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# SOY AND RELATED FERMENTATIONS.

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## INTRODUCTION.

Soy sauce is a dark-brown salty liquid made by the fermentation of soy beans with, as a rule, some additional starchy component. It is widely used as a seasoning throughout Japan, China, and Java, and has been introduced into the Philippines (*3*)[2] and Hawaii (*18*).[3] Where the occidental would use a vegetable or meat extract and salt, the oriental daily uses soy sauce. Americans are familiar with soy sauce as it is used in the Chinese-American restaurants and as the ingredient which produces the characteristic flavor of the Worcestershire type of sauce. The increasing popularity of highly seasoned foods in the United States may reasonably be expected to develop the use of soy sauce and of table sauces having a flavor more positively that of the ingredient, soy sauce.

The soy bean (*14*), an annual leguminous plant, native to southeastern Asia, has been cultivated in the East for more than 5,000 years. Rich in protein and oil, though deficient in starch, it has been used as a food and for technical purposes. It is now an important crop in commerce, large shipments being made to America and Europe. Although introduced into the United States in 1804, it has only recently been cultivated to any great extent in the South.

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[1] The experimental work here reported was conducted under the direction of Charles Thom, mycologist in charge, Microbiological Laboratory, Bureau of Chemistry.

[2] The italic numbers in parentheses throughout this bulletin refer to the bibliography on page 25.

[3] From a letter from C. W. Carpenter, September 23, 1918.

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The process of manufacture which produces soy-bean sauce, or, as it is called in Japan, shoyu, begins with the preparation of the ingredients and includes a preliminary mold fermentation, followed by a ripening in brine. The soy beans, having been cooked and mixed with prepared wheat, are inoculated with the shoyu mold or ferment. This mold is procured commercially in Japan under the name of "tane-koji," in which form it consists of starchy rice particles overgrown with the vegetative and yellow-green fruiting portions of the fungus. The action of the shoyu mold and its enzymes on a mixture of cooked soy beans and crushed roasted wheat, under specific conditions of temperature and moisture, produces in from three to four days a mold-fermented product known in Japan as "shoyu-koji."

The mold-fermented material is emptied into a strong brine, thus producing a mash. Constant daily attention is given to aeration, even distribution, and stirring of the solid ingredients. Progressive changes take place over a period of from six months to several years, until at last mature "moromi," as the mash is designated by the Japanese, is produced. These changes are due partially to the activity of bacteria and yeasts, but chiefly to the enzymes of the mold introduced into the mash with the koji. Purely chemical alterations in the ingredients also appear probable.

The rather thick, dark-brown mash resulting is siphoned or pressed to produce the soy sauce, which is brought to a boil, filtered, and in the more modern of the Japanese factories processed or partially pasteurized. The completed sauce is distributed in casks or bottles.

## WORK OF PREVIOUS INVESTIGATORS.

The first experiments in the Bureau of Chemistry were based on a study of available European literature. The principal aim of the early reports consulted was not to outline a process of manufacture. Rein (*17*) and Hoffmann (*5*) give a few pages of directions for making soy-bean sauce. Kellner (*6*) and Prinsen Geerligs (*16*) deal with chemical analyses of soy sauce and related products. The proper measures of soy beans and wheat to use in making soy sauce may be roughly estimated from a comparison of such analyses.

Yukawa (*27*) discusses the carbohydrates of the soy bean in their relation to the ripening of shoyu. Suzuki and Furuya (*21*) discuss the chemical changes which occur during shoyu fermentation. Mitsuda (*13*) has published a report on the carbohydrates in soy sauce.

The organisms concerned in the ripening of the mash or moromi are not well known, although attempts to gain this information have been made (*8*, *9*, *19*, *23*). Kita (*10*) discusses the whole process of making soy sauce. The more recent technical literature of Japan contains several references of importance to the shoyu industry, including a very practical piece of work on factory coefficients of shoyu by Kinoshita (*7*) and an elaborate manual for shoyu manufacture by Togano (*25*).

A report on soy manufacture in Kwangtung, China, has been published by Groff (*4*), and data prepared by Shih Chi Yien (*26*) have been made available to the department by Miss Cora D. Reeves of Ginling College, Nankin.

## EXPERIMENTAL WORK.

The Department of Agriculture had certain strains of the *Aspergillus flavus-oryzae* group of molds (*23*, *24*) known to be used in making soy sauce. Through the courtesy of W. T. Swingle, of the Bureau of Plant Industry, a can of commercial Japanese rice tane-koji designed for shoyu manufacture was also received. Dr. Gen-itsu Kita brought additional samples of shoyu tane-koji under sterile conditions directly from Japan. Provided thus with soy beans, wheat, and the mold ferment, experiments with soy sauce were undertaken by the Bureau of Chemistry in 1918.

### APPARATUS.

The apparatus was made according to specifications drawn by Doctor Takahashi, of the Imperial University of Tokyo, who worked in the bureau for a month.

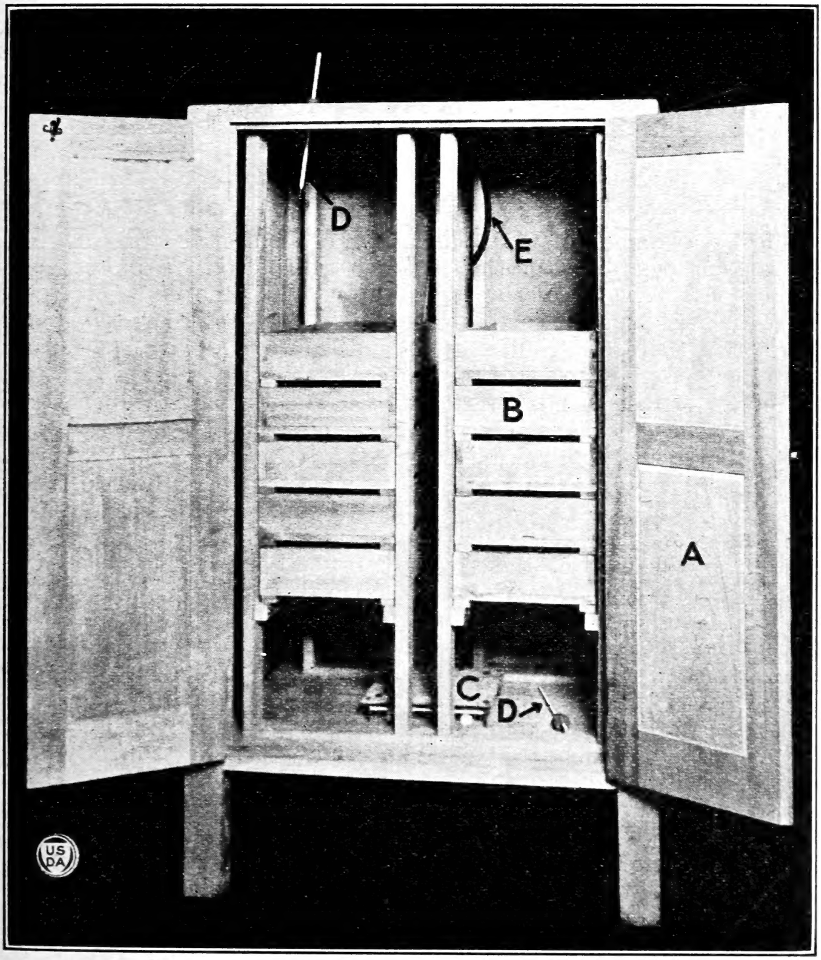



FIG. 1.—Experimental koji room used in Bureau of Chemistry. A, doors; B, trays on racks; C, electric plate; D, thermometers; E, tube for blast of cool air.

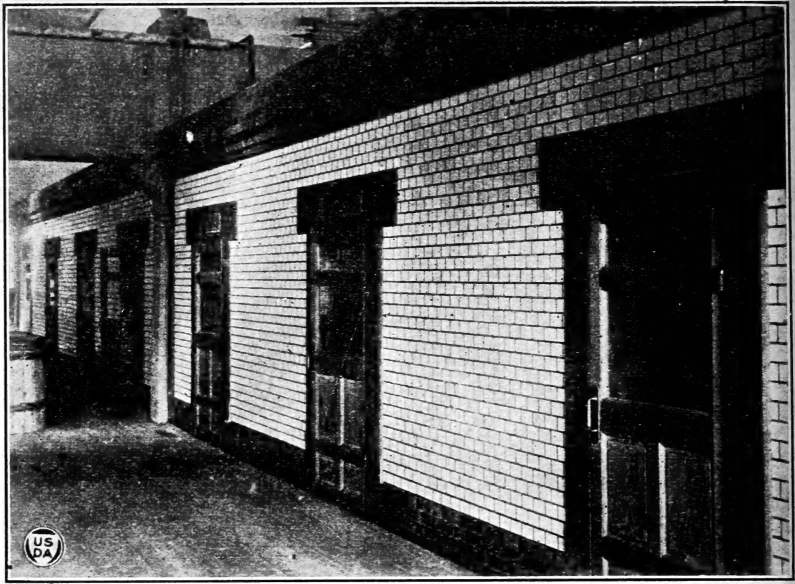


FIG. 2.—Japanese koji room (exterior).

