20. FERMENTED SOYBEAN FOODS IN JAPAN

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General Description

In 1967, 200,000 tons of soybeans and 180,000 tons of defatted soybeans were used for the production of fermented foods such as Miso, Shoyu and Natto. Miso is fermented soybean paste with rice or barley and ordinary salt. Shoqu is fermented soysauce made from soybeans, wheat and ordinary salt. Natto is fermented soybeans with *Bacillus natto* which produces characteristic sticky substance and flavor. The amounts of these fermented foods produced industrially are shown in Table 1.

The amounts of Miso, Shoyu and Natto consumed in Japan per head a year are 6.7 kg, 10.2 *l* and 760 g respectively.

Although soybeans may be highly nutritious from the view point of chemical composition, they have many problems to be solved prior to be used as general food. First, they are rather hard to be digested sufficiently for nutrition even after cooking or roasting by ordinary method. For example, the digestibility of the protein in cooked or roasted soybeans is only 65%. Furthermore, the carbohydrate is rather difficult to be digested comparing with that of rice or wheat consisted of starch as the principal constitutent. The carbohydrate of soybean consists of polysaccaride named arabinogalactan, oligeosaccharides such as sucrose, raffinose, and stachyose, and reducing sugars. Except sucrose at the level of 3% of soybean, these carbohydrates are rather unfavorable constitutents as food. It also seems that they are

Table 1. Produotion of fermented soybean foods in Japan (1968)

Food	Raw materials	Amount (metric ton)
Miso		533, 000
	Soybeans	169, 000
	Defatted soybeans	6, 600
	Rice	84, 400
	Barley	18, 200
	Salt	71, 200
Shoyu		1, 027, 000 (kl)
	Soybeans	14, 900
	Defatted soybeans	147, 320
	Wheat	126, 600
	Wheat bran	7, 700
	Salt	172, 200
Natto		
	Soybeans	90, 000
		47, 000

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apt to cause the flatulence by the microorganism fermentation in the colon. Secondary, soybeans have also unfavorable substances such as trypsin-inhibitors, hemagglutinins and saponins. Finally, the characteristic beany flavor is also a defect as food especially for western people who are not familiar with soybeans historically.

These problems are solved by the fermentation processes as follows:

The digestibility is remarkably improved by the processes as cooking, mashing and fermentation. On account of the enzymatic reaction of koji and salt resistant microorganisms during the fermentation of Miso and Shoyu, the constituents of soybeans come to partly digestible. For example, 60% of the protein in rice Miso is water-soluble form including polypeptides and amino acids which give paltable flavor to Miso. In addition, the digestive enzymes produced by *Bacillus natto* during fermentation of Natto are also useful to improve the digestibility of soybeans.

As the second problem, the unfavorable substances such as trypsin inhibitor, hemagglutinin and saponin are easily inactivated by the cooking with steam or water which is a necessary process to make start the fermentation. Finally, the components which give beany flavor to food are fairly complicated. But certain carbonyl compounds such as hexanal have been reported as the principal substances which cause the beany flavor. As the result of our investigations to detect flavors of fermented foods, it was revealed that the carbonyl compounds were eliminated easily by the fermentation proceeded by *Asp. oryrae* or *Rhibopus*. In fact, there is no beany flavor in the fermented foods as Miso, Shoyu and Natto.

Miso

About 550,000 tons of commercial Miso and 200,000 tons of home made Miso are made in Japan. The Miso are usually classified into three major types on the basis of raw materials from which Miso are made. Rice Miso made from rice, soybeans and salt, barley Miso from barley, soybeans and salt, and soybean Miso from only soybeans and salt are the major types. These types are further classified on the basis of taste into sweet, medium salty and salty groups, and each group is further divided into white, light yellow and red by color resulting in the production of many varieties as seen in cheese.

Miso is usually used as an ingredient for making Miso soup. Sometimes, it is also an ingredient of a sweet pasty food with sugar and general foods such as meat, fish, shellfish, vegetables and fruits, either solely or in adequate combination. Although the manufacturing method differs more or less from variety to variety, the principle is almost same. The process consists of washing and soaking soybeans followed by cooking, preparation of koji employing rice, barley and soybeans, mixing cooked soybeans with koji and salt, addition of starter consisting of salt resistant yeast and lactic acid bacteria, fermentation, turning over for mixing well and supplying oxygen in the air, blending Miso from different tanks, and packaging including sterilization if necessary.

