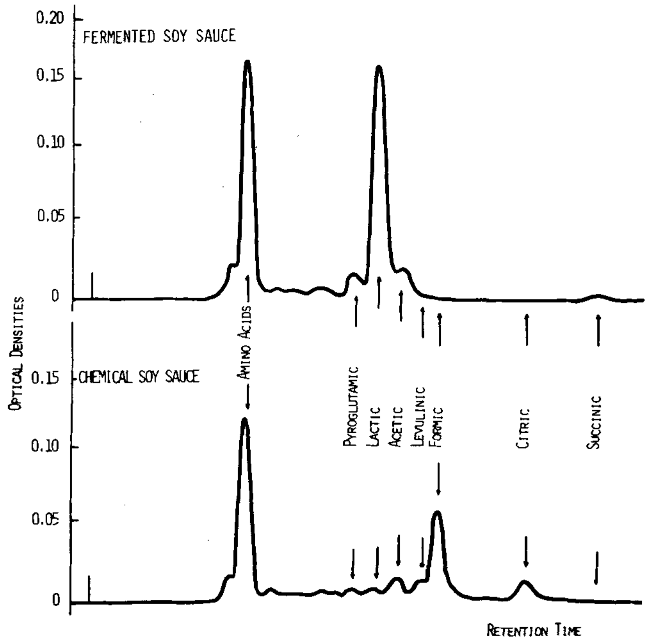

FIG. 1. Chromatograms of the organic acids in fermented and chemical soy sauce. Each sample analyzed is a representative soy sauce manufactured in the U.S.

is used as an extender for soy sauce in Japan.

Five types of traditional soy sauce in Japan are koikuchi shoyu, usukuchi shoyu, tamari shoyu, saishikomi shoyu and shiro shoyu. These are recognized by the Japanese government and each type is classified into three grades: special, upper and standard, determined by organoleptic evaluation, total nitrogen content, soluble solids other than sodium chloride and color. Special grade is assigned to the high quality soy sauce made only by the action of microorganisms. The use of chemical and/or enzymatic hydrolysates for special grade soy sauce is not permitted. Among the soy sauce products in Japan in 1979, ca. 70% are purely fermented products, ca. 25% are the products containing semichemical soy sauce, and the rest are the products containing chemical soy sauce. The total annual production of soy sauce reported by the Japanese Agricultural Standard (JAS) in 1979 was 1,252,431 kl.

The typical composition of the five types of soy sauce are shown in Table I. Among these, koikuchi shoyu represents Japanese fermented soy sauce which forms 85% total soy sauce production in Japan. It is an all-purpose seasoning characterized by a strong aroma, myriad flavor and deep, red-brownish color. Such properties of koikuchi shoyu are mainly derived from the use of equal amounts of wheat and soybeans in the koji. The second type of soy sauce is usukuchi shoyu, which is characterized by a lighter, red-brownish color and milder flavor and aroma. It is used mainly for cooking when one wishes to preserve the original flavor and

TABLE I

Typical Compositions of Five Varieties of Soy Sauce Recognized by the Japanese Government

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

color of the food itself. The ratio of soybeans and wheat in this type of soy sauce is the same as in koikuchi shoyu, but the fermentation is done under conditions such that the color development may be prevented. The third type is tamari shoyu, characterized by a little higher content of amino acids, though it lacks aroma. The feature of this product is that soybeans are the main material of the koji and only a small amount of wheat is used.

Saishikomi shoyu is produced by using equal amounts of wheat and soybeans in the koji and using raw soy sauce instead of the salt solution, which is mixed with the harvested koji. This type of soy sauce is characterized by aroma and full-bodied taste. Shiro shoyu is made by using a very high ratio of wheat to soybeans in the koji and further by being fermented under conditions which prevent color development. It is characterized by a very light yellow to tan color, though amino acid content is very low.

In the U.S. in earlier times, domestic and popular soy sauce was chemical soy sauce. Fermented soy sauce was imported, and it was basically for Orientals. Fermented soy sauce was unfamiliar to non-Orientals. However, the aroma and flavor of koikuchi shoyu have been recognized gradually by non-Orientals and a plant for its manufacture was opened in the U.S. in 1973 by Kikkoman Foods, Inc. Recently, koikuchi shoyu has been moving steadily into the U.S. market and production has reached 16,500 kl/year. Most recently, the annual consumption of fermented and chemical soy sauce in the U.S. is estimated as 17,850 and 25,500 kl, respectively.