The preliminary mold fermentation of shoyu is best carried on in shallow trays made of wood (fig. 1), each separated from the others by wooden legs placed on the corners of the bottom of the tray. The inside measurements of the trays were 17 by 8 inches, with a depth of 2½ inches. A soft wood, neither shellacked nor coated, was used. The trays, therefore, could absorb moisture to an extent sufficient to keep condensed water from running down upon the koji.

For experimental purposes a case, 38 inches long, 28 inches high, and 22 inches deep (fig. 1), was constructed to serve as a koji room, or compartment, for the trays. Sixteen trays were stacked in two tiers in this case, with 2 inches of air space between the two tiers of the trays and on the four sides, and a space of 3 inches from the uppermost tray to the top of the case and one of 9 inches from the bottom of the case to the lowest tray. This greater space at the bottom of the case prevented an electric plate which was introduced from heating any one tray too intensely. The electric plate kept the miniature koji room from becoming too cool in a cold season. It was insulated with asbestos to guard against ignition of the wooden case. The air space as a whole allowed for the absorption of moisture generated by the mold growth which occurred during the fermentation process. It was found, however, that sufficient space was not allowed for this purpose when all 16 trays contained immature koji where very active mold growth was naturally going on. The case, as well as the trays, was made of nonresinous wood. Its temperature and moisture, when not controlled by the fermentation process itself, were regulated by means of an electric thermostat, supplemented by a blast of cool air. As the case was not air-tight, a satisfactory circulation of air was thus secured.

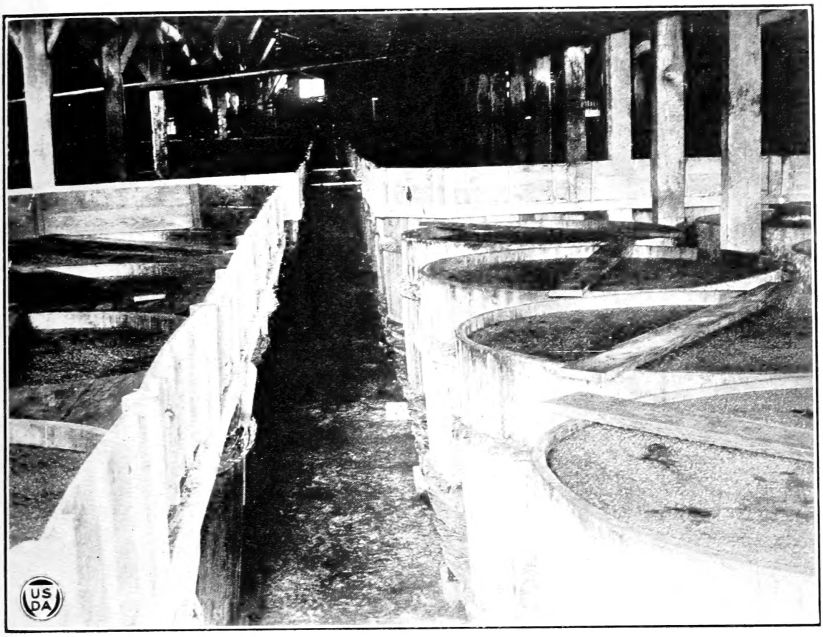
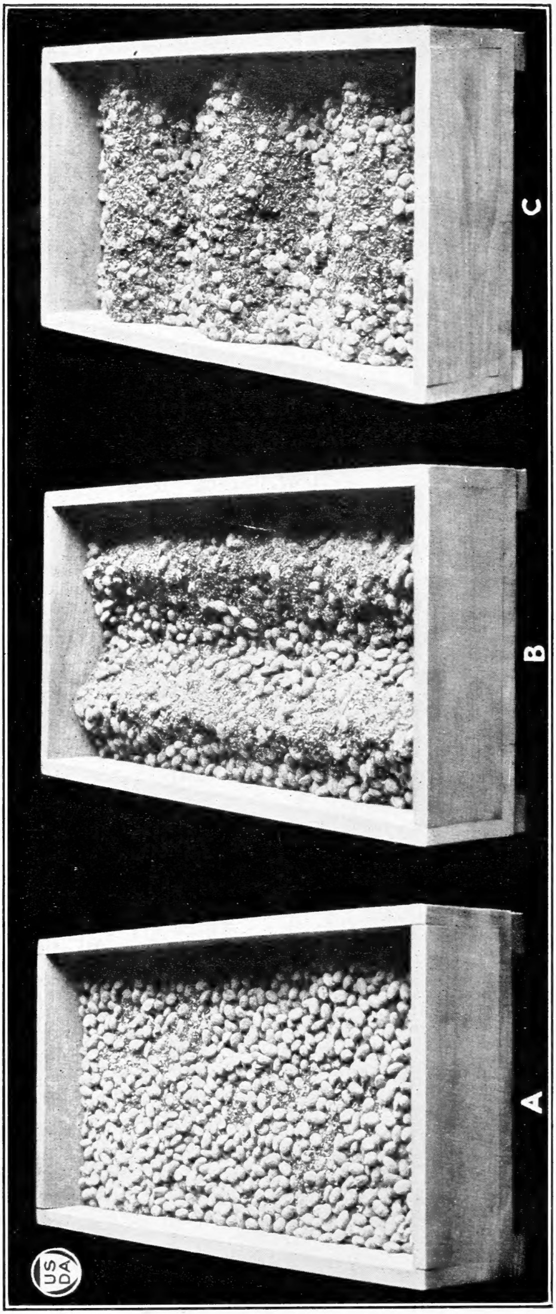


FIG. 1.—Tubs of shoyu-moromi in Japan, showing beans floating on the surface of the mash. (Reproduced by courtesy of G. Kita.)

FIG. 2.—Ripening of soy sauce mash in Ichang, Hupe, China. "In the center of the large jars, full of fermented soy beans and brine, strong small baskets have been placed, in which the soy sauce accumulates and is dipped out, ready for use. This method of obtaining the soy sauce is possibly local." (Reproduced from a photograph taken in 1917 by F. N. Meyer.)




(A) Shoyu-koji overgrown with white mycelium of mold ferment; (B) shoyu-koji after stirring and heaping in two piles; (C) shoyu-koji after second stirring and heaping in three piles.

The usual Japanese koji room (fig. 2) is 32½ feet long, 11 feet wide, and 7 feet high. Its walls are thick, and in the more modern factories are built of brick, which does away with fluctuations in the temperature from without. At one end of the room is an entrance and at the opposite end a window (fig. 3). In the ceiling several openings provide means of escape for the carbon dioxid and the damp air. Steam pipes along the floor make it possible to warm the room in cold weather. The ceiling is built with many layers of straw in order that the condensing moisture may be absorbed. One disadvantage of such a ceiling is that infection always occurs in the wet straw. A large area of infection directly over the piles of koji trays is detrimental to the production of sweet koji. In modern buildings, therefore, the surface of the ceiling is coated with cement. When a cement ceiling is used the condensed water drops on the trays of koji, which also is harmful.

The koji room is naturally always well inoculated with the spores of the mold used in making the koji for shoyu. The presence of these green Aspergillus spores is not detrimental unless the growth of the tane-koji alone is desired for the koji. The koji room may also become infected with other troublesome fungi. Insects, too, occasionally overrun the room. The burning of sulphur is useful in combating any infection of a koji room.

The fermentation or brining, which produces the moromi, is carried on in crocks or wooden vessels of any kind, such as tubs, barrels, or vats (Pl. I). According to Takahashi, the inside of wooden receptacles may be painted, paraffined, or even pitched, without injury to the mash.

FIG. 3.—Japanese koji room (interior).

## MATERIAL.

A yellow, round soy bean, such as the Yellow Mammoth variety, was selected as preferable for soy sauce, because it is rich in oil and in protein and has practically no starch.

An eastern-grown American wheat, the Stoner variety, which is rich in starch, was chosen by the Office of Cereal Investigations of the Bureau of Plant Industry as a variety close to that used in Japan. Only pure clean water should be used for soaking the beans and in making the brine. A biologically impure water, one contaminated with sewage organisms or factory waste, should not be used. The obnoxious bacteria in sewage-contaminated water become active readily in the beans during soaking and may disturb the growth of the mold ferment in the early part of the fermentation, later producing bad effects or off-flavor in the mash.

