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Industrial production of soy sauce

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SUMMARY

Soy sauce is a seasoning agent with a salty taste and a distinct aroma suggestive of meat extracts. The sauce is made by fermentation of a combination of soy beans and wheat in water and salt. This paper covers the method for production of fermented soy sauce, and that for acid-hydrolysis of defatted soy bean proteins. The microorganisms involved in soy sauce production, and biochemical and chemical changes in soy bean and wheat during fermentation influence greatly the sensory attributes and quality of soy sauce. Recent progress in industrialization of soy sauce manufacture is discussed.

INTRODUCTION

Of the many Oriental fermented products, soy sauce is the one most widely consumed in China, Japan, Korea and other Asiatic countries as a condiment and coloring agent in preparation of foods and for table use. It is a dark brown liquid, stable at ambient temperature, which does not require refrigeration during storage due to its low water activity and high salt content. Soy sauce and miso are flavoring agents having similar aroma and flavor. Soy sauce is a liquid whereas miso is a paste. Each is made by a two-step fermentation process from wheat flour and soy beans with a mixture of molds, yeasts and bacteria. The first step involves fermentation with mold to produce proteolytic and amylolytic enzymes in the Koji which is a culture starter. A good culture starter must give characteristic aroma and flavor to the soy sauce, have high proteolytic and amylolytic activities, and must be easy to culture. This is followed by a second fermentation with yeast and bacteria in the presence of 18–20% salt. The microorganisms used in these fermentation steps are not inoculated at the same time, but are applied sequentially.

Literature on soy sauce manufacture is largely in Chinese, Japanese, and other Oriental languages. Several references have been published by Fukushima [4,5,6], Noda [10], Nunomura and Sasaki [11], Wang and Hesseltine [20], Onaga et al. [13], Reed [15], Skinner et al. [16], Sugimori [18] and Yokotsuka [19]. This paper covers the methods for production of soy sauce made by fermentation of wheat and soy beans, and by acid hydrolysis of defatted soy beans. The chemical properties of soy sauce, and recent progress in technology concerning soy sauce production are also presented.

PRODUCTION OF FERMENTED SOY SAUCE

Soy sauce is made by fermentation of a combination of soy beans, wheat grain, water and salt [8,17]. The processes for production of fermented soy sauce consist of three major steps, namely, Koji production, brine fermentation, and refining. A flow sheet for manufacture is shown in Fig. 1.

Koji production

Koji is a source of proteolytic enzymes for converting soy bean proteins into peptides and amino acids, and amylase for hydrolyzing gelatinized starch into simple sugars. The substances converted by the enzymes in Koji become the nutrients for yeasts and lactic bacteria in the subsequent brine fermentation. In Koji production, defatted soybean flakes or soy beans are soaked in water to increase the moisture content and then cooked under pressure in a retort. Formerly, the soaked soy beans containing 60% moisture were cooked with saturated steam at 0.8–1.0 kg cm^{-2} gauge pressure for 40–45 min in a batch type pressure cooker. Currently, the soaked beans containing 30–45% moisture are cooked at 6–7 kg cm^{-2} gauge pressure (about 170 °C) for 20–30 s in a continuous cooker which allows high pressure and short time cooking. The wheat contains 8% moisture and is heated in a continuous roaster with hot air at 150 °C for 30–45 s at atmospheric pressure. It is then cracked in a machine into 4 or 5 pieces per kernel accompanied by smaller particles of wheat flour. In making regular soy sauce, the cooked soy beans (or defatted soy flakes) are mixed with an equal amount of roasted wheat and then inoculated with 0.1–0.2% of starter mold (*Aspergillus oryzae* or *Aspergillus sojae*) in wooden trays, each loaded with a 3–5 cm thickness of the fermenting Koji. The ratio of wheat to cooked soybean may vary, depending on the type of soy

This paper is dedicated to Professor Herman Jan Phaff in honor of his 50 years of active research which still continues.

