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REVIEW

Traditional healthful fermented products of Japan

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Abstract A variety of fermentation products, such as foods containing probiotic bacteria, black rice vinegar (*kurosu*), soy sauce (*shoyu*), soybean-barley paste (*miso*), *natto* and *tempeh,* are sold in food stores in Japan. These fermented food products are produced by traditional methods that exploit mixed cultures of various non-toxic microorganisms. These microorganisms include lactic acid bacteria, acetic acid bacteria, sake yeast, *koji* molds and *natto* bacteria. Many traditional fermented foods have been studied and their effects on metabolism and/or immune system have been demonstrated in animal and/or human cells. This review summarizes the scientific basis for the effects of these traditional food products, which are currently produced commercially in Japan.

Keywords Probiotics · Black rice vinegar · *Natto* · *Miso* · Soy sauce (*shoyu*)

Introduction

Traditional fermentation products, such as foods containing probiotics, black rice vinegar, soy sauce (*shoyu*), *miso, natto* and *tempeh,* are quite popular foods in Japan. These fermented foods have attracted attention all over the world as foods that might promote longevity. These foods are not only traditional fermented foods but also functional foods and thus experiencing a burst of popularity as health foods worldwide.

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Lactic acid bacteria in traditional fermented foods

The recent study of foods containing probiotics has consistently demonstrated their positive impact on the immune functions of animal and human cells [44]. Most of the probiotic strains of bacteria studied have been isolated from dairy products in western countries [12, 59]. With our colleagues, we have isolated several strains of lactic acid bacteria from non-dairy fermented foods that are produced in Asia. *Lactobacillus* species are the most common bacteria isolated from traditional Asian fermentation foods, which are made from rice, beans or vegetables. One such strain, *Lactobacillus plantarum* L137, isolated from *Burong Isda*, a traditional fermented food made from rice and fish in the Philippines can hydrolyze starch, a feature rarely associated with lactobacilli from dairy products [54, 55]. Thus, strain L137 is of interest for use in food processing, in particular, for the degradation of starch and lipid. Strain L137 contains 15 plasmids [54], one of which includes a gene for a starch-degrading enzyme, amylopullulanase (Kim H. J. et al., Abst. Meet. Soc. Nogei Kagaku, Japan, 2008). We have also investigated the operon that encodes acetyl coenzyme A carboxylase (*fabH-accBCDA*) from *L. plantarum* L137, which is essential for the biosynthesis of fatty acids [24].

Lactobacillus plantarum strain L137, isolated from a common component of the Philippino diet had a strong promotive effect on the immune response. Heat-killed L137 cells induced the production of interleukin 12 (IL-12) and interferon γ (IFN-γ) by spleen cells in vitro and stimulated production of IL-12 by peritoneal macrophages in vivo [43]. The cited paper was the first report of induction of the synthesis of IL-12 by a heat-killed lactic acid bacterium. Figure 1 shows details of the production of IL-12 and IFN-γ in response to various strains of heat-killed lactic acid bacteria. *L. plantarum* L137 had the most significant promotive effect on cytokine production by peritoneal macrophages and spleen cells of the strains tested. Moreover, heat-killed L137 cells suppressed production of IgE against a naturally fed antigen via stimulation of the production of IL-12 in mice [43]. The extent of suppression by heat-killed L137 cells was similar to that obtained by injection of pure IL-12. Christensen et al. [8] also showed that lactobacilli can differentially modulate the expression of cytokines in murine dendritic cells. Antitumor effects of lactobacilli have also been reported [22, 75], with L137 cells having anti-tumor effects via through restoration of the production of IL-12 after impairment of such production in tumor-bearing mice [42].

These phenomena can be explained by the hypothesis that lactic acid bacteria activate Th1 cells and suppress Th2 cells through the actions of peritoneal macrophages. A high ratio of Th1 to Th2 cells results in induction of the production of IL-12. The newly induced IL-12 and IFN-γ together suppress the production of IgE. Suppression of IgE results in desensitization to allergens since IgE is responsible for a variety of allergies. Despite a detailed analysis of the cellular fractions of L137 cells, such as the cell membrane, plasmids, chromosomal DNA and cytoplasm, we found no significant difference in terms of induction of cytokine synthesis between the various fractions from strain L137 and those from other strains of lactic acid bacteria. Thus, several cellular functions of L137 cells or entire cells seem to be required for stimulation of the production of cytokines. Heat-killed L137 cells are now produced commercially as an immunological supplement, "Lacdent", in Japan (House Wellness Foods, Osaka, Japan).