The mold ferment employed in shoyu-koji manufacture is *Aspergillus flavus* Link, occasionally *A. oryzae* (Ahlb.) Cohn, or strains intermediate between the two species (*24*). A mixture of several strains is frequently found in the tane-koji. The particular tane-koji used in the laboratory experiments was a strain (Bureau of Chemistry No. 4272.2) of the *A. flavus* group grown on rice grains and similar in morphology to Bureau of Chemistry No. 108,[4] a strain of *A. flavus*.

Commercial, not chemically pure, salt was used for the moromi. According to Japanese authority, experimental work has been successful with purified sodium chlorid only occasionally and commercial practice never. Foreign substances other than basic salts of calcium and magnesium, often found in even a fair grade of bulk commercial salt, will not interfere with shoyu making. This manufacturing process, like all others, however, should be conducted under sanitary conditions. Sea salt is used as a rule in Japan.

Certain Japanese manufacturers add cultures of pure yeast belonging to the genus Zygosaccharomyces (*23*) at the time of placing the first mold-fermented material in the brine.

## PREPARATION OF INGREDIENTS.

In preparing shoyu-koji the soy beans are soaked in running water for about 20 hours (fig. 4) and then cooked until they are rather soft. Unless the water is changed during the soaking a rapid fermentation, due to spore-forming rods, occurs. These bacilli as spores are on the beans as they come from the field. Beans soaked in unchanged water become warm, even hot, and sour at the bottom of a mass 5 to 6 inches deep in two or three hours at 22° C. (72° F.). Such beans, even after autoclaving, are sour to the taste. It is the customary factory practice in Japan to soak the beans with changes of water at intervals of several hours. In laboratory experimentation beans were placed in running water for 20 hours and cooked on the following day. The hours used as an illustration here may be changed to conform to factory working hours. The intervals given, however, should not be changed beyond reason.

[4] Obtained from former Centralstelle für Pilzkulturen, a collection now in the custody of Dr. Johanna Westerdijk, Phytopathologisch Laboratorium, Willie Commelin Scholten, Javalaan, Baarn, Holland.

After being soaked for 20 to 24 hours the swollen beans are cooked in an open kettle or under pressure until they are soft enough to be easily pressed flat between the thumb and finger. This desired softness can be obtained by autoclaving at 15 pounds pressure for 50 minutes and also by much longer cooking in an open kettle. Autoclaving under pressure has the advantage of sterilizing the material. A slight excess of water, just more than enough to cover them, is added to the beans before autoclaving. The beans are drained as soon as the autoclave runs down.

When the cooking and draining have been completed the beans are spread out to cool in about a 1-foot layer on a large traylike platform, being turned over from time to time to hasten the cooling. Or they may be spread in wire trays and cooled with the draft of air from an electric fan. The rapid cooling of the hot sterile beans prevents the growth of organisms collected from utensils and handling and further lessens the exposure incident to stirring or turning.

The wheat is first roasted and then crushed unevenly. The roasting should be continued until the wheat is crisp but not tough, and is browned to produce a slightly charred flavor. It is said that some manufacturers of soy sauce roast the wheat only slightly while others char the cereal. The browned wheat is believed by the Japanese to add flavor to the finished product through the formation of maltol due to activity of yeasts during the moromi stage of the fermentation. It also adds a desired brown color. In the roasting of the wheat practically all microorganisms are killed. If the wheat were browned slightly more than was the practice and heaped up hot in large quantities, as in shoyu factories of Japan,


FIG. 4.—Increase in volume of beans caused by soaking. Each pile contains the same number of beans.

all organisms would undoubtedly be killed. After roasting, the wheat is crushed, the crushing being carried to the extent of breaking the grains into large pieces. Furthermore, the crushing should be of such a character as to reduce some portions of the kernels to a fine powder or wheat dust. A supply of roasted wheat may be kept on hand and crushed as needed.

The sterile softened beans and the crushed and powdered wheat are mixed in large trays or on mixing tables (fig. 5). All surplus water having been drained from them, the beans are cooled down to below 28° C. before being mixed with the wheat and the tane-koji. If the beans are needed immediately they may be cooled with a draft of air directed over a thinly spread layer. If it is necessary to allow the cooked beans to stand for a while before being used, they may be spread out or heaped in low piles and covered with a cloth or canvas.

FIG. 5.—Inoculating soy beans and wheat with mold ferment for shoyu-koji in Japan. (Reproduced by courtesy of G. Kita.)

The beans and wheat need to be thoroughly mixed in such a manner that the beans are held apart. The angular pieces of wheat when evenly and thoroughly distributed among the beans serve as a mechanical means of separating the wet smooth beans which would naturally pack much closer. The interstices are filled with the finer wheat particles to a certain extent, but not enough to check aeration. It is well to have the wheat, rather than the beans, on the surface. Furthermore, these two ingredients need to be thoroughly mixed, so that the wheat dust may form a coat over each bean. The surfaces of the beans treated in this manner have a lower water content than when the precaution of thorough mixing is not taken. The dry wheat dust takes up moisture readily. The lower water content thus induced on the exterior of the beans is better adapted to mold growth than to bacterial growth.

### SHOYU-KOJI.

#### Ripening.

After the beans and wheat are thoroughly mixed, a very small quantity of previously molded material, such as mature rice koji (tane-koji), some shoyu-koji, or a pure mold culture, is thoroughly mixed into the ingredients. The whole mass is then distributed in the small flat koji trays (Pl. II) which are immediately placed in the koji fermentation room before they cool further. Each tray holds about 1.8 liters, or about 2 quarts, of raw material. The koji trays are placed in tiers along the wall of the room. (Fig. 3.) Unless provided with little legs, they are not placed closely over one another but in a zigzag fashion, so that there is free space between each tray and the next. The favorable aeration thus secured is extremely important in shoyu-koji fermentation because moisture and the lack of oxygen induce the development of mucors and bacteria, and are said to cause the diastatic enzyme to develop at the expense of the proteolytic enzyme.

In some localities in Japan no such trays are used, but a broad straw mat with which very good koji can be secured. The mat may be as large as or larger in area than the tray and the immature koji is spread on it to the same depth as on the tray.

If the trays have been previously used to produce clean molded material for shoyu, no further inoculation than that to be obtained from the spores already on the trays is necessary. When koji trays are used over again without being washed or disinfected, they should be carefully wiped with dry cotton or clean waste of some kind in order to remove small masses of koji or heavy spore inoculation. Otherwise, the fresh koji will be too heavily inoculated with spore material.

After the ingredients of the koji have been distributed in the koji trays, they are leveled to the depth of 3½ centimeters, or 1½ inches, or they may be heaped in one pile in such a manner that they do not touch the sides of the tray. Piling the beans is a good method when the temperature is slightly low, but care should be taken not to pile them to a depth of over 4 centimeters. A deeper pile heats excessively. Overheating is usually brought about by the development of undesirable bacteria, which by their rapid growth increase the condensation of moisture. Further bacterial activity is fostered by this condition.

The koji room or compartment is kept at a temperature of 24° to 25° C., with a definite humidity. The temperature of the koji is at the start between 24° and 28° C., if the mixing has not been too greatly delayed, or it may be as low as that of the mixing room. Eighteen hours after the trays have been stacked in the koji room they need to be examined. (Pl. II, A.) The koji should have at this time a temperature of from 27° to 29° C. Any higher temperature is due to serious bacterial contamination, or to unsatisfactory conditions of temperature and humidity in the koji room.

At the end of 18 hours the koji is stirred, the bottom being brought to the top, and the beans are broken apart. Thorough stirring is

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necessary, for the beans and wheat are now bound by the mycelium or white threads produced by the shoyu-mold. Every bean should be covered with this white growth. If the white mycelium is not distinguishable with the trained naked eye, the koji is too immature to be disturbed. This immaturity may be due to low viability in the spore material, to insufficient inoculum, to low temperatures, or to temperatures high enough to allow inhibiting bacteria to grow. The koji at its best has a heavy surface felt of mycelium, matting the beans and wheat together in a solid cake. There is, however, no objection to a scantier growth. Sporulation must not have begun. Even the slightest visible indication of fruiting at this age marks shoyu koji as being not of the highest grade. This immature koji should be spread out and exposed by stirring until it has cooled down to 24° or 25° C. (70° to 72° F.) or lower.