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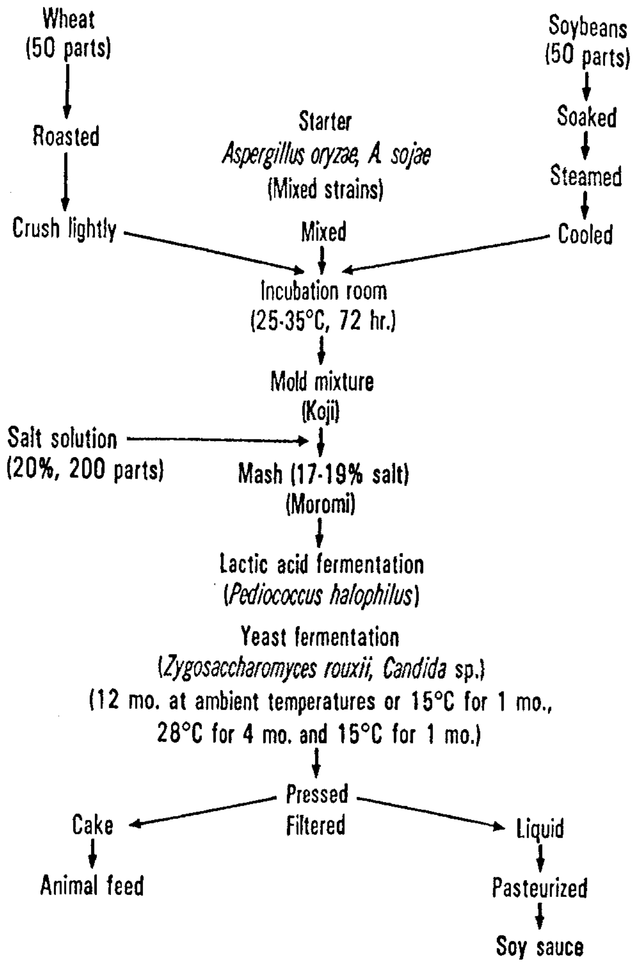

Fig. 1. Flowsheet for manufacture of soy sauce.

sauce to be prepared. After incubation at 25 °C for 72 h, the Koji becomes a greenish yellow mass as a result of mold growth and sporulation. It is necessary to stir the contents, to remove the metabolic heat formed, and to stir further to control the temperature at 25–28 °C. The important factors are selection of the best strains of *A. oryzae* and *A. sojae* and controlling the product temperature in that range during the Koji fermentation process. More recently, the Koji manufacturing process has been changed from a manual process to an automatic equipment process. This includes a continuous cooker for the soy beans, a continuous wheat roaster, mixer, cooler, automatic inoculator, mechanical mixer, temperature controllers, conveyors, and mechanical devices for turning the substrates during incubation. The inoculated mixture is put into large shallow perforated vats in closed chambers and forced air is circulated through the mass. After 3 days, *A. oryzae* or *A. sojae* grows and the culture mixture becomes green-yellow in color as a result of mold growth and sporulation. The automatic Koji-making system increases the protease activity of Koji and protects it from infection by undesirable microorganisms. This new device reduces the labor cost by 85–90% compared with the conventional manual method.

Brine fermentation

The second step in making fermented soy sauce is brine fermentation. It utilizes the lactic bacterium, *Pediococcus halophilus* and the yeasts *Zygosaccharomyces rouxii* and *Candida* species both of which tolerate a salt concentration of 20 g per 100 ml. The brine effectively prevents growth of undesirable microorganisms. The harvested Koji is mixed with 20% salt brine, and transferred by means of a spiral pump into deep fermentation steel tanks coated with epoxy resins on the interior. The resultant mixture is called moromi mash. It is important to control the microorganisms in the brine fermentation. The specially selected *P. halophilus* is cultured and added to the mash. To control its growth rate it is necessary to keep the fermenting mixture at 15 °C for the first month, allowing the pH of the mash to decrease slowly from 6.5 to 5.0. Then cultures of *Z. rouxii* and *Candida* species are added as a starter. The temperature of the moromi is allowed to rise slowly to nearly 28 °C until vigorous alcoholic fermentation starts. The temperature in the tank can be controlled by coil-type heat exchangers with mixing devices, thermocouples, and control systems.