In addition to the L137 strain, several strains of lactic acid bacteria have been commercialized in Japan as probiotics that prevent pollen allergies. A third of the Japanese adult population suffers from pollen allergies, mostly due to Japan cedar, in springtime (Allergen Research Report of the Advisory Panel to the Ministry of Health and Welfare, Japan, 1996). One of the commercialized probiotics, which

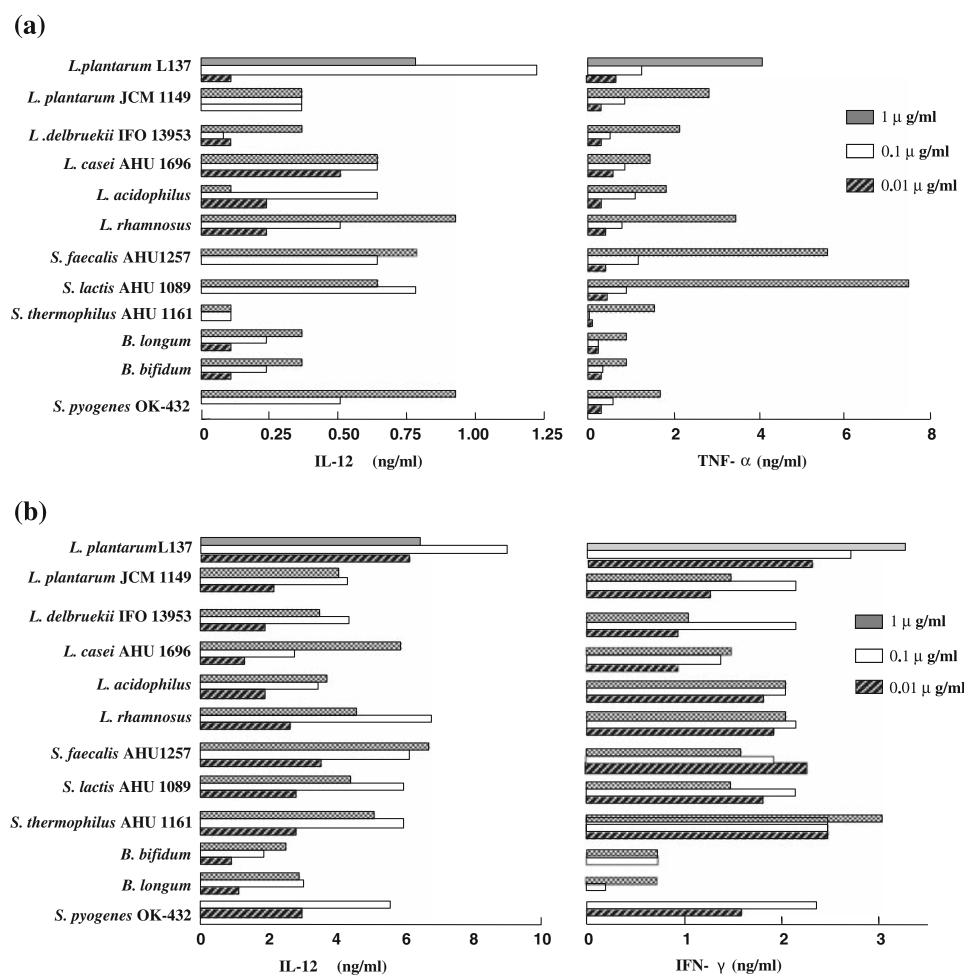

Fig. 1 Levels of cytokines in peritoneal macrophages and spleen cells after treatment with various strains of heat-killed lactic acid bacteria in vivo and in vitro, respectively. **a** Peritoneal macrophages; **b** spleen cells. Results are shown for the following genera: *L, Lactobacillus; S, Streptococcus; B, Bifidobacterium.* Each column represents the mean of results from three assays. Data are provided from Yamamoto Y (Takeda Food Industry, Osaka, Japan)