The koji, instead of being leveled at the end of the 18 to 20 hour period, is this time heaped in two piles, extending the length of the tray. (Pl. II, B.) The material should come in contact with the sides of the tray as little as possible, since condensed water on the surface of the saturated wood induces bacterial growth when absorbed by the tray or retained by the beans and wheat. The wheat, rather than the beans, should be at the surface of the two heaps. After the stirring is completed the trays of 18 to 20 hour koji are replaced in the koji room where they remain undisturbed until six or seven hours later. The temperature of the koji room may now be just under 30° C. with safety, while that of the koji itself should be between 27° and 29° C. The mycelial growth is now heavier, but no sporulation should be evident.

At the end of this second incubation period, the koji is stirred thoroughly a second time, the beans and wheat being broken apart. The mass is cooled below 24° C. After this stirring, four furrows, running the short way of the tray and forming three heaps, are made. (Pl. II, C.) The trays of koji are again placed in the koji room.

When examined at the end of 40 hours, 12 to 14 hours after the second stirring, the surface of the koji should be a clear "flavus" or yellow color from the fruiting heads of Aspergillus. This yellow color does not develop if the temperature overnight has been 50° to 55° C., causing a drying and apparent burning out of the surface, or if the moisture has been excessive enough to effect bacterial multiplication. The temperature of the best koji should now be not much above 35° C., although 35° to 36° C. is permissible. Koji with a temperature of 42° to 45° C. at the end of 40 hours may appear excellent in spite of the high temperature. This is not good factory practice, however, as the greater heat is due to bacterial activity. If the temperature of 40-hour koji is over 35° C., the product may be cooled to advantage by so breaking apart each heap as to form several cracks in the solid mass, or, if more extreme cooling is necessary, the koji may be be broken into chunks and turned over, leaving exposed as large a surface as is possible. In the climate of Washington, D. C., shoyu koji may be carried through the second stirring in 30 hours and be mature in less than 2½ days. Usually 24 hours is sufficient for the maturing of the koji, a stage in the fermentation which extends from the beginning of evident sporulation on the surface to mature sporulation throughout the interstices of the mass of beans and wheat. Mature koji has a clear yellow to yellow-green color on the surface and throughout the whole mass. If well ripened it may be lifted from the tray as one entire block.

The koji may become infected with *Rhizopus nigricans* if the atmosphere of the koji chamber is moist to the point of condensation as drops. A little Mucor or Rhizopus is disregarded in the material, unless a bad flavor or odor is also present. It is poor practice, however, to allow the Rhizopus to enter. If allowed to gain a foothold, its fruiting to any extent may be prevented by breaking up the koji into chunks and turning these chunks bottomside up. Instead of exposing a large surface, as in the case of bacterial infection, care should be taken to have only surfaces where Rhizopus has secured no firm footing exposed to the air. Trays of koji infected with Rhizopus should be stacked in a cool, dry place until the material is mature or needed for the shoyu-moromi.

### Proportion of Wheat.

Experiments were conducted to determine the proportions by weight of prepared beans and wheat necessary for shoyu-koji. The quantity of wheat by weight to be used was made the varying factor.

Soy beans were put to soak in running water. The water was always very cold and no warmth resulted among the beans, even at the bottom of the container. Initial bacterial contamination was thus made negligible. After being cooked for 50 minutes, at 15 pounds pressure, the beans were divided into four lots of 600 grams each. Roasted and crushed wheat was added to the beans in the proportions of 3 to 6, 2 to 6, and 1 to 6, there being one lot to which no wheat was added.

Where wheat was used with the beans in the proportion of 3 to 6, the resulting shoyu-koji was satisfactory and had a clean, sweet odor. Similarly, where wheat was used in the proportion of 2 to 6, the koji was excellent, with a sweet odor and fine fruiting of the mold below the surface of the mixture. The proportion of 1 part of wheat to 6 parts of beans did not afford conditions for satisfactory maturing of the koji. An odor of ammonia was present under these circumstances, as well as a slightly putrid condition. The mold, however, fruited in large quantities. This shoyu-koji was discarded as unfit for use in shoyu, for the ammoniacal odor and slightly putrid condition indicated that bacteria capable of rapidly breaking down protein were present to a large extent. Where no wheat was added to the beans the koji resulting under the method of fermentation followed was rotten and had a strong odor of ammonia. The mold was fruiting in a restricted fashion even in this instance. This shoyu-koji also was discarded as unfit for use.

These results indicate that koji for shoyu prepared after the Japanese fashion requires for a satisfactory development and ripening at least one part of prepared wheat to three parts of prepared beans by weight. This proportion of wheat is somewhat less than equal parts of the unprepared ingredients by volume. Successful shoyu-koji having been made day after day for many weeks in the Microbiological Laboratory, sufficient previous experience had undoubtedly been acquired to judge fairly these four experimental lots of koji.

### Color.

Mold-ripened soy beans and wheat are usually clear yellow when prepared by the Japanese method for shoyu-koji. This coloration is due to pigments of the spores or seedlike bodies of the mold. Occasionally the color of the mass of beans and wheat is yellowish green. The reason for this variation is not easily defined. Temperature, humidity, heaviness of seeding, and rate of growth undoubtedly affect the color development. A lack of green in the fruit of the *Aspergillus flavus-oryzae* group is invariable when the mold is growing on material free from carbohydrates under culture conditions.

The ingredients of soy sauce afford wheat starch in a quantity presumed to be sufficient only to stimulate mold growth. In the work conducted in the Bureau of Chemistry, microscopical evidence is against this supposition, since, in this work at least, the supply of wheat starch was not always exhausted. Even in the brined mash or moromi, innumerable starch grains as yet showing no signs of corrosion were found. The color variations in the *Aspergillus flavus-oryzae* group are more easily comprehended in the consideration of pure laboratory cultures than in that of the more unstable mold-fermented beans and wheat (*24*). Environmental as well as nutrient factors need to be investigated in order to understand the clear yellow color of shoyu-koji.

### Bacterial Count.

Condemnation of batches of koji may be readily based on the presence or absence of an ammoniacal odor or evidence of putrefaction. Any odor of ammonia or of putrefaction, any sticky condition, or any appearance of rottenness in koji is due to bacterial and not to mold activity. The bacteria involved in the ripening of shoyu-koji in these experiments belong to the *Bacillus mesentericus* and *B. vulgatus* groups (*2*).

The opportunities for bacterial contamination from the soy beans and wheat were considered. Soy beans as they came from the field were studied in plain broth. The organisms recovered were club-shaped rods (4.8 by 7μ), free spores (1μ in diameter), short rods (2.5 to 5 by 0.5μ), short thick rods (4.5 by 1μ), and germinating spores. Soy beans soaked for 24 hours in cold running water and then cooked for 30 minutes at 15 pounds pressure showed no growth of micro-organisms on culturing. Soy beans soaked for the same period in unchanged water having a temperature of 20° to 22° C. were cooked in a similar manner. Cultures developed short, thick rods.

Whole wheat as it came from the market, when cultured in a routine manner, developed spore-bearing rods with rounded ends, measuring 1 to 1.5 by 3 to 5μ. The same wheat when roasted and exposed in a room for cooling developed only mold. When the wheat sample was taken directly from the roaster no mold developed. The mold which developed undoubtedly came from the air of the laboratory which was thoroughly infected with molds from frequent handling of mold cultures and the making of large quantities of shoyu-koji. All bacteria discussed here are aerobic spore formers belonging to the *Bacillus mesentericus* and *B. vulgatus* groups.

### PEANUT PRESS CAKE KOJI.

A sample of commercial peanut press cake, with a water content of 8.6 per cent, contained 50,000 bacteria per gram. These bacteria were predominantly of the *Bacillus subtilis* and *B. mesentericus* groups (*2*). Three thousand grams of this cake cooked in 2,000 cubic centimeters of water for 50 minutes at 15 pounds pressure[5] was not sterile. The container used was a crock and the material was undoubtedly deep enough to prevent sterilization in such a container. When held overnight at room temperature (24° to 26° C.) this cooked cake became viscid, and smelled of ammonia.

Three parts of the peanut press cake was steamed in two parts of water for one-half hour. There was no excess of water. The wet cake was placed on a huck towel[6] in a wire basket, to a depth of 1½ inches, when treated with steam. A wet huck towel also covered the cake to prevent superficial drying. This material was autoclaved for one hour at 15 pounds pressure. No bacteria survived, as shown by the sterile cultures.

This last procedure was repeated with the following variation. The cake was heaped in the wire trays instead of being spread evenly in the tray. The bacterial count at 37° C. for 24 hours was 100 bacteria per gram. This last lot of peanut press cake[7] was mixed with 900 grams of roasted and ground wheat. Its further treatment was that already outlined.