After the alcohol fermentation is finished, the temperature is kept at 25 °C. Aeration stimulates microbial growth and mixes the contents. During the fermentation period, proteolytic enzymes from Koji hydrolyze the proteins in soy bean and wheat to form amino acids and low molecular weight peptides. Starch is converted to simple sugars which are fermented primarily to lactic acid, ethanol and carbon dioxide. During the brine fermentation, the pH of the mixture drops from 6.5 to 5.0 in the first month at 15 °C. This is followed by fermentation at 28 °C for four months. Sometimes it is necessary to add more pure cultures of *P. halophilus* and *Z. rouxii* and *Candida* species to the moromi mash during the fermentation.

Refining

The final process in soy sauce fermentation is refining which includes pressing, filtration, pasteurization and packaging.

The aged moromi is pressed in a vertical automatic press to separate the soy sauce from the residue. After pressing, the filtered raw soy sauce is pasteurized in a heat-exchanger at 70–80 °C for a few minutes to ensure clarity, to inactivate residual enzymes, and to inactivate any undesirable microorganisms. It may be necessary to clarify the soy sauce additionally by centrifugation or sedimentation. The sauce is treated with caramel as a coloring agent, and then packaged either in clean glass bottles, enameled gallon cans or in plastic containers.

The residue from the press can be extracted with more 20% salt brine to increase the yield. Much expertise is needed to produce a soy sauce that is attractive in flavor and taste, stable on storage at room temperature, and acceptable to the consumer. The quality assurance group must check the pH, acidity, amino nitrogen, salt content, color, microbial contamination, and sensory attributes: color, aroma and flavor of the product.

SOY SAUCE PRODUCTION BY THE ACID-HYDROLYSIS PROCESS

In addition to the fermentation method, soy sauce is also made by acid hydrolysis of defatted soya beans with food grade hydrochloric acid as a catalyst in a pressure cooker. The hydrolysate is neutralized with sodium hydroxide or sodium carbonate under careful supervision so that excessive alkali does not come into contact with the neutralized product. The acid-hydrolyzed soy sauce is refined by sedimentation, treatment wtih active carbon, filtration through a filter press to remove undesirable substances, and high vacuum to remove undesirable volatile compounds. However, the acid-hydrolyzed soy sauce is less attractive in aroma and flavor because of lack of aromatic substances such as esters, alcohols and carbonyl compounds which are derived from the fermentation process. In some countries, a combination of fermentation and acid-hydrolysis procedures are used for making less-expensive soy sauce. High quality soya sauce is made exclusively by the fermentation process.

Grades of soy sauce

In Japan, 'Special grade' soy sauce or 'Koikuchi' is made exclusively by the fermentation process, and is marketed under the Japanese Agricultural Standard (JAS) for this grade. It is an all-purpose seasoning characterized by a pleasant aroma, complex flavor, and a deep reddish brown color. It comprises more than 56% of the annual soy sauce production in Japan.

A second type is 'Upper grade' or 'Usukuchi shoyu' which is characterized by a lighter reddish brown color and milder aroma and flavor, and comprises about 24% of the total production. The product is used mainly for cooking when one wishes to preserve the original flavor and color of the foodstuff itself.

A third type is 'Standard grade' or 'Tamari shoyu' which is characterized by a slightly higher amino acid content, but lacks in aroma. The distinguishing feature of 'Standard grade' is that soy bean is the main raw material and wheat is not used or is used only in small amounts. This grade comprises 8% of the total production. The remainder belongs to the 'Non-JAS' grade.

The 'Special' and 'Upper' grades are derived mainly from the use of equal amounts of wheat and soy beans as the raw materials of Koji. The Japanese Soy Sauce Association indicates an annual production of 1.2 million tons of soy sauce in Japan.

CHEMICAL COMPOSITION OF SOY SAUCE

In general, good quality soy sauce contains 1.0–1.65% total nitrogen (w/v), 2–5% reducing sugars, 1–2% organic acids, 2.0–2.5% ethanol and 17–19% sodium chloride (w/v). About 45% of the total nitrogen is found in simple peptides, and 45% in amino acids.

The properties of some Japanese soy sauces are shown in Table 1. Koikuchi is a representative of the fermented soy sauce popular in Japan [4]. It is an all-purpose seasoning characterized by a strong aroma, attractive flavor, and a deep reddish brown color. More than 56% of Japanese soy sauces are of this type.

