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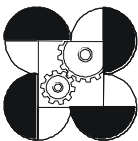
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SOY SAUCE MANUFACTURE



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‘Our Business is Industry...’

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SOY SAUCE MANUFACTURE

Soy sauce is the brown and salty product produced during the enzymatic hydrolysis of soy bean proteins into amino acids and small peptides to make it more flavorful. Soy sauce is called by many names such as *toyo* in the Philippines, *shoyu* in Japan, *ketjap* in Indonesia and *tou-yu* in China.

Manufacture

Soy sauce manufacture is composed of 6 stages, namely, preparation of starter, treatment of raw materials, *koji* making, mash making and aging, pressing and refining.

Stage 1: Preparation of starter

Cultures. Pure culture of *Aspergillus oryzae* is used in the preparation of soybean *koji* starter. The organism is maintained in Czapeck's slants and can be purchased at the Microbiological and Genetics Division (MGD) of ITDI. The pure culture should be stored at refrigeration temperature to maintain its purity.

Pure culture of *Saccharomyces rouxii* is also available at MGD. This organism converts the sugars formed during fermentation to alcohol.

Medium for growth. The medium for soy sauce starter is composed basically of rice bran, rice and water, and the formulation for the medium is:

Rice bran	50 g
Rice	11 g
Distilled water	72 g

This mixture is sterilized for 15 minutes at 250°F, after which it is allowed to cool to less than 37°C before inoculation with pure culture of *Aspergillus oryzae*. The mixture is then incubated at room temperature for 2-3 days prior to use. At this time, the rice bran turns yellow to green in color.

The medium for *S. rouxii* is prepared by hydrolyzing 400 g soy sauce cake or mash in 1 liter distilled water at 50°-60°C for 1-2 hrs. The hydrolysate is filtered, the pH adjusted to 5.0 with lactic acid, and 4.5 and 5% glucose powder and sodium chloride, respectively, is added and dissolved into the mixture. The mixture is then sterilized at 250°F for 15 minutes before inoculation with the culture. Use 1 test tube pure culture for every 500 mL medium. Incubate medium at room temperature for 2-3 days before adding to the soy sauce mash.

For commercial production, the starter is produced by a building-up process. Starting from a pure culture in the test tube, spores of *Aspergillus oryzae*, are successively inoculated into sterilized culture media in an Erlenmeyer flask. From the flasks, it is transferred to a much larger quantity of rice bran. The starter is then used as inoculum soybean *koji*. This process is generally done to minimize contamination and usage of pure cultures.

Follow the above procedure for *S. rouxii*.

The prepared starter should be used at once to minimize bacterial contamination and enzyme inactivation.

Stage 2: *Treatment of raw materials*

Soybeans. Whole or defatted soybean meal may be used in soy sauce manufacture. Whole beans are soaked in running water overnight to hydrate the beans, while defatted meal is moistened with water.

The protein in raw beans is present in an undenatured state and cannot be hydrolyzed by the enzymes of *koji* mold. To denature the protein for enzyme digestion, the beans are cooked by steaming under atmospheric pressure (45-60 minutes at 250°F).

Wheat flour. This is the major source of carbohydrate, and according to Yokotsuka (1986), about ¼ of the *shoyu* protein comes from wheat. The flour is roasted prior to use. If insufficiently roasted, the raw starch or β -starch cannot be digested by the mold amylase and becomes white particles in the presscake of soy sauce mash. If overroasted, the protein digestibility decreases. During roasting, β -starch is changed into α -starch in order to be digested by mold amylase.

Stage 3: Soybean koji making

The cooked soybeans and the roasted flour are mixed prior to inoculation with the starter. The conventional method involves cooling the materials by hand mixing, and the larger particles of whole beans are cooled more easily than are the smaller particles of defatted meal. Now, this can be done easily with the use of mechanical *koji* equipment in which the temperature is controlled by mechanical aeration.