The experiment was completely set up at 3 p. m. of April 1. On April 2 at 1.30 p. m. there was a delicate growth of mold, a very slight local viscosity (due to bacteria) near the wood of the trays, and a sweet nutty odor. At 3 p. m. further mold growth and a warmth to the material were noted. At 3.35 p. m. one (1) tray was hot (35° C.), two (2 and 3) were warm (30° C.), and the last (4) was becoming hot (31.5° C.). The temperature of the compartment was 27° C. The contents of trays 1 and 3 were viscid to slightly viscid. Tray 4 contained excellent koji from all external appearances. The growth of mold had caked the material. This peanut press cake koji was mature in less than 48 hours. The temperature of tray 1 at 5.30 p. m. of April 3 was 41.5° C. Its contents were slightly viscid, drying out, and gave a positive test for ammonia with fuming hydrochloric acid. The mold had grown throughout with a yellow color against the wood of the tray, and the bacterial count was 100,000,000 bacteria per gram. The material in trays 2 and 3 ran an approximate bacterial count of 700,000,000 bacteria per gram. The temperature of tray 4 at 9.30 a. m. of April 3 was a little more than 35° C., and the contents were yellow throughout from the fruiting of the mold, and not viscid. This was the best appearing tray. It showed little evidence of ammonia. Both its mold count and bacterial count were slightly lower than those of the others, being 30,000,000 molds and 88,000,000 bacteria per gram.

In these laboratory experiments a count of 300,000,000 or over per gram for bacteria in shoyu-koji was directly parallel to the

[5] Fifteen minutes were required for the autoclave to run up to 15 pounds pressure and 20 minutes for it to run down. The total period of cooking was therefore 85 minutes.
[6] A towel was used because the mesh of the basket was too large for pieces of cake.
[7] Prepared for koji by being ground into strings one-half inch in diameter by a food chopper driven by an electric motor.

condemnation of the product for poor physical qualities. Samples of shoyu-koji from one manufacturer gave a bacterial count of 11,600,000,000 per gram for koji well covered with the yellow-green fruit of the mold ferment, but not matted together with vegetative growth of the mold. This koji was dry and mature when received. Examination of the process as carried on by the factory producing the koji substantiated the impression that such a high count of bacteria must have been preceded by poor conditions in the immature koji. Mature koji showing little or no fruiting of the mold ferment, but instead a cobwebby growth of a Mucor, had a bacterial count of 14,300,000,000 per gram. In both instances the organisms belonged to the *Bacillus mesentericus* or *B. vulgatus* group. When koji is overgrown with Mucor and is moist and sodden with slime from bacteria, it has a very filthy appearance, as well as objectionable odors contingent upon rapid proteolytic changes.

It is evident that a koji made of practically sterile ingredients may develop ubiquitous bacteria, because of exposure necessary in manipulation. Several experiments indicate that the bacterial count in an acceptable product may be approximately two and one-half to three times as great as the mold count. The mold is present, however, in much greater quantity than figures would indicate. It is obviously the conspicuous organism. Furthermore, the structural difference between a mold and a bacterium permits a far more complete count of bacteria when using bacteriological methods. The bacterial colony can break apart at every segment, whereas the vegetative structure of mold can only be torn apart. Therefore, the mold count is really a spore count, not a count of the whole mass of mold material, as in the case of the bacteria.

A koji with a reasonably low bacterial count may be secured by using as a criterion for an acceptable product certain specific appearances, odors, etc. If viscosity, the odor of ammonia, putrefaction, poor mycelial or vegetative development of the mold, high ultimate temperature (over 40° C. at any time) are encountered, uncountable numbers of bacteria will be found. Koji of this character should be destroyed in such a way as to kill the microorganisms. Its retention around the workrooms is a menace to the clean koji.

### SHOYU-MOROMI.

The shoyu-koji when mature is emptied into a tub of brine. In the experimental work conducted in cooperation with Dr. T. Takahashi one-half of a 65-gallon wooden cask was used for the tub or vat. The brine was a solution of commercial salt at a strength of 20° to 22° B. The lot of mature koji ripening each day was emptied into the brine. With each lot was added fresh brine of the strength mentioned on page 17. The mash thus produced constitutes the shoyu moromi.

Each tub of moromi was inoculated on the first day with four flasks (450 cubic centimeters of wort extract broth and 5 per cent sodium chlorid in each) of a yeast culture, Zygosaccharomyces sp. Each day a new batch of shoyu-koji was started and a mature batch was emptied into the mash vat, along with eight quarts of the brine. Mature koji, mixed with a brine solution, forms the shoyu-moromi, the ripening of which follows the mold fermentation. Commercial salt was used to form the brine solution. In Japan sea salt is used. It is said that one part of the sea salt to two parts of water by volume gives a brine of from 20° to 22° B., presumably because of the coarseness of the native Japanese sea salt.

### RIPENING.

The first experiment in shoyu making conducted in the Bureau of Chemistry provided three tubs or half barrels of moromi. Two (A and B) were kept at the average temperature of the laboratories, 20° to 24° C. These two tubs contained shoyu-moromi made from a brine with an original salt content slightly greater than 22° B. The third tub (C) contained at the start a mash formed from a brine having a salt content just under 20° B. and was held at a temperature between 25° and 27° C. These mashes were stirred daily for a period of one hour. Also a blast of air was bubbled into the material in order to supply oxygen for the microorganisms present. Small portions of each mash were pressed during the course of a 10 months ripening period, in order to extract the sauce for examination. An idea of the progress of the fermentation was thus secured. After the ripening was considered as completed, the entire mash was pressed in each of the three batches. No heat was applied to the mashes or to the extracted sauces.

### QUALITY.

The three samples of shoyu secured by pressing the three lots of shoyu-moromi were subjected to chemical analysis and judged for flavor and taste. The quality of shoyu has an intimate connection with the method of fermentation. According to Japanese scientists, after the moromi fermentation or the ripening in brine is over, alcohol and esters are formed. An excess of acids is also removed, if the process is conducted in a proper manner, the organisms involved in the moromi fermentation consuming the larger part of any unpalatable nitrogenous matter present in the mash (*21*).

Mash C, having the weaker brine as a component part and kept at a higher temperature, produced an unattractive shoyu, even after ripening for nine or ten months. Not only was its flavor weak and unattractive, but it had none of the taste characteristic of Japanese shoyu. The keeping qualities of the shoyu from mash C were not satisfactory. The flavor deteriorated on standing at room temperature. The shoyu from both mash A and mash B was superior to that from mash C. The keeping quality was satisfactory in both shoyu A and shoyu B. The flavor and taste of these two lots of shoyu was neither universally approved nor condemned. As shoyu B was rather mediocre as compared with shoyu A, criticism was centered on A alone. Samples of A were sent to several Japanese scientists for comparison with Japanese shoyu which they considered acceptable.

Samples were given to Japanese visiting the laboratory and to others interested in soy products and sauces whenever opportunity presented itself. Doctor Takahashi stated that shoyu A compared favorably with the best commercial shoyu of Japan, while others thought that it resembled the heavy soy made by Japanese housewives in the country districts.

Agreement as to exact requirements for the best shoyu seems not to have been reached, even among Japanese themselves. The highest quality of shoyu in Japan is said to go under the label "Mogi" or "Kikkoman," but chemical analyses in König (*11*) and in the Bureau of Chemistry records indicate no consistent chemical composition.

## BACTERIOLOGICAL EXAMINATION.

The well-known rôle of many varieties of microorganisms, for example in the dairy industry, makes it impossible to disregard the activity of bacteria and yeasts in the ripening of the salt shoyu mash or moromi. The established importance of the mold *Aspergillus flavus* and its enzymes to the process is, however, recognized. The microorganisms present in the mash in a living condition may exert a positive influence. A direct examination of the mash shows a variety of bacterial forms. Stained slides indicate that certain of these bacteria are living actively, while the majority are in spore form. Plate counts may be used to demonstrate how many viable bacteria of certain types are in the mash. Total plate counts made on the ingredients of the sauce, on the immature and mature koji, and on the mash at intervals during the course of this ripening also determine at what points in the making of particular lots of sauce the bacterial increase occurs and its rate of increase. The bacterial forms developing in moromi made in the Bureau of Chemistry seemed to be predominately of one group, *Bacillus mesentericus*. Other recognizable groups of bacteria and yeasts appeared only intermittently.

In January, 1920, two lots of sauce were started. One was composed of soy beans and wheat and the second of peanut press cake and, for the most part, wheat as a starchy substance. These may be designated as moromi 1 and moromi 2. Fresh koji and brine were added to these mashes for more than three months. With the assistance of Dr. Edwin LeFevre, of the Bureau of Chemistry, a series of smears and later dilution cultures were made of these two mashes. Smears were made as a means of preliminary investigation. Apparatus was devised by means of which samples could be secured at any one depth without contamination from other depths. The results of this examination are given in Tables 1 and 2.

