Sufu and Lao-Chao

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Pure culture fermentation methods were developed for two centuries-old Oriental fermented foods, one a soybean product and the other a rice product. Both possess the kind of texture, appearance, and mild flavor to which the people of many areas of the world are accustomed. The changes during fermentation processes are described.

A diet composed of only rice and vegetables can be rather flat. The people in Asia, however, have used fermented products made from soybeans and cereals to add pleasure and variety to their otherwise monotonous diet. The microorganisms used in these fermentation processes modify the original materials physically, nutritionally, and organoleptically. Among these changes, perhaps flavor is one of the most important contributions from the fermentation. In fact, most of the so-called traditional Oriental fermented foods are used as flavoring agents. The flavor of these fermented foods not only makes the rice-vegetable diet endurable but, by stimulating the appetite, contributes nutritional value to people who may otherwise not consume enough food.

Traditionally, preparation of fermented foods is a centuries-old Oriental household art. Americans and Europeans know little about these products and their involved technology. Not until the late 1950's were studies on Oriental food fermentation begun in the United States at the Northern Regional Research Laboratory and at the New York State Agricultural Experiment Station, Geneva, N.Y.

The original objective of the Northern Laboratory research in this field was to solve some problems involved in the use of American soybeans by the Japanese for miso fermentation. Miso is a fermented product made of soybeans and rice. The product has the consistency of peanut butter and has the color and flavor of soy sauce. The results of our research not only increased the use of American soybeans by Japanese miso fermentation industries, but also stimulated our interests in the field of Oriental food fermentation.

Some of our results and those obtained from projects abroad were presented before the American Chemical Society about 3 years ago (Hesseltine and Wang, 1967). Now we can give additional information on sufu and introduce another fermented product, lao-chao.

SUFU

The detailed information on sufu was obtained mainly from Dr. N. Wai, The Institute of Chemistry, Academia Sinica of Taiwan, who carried out a study on sufu fermentation (Wai, 1968).

Sufu is a Chinese name for a fermented product of soybean curd. Because of the numerous dialects used in China and the difficulties of phonetic translation from Chinese to English, sufu has appeared in literature under many different names. These names confuse Americans as well as Chinese. We have found the following synonyms for sufu in the literature: sufu, tosufu, fu-su, fu-ru, toe-fu-ru, tou-fu-ru, teou-fu-ru, fu-ju, fu-yu, and foo-yue. Sufu is the name for this product that first appeared in the literature. Literally, sufu means "milk molded," and "to sufu" means bean milk molded. In the Western world, sufu has been referred to as Chinese cheese by scientists and "bean cake" by Chinese grocers. These various names, indeed, give a good description of the product. Sufu, therefore, is a soft cheese-type product made from cubes of soybean curd (tofu) by the action of mold.

The process of making sufu was considered a natural phenomena. Not until 1929 was a microorganism, believed to be responsible for sufu fermentation, isolated and described by Wai (1929). He identified the microorganism as an undescribed species of *Mucor* and proposed the name *Mucor sufu*. More than 30 years later he reinvestigated the microorganism in sufu fermentation; as a result, a pure culture fermentation for making sufu was developed. Three steps are involved in sufu fermentation (Figure 1): Preparation of soybean milk and soybean curd; molding; and brining and aging.

For preparation of soybean curd (Wang, 1967a), soybeans are washed, soaked, and ground with water. When the soluble proteins, along with some lipids and other soluble constituents, are extracted by water, so-called soybean milk results after removing the residue by filtering. The milk is then heated to boiling to inactivate growth inhibitors and to remove some of the beany flavor. The curdling process is initiated by the addition of salts, such as calcium sulfate and magnesium sulfate. After this material is pressed to remove excess whey, a soft but firm cake-like product results which can then be cut into cubes of desired sizes. This product is commonly known as tofu. So, we see that tofu is to soybean milk what cottage cheese is to cow's milk, except that the curdling of soybean milk is traditionally brought about by calcium salt and only occasionally by acid.

Nutritionally, soybean curd and soybean milk have the same importance to the people of Asia as cheese and cow's milk do to the people of dairy countries. Asians prefer the salt-precipitated curd, not only because it has the desired texture, but also it serves as an important source of calcium. Tofu, unlike cheese from cow's milk, is usually consumed without the action of microorganisms. Sufu is the only traditional fermented product of bean curd. Tofu has a bland taste and can be consumed directly—flavoring with any flavor one desires such as soy sauce and miso, or cooked with other food, such as meat, seafood, vegetables, and also used as an ingredient in soup. Tofu used for sufu fermentation has a water content of 83%, protein 10%, and lipids 4%.