SOY SAUCE: MANUFACTURING

The manufacturing process of fermented soy sauce basically consists of three major processes: koji-making, brine fermentation and refining, as shown in Figures 2-4. The koji-making process is unique for the manufacturing of fermented foods, i.e., soy sauce, miso, sake (rice wine), shochu (rice wine spirits) and yonezu (a rice vinegar). Koji-making is a traditional technique of the Orient. Koji is a source of enzymes for converting the carbohydrates and proteins of the materials into sugars, peptides and amino acids. The substances converted by the enzymes of koji become the nutrients of lactic acid bacteria and yeasts in the subsequent brine fermentation. In koji-making, soybeans, or more commonly, defatted soybean flakes or grits, are moistened and cooked under pressure. Soybean cooking was done in a batch-type cooker previously, but recently it is being done in a continuous cooker which allows high pressure-short time cooking. On the other hand, the wheat is roasted by continuous roasting and then cracked into 4-5 pieces. In koikuchi, usukuchi, or saishikomi shoyu, the defatted soybeans are mixed with equal amounts of roasted wheat and then inoculated with a purely cultured starter of *Aspergillus oryzae* or *sojae*, which is called "seed mold." In tamari shoyu, however, the ratio of wheat to soybeans must be less than 10%. In shiro shoyu, on the contrary, a very high ratio of wheat to soybeans must be used. In usukuchi and shiro shoyu, special species of *Aspergilli* are used which do not cause strong color development during brine fermentation. In traditional koji-making the mixtures of soybeans and wheat inoculated with a seed mold were put into small wooden trays and kept for three or four days in a koji-making room. During the growth of *Aspergilli*, the temperature and moisture of the cultured mixture were controlled by manual operations containing the stirring of the mixture several times. In recent years, however, automatic koji-making processes have been developed to replace this traditional process 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 the shallow, perforated vat, and the forced air is circulated through the mass. After three days, *Aspergillus oryzae* or *sojae* grows and the culture mixture becomes a green-yellow as a result of the sporulation. We call it "koji."

The second step of the manufacturing of fermented soy sauce is brine fermentation. This also is a unique way to make foods through special fermentation using osmophilic lactic acid bacteria and yeasts. The presence of the brine (16-19 g salt/100 ml) in this fermentation effectively excludes undesirable microorganisms. The harvested koji is

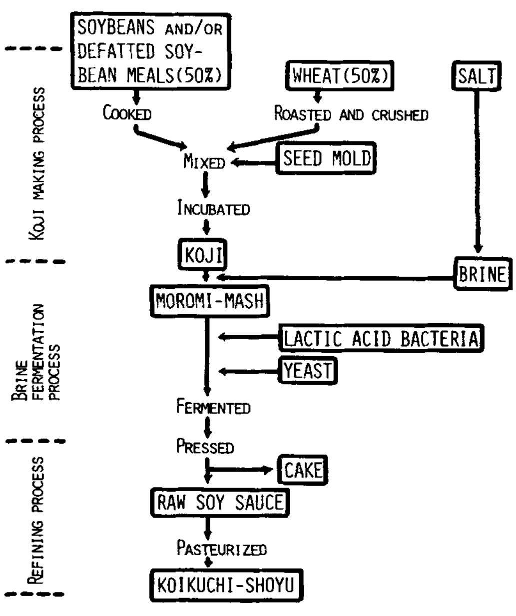

FIG. 2. Manufacturing process of koikuchi shoyu, a representative of Japanese soy sauce.

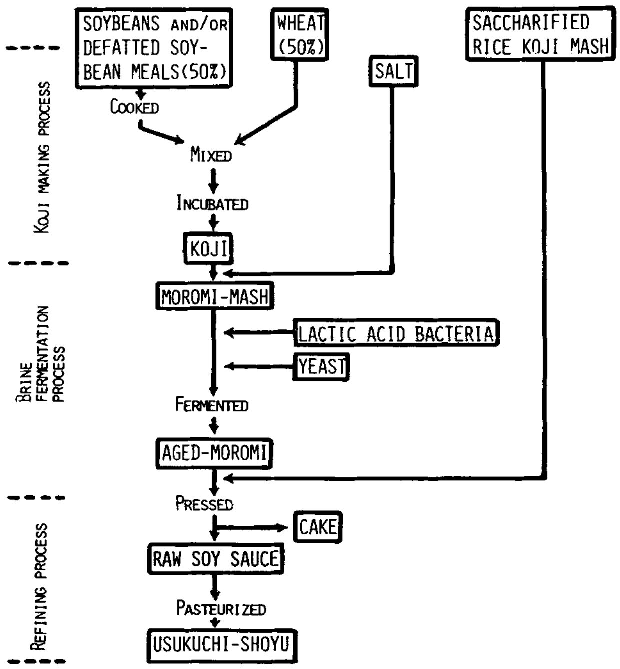

FIG. 3. Manufacturing process of usukuchi shoyu.