TABLE 1.—*Results of examination of smears made from moromi.*[1]

| Date examined. | Moromi 1 (Jan. 15–Feb. 18). | | | Moromi 2 (late Jan.–Feb. 18). | | |
|---|---|---|---|---|---|---|
| | Surface. | Below surface. | Center. | Surface. | Below surface. | Center. |
| Feb. 13 | A few spore bearers; micrococci. | Numerous spore bearers; yeasts. | ........ | Spore bearers quite numerous; yeasts. | Numerous spore bearers. | ........ |
| Feb. 18 | Spore bearers present. | ........ | Very few spore bearers. | .....do........ | A few spore bearers; yeasts. | ........ |

[1] Gradation of terms used: Very few, few, present, numerous, quite numerous, very numerous.

TABLE 2.—*Dilution plate counts of moromi 1 and 2.*[1]

| Date of examination. | Bacteria.[2] | | | | Molds.[2] | | | |
|---|---|---|---|---|---|---|---|---|
| | Below surface. | | Bottom. | | Below surface. | | Bottom. | |
| | Moromi 1. | Moromi 2. | Moromi 1. | Moromi 2. | Moromi 1. | Moromi 2. | Moromi 1. | Moromi 2. |
| 1920. | | | | | | | | |
| Feb. 26 | 300 | 900 | 200 | 1,400 | 3 | 0 | 3 | 0 |
| Mar. 1 | | 500 | | | | 0 | | |
| May 3 | 220 | 800 | 400 | (3) | 0 | 0 | 10 | 20 |
| Mar. 15 | | | 1,450 | (3) | | | 0.2 | 0 |
| Oct. 3 | | | 140 | 750 | | | 0 | 0 |

[1] Moromi 1 was started on January 15 and moromi 2 later in January.
[2] The factor is 1,000,000 in each instance.
[3] Uncountable.

## PROPORTIONS OF INGREDIENTS.

Table 3, prepared by Doctor Takahashi, gives the proportions of beans, wheat, salt, and water used in grades of Japanese soy sauce. The salt specified is Japanese sea salt, which contains more impurities and is coarser than the commercial salt used in America. The figure for salt should accordingly be reduced at least 4 per cent when using such salt as is to be procured in the United States.

In this table 100 is the unit of volume of ingredients to be used. The beans and wheat are measured in their raw condition. The volume of the beans increases with soaking and cooking, and that of the wheat with roasting and crushing. The prepared beans and wheat increase approximately 25 per cent in volume. The volume ratios of prepared ingredients to a brine made from Japanese sea salt will then be as follows: For common shoyu, 100:132; for good shoyu, 100:118; for excellent shoyu, 100:105. The relation of the salt to the water is as 1 to 2 in all three classes of sauce. The point to be noticed is that the volume of water in the mash as a whole decreases as the quality of the mash increases. This indicates a slower and more restrained fermentation with which it is possible, through more carefully controlled conditions, to obtain a product with a more uniform and delicate flavor. Where the added water in a mash which is to be ripened into good soy sauce constitutes 2 parts to 5 parts of the other ingredients, in common sauce it is greater in quantity, and in an excellent grade of sauce it is less.

TABLE 3.—*Proportions of ingredients by volume for grades of shoyu.*

| Ingredients. | Grade. | | | Ingredients. | Grade. | | |
|---|---|---|---|---|---|---|---|
| | Best. | Good. | Common. | | Best. | Good. | Common. |
| Soy beans | 100 | 100 | 100 | Salt | [1] 90 | 100 | 110 |
| Wheat | 100 | 100 | 100 | Water | 180 | 200 | 220 |

[1] The proportions 100 : 100 : 70 : 200, using salt commonly procured on the market of the United States, appear to give a rather excessive amount of salt in the mash of moromi. Such salt is lower in impurities and being fine packs closely when measured by volume.

## YIELDS.

The approximate yields of shoyu obtained in experiments corresponding closely to Japanese practice, computed from records in an article by Kinoshita (*7*), are shown in Table 4.

TABLE 4.—*Quantity of raw shoyu and of residue obtained in each step in the filtration of shoyu moromi.*[1]

WITH PRESSURE OF 1,250 POUNDS.

| Vat No. | Quantity of mature moromi. | Filtrate obtained in 2 hours without pressure. | Filtrate obtained by pressing for 17 hours. | Filtrate obtained by pressing for 23 additional hours. | Total filtrate obtained.[2] | Total filtrate in percentage by volume of moromi taken.[3] | Residue. |
|---|---|---|---|---|---|---|---|
| | *Liters.* | *Liters.* | *Liters.* | *Liters.* | *Liters.* | *Per cent.* | *Kilograms.* |
| 1 | 275 | 87.8 | 119.0 | 9.2 | 216.0 | | 58.4 |
| 2 | 267 | 93.4 | 103.0 | 8.1 | 204.5 | | 57.8 |
| 3 | 260 | 75.8 | 124.0 | 7.2 | 207.0 | | 56.7 |
| 4 | 256 | 68.6 | 128.0 | 8.5 | 205.1 | | 56.1 |
| 5 | 273 | 93.4 | 117.0 | 4.5 | 215.0 | | 57.4 |
| Average | | 83.8 | 118.0 | 7.5 | 209.5 | 78.7 | 57.3 |
| Per cent | | 31.5 | 44.54 | 2.8 | | | |

WITH PRESSURE OF 1,500 POUNDS.

| Vat No. | Quantity of mature moromi. | Filtrate obtained in 2 hours without pressure. | Filtrate obtained by pressing for 17 hours. | Filtrate obtained by pressing for 23 additional hours. | Total filtrate obtained. | Total filtrate in percentage by volume of moromi taken. | Residue. |
|---|---|---|---|---|---|---|---|
| 1 | 238 | 63.2 | 117.0 | | 180.2 | | |
| 2 | 245 | 70.4 | 124.0 | | 194.4 | | |
| 3 | 248 | 74.0 | 110.0 | | 184.0 | | |
| 4 | 244 | 72.0 | 115.0 | | 187.0 | | |
| 5 | 246 | 63.2 | 121.0 | | 184.2 | | |
| Average | | 68.6 | 117.4 | | 186.0 | 76.0 | |
| Per cent | | 28 | 48 | | | | |

WITH PRESSURE OF 1,500 POUNDS.

| Vat No. | Quantity of mature moromi. | Filtrate obtained in 2 hours without pressure. | Filtrate obtained by pressing for 17 hours. | Filtrate obtained by pressing for 23 additional hours. | Total filtrate obtained. | Total filtrate in percentage by volume of moromi taken. | Residue. |
|---|---|---|---|---|---|---|---|
| 1 | 1,530 | 453.0 | [4, 5] 635.0 | 59.5 | 1,147.5 | | |
| 1 | 1,415 | 478.0 | 588.0 | 52.4 | 1,118.4 | | |
| 2 | 1,525 | 455.0 | 682.0 | 55.7 | 1,192.7 | | |
| 2 | 1,425 | 435.0 | 642.0 | 53.2 | 1,130.2 | | |
| Average | | 455.2 | 636.7 | 55.2 | 1,147.2 | 77.7 | |
| Per cent | | 30.8 | 43.2 | 3.7 | | | |

[1] A hydraulic press was used for pressing the material.
[2] As all bags used in the laboratory for the filtration of moromi were washed and dried before every experiment, the quantity of moromi absorbed by the bags might be greater than that of the practical factory.
[3] Per cent by volume of total quantity of moromi.
[4] The interval here was 18 hours.
[5] The sacks in which the moromi was placed for pressing were rearranged or restacked at this point.

## CHINESE SOY SAUCE.

The primary difference between the soy sauce of Japan and that of China, judging from the department's contact with Japanese and Chinese scientists and travelers, is that Japan has a generally standardized commercial product, while the soy of China is made on a smaller scale, varying with the locality and the producer to a much greater extent. Small-scale production, however, gives a choice product of uniform quality from certain factories or localities. The underlying principles of the Japanese and Chinese soy industries are the same. The Chinese soy-sauce manufacture has been discussed by Prinsen Geerligs (*16*).