To prepare tofu for fermentation, the cubes (2.5 × 3 × 3 cm) are first soaked in a solution containing 6% NaCl and 2.5% citric acid for 1 hr, and then subjected to hot air treatment at 100° C for 15 min. This treatment prevents growth of contaminating bacteria but does not affect growth of the

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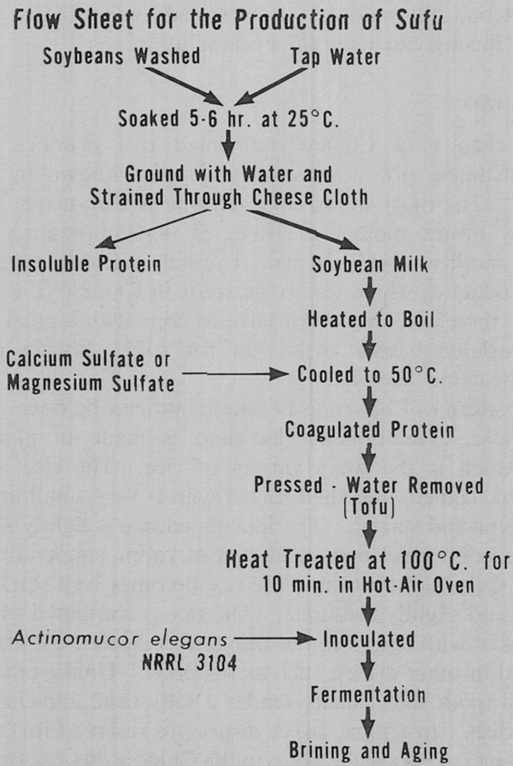

Figure 1. Flowsheet for the production of sufu

mold used in making sufu. The cubes are then placed in a tray with pinholes in the bottom and top to aid air circulation. They also should be separated from one another, because mycelia must develop on all sides of the cubes. The cubes are inoculated over their surfaces by rubbing with pure culture of an appropriate fungus grown on filter paper impregnated with culture solution, and then incubated at 20° C for 3 to 7 days, depending upon the fungus used. At this time the cubes are covered with white mycelia.

It is clear that the mold used in this fermentation has to have certain qualifications. The organism perhaps utilizes the carbon in lipids as an energy source, because proper types of carbohydrates are not readily available in the substrate. The organism must develop enzyme systems having high proteolytic and lipolytic activity, since the mold grows on a protein and lipids-rich medium. The mold must have white or light yellowish-white mycelium to ensure that the final product has an attractive appearance. The texture of mycelial mat should also be dense and thick so that a firm film will be formed over the surface of the fermented tofu cubes to prevent any distortion in their shape. Of course, it is also important that the mold growth does not develop any disagreeable odor, astringent taste, or mycotoxin. Dr. Wai and his coworkers confirmed that *Actinomucor elegans*, *Mucor hiemalis*, *Mucor silvaticus*, and *Mucor subtilissimus* possess all these characteristics and can be used to make sufu having good quality. Among them, *A. elegans* seems to be the best organism and is the one used commercially. As reported by Dr. Wai, freshly molded tofu tastes very bland, and its constituents remain the same as tofu except that the moisture content is slightly decreased.

The last step in making sufu is brining and aging. The molded cubes can be placed in various types of brine solutions according to the flavor desired. A basic and most common brine suitable to Chinese taste is one containing 12% NaCl and rice wine containing about 10% ethyl alcohol. The mixture is then allowed to age for about 40 to 60 days or longer. The product is then bottled with the brine and marketed as sufu. Figure 2 shows rows of freshly molded tofu as compared to tofu itself; Figure 3 is the final product, sufu.

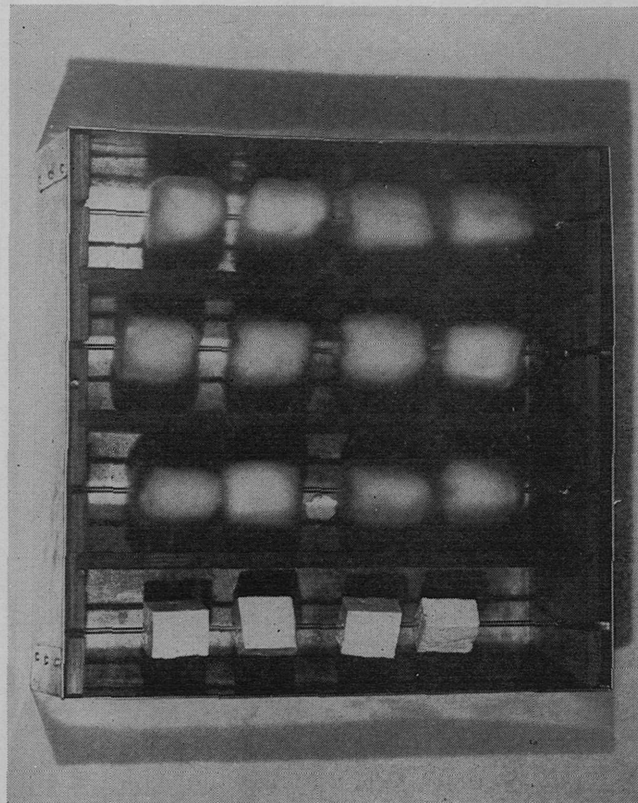

Figure 2. Luxuriant growth of *Actinomucor elegans* over cubes of tofu, as compared to the uninoculated cubes (bottom row)