transferred to deep fermentation vessels with a salt solution of 22-25% (w/v) by a snake pump. In saishikomi shoyu, however, raw soy sauce is used instead of a fresh salt solution to obtain a thicker taste of soy sauce. The resultant mixture is called moromi or moromi mash. The moromi mash is held for 6-8 months under appropriate temperature controls with occasional brief aeration to mix the contents and to stimulate microbial growths. In usukuchi and shiro shoyu, however, the period of brine fermentation holding time is shorter, because of the prevention of the color development. During the fermentation period, the enzymes from koji hydrolyze most of the proteins 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 to 4.7-4.8. The high salt concentration effectively limits the growth to a few desirable osmophilic types of microorganisms, i.e., at the first stage of moromi mash, *Pediococcus halophilus* (a lactic acid bacteria) grows and produces lactic acid which causes a drop of the moromi pH; in koikuchi and usukuchi shoyu, *Saccharomyces rouxii* (a type of yeast) grows, accompaning the decrease of the moromi pH, and as the result a vigorous alcoholic fermentation occurs, particularly in koikuchi shoyu. In tamari shoyu, however, alcoholic fermentation does not occur substantially, because of its sugar shortage and the inhibiting effects due to high nitrogen contents. Therefore, tamari shoyu lacks the aroma characteristic of ester-type compounds. At the middle and last stages of moromi fermentation, *Torulopsis* strains often grow, which are a group of salt-resistant yeasts. These strains produce phenolic compounds and add some aroma to soy sauce. However, *Torulopsis* strains do not exist in the moromi under the same conditions. The growth of *Torulopsis* can be controlled. Recently, the techniques to control the microorganisms during the brine fermentation were established in koikuchi shoyu. This is done by using pure-cultured *Pediococcus halophilis* and *Saccharomyces rouxii.* This technique gave us constant production of a desirable quality koikuchi shoyu.

The final process of soy sauce making is refining, which includes filtering and pasteurizing. In koikuchi shoyu, the aged moromi is put into a cloth and then pressed with a hydraulic press machine until the water content of the residue becomes less than 25%. Recently, automatic vertical or horizontal filter presses have been used commercially in Japan. After pressing, the filtered raw soy sauce is heated to 70-80 C by a plate heater in the case of koikuchi shoyu. This heating is very important to develop a red-brownish color and sharp aroma, and to inactivate most of the enzymes. After clarifying the resultant soy sauce by sedimentation, the clear supernatant is bottled and packed. In usukuchi shoyu, however, a digestion mixture of rice koji is added to the aged moromi and then the pressing of the resultant moromi is carried out, as shown in Figure 3. The purpose of the addition of the digestion mixture to usukuchi shoyu is to make its flavor bland. The heating and clarification of usukuchi shoyu are done in the same way as koikuchi shoyu, except the heating temperature is lower. In tamari shoyu, the separation of soy sauce from moromi is done with natural gravity filtration, followed by the leaching of the residue with a salt solution. A preservative such as sodium benzoate is added to the resultant filtrate and/or leaching solution and then bottled. In tamari shoyu, pasteurization is not done in principle. In general, preservatives have been used widely not only in tamari shoyu, but also in most types of soy sauce. Recently, however, more than 50% of the soy sauce on the market in Japan has been bottled asceptically without using preservatives such as sodium benzoate.

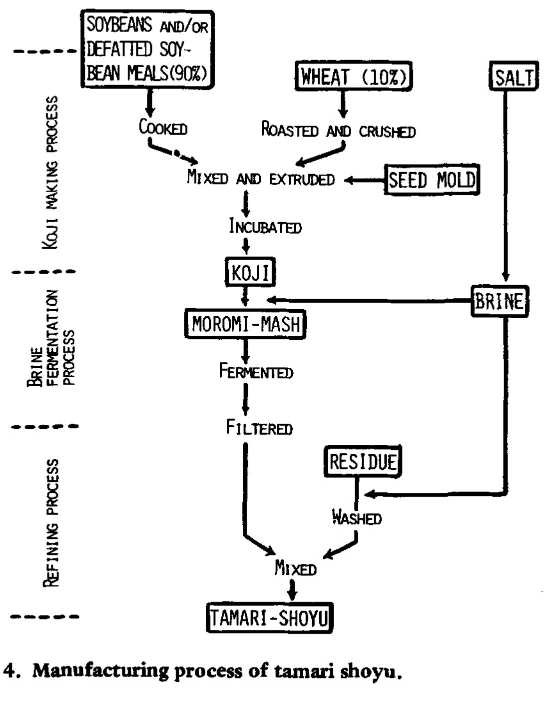

FIG. 4. Manufacturing process of tamari shoyu.

Tamari-type soy sauce is widely produced in Southeast Asia, including Indonesia, Malaysia, the Philippines, Singapore and Thailand. The manufacture of soy sauce in these areas is a very old industry originated by the Chinese. According to the reports presented in the ASEAN Workshop on Soy Sauce held in Singapore in 1978, most soy sauces in these areas are manufactured in the small-scale factories, which blindly follow Chinese traditional techniques handed down from generation to generation. Basically, there are no differences in the manufacturing methods among these factories, with the exception of one or two modern factories with large-scale equipment. In manufacturing, soybeans are boiled in a large caldron, drained in bamboo baskets, and then mixed with a small amount of wheat flour. The resulting mixture is spread onto a bamboo tray and kept in the incubation room for one week to make koji. The inoculation of the mold is usually done spontaneously in the incubation room or by being mixed with a previous batch of koji. Because a seed mold which is cleanly prepared by the inoculation of a pure-cultured mold is not used, the harvested koji usually consists of more than one kind of mold. The koji thus prepared is placed in large earthenware jars of 45-70 ℓ with a brine solution. The full jars are left outside and the moromi mash is exposed to the sun for 1-6 months, which varies, depending on the factory. After the end of the brine fermentation period, the bamboo baskets are dipped in the moromi mash and the liquid accumulated in the baskets is transferred to another earthenware jar and exposed to the sun for several weeks. The first grade soy sauce is made from this liquid. Fresh brine is added to the residue two more times to extract the second and third grade soy sauce, followed by exposure to the sun for a few weeks. Caramel and monosodium glutamate are added to the second and third extracts. The soy sauce is bottled after the addition of sodium benzoate. Pasteurization is done before bottling in some factories. One of the special features in the manufacturing process of soy sauce in these areas is to expose the moromi mash to the sun. It is unclear, however, why such a procedure is needed. This is never seen in the manufacturing processes of soy sauce in Japan, because such a process has an adverse effect on the qualities of soy sauce.