The 'Upper grade' or 'Usukuchi shuyu' is used mainly in the western part of Japan. The 'Standard grade' or 'Tamari shuyu' sauce is characterized by a higher total nitrogen and formol nitrogen compared with the other varieties. It is consumed largely in the Nagoya region of Japan. The Saishikomi and Shiro soy sauces are produced and consumed only in isolated localities or for special industrial uses.

The amino acid composition of the soy bean, wheat and a representative Koikuchi soy sauce is presented in Table 2. Glutamic and aspartic acids are the major amino acids present in the fermented soy sauce. A total of 18 amino acids are present in the sample. Arginine, lysine, phenylalanine, serine, threonine, leucine, isoleucine, valine, alanine and proline are present in significant quantities in the sauce. A comparison of the amino acids present in the fermented soy sauce with those in the soy bean and wheat indicates the following changes: a) arginine is converted into ornithine in the fermentation process; b) tryptophan and cystine may have been used during the fermentation as nutrients; and c) tyrosine decreases in the moromi mash.

Sugars in fermented soy sauce have been reported by Yokotsuka [19]. A representative Japanese fermented soy sauce contains the following sugars % (w/w): arabinose, 0.08%; glucose, 2.05%; mannose 0.06%; galactose, 0.17%; xylose, 0.06%; disaccharide, 0.65%; polysaccharide, 1.15%; unidentified sugar 0.23%; total sugar, 4.45% (as glucose). Organic acids: acetic acid, 0.16%; citric acid, 0.04%; formic acid 0.02%; lactic acid, 0.68%; succinic acid, 0.05%; total 0.95%.

Volatile flavor compounds

Forss and Sugisawa [3] reported 150 volatile compounds in soy sauce. Aishima [1] presented gas chromatographic profiles of soy sauce aroma. He observed more than 200 peaks in the chromatograms by use of distillation-extraction techniques and using various types of glass and fused silica capillary columns. These volatile compounds included: 1) hydrocarbons, 2) alcohols, 3) esters, 4) aldehydes, 5) acetals, 6) ketones, 7) furans, 8) lactones, 9) furanones, 10) pyrones, and 11) pyrazines. Of these aromatic compounds, HEMF (4-hydroxy-2 (or 5)-ethyl-5 (or 2)-methyl-3-(2H)-furanone) appears to be a very important flavor component of fermented soy sauce. HEMF possesses a strong flavor and resembles the aroma of the moromi mash of Koikuchi soy sauce. Nunomura and Sasaki [11] reported that HEMF is the most abundant aroma component, comprising 200–300 p.p.m., in Koikuchi soy sauce.

The volatiles are formed by microorganisms during the Koji and brine fermentation as well as the heating and pasteurization process. The same authors reported on the quantitative aspects of 12 major flavor components in Japanese soy sauces as follows: isobutyl alcohol, 3.07–18.35 p.p.m.; *n*-butyl alcohol, 1.41–11.48 p.p.m.; isoamyl alcohol, 4.47–22.0 p.p.m.; acetoin, 5.08–8.44 p.p.m.; ethyl lactate, 7.35–27.12 p.p.m.; furfuryl alcohol, 4.35–10.07 p.p.m.; methionol, 2.60–4.47 p.p.m.; 2-phenylethanol, 3.71–10.25 p.p.m.; HDMF (4-hydroxy-2,5-dimethyl-3(2H) furanone), 1.83–5.39 p.p.m.; 4-

TABLE 1

Typical compositions of five varieties of soy sauce

	Be	NaCl (g per 100 ml)	Total nitrogen (g per 100 ml)	Formol nitrogen (g per 100 ml)	Reducing sugar (g per 100 ml)	Alcohol (vol per 100 ml)	pH
Koikuchi	22.0	16.9	1.57	0.94	3.0	2.3	4.7
Usukuchi	22.2	18.9	1.19	0.80	4.2	2.1	4.8
Tamari	29.9	19.0	2.55	1.05	5.3	0.1	4.8
Saishikomi	26.9	18.6	2.39	1.11	7.5	trace	4.8
Shiro	26.9	19.0	0.50	0.24	20.2	trace	4.6

Source: [4].