As raw materials whole soybeans are widely used and defatted soybeans are not employed except special use. As for the whole soybeans for making Miso, Japanese domestic soybeans are most suitable especially for making Miso of high quality, followed by Chinese soybeans and U.S. soybeans in this order. The characteristics of suitable soybeans were revealed as the results of our evaluation test employing 101 samples of U.S. soybeans and 24 varieties of Japanese soybeans. The desirable quality of the suitable soybeans should be soft with bright color when cooked. Such soybeans have high water-absorbing capacity when soaked in water for a certain time. Among the principal constituents of soybeans, protein and oil have no correlation to the water absorbing capacity but carbohydrate has keen correlation. Soybeans rich in carbohydrate show generally high capacity of water-absorption. Soybeans of large size are generally preferable since the patr of hull is less than that of small soybeans. Soybeans of paleyellow hull and hilum are acceptable especially for making Miso of white or light yellow color.

Soybeans are washed in water and soaked to absorb enough water for cooking. The soybeans are then cooked in water or steam at a temperature of 115°C for 20 min in a closed cooker capable to raise the temperature over 100°C. For koji preparation, milled rice is cleaned and washed in water and then soaked for 15 hours at 15°C. The soaked rice is steamed in an open cooker for 40 min. When cooled to 35°C, tane-koji, sporse of *Asp. oryzae* prepared at a laboratory, are sprayed over the rice and mixed well. At present, koji-fermenters of various types are widely used. After the inoculation of tane-koji, it takes about 40 hours for making koji.

Koji is first mixed with salt to stop the further growth of mold resulting in raising the temperature of koji. The mixed koji is then further mixed with the cooked soybeans and starters consisting of cultured yeast and lactic acid bacteria in order to promote the fermentation. Fermentation is proceeded at the temperature of 25–30°C in a fermenting vat. The period of fermentation is widely varied depending upon the variety of Miso and the plant where Miso is produced. For example, it takes about one week with white Miso, 1 to 3 months with rice salty Miso, and over one year with soybean Miso.

Characteristics of Miso as Food

The constituents of some varieties of Miso are shown in Table 2.

As stated before, Miso has many characteristics as proteinous food especially in its acceptability and digestibility. Furthermore, the following characteristics are worthwhile to be noted here. First, it has a so strong antioxidative activity that vitamin A added for enrichment of Miso can be preserved without any antioxidant such as BHT and BHA widely used for edible oil and fat products. In addition, the peroxide value of the oil at the level of 5% in Miso which fermented for 6 months was only 0.6 ME/kg, indicating that the oil in Miso was preserved well from oxidation. Secondarily, Miso has a strong buffer activity caused by the protein, peptides, amino acids and phosphoric acids as well as various organic acids produced by the fermentation of Miso. This property plays a very important role as a seasoning capable to use for many varieties of foods to which give palatable flavor.

Thirdly, it has a long storage life even at the room temperature. It is safe from the contamination of any pathogenic bacteria and microorganisms which might cause putrefaction. This property is caused by the salt and some substances produced by the fermentation including many varieties of organic acids and amino acids.

Table 2. Constituents of some typical Miso

Variety	Moist (%)	Protein (%)	Reducing sugar (%)	Fat (%)	Sodium Chloride (%)
White Miso	44	8	33	2	5
Edo sweet Miso	46	10	20	4	6
Salty light yellow Miso	49	11	13	5	12
Salty red Miso	50	12	14	6	13
Barley sweet Miso	46	11	15	5	11
Barley salty Miso	48	12	11	5	12
Soybean Miso	47	19	2	10	10

Advances in Miso Manufacturing

Application of starters

Although many varieties of microorganisms have been found in Miso, only the halophilic strains are important for the fermentation of Miso which contain 12% of salt. Among these microorganisms, halophilic yeast including *Saccharomyces rouxii* and *Torulopsis,* and lactic acid bacteria including *Pediococcus halophilus* and *Streptococcus faecalis* have been revealed useful for the fermentation. As seen in the manufacturing cheese, starters consisting of these useful microorganisms are added to green Miso to promote the fermentation resulting in the remarkable improvement of the flavor of finished Miso reducing the period necessary for ripening.

