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A combination of soybean and skimmed milk reduces osteoporosis in rats

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ABSTRACT

The potential application and nutritional advantages of milky miso (MM) as a functional food were investigated. MM is a mixture of miso, a traditional, fermented soy paste, and skimmed milk. The data suggested that the total and free amino acids were higher in the mixture than in the traditional miso product alone. The newly developed product greatly improved the nutritional profile of animals and supplemented the calcium deficiency in soybean. Bone mass density (BMD) was significantly enhanced in Wistar rats that were given MM orally. Pyridinoline (type-I collagen) was remarkably reduced in the urine of the same rats fed MM compared to rats of groups fed ordinary miso. The results supported our hypothesis that MM is a useful product that can be produced and consumed on a large scale, particularly in Southeast Asia. The results also suggested that MM may be an ideal source of some novel peptides that have marked effect clinical benefits, in particular to increase BMD in young rats and potentially to children in humans.

1. Introduction

Commercial interest is increasing in functional foods that are mixtures of plant and animal products manufactured using traditional techniques. Research on functional foods has become a major area of interest in food science. To assess the relationship between functional food intake and bone health, many risk factors must be analyzed, including dietary, lifestyle-related, and other confounding factors such as age, body mass index, carbohydrate and protein intake, calcium and phosphorus levels, and the levels of other nutrients such as vitamins. However, a significant correlation exists between bone mass density (BMD) and human diet specificity and lifestyle-related habits. Diet and lifestyle are modifiable risk factors and prevention is an accepted strategy for lowering the socioeconomic impact of these factors (David, 2007).

Osteoporosis is a common disease in elderly people worldwide and is considered to be an indicator of significant public health problems in Japan. The risk factors for osteoporosis in the Japanese population have been extensively documented. However, improving the nutritional profile of people with osteoporosis through the use of functional foods has not been

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well studied. Cereals and legumes are important in the human diet (Sindhu & Khetarpaul, 2001). Soybeans and rice are of great importance worldwide, particularly in Asian, African, and Latin American countries that are on the hunger map, as well as in developed countries from the same regions. In Asia, soybeans are consumed in various forms, including soymilk, tofu and fermented products such as miso, tempeh and sufu (Kim, Kim, Hahn, & Chung, 2005). Beans and cereals form the staple diet of the populations in these regions. Miso soup (miso shiru) is one of the most important food items in Japan (250 mg/kg weight/person/day). This soup is made of a soybean paste that has been fermented under specific chemical and environmental conditions. It lacks calcium but is rich in proteins, and contains nonpathogenic microbes that help in the digestion of other food components. The daily standard intake of calcium is not appropriately achieved in the Japanese diet. Consequently, in this population, BMD is reduced and bone regression from lack of calcium occurs with increasing age.

A majority of people consider milk and its products to be dependable sources of calcium, complete proteins, riboflavin, and vitamin A (McDonald, 2008). Dried skimmed milk is made by removing fat and almost all moisture from milk followed by pulverization. Dried skimmed milk is more than 96% milk solids, 4% or less moisture, and has little fat (about 0.6%). It is about 36% good-quality protein and about 51% lactose, and is high in vitamin B complexes, especially riboflavin. In addition, it is about 8% good-quality minerals, mainly calcium. Since soybeans are rich in protein, a novel product was developed using soybeans and skimmed milk. We suggest that this product would be useful, particularly in Japan, where many people consume miso soup on a regular basis.

To minimize the risk of osteoporosis in elderly people and infants, we attempted to take advantage of the functional properties of skimmed milk and soybeans by developing a new product named milky miso (MM). This product consists of miso paste and skimmed milk. It was found to be effective in improving rats bone conditions and decreasing the incidence of lifestyle-related diseases, especially in elderly women.

Milk fermentation by lactic acid bacteria generates several peptides, including some with potential bioactivity (Quiros et al., 2007). Fermented milk products are an excellent source of peptides with potential bioactivity, in addition to providing both energy and nutrients. Korhonen (2009) reported that milk proteins are potential ingredients of health-promoting functional foods targeted at diet-related chronic disease, such as cardiovascular disease, diabetes type II and obesity. Numerous peptides with bioactive properties have been isolated from fermented dairy products (Donkor, Henriksson, Singh, Vasiljevic, & Shah, 2007). Traditional foods such as fermented soy milk and fermented cereal-dairy mixtures are important in the diets of many people in the Middle East, Asia, Africa, and some parts of Europe (Ibanoglu & Ibanoglu, 1999). A food is regarded as functional if it is satisfactorily demonstrated to have beneficial effects on one or more target functions of the body, beyond nutritional effects, in a way that is relevant to either well-being and health, or disease risk reduction (Lattanzio, Kroon, Linsalat, & Cardinali, 2009). However, little information is available on the effects of dairy products on the nutritional, functional, chemical, and sensory properties of miso products.

This study was designed to determine if combinations of plant and animal products, specifically beans and dairy products, would be considered as functional foods. The objective of this study was to develop a novel product from soybeans and skimmed milk and determine its nutritional and functional effects on lifestyle-related diseases such as bone frailty.

