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Manufacturing Digestible Proteinous Foods from Oilseeds and Pulses by Enzymic Treatment

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Improvement of Miso manufacturing

Miso and shoyu made from soybeans by fermentation have been important food in Japan from ancient times. Although highly nutritious, soybeans consiting of 40% of protein, 20% of fat and oil, and 25% of carbohydrate are so hard in consistency that they are rather difficult to be digested sufficiently for nutrition, even after cooking by usual methods. In addition, they have an unfavourable beany flavour as a food. In order to improve these defects as food, a mechanical grinding method and fermentation processing have been devised a long time ago in Japan. Miso and shoyu are from same origin, the prototype of which is considered to have been introduced from the Chinese continent about 1,200 years or more ago. At present, shoyu is different from miso in method of manufacturing, consistency, and the way of usuage. Shoyu is considered as a salty seasoning for kitchen and table use.

Although there are many variety differences in the raw materials from which miso is made, recipes of raw materials including soybeans, rice, barley and salt, flavour as well as color, the principle of manufacturing can be outlined as follows:

Milled rice is cleaned and soaked in water over-night at 15℃ or more, and then cooked in steam for 40 minutes. The cooked rice is then cooled to 30℃ to be inoculated with tane-koji (spores of *Aspergillus oryzae* purely cultured) for fermentation in a koji-chamber or koji-fermenter, of which the temperature can be controlled at 30℃. Fermentation takes about 40 hours, or until all the rice is covered by the mycelium of the mold and develops into a mat of molded rice. Soybeans are cleaned and soaked in water under the same conditions as rice, and then cooked in steam or water at 115℃ for 30 min. After cooling, cooked soybeans are mixed well with koji and salt. The mixed materials are passed through a big meat chopper, then packed into a vat or tank for fermentation. Weights equivalent to 20% of the total green miso are placed on the surface, which is covered with a plastic film sheet. During fermentation of miso, it is transferred from the first vat to another with a purpose to stir and mix homogeniously. At atmospheric temperature, it takes six months or more for green miso to be ripened well. The period for ripening is considered too long for modern industry, and an important problem to be solved as soon as possible is how to shorten the length of the ripening period. As a result of investigations on the mechanism of ripening, at least following steps have been revealed as necessary for ripening. First, the enzymes of koji act to hydrolyze the constituents of soybeans and rice or barley, resulting in the production of water-soluble protein up to 60% of the total protein, amino acids up to 30%, reducing sugar up to 75% of the total sugar (including starch and other polysaccharides). These products are important constituents of the flavour of miso as well as the media for microorganisms useful for ripening. Since this step is carried out principally by the enzymes of koji, such as proteinase and amylase, it is possible to shorten the time of reaction by raising the temperature of green miso up to the optimum temperature for enzyme reaction. In fact, ripening was easily attained by raising the temperature to 45°C, and within a week the same degree of hydrolization mentioned above was achieved. However, the flavour of the product is quite different from that of miso fermented naturally. Consequently, a second fermentation was revealed necessary for full ripening, and it is carried out by using yeasts and bacteria. As the result of screening tests of useful microorganisms, certain strains of *Saccharomyces rouxii* and lactic acid bacteria, including *Pediococcus soya* and *Streptococcus faecalis* were isolated to be employed as a starter, the same as in cheese production. Green miso to which the starter is added at the level of 10^5 per one gram is fermented at 30°C, which is the optimum temperature for the microorganisms. By this new method, the time of ripening was remarkably reduced to obtain well fermented miso of pleasing flavour.

Production of new type miso of low salt and high protein

Although miso is one of the excellent foods from the point of nutrition, palatability and keeping quality, the principal way of application as food is restricted to making miso soup. Thus, consumption by a person in a day can not be enlarged to any extent, since it contains high level of sodium chloride (up to 12% or more). Consequently it might be necessary to reduce the level of NaCl as low as possible in order to increase consumption. However, the reduction of the salt in ordinary processing is apt to cause an abnormal fermentation, or even putrefaction, during fermentation. As the result of investigation, it was cleared that the following relation must be kept among the raw materials from which miso is made safely.

$$N = \frac{5S-R}{10}$$

N : weight of salt
S : weight of soybeans
R : weight of rice or barley

From another point of view, the lowest level of sodium-chloride in water of miso, ranging from 40% to 55%, must be kept up to 18.8% or more in order to prevent acidification, which causes off-grade miso. According to these facts, mere reduction of salt results in failure. As the result of investigation, the following process was developed with success. The principle is to mix the enzyme hydrolyzate of defatted soybean with miso to be fermented for a short time for ripening. Practically, dehulled and defatted soybeans are sprayed with the same amount of water, and then cooked in steam under 0.7 kg/cm² for 40 min. Enzymes are added to the cooked material at the level of 0.2% of the raw material to be fermented at 40°C for 3 to 4 hours. Among several enzymes tested, Takadiastase-SS made from *Aspergillus oryzae* gave satisfactory results. The hydrolyzed product is then mixed well with the same amount of miso and allowed to ferment at 30 to 35°C for 3 to 5 days. Constituents of miso thus fermented are shown in the following table.