The soy sauce manufacturing method which is used in mainland China at present is quite different from the traditional Chinese method just described. This method was developed recently in China and therefore it is also different from the Japanese method. Defatted soybean meal and wheat bran are used in a ratio of 60:40, respectively. The koji manufacturing time is reduced from 48 to 24 hr by the improvement of the strain of the mold. The duration of the brine fermentation was also reduced to about 3 weeks by using a special fermentation method in which the koji is mixed with a smaller volume of the brine (about half the usual amount) having about three-fourths as much salt. The very firm moromi mash is kept for 3 weeks at 40-45 C. After 3 weeks, the moromi mash is transferred to a separation tank, mixed with a brine, and then heated over 80 C, followed by the separation of the liquid from the bottom of the tank by gravity. The soy sauce is mixed with benzoic acid, clarified, bottled and shipped. This method is very economical, but the ratio of the amino nitrogen to total nitrogen is not high. Also, the lack of the alcoholic fermentation due to both the use of wheat bran and the reduction of the brine fermentation time gives the soy sauce a different flavor than that of koikuchi shoyu, which is characterized by the aroma derived from an alcoholic fermentation.

There were two marked advances in fermented soy sauce technology during the last two decades in Japan: the improvement of the treating method of soybeans, and the mechanization of the processes, particularly the mechanization of koji-making. The improvement of the treating method of soybeans is based on findings that the degree of hydrolysis of soybean proteins by proteases of *Aspergillus* species is greatly influenced by cooking conditions (25,26). The relationship between the cooking conditions and the yields of soy sauce have been studied (27). The higher the pressure, that is, the higher the temperature, the higher the yields become, whereas the longer the time, the lower the yields. The total nitrogen and amino nitrogen increase with increased yields. Furthermore, the development of new, automatic koji-making equipment enables maximization of enzyme production. In the past, koji was made in small wooden trays. The wooden tray method does not allow control of temperature and humidity to maximize the enzyme production. A comparison between a wooden tray and an automatic equipment method shows that use of the automatic koji-making system in soy sauce production not only increases the protease activities of koji, but also prevents koji from being infected by the undesirable microorganisms which can give soy sauce unfavorable odor. As a result, a high quality soy sauce with plenty of favorable aroma can be produced consistently. A new technique to control the microorganisms of moromi mash has been developed. The mechanization of the moromi pressing has saved many hours of labor. Thus, soy sauce manufacturing has emerged from tradition into the modern industrial world.

MISO

Miso is also a fermentation product of soybeans and cereals in the presence of salt. Miso is a fermented soy paste, not a liquid like soy sauce.

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 "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.

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 a soup base. While the average annual consumption of miso is 7.2 kg/person in Japan, 80-85% of this is consumed in miso soup preparations. The balance is used as seasonings for various types of foods.

There are many varieties of miso in Japan as well as 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 II, miso can be classified into three major types on the basis of the raw materials used, 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, forming 81% of the total miso consumption.

The manufacturing methods for miso differ by type of miso, but the basic process is really all the same, as shown in Figure 5, 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, i.e., the mixture of soybeans and wheat, whereas the koji of miso is made by using only carbohydrate mater-

TABLE II

Chemical Composition of Major Types of Miso in Japan

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

ials, that is, rice or barley. The soybeans are used in miso-making without the inoculation of koji mold. This point is the essential difference between shoyu and miso. The proteins in soybeans are hydrolyzed by the enzymes of the koji made on the cereals other than soybeans. Therefore, the degree of the hydrolysis of soybean proteins is low in miso. The only exception is soybean miso, which is the same as Haccho-miso or tamari-miso. In these products, soybeans are used for koji making. These products belong to a category of shoyu from the biochemical point of view, though they are semisolid products. The other difference is that miso is a solid paste, and therefore the manufacture 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 similar and sometimes the same as in shoyu manufacturing.

Recently, miso manufacture has been automated and made continuous. Particularly, the use of a rotary fermenter is used in preparation of rice or barley koji. Cooked and mold-inoculation rice is put into a large trommel of this rotary fermenter in which 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.