Application of new type cookers and koji-fermenter

For cooking soybeans, a batch-type rotary cooker capable to raise the cooking temperature up to 120°C have been widely employed. Even cooking is possible by rotating the cooker during steaming. When cooking is finished the temperature of the soybeans can be lowered instantly by reducing the inner pressure with a vaccum pump. For the cooking of rice or barley, a continuos cooker with a belt conveyer of stainless steel net is also widely used in order to save labor and time. Although there are many types of koji-fermenter, rotary drum fermenters are employed at Miso factories. The temperature and relative humidity of the circulating air can be regulated automatically so as to give the most favorable air condition for the development of the mold.

Development of new type Miso

With the purpose to reduce the concentration of salt over 12% and raise the level of protein, a new type Miso was investigated at our laboratory. The enzyme preparation, Takadiastase SS prepared by *Asp. oryzae* is added to the cooked dehulled and defatted soybeans at the level of 0.2% of the cooked materials for digestion. The soybean mash is then mixed with ordinary Miso and allowed to stand for several days for ripening. The new type Miso contains 53% moisture, 6.3% sodium chloride and 17.6% protein.

Dehydration is one way to improve the defect of Miso caused by the sticky property. The flavor, however, is apt to be affected by the temperature over 40°C for dehydration. Vacuum drying carried out at our laboratory employing several varieties of Miso gave a satisfactory result. This method is now practiced at some factories to make dehydrated Miso for making instant Miso soup in a mix with with freeze-dried vegetables, baked wheat gluten, and seasonings. Although the amount of dehydrated Miso is less than 10,000 tons, its production may increase in the future.

Problem of mycotoxin

There has been much concern regarding mycotoxins, especially aflatoxins formed by some strains of *Asperillus flavus.* Since *Aspergillus oryzae* is a species closely related to *Aspergillus flavus,* there was a suspicion of contamination of the toxin in Miso. According to Manabe and Matsuura, our colleagues, however, there were no strains which produce aflatoxins among the 238 strains collected from factories which prepare koji. Furthermore, no aflatoxin was detected in 28 strains of rice koji from Miso factories, 108 industrial Miso samples, 30 home made Miso samples, and 20 Shoyu samples collected throughout Japan.

Shoyu

The origin of Shoyu and Miso is seemed to be same. According to a literature published in 1489, the juice from mash resembles to soft Miso became to be used as a liquid seasoning to give delicate flavor to food. Therefore we can find many similarities between them in many respects including raw materials, microorganisms, principle of fermentation, and constituents. Consequently, only the principal differences are cited here to avoid duplication. Defeatted soybeans are generally used for making Shoyu instead of whole soybean from economical reason. Therefore, there is no problem to select suitable varieties of soybeans as done in Miso manufacturing.

Although there are many advances in the manufacturing method, the improvements done in the ways of cooking soybeans and fermentation of mash are the most noteworthy. A shorter cooking time at higher temperature for cooking defatted soybeans has a key to improve the processing method. This new method effective to raise the digestibility of protein in raw materials made a great contribution to increase the yield of Shoyu. According to the old fashion way, the yield was only 65% of the protein, but nowadays it was raised up to near 90%. In order to digest the constituents of raw materials, there are two ways practiced at Shoyu factories, one is enzymatic way by koji and the other is chemical way by hydrochloric acid. According to Japanese Agricultural Standards (abbreviated as JAS) the label "genuine fermented" is allowed to stick only to the Shoyu prepared by enzymatic way. There are some differences in amino acid composition between chemically hydrolyzed Shoyu and fermented Shoyu. There is no tryptophan, and very little serine and methionine exist, in the former, and little arginine in the latter. Chemical Shoyu usally contain oxalic, formic and levulinic acids and has a characteristic odor. Therefore, the determination of levulinic acid is a key to distinguish chemical Shoyu from genuine fermented Shoyu.

Shoyu is classified into three major types, namely "koikuchi" meaning deep color, "usukuchi" meaning light color and "tamari" prepared from only soybeans and salt.

Ninety percent of the total production is occupied by "koikuchi" type. Chemical analysis of "koikuchi" Shoyu of good quality indicates 1.5-1.8g of total nitrogen, 3-5g of reducing sugar, 1-2g organic acids consisting of lactic acid and acetic acid as major component, 18g of sodium chloride in 100 ml of Shoyu. It also contains many kinds of substances which give the characteristic flavors.