TABLE 2

Distribution of amino acids in soy bean, wheat and Koikuchi soy sauce

Amino acid	Soybean (%)	Wheat (%)	Raw materials of koikuchi-shoyu (%)	Koikuchi-shoyu (%)
Arginine	8.42	4.71	7.58	2.6
Histidine	2.55	2.12	2.45	2.5
Lysine	6.86	2.67	5.90	6.5
Tyrosine	3.90	3.19	3.74	1.0
Tryptophan	1.28	1.13	1.25	–
Phenylalanine	5.01	4.43	4.88	4.2
Cystine	1.58	1.80	1.63	0.9
Methionine	1.56	1.74	1.60	1.4
Serine	5.57	5.22	5.49	5.3
Threonine	4.31	2.76	3.96	4.2
Leucine	7.72	6.52	7.45	7.3
Isoleucine	5.10	3.78	4.80	4.8
Valine	5.38	4.69	5.22	5.5
Glutamic acid	21.00	29.30	22.89	22.5
Aspartic acid	12.01	4.85	10.38	10.5
Glycine	4.52	3.94	4.39	3.9
Alanine	4.51	3.37	4.25	4.4
Proline	6.28	9.94	7.11	6.5
Ornithine	–	–	–	5.7

EG (4-ethyl-2-methoxyphenol(4-ethylguaiacol)), 1.12–3.67 p.p.m.; HEMF (4-hydroxy-2 (or 5)-ethyl-5 (or 2)-methyl-3(2H)-furanone), 177.8–418.7 p.p.m.; HMMF (4-hydroxy-5-methyl-3(2H)-furanone), 84.54–153.6 p.p.m.

Color, flavor and aroma are three important criteria for evaluating the sensory quality of soy sauce [13]. These authors applied chemical and physical tests to analyze the salt content, amino acids, sugars, total nitrogen, and % amino nitrogen in samples collected in California. Similar approaches were used by Forss and Sugisawa [3], Fukushima [4], and Yokotsuka [19]. Aishima [1] studied the gas chromatographic profiles of soy sauce volatiles as related to organoleptic characteristics by multivariate analysis.

BIOTECHNOLOGY IN SOY SAUCE RESEARCH

Biotechnology and genetic engineering have been widely used by food researchers [9], microbiologists [12], and geneticists [2]. Osaki et al. [14] investigated a shortening of the brine fermentation period through fermentation in a bio-reactor on a pilot plant scale. The first step in this process is digestion of the defatted soy beans by proteolytic and amylase enzymes in Koji at 55 °C in a salt brine 10% (w/v). The resultant hydrolysate is fermented in bio-reactors with cells of *P. halophilus* entrapped in calcium alginate beads [14] or in a mixture of gel beads of alginate and silica [7]. In the reactor with immobilized *P. halophilus*, the viable cells in gels at steady state showed a decimal increase of 2.5. The number of viable cells in gels increased 1000–10 000 times over numbers in the effluent with a holding time of 40 h. The production of lactic acid reached a satisfactory level of 10 g L^{-1} after 28 days.

In the subsequent alcoholic fermentation by *Z. rouxii* or *Candida versatilis*, using a bioreactor, the difference in the number of viable cells between gels and effluents decreased gradually with time. About 2% ethanol was produced continuously in 40–80 h while the pH level reached 4.8–5.2. These results indicate that both lactic acid and alcoholic fermentation can be achieved in a very short time with a satisfactory level of production. However the aroma pattern of the fermented samples differed slightly from that of commercial soy sauce. The bioreactor samples were higher in isobutyl alcohol, isoamyl alcohol and 2-phenylethanol, but lower in HEMF which is an important aroma component of Koikuchi soya sauce.

Despite the reduction in time of brine fermentation in the bioreactor, the Japanese Government has ruled that soy sauce produced without the aging process is not a fermented product, because the product does not meet the criteria for 'Special' grade soy sauce. It is hoped that soy sauce researchers will continue to work on the subject to improve the protease and amylase activity of Koji through protoplast fusion, mutation, and genetic engineering studies of the genes involved in soy sauce microorganisms. Future advances will depend on the development of better species for improvement of aroma, flavor, and color of fermented soy sauce.

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