contains *Lactobacillus brevis* KB290, is called Labre (Kagome, Tokyo, Japan). KB290 cells were isolated from a traditional pickle made from cress, *Suguki*, which is cultivated in the Kyoto area. These cells induce the synthesis of IFN-α in humans [29] and increase the levels of both IFN-α and NK cells in mice [77]. The genomic sequence of *L. brevis* ATCC367 has been reported [36], and the genome size of KB290 cells is 0.2 megabase pairs (Mbp) larger than that of strain ATCC367 [77]. Many lactic acid bacteria have been isolated from traditional plant-derived fermented foods, such as *miso,* soy sauce, *sake,* and *shochu* (Japanese whiskey). A lactic acid bacterium isolated from soy sauce was identified as *Tetragenococcus halophilus* [60]. *Leuconostoc mesenteroides* and *Lactobacillus sakei* were isolated from by-products of *sake* brewing [19]. Strains of *Leuconostoc citreum,* which can catalyze fermentation at 4°C, were isolated from the rice *koji* that is used for brewing of *sake* [33]. However, immunological activation is not specific to lactobacilli that originate from plant sources, and have been found properties to be associated with strains from variety of source *Lactobacillus paracasei* strain KW3110, isolated from a dairy product, for example, stimulated the synthesis of IL-12 and repressed that of IL-4, suppressing of production of IgE against naturally fed antigens, which resulted in desensitization of mice against the antigens [11].

To enhance the functions of a probiotic strain, we developed an expression vector for *L. plantarum* that was based on one of its endogenous plasmids, pLTK2 [18], and included a native promoter from strain L137 [24]. As transgene, we chose the *choA* gene, for cholesterol oxidase, which has been widely used for degradation of cholesterol in bacteria [41]. Using this expression vector, we were able to produce a cholesterol-degrading enzyme in both *L. plantarum* and *L. casei* [25]. An edible vaccine is also been developed using L137 cells. In developed countries, more than 70% of all cases of asthma in children are caused by house dust mites. To desensitize children against mite allergens, the *Del f10* gene [1], for one of the main allergens from house dust mites, was expressed in cells of *L. plantarum* NCL21, which is a derivate of L137. The Der f10 antigen produced by *L. plantarum* NCL21 had high IgE-binding activity in sera from patients and healthy persons (Yoshida M et al., Abstr. Meet. Soc. Biotechnol., Japan, p. 53, 2005). Thus, *L. plantarum* L137 also has potential as the source of an edible vaccine for desensitization to a mite allergen.

Black rice vinegar, *kurosu*

Two Japanese traditional rice vinegars, *komesu*, which is a polished amber rice vinegar, and *kurosu*, which is an unpolished black rice vinegar, are both produced by a traditional static fermentation process. The process is not costly in terms of factory investment but considerable time is required to achieve adequate fermentation. These vinegars are known for their health benefits via the prevention of inflammation and hypertension. *Komesu* and *kurosu* are produced by the same process, namely, saccharification of rice starch by a *koji* mold, *Aspergillus oryzae*; alcohol fermentation by a *sake* yeast, *Saccharomyces cerevisiae*; and oxidation of ethanol by acetic acid bacteria to yield acetic acid. For the static fermentation process, *moromi,* which is a mixture of alcoholic liquid media that contains acetic acid and acetic acid bacteria, is fermented in large covered containers. During fermentation, no strict sterilization measures are employed and no purified strains are introduced. After a few days, a crepe-like skin of acetic acid bacteria covers the surface of the *moromi*, at which time the fermentation process begins and then continues for about a month. The surface layer of acetic acid bacteria that covers the surface of the *moromi* is removed by scooping it up in a meshed strainer, and it is then gently floated on a new batch of *moromi* (Fig. 2). We found that optimum strains of acetic acid bacteria have become naturally established as almost pure during more than 100 years of continues *kurosu* fermentation. These acetic acid bacteria were identified as *Acetobacter pasteurianus* on the basis of sequences of 16S rDNA, physiological characteristics, and patterns of DNA fragments after analysis by the polymerase chain reaction (PCR) [48].

Since *kurosu* is produced from unpolished rice, it is characterized by higher levels of amino acids and organic acids than other vinegars, with the exception of balsamic vinegars. An extract of *kurosu* was recently shown to suppress lipid

Fig. 2 Transfer inoculation with a crepe-like skin of acetic acid bacteria. A layer of acetic acid bacteria, covering the surface of *moromi,* is shown as it is being transferred to a new batch of *moromi* (Photograph courtesy of Nishikawa Y, Tamanoi Vinegar, Nara, Japan)

peroxidation more effectively than extracts of other vinegars since the former has stronger anti-oxidative activity in a radical-scavenging system [50, 66]. This extract was also shown to prevent hypertension in rats, lowering their blood pressure [51]. *Kurosu* also exhibited anti-tumor activity in a mouse skin model of carcinogenesis and it had a suppressive effect on the growth of a variety of lines of cultured tumor cells [47, 64, 65]. Vinegars produced from sweet *shochu* (Japanese whisky) post-distillation slurry by *Acetobacter aceti* had antitumor activity when administered orally to mice in a mouse model [40]. They inhibited the activity of angiotensin I-converting enzyme and repressed the formation of advanced end products of glycation [78].