Natto

Natto is a unique fermented soybean food by *Bacillus natto* a strain of *Bacillus subtilis*. When fermented by *Bacillus natto* the surface of soybeans are covered with the characteristic viscous substances consisting of polymer of glutamic acids.

In 1960, an investigation to develop a new type food from soybeans was carried out at the NATIONAL FOOD RESEARCH INSTITUTE meeting by the demand of UNICEF to supply nutritive proteinous foods for children. As the results, the outline of the recommended process to make the new food is as follows: Soaked soybeans are cooked in an autoclave at 121°C for 30 min. When cooled down to 60°C, a starter of *Bacillus natto* is mixed well with the cooked soybeans for the fermentation at 42°C for 8 hours. Thus fermented soybeans are passed through a chopper with many perforations of 8 mm in diameter to be spread over trays for dehydration in vacuum. The dried materials are made into powder capable to pass through 40 mesh.

As the result of animal feeding experiment, absorption rate and biological value of the new food was 83% and 63 respectively, indicating remarkable improvement in comparison with those of raw materials.

Conclusion

We can find the fact in an ancient literature that the technology to make foods from soybeans by the aid of microorganisms was originated in China continent. These soybean foods were introduced in Japan at least 1200 years ago. As the results of modifications of the prototype, Miso and Shoyu of nowadays have been produced. As stated before, although soybeans are highly nutritious they must be processed adequately prior to be human foods. Fermentation processing succeeded to improve the defects with comparatively simple facilities and equipments.

Furthermore, we can produce foods of delicate flavor suitable for seasoning of general foods by fermentation. Many advances have been done in the field of the traditional fermented foods especially after the war. The new technology is expected to be widely utilized also in foreign countries to improve the processing or to develop new foods from not only soybeans but also other legumes.

Discussion

T. C. Tung, Republic of China: According to your statement, there were no strains which produce aflatoxins among the 238 strains collected from factories which prepare 'koji'. Does it mean that *Aspergillus oryzae* can not produce aflatoxin at all?

Answer: Judging from the results so far obtained by several researchers, it can be said that there is very little possibility for *Aspergillus oryzae* to produce aflatoxin. When discussions are made on this problem, however, taxonomical problems on *Aspergillus* should be cleared out first of all.

P. P. Kurien, India: How much of the Miso proteins N are in the form of N.P.N.?

Answer: Although the value deviates to some extent from variety to variety, the level of N.P.N. in the total nitrogen of salty rice Miso is approximately 50%. Sixty % are water-soluble, and 25% are in the form of amino-nitrogen of the total nitrogen.

K. Hanada, Japan: Are there any good or bad effects of oil contained in soybean seeds to the quality of "Miso" and "Shoyu"?

Answer: Whole soybeans have been widely employed for making Miso of high quality instead of defatted soybeans which were the principal raw materials about 25 years ago. This fact indicates that the oil in soybeans does not give unfavorable effect on the quality of Miso. Although there is no detailed investigations on the effect of the oil, it might be supposed that the oil will give good effect on the quality, especially on the flavor when partly hydrolyzed to fatty acids which form esters with ethylalcohol during fermentation of Miso.

C. P. Cheng, Republic of China: I just want to make supplement to Dr. Jain's question to Dr. Ebine. For the Chinese, soybean are frequently consumed in the form of seeds, either dried or freshly green. They are cooked with meat or boiled solely with salt and some spices.

Answer: Also in Japan, soybeans of freshly green are served as food after just boiling and even fully matured soybean seeds are consumed after cooking in water for a long time, although the amount is not so large in the comparison with that employed for processing. However all the soybeans are not always soft when cooked by ordinary method, especially imported soybeans are employed. For such hard beans, cooking and fermentation processing are favorable to improve the digestibility.

T. Sanbuichi, Japan: It seems that recently the Natto (fermented soybean) and Tofu (soybean curd) have been greatly spoiled with their tastes because Chinese soybeans have come to be used abundantly in place of Japanese as the raw materials for their productions. How about your opinion on this problem?

Answer: Although there are some varieties in Chinese soybeans which are comparable to Japanese one, it can be safely said that Japanese soybeans are generally more suitable for making Natto or Tofu of high quality. As stated in my paper, Japanese soybeans are generally rich in carbohydrate which gives relatability to the cooked soybeans and nutrients for microorganism indispensable for fermentation. In addition, Japanese soybeans are generally soft when cooked resulting in an adequate consistency of cooked soybeans as food.