One Chinese method of extracting soy sauce by a system of multiple drawings is also described by Groff (4). The complexity of this method and the differentiation of the ripening soy into various drawings or siphonings are indicated by the following abstract:

The jars of molded beans and flour in brine are placed in a yard to sun. The sunning continues for from two to six months, the longer the period the better. Most of the soy sauce shops, however, siphon the first drawing after three to four months. The sunning process results in the evaporation of the liquid in the jars, and three days before siphoning off the soy, salt solution is added to fill the jar. The first drawing (teng ch'au) is allowed to settle and its clearer portion is siphoned off, thus reducing the initial quantity of the first drawing. The first drawing is placed in clean jars and allowed to sun again for from one to six months. Some soy of this type is at times allowed to sun for three years, but such a procedure is too expensive to be a regular practice. The material that remains in the original jar after the first siphoning is called "first salted" (teng shi), and may be sold as a separate sauce, or used as the base of several different sauces or as a base for the second drawing (i ch'au).

When used as a base for the second drawing, a salt solution is poured on "the first salted" or the material which remains in the jar from the first drawing. The jars are again placed in the sun for from one to two months. Salt water is added three days before the drawing. After settling, the soy is drawn off and placed in the sun for from one to two months. This soy is called "second drawing." The material which remains in the jar is called "beginning salted" (ün shi), and may be used as the base of a number of other soys or as the base for the third drawing (sam ch'au).

The same method is used in making the third drawing, and the material which remains is called "middle salted" (chung shi). This may be sold as a sauce, or it may be used as the base of different soys or of a fourth drawing (sz ch'au).

The fourth drawing is made in the same way as the previous ones, and the material left in the jars is also called "middle salted." It is used as the base of a number of very cheap soys.

The sunning method (Pl. I) takes so much time that many manufacturers boil the second, third, and fourth drawings instead of sunning them. This makes a soy decidedly inferior in quality. When soy is obtained by boiling, it is drawn from the solid constituents and boiled for two to four hours, after which it is allowed to cool and salt is added. Of the four drawings of soy, the only one that is always sold as it is drawn is the first drawing. The others are blended before going into commerce. Candied molasses is added to the cheapest types of sauce as a coloring and sweetening agent.

## PEANUT SAUCE.

The possibility of substituting peanut press cake for soy beans in sauce was investigated. A koji fermentation was carried out as with soy beans and wheat. The physical differences between soy beans and peanut press cake required that the procedure of preparing the protein ingredient of the koji be altered. The method finally adopted consisted of wetting three parts of dry cake with two parts of water and steaming it in the autoclave under pressure (p. 13). After having been brought to a suitable water content and practically sterilized, the cake was ground into strings about one-half inch in diameter in an electrically driven food chopper. The roasted and ground wheat was quickly mixed with the strings of peanut press cake, the mixture was inoculated with the mold ferment desired, and the koji process was carried out as usual. The ripening of the peanut press cake koji was completed in a shorter period than when whole soy beans and wheat were used.

The texture of the peanut press cake presents broken cells of peanut tissue where food materials are at once available to the mold.

The peanut also contains starchy substances and in the broken cells composing the press cake these starchy compounds are at once available as food for prompt mold growth. When the usual Japanese shoyu-koji process is followed, the soy-bean tissue is practically unbroken, except that the seed coats are usually split at some point. The mold penetrates the seed coats, the germ, and the cell structure of the cotyledons to a limited extent. The food substances, however, are not as immediately obtainable in the uncrushed soy bean as in press cake.

The control of bacterial activity is much more difficult in a koji made of press cake than in one made of whole seeds. The food materials made available by crushing the plant cells are directly accessible to bacteria unavoidably introduced in cooling, distributing, and seeding the koji. The life cycle of the bacteria is shorter, and therefore their work is more speedily accomplished than that of the molds. When mold fermentation is desired, bacterial growth must be inhibited and obstructed at every possible point. Water content of the koji mixture, temperature control, and degree of humidity permitted are the important factors. The degree to which bacterial activity is controlled in a koji where mold fermentation is desired is an indication of the quality of that koji.

The peanut press cake koji was made into a moromi having an initial salt content of 11 to 12 per cent sodium chlorid.[8] Evaporation through January to September, 1920, increased the salt content gradually. The extent of evaporation in the mash was such that the salt content of the sauce was about 19 per cent sodium chlorid.[9] The sauce was secured from the mash by pressing.

The quality of the peanut sauce was very satisfactory. The taste of the peanut was retained to such an extent that those accustomed to judging peanut products by tasting were not deceived, even when uninformed as to the ingredients of the sauce. The peanut sauce, although not having the flavor desired by the Japanese in shoyu, was very acceptable, combining the natural taste of the peanut with a mild meaty flavor.

## RELATION OF ENZYMIC ACTIVITY TO SOY PROCESSES.

Certain changes occur in the mixture of mold, beans, and wheat after it has stood in the brine as moromi to ripen for a period of time. Doubtless these are not due to any activity of the salt, which is a restrictive agent. The essential factors are the diastatic and proteolytic enzymes produced by the mold. Having become satisfied of the certainty of these two points, inquiry may be made as to what part the enzymes of the bacteria play. Bacteria are undoubtedly present and to a certain degree they are active. Increasingly great numbers of bacteria in the koji cause an undesirable flavor in the final sauce. The same may be said of uncontrolled bacterial activity in the mash. In other terms, low salt content, below 16 per cent, and higher temperatures, above 22° to 24° C., influence the flavor of the mature soy sauce to such a degree that the flavor deemed desirable in a good sauce by the oriental people is not usually secured.

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[8] Analysis by J. F. Brewster, formerly of Bureau of Chemistry, United States Department of Agriculture.

[9] Analysis by C. E. Goodrich, Bureau of Chemistry, United States Department of Agriculture.

The question of the influence of microorganisms and their enzymes on such a complex product as the mash of soy sauce is hard to solve, even with the aid of experimental evidence. The question can, for the most part, at present be answered only indirectly. It may seem that a consideration of enzymic activity can have little practical relation to the process of shoyu manufacture. The whole process of shoyu-koji fermentation, however, emphasizes the building up of the mold enzyme, protease. Other organisms are able to form protease in such a manner as to break down proteins more rapidly during their active growth than the yellow-green Aspergilli, but the flavor produced by bacterial proteolysis is not that of soy sauce. While water-soluble protein is formed by the activity of the mold ferments used for shoyu, as well as by certain accompanying bacteria, investigation has proved that the delicate manipulation essential for a clean koji fermentation causes a satisfactory accumulation of enzymes in the mature koji. The activity of the mold enzymes in the moromi or brined mash depends intimately upon how the salt content of the mash is built up.

The vegetative portions of the mold growth and the billions of spores produced in the shoyu-koji are present throughout the moromi because the moromi is stirred daily. Do the intra-cellular enzymes (*1, 15*) of the mold eventually come out into the mash from a goodly proportion of the mold material and thus aid in the gradual breaking down of the higher proteins into simpler protein as is desired in producing a well-flavored shoyu? Kopeloff (*12*) has indicated that intra-cellular enzymes of mold spores (*Aspergillus Sydowii*) are the cause of deterioration in sugar. The selection of the strains of the shoyu mold ferment best adapted for shoyu-koji may be based on tests of enzymic strength of the particular mold strains. In fact, K. Oshima (*15*), working in the Bureau of Chemistry, has demonstrated that of the strains of the *Aspergillus oryzae-flavus* group only those actually related to the shoyu industry have marked proteolytic strength.

## MANUFACTURE IN THE UNITED STATES.

The variations in the flavor of oriental soy sauce should reduce the task of American manufacturers of this product. The American product would not be compelled to compete with a product having only one recognized standard of flavor. If the sauce the manufacturer developed had an individual flavor of its own, there would be less prejudice to break down when he placed it on the market. All of which, however, should not lead to satisfaction with an output lacking uniformity nor to the acceptance of flavors produced by hurried or improper processes of ripening. It has been shown that unsatisfactory flavors in soy sauce can readily be correlated with a predominance of bacterial instead of mold activity. A low brine content in the moromi while fermentation is in progress, as well as too high a temperature during this period, produces conditions favoring these undesirable flavors.

There are many difficulties in conducting a process like the manufacture of soy sauce. If this were not true the process would not be regarded as secret, as it so generally is in the Orient. In Japan the process of shoyu manufacture is conducted in relatively modern factories. The reasons for the practices followed in the various steps in the ripening of shoyu-koji have been but partially worked out. The established practices are based upon accumulated manufacturing experience rather than upon carefully planned investigations. Available scientific studies on the moromi fermentation or the brining have up to the present been either futile or inadequate from a practical point of view.

The great obstacle in the way of developing a soy-sauce industry in the United States lies not only in the fact that soy sauce is not an everyday necessity, as it is with the people of the Far East, but also in the very little realized truth that a properly flavored and uniform output can be readily produced only at comparatively great expense and after a certain amount of experimentation has been conducted. The majority of soy-sauce makers and manufacturers in the Orient employ purely rule-of-thumb methods which have been handed down and individually perfected by more or less successful experience. Accurate knowledge of the reasons for the steps involved in the process as practiced is not common.