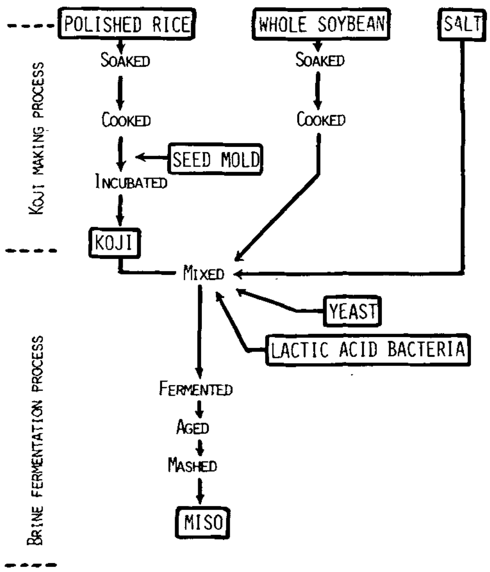

FIG. 5. Manufacturing process of rice miso.

OTHER FERMENTED PRODUCTS

Tempeh and natto are other important fermented soy protein foods. Besides these, sufu and fermented soy milk beverages are described in the section on products related to tofu. Tempeh was originated in Indonesia and is consumed in large amounts there, as well as in Malaysia and the Philippines. Recently, tempeh has been sold in some health food stores in the U.S. Unlike most of the other fermented soybean foods which usually are used as flavor agents or relishes, tempeh serves as a main dish in these countries. Tempeh is not produced or consumed in Japan. Tempeh is a cake-like product made by fermenting soybeans with *Rhizopus.* A typical process for making tempeh follows. Soybeans are soaked in water, dehulled and boiled for 1 hr with excess water. The cooked soybeans are spread out for surface drying and then mixed with a previous batch of tempeh. The inoculated soybeans are wrapped with banana leaves and fermented at room temperature for 1 day. During the fermentation, the soybeans are covered with white mycellia which bind the mass together as a cake. The cake of tempeh is sliced and fried, or if preferred, dipped in brine or spices before frying. It can also be baked or added to soup. By itself, tempeh has a bland flavor. Recently, in a larger scale fermentation of tempeh, the pure cultures of molds such as *Rhizopus oligosporus, R. oryzae, R. arrhizus,* and *R. stolonifer* have sometimes been used.

Natto is a traditional fermented food of two types: "itohiki-natto" and "hama-natto." Hama-natto is a completely different product from itohiki-natto in the appearance as well as in the manufacturing method, though the name "natto" is attached. Hama-natto is a food fermented by *Aspergillus* species and resembles soybean miso in taste. This product belongs to a category of shih and shoyu along with soybean miso. The production of hama-natto is limited, as it is produced only in certain localities. On the other hand, itohiki-natto is very popular and produced in large amounts. 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 maintained, and the surface of the particles is covered with a very viscous substance, which consists of glutamic acid polymers produced by *B. natto.* The manufacturing of natto is very simple, and the time of fermentation also is short. A small quantity of the inoculated, cooked whole soybeans is put into a small plastic tray with cover and packed. The resulting packed tray is kept at 40 C for the fermentation by *B. natto.* After 14–18 hr, the packed tray is cooled to 2–7 C and shipped to the market. Natto is a low-cost nutritious protein food, and its annual production is ca. 124,000 tons in Japan. Natto is tasty and does not have much odor, but its viscous fluid is distinctive; each soybean is covered with a viscous, stringy substance produced by the bacteria. Natto usually is served as is with shoyu and mustard.

TOFU AND RELATED PRODUCTS

Regular and Silken Tofu

Over the centuries, various types of the products have been developed from regular tofu (momen-tofu), such as silken tofu (kinugoshi-tofu), dried-frozen tofu (kori-tofu), and deep-fried tofu (aburage). However, regular and silken tofu are basic forms of tofu. Regular tofu is a soybean curd, which is white, soft and fragile. Silken tofu is almost the same in the appearance, but the texture is much softer, smoother, and more fragile than that of regular tofu. Tofu can be served not only with soy sauce and spices without further cooking, but also as an ingredient in an entire range of dishes. In Japan, nearly 500,000 tons of soybeans are used for the production of tofu and tofu-derived products annually. Tofu is so bland in flavor that it can be used in the national cuisine of countries worldwide. In the U.S., tofu is growing in popularity among the non-Orientals. Today, tofu can be found in many supermarkets, but some of the products are not always of good quality.

The manufacturing processes of regular tofu basically consist of three steps: (a) a soy milk manufacturing process, (b) a coagulation process, and (c) a pressing process, as shown in Figure 6. Whole soybeans are washed, soaked in water for one night and then ground with a small amount of water. After the addition of water, the final solid content of filtered soy milk may become 5–6%, and the slurry is cooked for 3 min at 100 C and then filtered with a cloth. This is the first process of regular tofu making, in which a heated soy milk is produced. The second step is a coagulation process of the heated soy milk. The heated soy milk is cooled to 70-80 C and then followed by the addition of a powdered calcium sulfate ($CaSO_4 \cdot 2H_2O$) suspended in a small amount of water. When the solid content of soy milk is 5-6%, the coagulation does not occur as a whole by the addition of the coagulant, but it occurs locally to release the whey. After the removal of the whey by discarding, the coagulates are transferred to a perforated wooden or aluminum box and then pressed to the formation of the tofu curd. The pressed curd, from which an excess whey was squeezed out, is taken out from the box and put into running water for several hours for both the removal of the excess calcium sulfate and the cooling of the curd. The curd is cut into a block of 300-500 g and sold in a fresh state. This is a traditional way of a regular tofu production. Ten kg soybeans produce ca. 40-50 kg fresh tofu, but about a half of the soybean solids is wasted as the residue and the whey.