Constituents of new type miso and ordinary miso (%)

Sample	Moist	NaCl	Crude protein	Soluble-N	Amino-N
New type	53	6.3	17.6	1.4	0.35
Ordinary	49	12.5	11.4	1.1	0.25

N: Nitrogen, Crude protein = Total nitrogen × 5.71

However this new type low salt miso must be kept under 10°C for storage, since the keeping quality is poor due to the lower level of NaCl. Freeze drying gave satisfactory results on the quality of storaged miso. Practically, it was spread over a drying stainless steel plate in a depth of 5mm and dried in a vaccum of 0.5 to 0.01mm Hg for 3 hrs. The constituents of miso thus dried is: Moist 6%, total-N 4.63 (26.4% as crude-protein), soluble-N 2.6%, amino-N 0.6%, reducing sugar 18.9% and NaCl 14%.

Fermentation of pulses

This work was started in 1963 in a cooperation with Dr. T. N. Rao, Central Food Technological Research Institute, Mysore, India. Several varieties of pulses grown in India, such as Horse gram, Green gram, Black gram, Bengal gram, Cow pea, Thur Dhall, Field bean and ground-nut were employed for this investigation. As for conditions of pretreatment for fermentation, soaking in water at 20°C for 15 hrs. and cooking at 110 °C for 30 min. were ascertained satisfactory. Some varieties, including Horse gram, Cow pea, Bengal gram and Field bean, are comparatively hard and it is necessary to dehull them prior to cooking. Horse gram is too small in size to be dehulled easily. Dehulled pulses were treated under the conditions mentioned above, and mixed well with half amount of rice koji as stated in case of miso. The level of water was adjusted to 50% and that of salt to 5% for fermentation at 28 °C for 5 days. Color, flavour and texture of fermented products were remarkably improved by dehulling. Bengal gram showed the highest rate of soluble protein to total protein, and score of organoleptic evaluation, indicating a most promising raw material for fermentation. The protein solubility of Field bean, Green gram, Cow pea and Thur Dhall ranged from 25% to 30%, and that of Black gram was lowest. The flavour of fermented Thur Dhall was rather peculiar, and its evaluation was distinctively differenciated by the test panel. Green gram and Field bean were evaluated comparatively high. Dehulled Green gram is apt to be crushed during processing. Field bean is difficult to dehull mechanically, since its shape is flat. As a result of evaluation, Bengal gram and Thur Dhall were revealed as promising for making fermented food of good quality. Constituents

Constituents of fermented products (%)

Sample	Moist	NaCl	Protein	Soluble-protein	Amino-N	Reducing sugar
Thur Dhall	48	4.6	8.1	2.1	0.23	13.7
Bengal gram	44	4.6	10.1	4.2	0.29	17.7

As seen in the above table, protein contents of fermented foods from Bengal gram and Thur Dhall are not as high as that of miso. Therefore, the use of soybean or ground-nut as a supplement of protein is recommended when high protein food is required. For instance, the following recipes were tried:

Raw materials	A	B
Defatted soybean	50	50
Ground nut	75	75
Bengal gram	275	0
Thur Dhall	0	275
Rice koji	250	250
Salt	75	75

The constituents of fermented produucts are shown in the following table.

Items	A	B
Moisture (%)	44.5	47.0
Protein (%)	11.2	8.7
Fat (%)	2.3	2.7
Carbohydrate (%)	36.0	35.1
Reducing sugar (%)	14.6	15.1
Ash (%)	6.3	6.5
NaCl (%)	5.0	5.4
Protein solubility (%)	51.0	55.0

The level of NaCl could be reduced down to 5 % by this method. However, it is still too high when dehydrated. In order to reduce the NaCl content, some spices which have antibiotic effects were employed partially in place of salt. Ground meal of pepper and chilli were extracted with 10 times of ethyl alchool (95 % by volume) at room temperature for ten days and filtered with filter paper. Three hundred grams of cooked ground nut, 250 g of cooked Bengal gram, 200 g of cooked defatted soybean, 300 g of rice koji, 22 g of NaCl, and 10 g of the extract of spices were mixed well and packed into bottles to be fermented at 28°C for 5 days. Constituents are shown in the following table: (Page 24)

Although the effect of the spices as antibiotics was not definitely clear, total sugar and the reduced sugar of the fermented products to which the spice extracts were added remained at a higher level than that of control, indicating less development of micro-

Constituents of fermented products

Item	Chilli product	Pepper product	Control
Moist(%)	45.0	45.0	45.0
Protein(%)	11	11.3	10.6
Fat(%)	5.6	5.6	5.0
Carbohydrate(%)	35.4	35.6	36.3
NaCl(%)	1.9	1.9	1.9
Soluble-N/Total-N(%)	0.57	0.56	0.45
Total sugar(%)	26.3	21.1	20.3
Reducing sugar(%)	16.4	16.4	14.8
Peroxide value(%)	1.8	0.3	2.4

organisms in them. However, pepper product gave a grayish color, resulting in a lower score in the organoleptic evaluation. It is interested that the peroxide value of the spice product was lower than that of control without spices.