At present, *kurosu* vinegar is experiencing a burst of popularity as a health drink in Japan.

Fermented soybean-barley paste, *miso*

Miso is a common Japanese food or seasoning, and the first reference to *miso* appeared on the ancient Chinese text 'Syurai' around 700 BC. *Miso* is produced by fermentation of soybeans, rice or barley with *koji* mold (*A. oryzae*), which is cultivated on steamed rice or barley under solid-state conditions, and then mixed with salt. In some cases, *Saccharomyces cerevisiae* and lactic acid bacteria are used in addition to *koji* mold. Although the most common type of *miso* is made from soybeans, many kinds of *miso* are produced, with variations the ingredients, the temperature and duration of fermentation (from one week to 20 months), the salt content (5–13%), the variety of *koji,* and the fermentation vessel.

The typical product is a thick paste that is used in *miso* soup, a Japanese culinary staple, which is prepared with vegetables, *tofu* (pressed protein curds from steamed soybeans), and dried sardines, seaweed or shellfish. Since *miso* contains a high level of protein and is rich in vitamins, amino acids, organic acids and minerals, it has played an important nutritional role in Japan. More than 95% of the Japanese population enjoyed *miso* [23], and *miso* is part of the daily diet of much of the Japanese population.

An epidemiological survey suggests that people who eat *miso* taken soup every day reduce their risk of gastric cancer [13]. The estrogenic effects of isoflavones in soybean products might limit growth of tumors in the mouse liver [15]. A study of human pulmonary adenomatosis cells and human gastric cancer cells revealed that fatty acids, such as oleic and linoleaic acids and their esters, which are found in *miso,* inhibit the proliferation of these cancer cells [74]. Since *miso* contains vitamin E, saponin, isoflavones, lecithin, choline, and dietary fiber from soybeans; vitamin B_2 from *koji* mold; and vitamin B_{12} from lactic or propionic acid bacteria, Japanese peoples believe that it is good for their health and, indeed, *miso* does lower cholesterol levels [14]; it might even have anti-aging effects and prevent arteriosclerosis [23].

We might anticipate that the high level of salt in *miso* might cause high blood pressure, although one cup of *miso* paste soup (average 150 g) contains 1.2 g NaCl, which is less than that in other favorite Japanese foods. For example, one piece of salted salmon contains approximately 5.7 g of NaCl/piece (70 g), whereas three pieces of yellow pickled radish (30 g) contain about 7.1 g of NaCl [23].

Soy sauce, *shoyu*

Soy sauce or *shoyu,* in Japanese, is a liquid seasoning that is currently used in cooking worldwide. The varieties of soy sauce that are produced depend on the various types and ratios of raw materials used, the microorganisms employed and the conditions of fermentation [21, 79]. Many other sauces that are produced in China and Southeast Asia are made from fish or grain, and they resemble *shoyu* visually. However, these sauces are not categorized as soy sauce. *Shoyu* was first made, according to legend, by a Buddhist bishop, Kakushin, at Yuasa, Wakayama, Japan, in 1254. Traditional soy sauce is produced in wooden tanks by static fermentation for 1 to 2 years. However, nowadays, most soy sauce is produced in computer-controlled systems, from the cooking of raw materials to bottling. Moistened soybeans are cooked at high pressure and high temperature, while wheat is roasted and ground. Then almost the same amounts, by weight, of soybeans and wheat are mixed with a small amount of seed *koji* mold, *A. oryzae* or *Aspergillus sojae* [31]. The mash, which contains *koji,* NaCl [15–17% (w/v)] and water, is called *moromi,* and is fermented in large enamel or stainless tanks for 6–8 months at room temperature or higher. The *moromi* also contains halotolerant lactobacilli, for example, *Pedicoccus soyae* nov. sp. [60] or *Tetragenococcus halophilus,* and halotolerant yeasts, such as *Zygosaccharomyces rouxii* and *Candida famata* [61]. Other microorganisms, such as *Aureobasidium pullulans* [62] and *Metschnikowia koreensis* [63], which contribute to the production of polyols from starch, have also been isolated from the mash. Finally, the aged *moromi* is pressed and the liquid exudate is pasteurized to yield soy sauce.