The other type of tofu is called silken tofu (kinugoshi-tofu in Japanese). Silken tofu also is a traditional product, which is characterized by a very soft and smooth texture. Traditional silken tofu was a special type of tofu, and therefore the total amount of the production is very small. The manufacturing process of silken tofu consists of two steps, i.e., a soy milk manufacturing process and coagulation process, as shown in Figure 6. It lacks a pressing process, because the soy milk coagulates as a whole without separating any whey by the addition of the coagulants. The coagulation as a whole occurs, when the coagulants are added to a high concentration of soy milk whose solid content is ca. 10%. Therefore, in silken tofu manufacture, soaked soybeans are ground with water to make soybean slurry, followed by a further addition of water, so that the solid content of the soy milk after filtration may become ca. 10%. The slurry mixture is heated for 3 min at 100 C and filtered. The filtered soy milk is put into an unperforated box and calcium sulfate is added at 70 C or a little higher temperature. After

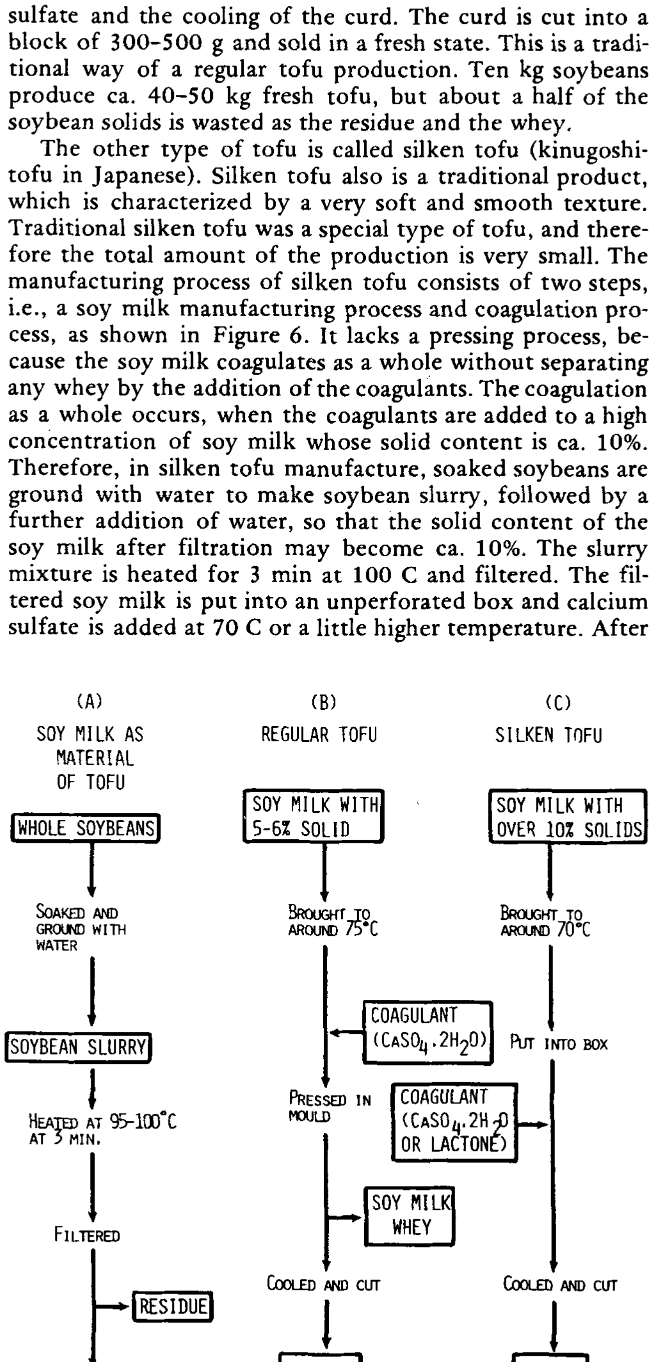

FIG. 6. Manufacturing process of regular and silken tofu.

40-60 min, the soy milk is coagulated as a whole to form a uniform curd with a soft and smooth texture. Regular tofu loses a part of the whey components in soy milk, such as soluble proteins, sugars and vitamin B, during the processing, but silken tofu contains the whole components of soy milk. Recently, glucono-γ-lactone has been used widely as a new coagulant, instead of calcium sulfate or in the mixture with calcium sulfate, because it needs less skill to form a high quality tofu curd. In general, tofu contains 88% moisture, 6% protein and 3.5% oil, but its curd is very fragile and perishable. Therefore, the plant scale for tofu manufacturing is usually very small. Thirty thousand small tofu plants still lie scattered in Japan and each plant only uses 40-50 kg of soybeans on the average/day (28).