Application of enzyme product

In the former work to make digestible protein food, rice koji was principally employed as an enzyme sauce, modifying the process of miso manufacturing. However rice koji is made by fermentation of *Aspergillus oryzae* in an opened fermenter microbiologically. At present, it is very difficult to make koji of pure culture on an industrial scale. In fact, many bacteria can be found in finished koji, indicating possible danger of abnormal fermentation when it is used for processing of fermented food, although they are not always harmful. Consequently, it is an easy and safe way to employ enzyme products instead of koji itself for hydrolyzation of protein from pulses and beans, even though economical problems remain.

Inorder to prevent microbial contamination of the product, reduction of the pH value to the acid side was found effective. Fortunately, enzyme sauce, including acid proteinase, was obtained from microorganisms. This work is being carried out in cooperation with Mr. K. R. Sreekantiah, Central Food Technological Research Institute, and its detailed report will be published in the near future. As raw materials, Green gram, Bengal gram, Field bean, Black gram, sesame cake and ground-nut were examined primarily. At the result, protein of Green gram, Field bean, Black gram and ground-nut was found very easy to solubilize by just cooking, indicating no necessity of further enzyme treatment. Therefore, Bengal gram and sesame cake were subjected to the enzyme treatment to obtain a digestible protein product. These raw materials were soaked and suspended in ten times the products weight of water to be cooked for 20 min. under atmospheric pressure. Cooked materials were hydrolized with an enzyme equivalent to 0.15% of the raw materials at 45℃ for 5 hours, after adjusting the pH value to 3 by citric acid or hydrochloric acid. As an enzyme sauce, *Tramates sanguina* which produces strong acid proteinase and cellulase, was employed. After hydrolysis, protein solubility of Bengal gram and sesame cake was increased up to 86% and 64%, respectively. The ratio of amino-nitrogen to total nitrogen was 17% and 9%. The filtrate of hydrolized products are evaporated at low temperature and finally freeze-dried to obtain a digestible protein product. Generally, it is apt to happen that protein hydrolizate by enzyme treatment is accompanied by bitterness, which can not be tolerated in food. However, it is of interest that there is no bitterness in the products made by this method of employing acid proteinase.

One of the most important problems for human beings is that the increase of population in the world would be greater than the increase in food production. Sharp increase in the amount of available food is of primary importance. Along with the advance of the science of human nutrition, the necessity of protein for human health is clear. Although it is said that most of the foods derived from animal sources are generally more nutritious than those from vegetable sources, entire requirement of the protein needs of human beings cannot be met by animal sources alone. The role of vegetable food as protein sources are important and should be taken into consideration. However, utilization of certain pulses and oil seed cakes as food sources present some difficulties, since they may not be easily digestible and some may also contain inhibitors which interfare with the animal metabolism. Hence, the development of a process to obtain predigested high protein foods might be necessary. With this purpose, enzymic treatments was outlined in this paper. Although the reaction of an enzyme is milder than that of acid hydrolysis, which produces by-products unfavourable as food, it often gives bitterness to the hydrolized product due to the production of certain polypeptides. This problem could be solved by fermentation in the presence of salt or by hydrolysis at lower pH employing acid-proteinase.

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A Book Review of "Crop Science in Rice"

L Theory of yield determination and its applicationL

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For obtaining high yields of rice, it is of great importance to make a correct diagnosis of the plant itself and its growth, because through diagnosis one can easily make clear the defects of rice growth and find out the key to improve cultivation methods. Numerous books on rice have been published in Japan, but there are few books except this one that were written from the viewpoint above. The author of this book has been working with rice for thirty years at the National Institute of Agricultural Sciences and Prefectural Agricultural Experiment Stations in Japan, and has released many valuable scientific papers on rice.

Several years ago (1959), he published a book "The Theory and Technique of Rice Cultivation" in Japanese. The book has been in such great demand in Japan, that the tenth edition has already been issued. Based on the Japanese edition, this English edition has been re-written with some additional notes acquired from his experiences and experimental results on Indica rice during his stay in Malaysia from 1960 to 1962, when he offered technical assistance on rice as FAO expert, and also from further additional results of his experiments in recent years.

The book is not composed of the many results of researches in the past by other researchers at home and abroad, but chiefly of the results of the author's own research, the fruit of his investigation so laboriously conducted based on his own ideas. Accordingly, the book is marked by his own strong individuality. The author often expressed his regret that many books on rice cultivation seemed to be mere collections of individual phenomenon of findings not only unrelated with each other, but also not considered from the viewpoint of the whole growth process of the rice plant, and that there were few books which intended to clarify systematically the whole growth process and yield determination.

The author has written the present book with the intention to bridge such gaps as described above. Therefore, the reader will easily recognize in every page the originality of the author as well as his desire to satisfy