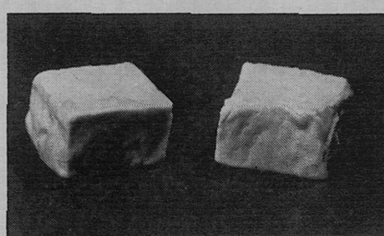

Figure 3. Sufu in its final form as removed from brine

Freshly molded tofu is bland in taste. The flavor and aroma of sufu develop during the brining and aging process. At that time, the enzymes elaborated by the mold act upon their respective substrates, yielding various hydrolytic products. Since sufu fermentation has a rather simple substrate—55% protein and 30% lipids on a dry basis—it is likely that the hydrolytic products of protein and lipids provide the principal constituents of the mild characteristic flavor of sufu. A possible explanation of the functions of the brine constituents and the changes in soybeans occurring during aging has been proposed.

The soybean proteins are digested by the proteases produced by the mold into peptides and amino acids. Free amino acids, such as aspartic acid, glutamic acid, serine, alanine, and leucine/isoleucine, were found by Wai in the water-soluble fraction.

The soybean lipids are also digested to some extent to fatty acids. The added alcohol reacts with the fatty acids chemically or enzymatically to form esters providing the pleasant odor of the product. Ethanol also prevents growth.

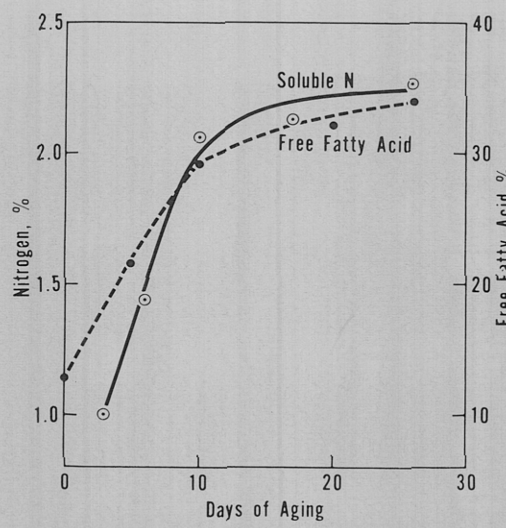

Figure 4. Time course of changes in soybean proteins and lipids during aging of sufu fermentation

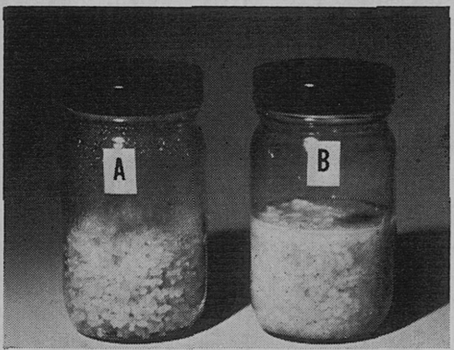

Figure 5. Steamed glutinous rice before (A) and after (B) lao-chao fermentation

The added salt imparts a salty taste to the product, as well as retards the mold growth and the growth of contaminated microorganisms. Most important, the salt solution releases the mycelia-bound proteases. In sufu fermentation, the mold growth is limited to the surface of the cubes, and the mycelium does not penetrate into the tofu cubes. The enzymes produced by the mold, on the other hand, are not extracellular (Wang, 1967b). They are loosely bound to the mycelium, possibly by ionic linkage. However, the enzymes can be easily eluted by NaCl or other ionic salt solutions, but not by water. Therefore, the NaCl brine solution also serves to elute the enzyme from mycelia which, in turn, penetrate into the molded cubes and act upon the substrate protein.

Traditionally, it is said that the flavor and aroma of sufu improve as the aging process progresses. From a time-course study of the enzyme digestion, Wai demonstrated that enzyme digestion occurred mostly during the first 10 days of aging (Figure 4).

Other additives, either to give color or flavor, are frequently incorporated into the brine. Red rice and soy mash are added to the brine, imparting to the product a red color; when these ingredients are added, the final product is known as red sufu or hon-fang. Fermented rice mash or a large amount of wine can be added to the brine, so that the product has more of the alcoholic fragrance. This product is known as tsui-fang or tsue-fan, which means drunk sufu. The addition of hot pepper to the brine would make hot sufu. Rose sufu can be made by aging in a brine containing rose essence. Therefore, the taste and aroma of sufu, in addition to its own characteristically mild one, can be easily enhanced or modified by the ingredients of the brine solution.