Soy sauce promotes the secretion of gastric juice in humans [32]. It contains certain bioactive components in addition to its taste components, such as amino acids and polyols [56–58] and aromatic compounds, and various biological activities of soy sauce have been reported. Such activities include anticarcinogenic activities, as indicated by inhibition of benzopyrene-induced forestomach neoplasia [6, 15, 20] and antimicrobial activity against *Shigella flexneri, Salmonella typhi, S. paratyphi, S. enteritidis, Vibrio cholera* [2], and *Escherichia coli* O157:H7 [37]. Soy sauce also has antioxidant activity [3, 34]. It contains 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl–3(2H)-furanone [45] and related compounds as flavor components [20], and these compounds have potent antioxidant and anticarcinogenic activity. Soy sauce also exhibits anti-platelet aggregation activity, which may be due to β-carbolines [72]. Soy sauce decreases blood pressure [17], and inhibition by soy sauce of an angiotensin I-converting enzyme has been demonstrated [26]. Isoflavone derivatives from soy sauce can inhibit the activity of histidine decarboxylases, which produce histamine, a mediator of inflammation, allergy and the secretion of gastric acid [27, 28]. Such active compounds are formed during digestion of soybeans and wheat and by the enzymatic reactions that occur during fermentation. During fermentation and subsequent aging of soy sauce, the proteins in soybeans and wheat are completely digested to short peptides and amino acids by microbial proteolytic enzymes, and none of the allergens present in the raw materials are present in soy sauce [52, 73]. About 1% (w/v) of the polysaccharides originating from the cell walls of soybeans are present in soy sauce and these polysaccharides have potent antiallergic activities both in vitro and in vivo [30]. The oral administration of polysaccharides from soy sauce was shown to be effective as a treatment for patients with allergic rhinitis. Thus, soy sauce is not only a traditional seasoning but also a functional seasoning. The daily consumption of soy sauce in Japan has been estimated to be close to 30 ml per person [survey from the Japan Soy Sauce Brewers Association, 1988]. Thus, on average, the Japanese consume approximately 4.5 g of NaCl per day per person as soy sauce. For this reason, the soy sauce containing a reduced concentration of sodium has been developed for consumers who are prefer to limit their intake of salt.

Koji

The use of *koji* for the saccharification of starch from grain or soybeans is a key feature of the preparation of traditional fermented foods and seasonings in Japan. A mixture of steamed rice or other grains and mold spores is placed on a large porous plate or cloth or in a wooden container through which temperature- and moisture-controlled air is passed to provide appropriate conditions for mold growth and the production of the enzymes (Fig. 3). The most popular mold for *koji* cultivation is *A. oryzae,* which is used in the production of *sake, shochu, miso, shoyu,* and a variety of pickles known as *tsukemono*. When *sake* is brewed from rice, the steamed rice is saccharified by *koji* mold and then sugars are converted to alcohol by *sake* yeast. In this process, saccharification and alcohol fermentation occur in parallel. The final alcohol content of *sake* reaches 18–20% without distillation, which is a world record as the highest ethanol content to be obtained by fermentation without distillation. *Shochu* is popular alcoholic drink that is distilled from *sake* or related alcohol in beverages. Generally, the alcohol content of *shochu* is close to 25%. Many varieties of *shochu* are produced from sweet potatoes, buckwheat (*soba*) and other grains including rice. In addition to *A. oryzae, A. sojae, Aspergillus usami, Aspergillus awamori, Aspergillus kawachii,* and *Rhizopus* spp. are occasionally used in the preparation of *koji* for the productions of these fermented foods and beverages.

The genome of *A. oryzae* [35] is 25% longer than that of the model fungus *A. nidulans* and the pathogenic fungus *A. fumigatus* [49]. The genome of *A. oryzae* has 50% more genes that are involved in the formation of secondary metabolites than the latter two species. These results might explain why *A. oryzae* not only has high enzyme productivity but also has an unusually strong ability to convert starches, proteins and lipids to a variety of bioactive compounds, as mentioned earlier in the discussion of individual fermented products. The genomic sequence also confirmed that *A. oryzae* cannot generate aflatoxin, a strong carcinogen that is produced, for example, by *Aspergillus flavus* and *Aspergillus parasticus* [39].