The soybean-wheat mixture ($\leq 37^{\circ}\text{C}$) is inoculated with 0.1-0.2% of starter mold. The mixed materials are cultured in clean plastic pail or plastic *batya* covered with sterilized *katsa*. This stage is called budding or spore formation stage. After 18-24 hours, or when the temperature of the mixture reaches 37°C , the materials are cooled down by hand mixing and transferring the inoculated mixture in small wooden boxes or

bistay lined with sterilized *katsa*. At this point, the thickness of material in the boxes or *bistay* should be controlled. To produce more protease, the temperature of the mixture should be kept below 37°C. This can easily be done by mixing either by hand or with the use of sterile turner. The incubation room should be kept clean and the temperature maintained at 28°-30°C by windows or by an air-conditioning system. The mixture should be cultured for a total of 72 hours.

The use of mechanical *koji* equipment reduces the required cultivation time from 48 to 72 hours, increases the enzymatic activities of *koji* and reduces bacterial contamination.

Stage 4: *Mash making and aging*

This stage consists of harvesting the soybean-wheat *koji*, preparation of brine or salt solution, brining of mixture and fermentation. The harvested soybean-wheat *koji* is placed into appropriate fermentation containers (plastic drums, clay pot, fabricated steel containers or cemented tanks lined with fiberglass). Brine or 20% salt solution is poured and mixed into the mixture. The *moromi* or mash is allowed to undergo hydrolysis (usually under the heat of the sun) for at least 6 months with occasional agitation. Agitation (by compressed air in commercial production) mixes the dissolve contents uniformly and promotes microbial growth. During this period, the enzymes from the *koji* mold hydrolyses most of the protein to amino acids and peptides. The pH drops from 6.5-7.0 to 4.7-4.9. The lactic acid fermentation is then gradually replaced by yeast fermentation. Pure cultures are sometimes added to the mash. The salt concentration of the mash stabilizes at around 17-18% after 1-2 months. The high salt concentration of mash limits the growth of microorganisms to a few desirable types.

Stage 6: *Pressing of mash*

The aged mash is filtered under a high hydraulic pressure through cloth. The soybean cake is collected, and sometimes, it is allowed to undergo further hydrolysis by mixing with 20% brine for at least 2 weeks. The collected second extract is used as diluent to the first extract. The pressed cake is used as animal feed.

Stage 7: *Refining*

The collected raw soy sauce (1st extract) is stored in a tank. After settling, the mixture is divided into 3 layers, the oil on top, the soy sauce in the middle and the sediment at the bottom. The clear liquid is further clarified with the use of filter aid. The protein and salt of raw soy sauce is adjusted as to standard before pasteurization at 70°-80°C. The pasteurized product is stored in semi-closed tank to allow the coagulum produced during heating to settle. The soy sauce produced is then bottled and sealed.

Philippine National Standard (PNS 274:1993)

1. *Physical characteristics*

Soy sauce shall possess the color, taste, and aroma characteristic of the product. It must be free from scums and other foreign matter.

2. *Chemical composition*

Soy sauce shall conform to the following requirements:

Characteristics	Fermented	Hydrolyzed	Blend
pH	4.3 – 5.0	4.3 – 5.0	4.3 – 5.0
Salt as NaCl, %	15 – 25	15 – 25	15 – 25
Total solids (excluding NaCl, %, minimum)	5	5	5
Total nitrogen, %, minimum	0.6	0.4	0.6
Amino nitrogen, %, minimum	0.20	0.14	0.20
Total halophilic yeast count, cfu/ml, maximum	20	20	20

3. *Labeling*

Each container shall be declared with the following information:

- a. Name of product and the descriptive name whether fermented, hydrolyzed or blended, as in the following examples:

soy sauce

prepared from fermented soy bean

soy sauce

prepared from hydrolyzed soy bean protein (or plant protein)

soy sauce

blend of fermented soy bean and hydrolyzed protein

- b. Brand name or trade mark may be indicated
- c. Name and address of the manufacturer
- d. Net content in mL
- e. List of ingredients declared in descending order of proportion
- f. Country of origin
- g. Lot identification mark