LAO-CHAO

Lao-chao is a Chinese fermented rice product. Like many Chinese products, lao-chao is also known by other names. The most well-recognized one is chiu-niang, which literally means mother of wine, or tien-chiu-niang, which means mother of sweet wine. The only scientific report on this product of which we are aware is in Chinese (Liu *et al.*, 1959); therefore, we do not have to cope with a great array of translated names as we do for sufu. Frazier (1967) used the name "lao-chao."

Preparation of lao-chao is simple and can be done in any household. Traditionally, lao-chao is made of glutinous rice, which is the waxy variety of rice. The rice is first steamed, cooled, and then mixed with a very small amount of commercial starter. The inoculated rice is lightly packed in a covered bowl and incubated at room temperature for 2 to 3 days. At that time, the rice becomes very soft, juicy, sweet, and slightly alcoholic. The rice is consumed as such, or cooked with eggs or other things as a dessert. It also can be used in other dishes, such as seafoods. Unlike other fermented foods that usually render a salty taste, lao-chao is a sweet dish. In China, sweet dishes are reserved for special occasions. This special place in the Chinese diet has brought about the relatively high price of the product, although it is not made expensively. Lao-chao, therefore, is not consumed daily by all segments of people. However, lao-chao has a unique place in the diets of new mothers. They believe that lao-chao helps them regain their strength.

The commercial starter used for lao-chao fermentation is known by the Chinese as chiu-yüeh or peh-yüeh. In our laboratory, we have often referred to it as Chinese yeast ball. It is a grayish-white ball made with rice flour and contains yeast and fungi.

Recently, we began to investigate the lao-chao fermentation. Drs. Hesseltine and J. J. Ellis studied several samples of the commercial starter obtained from Taiwan. They consistently found members of Mucoraceous fungi, including *Rhizopus oryzae*, *Rhizopus chinensis*, and *Chlamydomucor oryzae*, and a yeast which Dr. L. J. Wickerham identified as a species of *Endomycopsis*. By following the traditional method, we have made a satisfactory product (Figure 5) by using *R. chinensis* NRRL 3671 and *Endomycopsis* sp. NRRL Y7067 instead of a commercial starter. *C. oryzae* and the yeast, on the other hand, make a somewhat inferior product.

Lao-chao has a taste of sweet tartness and a distinctive fragrance of fruit aroma. The juice of lao-chao made in our laboratory contains about 1 to 2% of ethanol and 20 to 30% of reducing sugar measured as glucose. A mixture of esters, perhaps, gives rise to the fruit aroma of the product. We suggest that the following changes occur during fermentation.

Starch is the most important constituent in this fermentation. The amylase and other glucosidases produced by the organism break down starch to glucose, which is then used by the yeast. Although *Endomycopsis* is one of the few yeasts that will produce amylases and utilize starch, the utilization of starch by the yeast may not be important or efficient in this fermentation because the yeast is highly oxidative and the fermentation condition is semianaerobic. The lipases break down the lipids of rice to fatty acids, which react with alcohol to form a mixture of esters, providing the pleasant odor. However, strains of *R. chinensis* are known to produce large amounts of lactic acid. The lactic acid-producing strains of *Rhizopus* organism yield equal molar ratio of lactic acid, alcohol, and CO_2 when grown under anaerobic conditions. A mixture of esters, therefore, can be formed without the yeast fermentation. In fact, steamed rice inoculated with the spores of *R. chinensis* yielded a product having a very pleasant fruit aroma; but, it was dry and had a moldy look. Yeast seemed to stimulate the utilization of sugar and the fermentation process. On the other hand, *C. oryzae* does not give so pleasant an aroma as *R. chinensis*.

When nonwaxy rice was used, black spore formation was abundant and resulted in an unsatisfactory product. Presumably, the nonwaxy rice is not sticky enough to create the kind of anaerobic condition that the mold requires for lao-chao fermentation.

The strains of *R. chinensis* used in lao-chao fermentation produces an antibacterial compound. *R. oligosporus*, the mold used in tempeh fermentation, which is an Indonesian soybean product, also produces the same compound (Wang *et al.*, 1969). The compound is active against many Gram-positive bacteria, including some typical intestinal clostridia. As we know, it is well established that antibiotics, in addition to minimizing infection, also have a growth-stimulating effect on animals fed an inadequate diet. Therefore, our finding that antibiotics are produced by the molds used in lao-chao and tempeh fermentations will help to explain the true nutritional value of tempeh and lao-chao.

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