Natto

Recently, the Western world has started to pay attention to *natto*, which is a fermented product made from soybeans

Fig. 3 Traditional method for cultivation of *koji* with *Aspergillus oryzae.* A brewing artisan, *Toji,* is inoculating spores of *koji* mold on steamed rice (*left panel*) and the *koji* mold is cultivating in wooden containers (*right panel*) (Photograph courtesy of Miyashita N, Yaegaki Bioindustry, Himeji, Japan)

cultured with *Bacillus subtilis* (synonym, *B. subtilis* subsp. *subtilis*; former name, *Bacillus natto*). *Natto* has been popular in Japan for more than 400 years. *Natto* has a high nutritive value and is easily digested. In addition, *natto* has antibacterial effects. In the olden days, food poisoning was more common in Japan and people ate *natto* in efforts to prevent cholera, typhoid and dysentery. Approximately three quarters of the Japanese population eat *natto* at least once a week and half eats *natto* once every 3 days on average [16]. To prepare *natto* soybeans are immersed in water and then steamed. The steamed soybeans are inoculated with spores of *B. subtilis.* The original source of the *natto* bacterium was rice straw. Traditionally and, sometimes, nowadays, *natto* is made from steamed soybeans covered with rice straw, without the inoculation of bacteria. During for approximately 20 h fermentation, the starch and proteins of the soybeans are converted to a mixture that contains amino acids, vitamins, and enzymes.

Natto contains saponin and isoflavones, which come from soybeans, as well as the fibrinolytic enzyme, vitamin K_2 and dipicolinic acid, which are generated by *natto* bacteria. The concentration of vitamin K_2 increases to 124 times that in the soybeans at the start of the fermentation by *natto* bacteria [76]. Vitamin K_2 stimulates the formation of bone. After the growth of bacteria ceases (after approximately 15 h-fermentation), *natto* becomes sticky as a result of formation of poly-γ-glutamic acid [16], which also stimulates absorption of calcium. These effects of *natto* might help to prevent osteoporosis in older women in Japan. The polymer is marketed as a dietary supplement in Ajinomoto, Tokyo, Japan (Fujishima Y., Abstr Third Annu World Cong Indust Biotechnol Bioproc, p. 65 Toronto 2006). A fibrinolytic enzyme, designated nattokinase [69], not only breaks up blood clots (thrombi) but also breaks down built-up fibrin that has been associated with heart disease [10, 68]. No other enzyme came close to nattokinase in studies of 173 different foods that were examined for their potential thrombolytic activities [76]. Dipicolinic acid, found in *natto,* has anti-bacterial activity against *E. coli* O157 and against *Helicobacter pylori* [53, 70], the causative agent of stomach ulcers. *Natto* has attracted attention all over the world as a food that might promote longevity.

Tempeh

Tempeh is a traditional Indonesian fermented soybean-based food that is now being produced in Japan because of its superior nutritive qualities and metabolic regulatory functions [38, 46]. Traditional *tempeh* is produced from boiled and dehulled soybeans that are fermented with *Rhizopus* sp. After fermentation for 1–2 days, the resulting *tempeh* is pressed into blocks that are either yellow or black. The color depends on the species of soybean and mold used. Recently, improved methods for *tempeh* production have been developed that raise levels of γ-aminobutyric acid [4, 5] and isoflavones [46] in the final product. γ-Aminobutyric acid has antihypertensive effects. By adding soybean germ (hypocotyls) that contains large amounts of isoflavones, Nakajima et al. [46] prepared a new isoflavone-enriched *tempeh.* This granular fermented soybean-based food might be useful as a nutritional supplement for the elderly [71]. Isoflavones have estrogen-like functions and, thus, may be expected to alleviate symptoms of osteoporosis after menopause [7] and to suppress the onset of arteriosclerosis because of it improves the metabolism of lipids, such as cholesterol [9, 67].

These variety of traditional fermented foods consumed in Japan probably contributes to Japanese longevity, which is the world's greatest. Although genetically modified (GM) soybeans and varieties of grain have been approved by the Japanese Ministry of Agriculture, their products must be labeled as GM foods. Consumers are, however, not eager to accept such products. In Japan, therefore, all fermented foods are still produced, for the most part, from non-GM ingredients.

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