Recently, however, the application of modern techniques for tofu-making has made possible large-scale production of tofu. The modernization of tofu manufacturing started from silken tofu, because of its simplicity of the manufacturing processes. Different from a traditional silken tofu, this tofu is packaged in a plastic container. About 10% solid soy milk which was heated at 100 C for 3 min is cooled to room temperature and then glucono-γ-lactone is added to this cooled soy milk. Glucono-γ-lactone itself does not have the ability to let soy milk protein coagulate, but it is converted from the inactive form of lactone to the active form of gluconic acid by heating. Therefore, glucono-γ-lactone does not work in the soy milk cooled to an ambient temperature. The soy milk mixed with glucono-γ-lactone runs into a ca. 250-300 ml plastic container and is sealed with a film-sheet cover using high-frequency-wave heating. Then, the container is heated in a water bath at 80-90 C for 40-60 min. The glucono-γ-lactone is activated by this heating and as the result the soy milk is coagulated as silken tofu right in the package with no separation of curd and whey. Packaged tofu is not damaged during distribution, because it is firmly held in the package. Further, packaged tofu is a sanitary product because it is heat-treated in the sealed package. However, it must be kept in a refrigerator, because heat-tolerant microorganisms were still alive at 90 C heating. At present, there are several factories in Japan which produce 50,000-100,000 tons of packaged tofu per day (28).

Most recently, ever fresh packaged tofu which can be stored at 25 C for 3 months entered the market. This is an application of the principle of high temperature-short time heating, which has been used for long-life milk in dairy products. In the manufacturing of this product, soy milk containing ca. 10% solid is heated at 130 C for 1 sec and then cooled to an ambient temperature, followed by the addition of the glucono-γ-lactone solution from which microorganisms were removed by milipore filter. The mixture is followed by the asceptic packaging into the plastic containers and then heating for curd formation. Ever fresh tofu is essentially silken tofu and is manufactured in Japan at present, but all the products are exported because of the opposition of the many small-scale tofu makers in Japan (28).

Now, the mass production for silken tofu is easier than that of regular tofu, because silken tofu does not have a pressing process in regular tofu making. Recently, however, a continuous mass production system for regular tofu has been developed. In this system, all the processes are done continuously. The coagulation is done in a moving box and the pressing is held in a large, moving frame. The large, pressed tofu curd on the moving frame is cut into small pieces and put into plastic containers continuously. With the increase of the production scale in regular tofu making, a large amount of whey as a waste has become a serious pollution problem.

Another product relating to regular and silken tofu is an instant powdered tofu. The instant tofu powder is actually a spray-dried soy milk. This product was originally made by Japan Protein Industry (Nihon Tanpaku Kogyo) ca. 15 years ago and was mainly used as a raw material in tofu manufacturing. Tofu makers can make either regular or silken tofu in a shorter time from the powder. Recently, however, the powder was placed on the market as an instant powdered tofu by Hausu Foods Co. In the manufacture of the tofu powder, temperatures must be kept below 60 C to prevent denaturation (29,30). At present, the quick-making tofu powder is sold widely in Japan and the consumer can make a silken tofu very easily by redispersing it into water, heating over 95 C for 3 min, adding a coagulant, and then letting it stand for 40 min. This product is now available in the U.S. market.

Dried-Frozen Tofu

Dried-frozen tofu is called "kori-tofu" in Japanese. As shown in Figure 7, the tofu which was made to a firm texture (water content–80%) by using calcium chloride instead of calcium sulfate as a precipitant is cut into square blocks and frozen rapidly to -10 C within 3 hr. This frozen tofu is kept at -1 to -3 C for 2–3 weeks and then thawed. After that, the thawed tofu is squeezed to remove water. Through the aging in the frozen state, the unique sponge-like texture is formed. Consequently, most of the water can be squeezed out after thawing. The removal of the water remaining in the pressed curd can be removed by hot air drying with no case hardening, because of the sponge-like texture. The dried-frozen tofu thus obtained swells two times in volume and six times in weight by soaking in hot water. Usually, a small amount of ammonia gas is added before packaging to the product after drying in order to facilitate the swelling in the soaking before cooking. A piece of dried-frozen tofu is usually a 20-g square and contains 53–54% protein, 25–26% oil, 7% carbohydrate, 2.6% ash and 10% water. As dried-frozen tofu is preservable and easy to transport, the production scale is much larger than that of fresh tofu. There are several plants using 10–20 tons of soybeans/day (28).

Dried-frozen tofu is not fragile like fresh tofu. Before using, it must be reconstituted by soaking in hot water and then squeezing the water out. The resulting dried-frozen tofu is cut and cooked with seasonings.