The possible manufacturer of soy sauce in America needs to remember that an attempt at transplanting an old, established fermentation practice to a new land carries with it difficulties due to new atmospheric and climatic conditions. Further, in bringing to this country a process which has arisen in a land where human labor is cheaply obtained there will be economic and technical factors to be adjusted to the new conditions. Imported technical assistance may secure a successful product, but it admits of no interpretation of the cause of failure, should such failure arise.

Soy bean seed is used as a food as well as for its oil and meal. Of the almost innumerable ways in which soy beans are used in the Orient as more or less elaborately prepared foods, soy sauce seems to offer prospects of more immediate adoption in the United States than any other product. Soy sauce and related substances, such as red miso and white miso among Japanese bean products and the various sauces and mold-fermented bean cheeses among Chinese foodstuffs, are highly relished in the Orient. Occidentals who have had the good fortune to become acquainted with the seasonings of oriental cookery readily adapt soy sauce and other soy bean products to their home dishes. Soy sauce has already gained a strong foothold with frequenters of Chinese-American restaurants.

Table sauces containing soy sauce as an ingredient are to be had in a great variety of grades and flavors. They also present an unlimited field for further variation. Concentrated forms of seasoning, such as yeast and vegetable extracts suitable as meat substitutes in flavoring soups and other prepared dishes, are receiving consideration by manufacturers. Soy sauce is of value in any table sauce and it can easily rank with yeast or vegetable extracts when prepared in concentrated form. Soy sauce, as developed in the laboratory of this department and concentrated under a vacuum, made delicious bouillon, especially when flavored with a little celery seed extract and garlic. It need scarcely be said that the method of concentration to be employed, as well as that of removing excess salt from a concentrated soy sauce, is one which modern machinery can ably cope with. United States Patent 1,332,448, of Sadakichi Satow, of Sendai, Japan, specifies a dry powder form of soy sauce.

The manufacturers of table sauces and condiments interested in soy sauce are among the largest and best known firms of the United States. Their evident desire for information in regard to the work of the department on soy sauce has led in part to the preparation of this bulletin. The experimental work of a purely laboratory nature included in this bulletin is indicative of the stage which the soy sauce industry has reached and suggestive of what problems the prospective soy-sauce manufacturer in a new country must contend with, if he is to carry on the fermentation process in anything but a blind or haphazard way. Several manufacturers at present have soy sauce experiments under way in their laboratories. One company at least in the United States manufactures a wholly domestic product.

## RELATED FERMENTATIONS.

Soy sauce is only one of the mold-fermented food products originating in the Orient, the majority of which are ripened by means of the molds represented by the yellow-green group of Aspergilli.

Miso, one of these products, is one of the most common breakfast foods for children. There are two types of miso, white or shiro miso and red miso. Miso is prepared from a koji ripened by means of the *A. flavus oryzae* group of molds. The soy beans are cooked in miso before the fermentation is undertaken. The treatment subsequent to the cooking and preparatory to the fermentation doubtless varies in different localities. It is said that the beans may be made into a paste before being ripened by the mold. As bought in this country, however, miso shows the beans intact. White miso is said to be made from a koji of soy beans or soy beans and a starchy material, as rice or barley. The koji is ripened as is shoyu-koji and placed in a weak brine for 10 days. Unfinished rice wine may be added to improve the flavor and to preserve the product, which is rather perishable. Red miso is prepared in the same way as the white miso, but is ripened for from one to three months in a stronger brine. White miso has been bought in the United States in two forms. One type is very salty and therefore less perishable than the other. Probably because of longer fermentation red miso is dark red. It is very cheap, whereas white miso is expensive.

In China the curd, or to-fu, made from soy-bean milk, is ripened with a mold preparatory to a ripening in brine. Such products are commonly termed cheese by travelers. The to-fu is cut into square, rather thick pieces which are arranged on the narrow face in rows upon traylike racks. The racks are stacked zig-zag fashion, or so that aeration is possible under damp conditions. The squares of bean curd become overgrown with a mold. The final cheese as received in the United States shows the mold on the squares of curd as white mycelium with no fruit. After the development of the mold on the curd the squares of to-fu are placed in brine for further ripening. At the completion of this ripening the product is utilized as a food product. It comes into this country commonly as canned white or red squares of fairly salty bean curd, covered with a salty liquid which is thick because of the crumbling from the curd itself. The red color in such mold-ripened and brined to-fu is due to red rice, made by changes produced upon rice kernels by the mold *Monascus purpureus* Went.

Natto, commonly called cheese, is a Japanese fermented food product prepared from soy beans. Although its manufacture varies, probably with the locality, it is a common practice to boil the soy beans until tender and then leave them in a warm place for 24 hours or until they have fermented through the activity of such bacteria as those of the *Bacillus mesentericus* or *Bacillus vulgatus* group. Pure starters of the natto bacillus, a member of this group, may be obtained from Hokkaido Imperial University in Sapporo, Japan. Experiments show that, while the natto bacillus breaks down protein rapidly as compared with the shoyu mold, it does not break it down as rapidly as a strain of the related group picked up accidentally. The making of natto may therefore be classified as a controlled fermentation, as a weakly proteolytic strain of such groups of bacilli as mesentericus, vulgatus, and subtilis is generally selected. In the ripe natto the soy beans are rather viscid, but protein decomposition should not have gone far enough to develop an offensive odor.

In the United States at least, certain enzymic products for industrial uses known by such trade names as polyzime, oryzyme, etc., owe their origin to the growth of these molds on cereals, bran, sawdust, or similar substrata. The mold is grown in trays and kept in a koji room where specific conditions of temperature and moisture are maintained. The enzymic substance produced by suitable development of the mold is marketed either by selling the gross material, by leaching and selling it in liquid form, or by extracting the desired properties and selling the product as a concentrated powder. Such enzymic materials or solutions contain both diastatic and proteolytic enzymes. They are used by the textile trade and pectin industries. Manufacturers also recommend their use in clarifying fruit juices, in place of soap in laundries, and as a partial substitute for yeast leaven in bread baking. The diastatic enzymes of these products are of value in desizing textiles when in order to weave it has been necessary to oversize the warp threads. It is claimed also that the application of these enzymic solutions improves the texture of the fibers in both warp and fill of a cotton cloth and may be substituted for other means of mercerizing. The proteolytic enzymes are supposed to improve the texture of cotton and silk fabrics, but their usual value to the textile trade is in degumming silk fibers. The thread spun by the silk worm is composed of two fibers bound together by a gum. Treatment with a proteolytic enzyme removes the binding material and furnishes two silk fibers in the place of one.

Recently the use of these mold enzymes in clarifying pectin substances for the jelly and jam trade has risen in importance above their other uses. The liquor pressed from apple pomace and utilized by makers of pectin contains proteins and starch which would cause cloudiness if introduced in jellies. The application of the mold enzyme reduces the proteins and starches to substances which cause no cloudiness and yet are not objectionable to the jelly maker.

*Aspergillus Wentii*, a mold closely related to the yellow-green Aspergilli, has been connected in the literature with fermentation processes. Experiments undertaken in the Bureau of Chemistry have shown that certain strains of *A. Wentii* have value for their power of inverting sugar and producing invert sugar on a scale suggestive of industrial application.

## SUMMARY.

Soy sauce is a brown, clean salty liquid used in the Orient as a universal flavoring. It takes the place of the vegetable or meat extracts and of salt common in the United States.

This sauce is made by a fermentation process in which specially prepared soy beans and wheat or a starchy material are first treated with a mold ferment from the *Aspergillus flavus-oryzae* group. The mold-ripened beans and wheat undergo a brining process where the activity of the mold ferment and of other occasional microorganisms, as yeast, continues under controlled conditions for from six months to a year.

Preliminary experiments indicate that peanut press cake is suitable for use in making sauces similar to soy sauce.

Soy sauce can be made in this country in so far as the process itself is concerned.

Soy sauce is valuable in itself as a seasoning agent and also as an ingredient of other table sauces.

It provides another means of utilizing soy beans, the growing of which in this country is being promoted.

The manifest interest of American manufacturers of condiments in soy sauce makes reasonable the present study and its further pursuance. It seems impracticable at this time to undertake in the United States the production of soy sauce, except in conjunction with some already established and related industry.

The mold ferments of the *Aspergillus flavus-oryzae* group are used to ripen Japanese and Chinese soy bean food products other than soy sauce. These same mold ferments are used in making enzymic preparations procurable on the market, in crude, liquid, or concentrated form, of a certain value to the textile industry and to the jelly and jam trade. Several other claims are made for such enzymic preparations.

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