Deep-Fried Tofu

Several kinds of traditional tofu products are made by deep-fat frying. When fresh tofu is deep-fat fried, it swells and forms a texture which is porous, meshy and elastic, quite different from original tofu. It is possible to prepare a product having various chewing characteristics, depending on the water content before frying, the temperature and time of frying (28). "Aburage" is the most popular type of deep-fried product. It is now made in large-scale, because it is more preservable and more easily transported than fresh tofu. Aburage is made by two-step frying at 120 C in the first step and 180–200 C in the second step. This tofu expands to 3 times the original volume. The color of the surface is yellowish-brown. The product which is made by one-step frying at 180–200 C from well-pressed regular tofu is called "namaage." Another product is called "ganmodoki," and is made by the two-step frying method from molded, mashed tofu mixed with ground vegetables and some plant seeds. The texture of ganmodoki is quite different from the original tofu and it resembles the meat of poultry. The word "ganmodoki" means "wild goose meat analog" in Japanese. New processes of these deep-fried products have been developed in which isolated soybean protein products

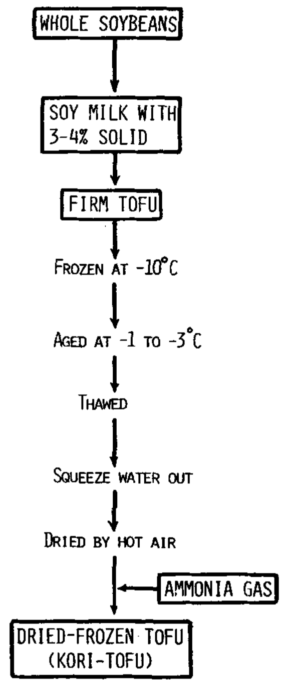

FIG. 7. Manufacturing process of freeze-dried tofu (kori-tofu).

are used instead of fresh tofu (28). The time and labor to make tofu can be saved by using isolated soybean proteins. According to recent research (31) the expansion of tofu in deep-frying is very much influenced by the amount of air present in tofu and the temperature of soy milk before making tofu curd.

FERMENTED TOFU

A fermented tofu called "sufu" in China is a cheese-like product. It has been widely consumed as a relish by Chinese people, but is not used in Japan. Sufu-making consists of three major processes: tofu-making, mold fermentation and brining. The tofu in sufu-making is made with water content less than 70%, i.e., the texture of the tofu is much firmer than that of regular tofu. The tofu thus prepared is cut into 3-cm cubes and heated. Heating is for pasteurization of the cubes and reduction of the water content on the cube surface, on which mold strains are inoculated. In mold fermentation, the molds belonging to the genera *Mucor* or *Actinomuccor* are usually used, but the molds belonging to the genus *Rhizopus* are also used. Examples of molds used are *Actinomuccor elegans, M. hiemalis, M. silvaticus, M. praini, M. subtilissimus* and *Rhizophus chinensis.* The mold fermentation time differs, depending on the varieties of mold. It takes ca. 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 morami mash, fermented soy paste or 5% NaCl solution containing rice wine having ca. 10% ethyl alcohol. The time of aging ranges from one to 12 months, depending on 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 can be used in the same way as cheese.

OTHER SOY MILK PRODUCTS

Soy milk itself is popular in China. In Japan, however, only a few people drink it, because the soy milk made the traditional way is not as bland as cow's milk and it has some off-flavors, such as green beany, rancid and/or throat-catching chalky flavor. On the other hand, soy milk changes into a very bland product once it is coagulated into a curd such as tofu. As a matter of fact, drinking soy milk is like eating tofu nutritionally. Recently, however, the techniques to make soy milk have progressed and packaged soy milk has appeared on the market in Japan. A hot grinding method (32) for soy milk manufacturing has also been introduced. There are some soy milk products to which fruit or vegetable flavors are added to mask the off-flavor. With these soy milk products, new types of beverages derived from soy milk are being marketed, such as fermented soy milk beverages. According to the recent studies on fermented soy milk beverages (33-35), the off-flavor found in soy milk disappears by both the fermentation and the addition of oil, sugars and stabilizers. The basic process of the fermented soy milk beverage is shown in Figure 8. *L. casei, L. acidophillus* and *L. bulgaricus* are usually used as the starters. An organoleptically rejectable astringent taste accompanied by a powdery or gritty sensation which is sometimes developed during the fermentation, can be repressed or masked effectively by addition of propylene glycol arginate with calcium lactate (35).

Another unique, traditional soy milk product is called "yuba." In yuba-making, soy milk is heated at a temperature just below the boiling point in a flat pan. The coagulated film formed on the surface of soy milk is scooped up successively by a fine stick and dried at room temperature. The yuba thus prepared contains 52-53% protein, 24% fat, 12% soluble carbohydrate, 8-9% moisture and 3% ash. For further reading, see refs. 36-41.

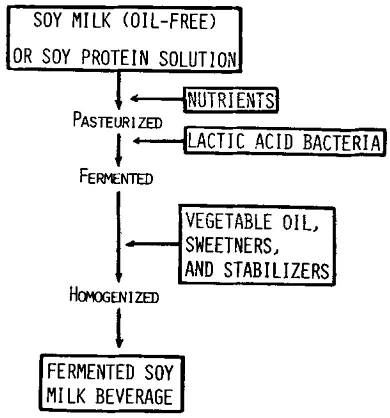

FIG. 8. Manufacturing process of fermented soy milk beverage.

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