2. Materials and methods

2.1. Materials

2.1.1. Miso preparation

First, soybeans were purchased from a local market in Miyazaki province (harvested in the southwest region of Japan; Saga province), and then cleaned, washed and soaked in water overnight. They were steamed in a cauldron for appropriately 60 min. After cooling to 40–44 °C and mashing into a paste, they were inoculated with *Aspergillus oryzae* spores. Miso is generally prepared with *koji* (barley grains fermented by fungi belonging to the *Aspergillus* genus;), steamed beans, water, salt, and a sufficient quantity of starters (*halotolerant* yeasts and lactic acid bacteria) and finally skimmed milk (a product of Holstein cows). The mixture was placed in a fermentation tank and left for about 2 months at 25–30 °C (Fig. 1).

2.1.2. Microflora and mechanisms

The main flora of *koji* is a fungus species related to *A. oryzae*. The amylase produced by this mold hydrolyzes starch, the main ingredient of miso. Amylase activity produces large amounts of glucose, which is fermented by *halotolerant* lactic acid bacteria and yeast, producing ethanol and other substances such as ethyl esters (organic acids of higher fatty acids) and aldehydes. Various higher alcohols are also produced from the amino acids. Glycerin is a byproduct of alcohol fermentation and is responsible for the taste and improved color and flavor of miso. Immediately after fermentation, *Pediococcus halophilus*, a lactic acid bacteria, becomes active and lowers the pH, preventing growth of unfavorable bacteria. This also modifies the flavor and color. Finally, the yeasts *Schizosaccharomyces rouxii* and *Torulopsis versatilis* promote fermentation, creating the unique taste of miso (Murooka & Yamashita, 2008).

2.1.3. Skimmed milk

Drinking fresh milk or reconstituted powder milk is an effective way to obtain dietary calcium, a nutrient lacking in many Japanese diets. Dried milk is prepared from defatted cow's milk. Skimmed milk was obtained from Yotsuba Co. Ltd. (Sapporo, Japan) in the form of a powder that contained 3.8% H_2O, 36.3% proteins, 51.5% carbohydrates, 7.8% minerals and 0.6% fat.

2.1.4. Animals

Animals used in this study were maintained according to the guidelines of the Institutional Animal Care and Utilization

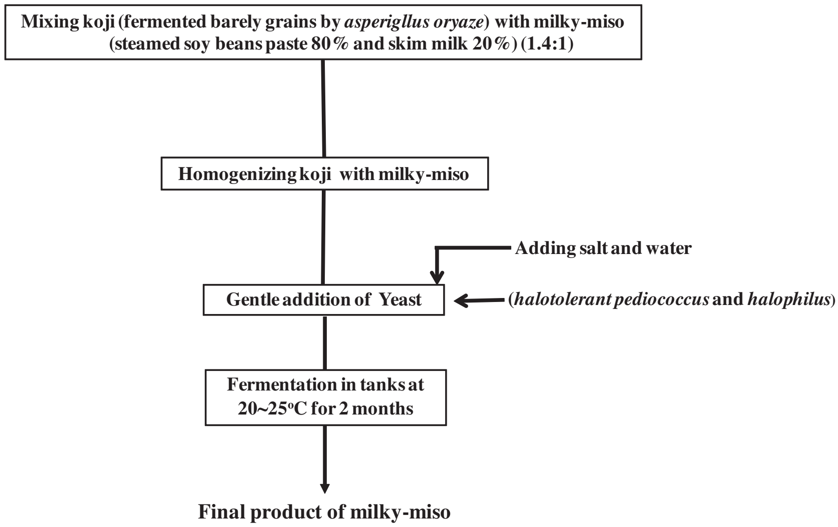

Fig. 1 – Milky miso preparation scheme.

Committee, in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals. Five Wistar rats were used for each treatment including a control group (5 groups × 6 rats). Rats aged 4 weeks were from Charles River Japan, Inc. (Yokohama, Japan). They were kept in a room with a 12 h light–dark cycle (lights on from 07.00 to 19.00 h). Temperature and humidity were controlled at 23 ± 1 °C and 50 ± 10% (Ahhmed, Namba, Nakamura, & Muguruma, 2009; Katayama et al., 2008; Muguruma et al., 2008). Diet (CRF-1, Charles River Japan) and tap water (0.2 μm-filtered) were available *ad libitum*. Upon arrival date of the rats, the experiment was initiated by starving the animals for 5 h (re-preparedness). BMD was measured before and five times over the course of the experiments. Table 1 shows the design of the animal experiments, with doses of MM and other materials that were orally administered to the rats.

2.2. *Methods*

MM paste consists of 80% steamed soybeans and 20% skimmed milk powder. MM paste was mixed with *koji*, at 1.4:1 *koji* to MM paste (Fig. 1). Subsequently, 10% salt was added with an appropriate quantity of yeast. Finally, the preparation was fermented at 25–30 °C for 2 months to obtain fermented MM. The miso paste could be refrigerated or even kept at room temperature for several months without biological or chemical deterioration.

2.2.1. *Chemical composition analysis*

The moisture, ash, fat, and nitrogen composition of MM was determined according to the methods described by Nielsen (2003).

2.2.2. *Amino acid composition analysis*

Amino acid compositions were determined by a method similar to Taheri, Abedian Kenari, and Gildberg (2009). Total amino acids were determined after hydrolysis with 6 M HCl for 16 h at 110 °C, and free amino acids were estimated by homogenizing 1 g of sample with 3 ml of 3% sulfuric acid. The mixture was kept in ice-cold water for 60 min and centrifuged at 1680*g* for 15 min at 4 °C. The supernatant was filtered through a 0.45-μm membrane filter. The sample for total ami-

Table 1 – Chemical analysis of milky miso at different stages of fermentation.

Chemical analysis	1st month of fermentation		2nd month of fermentation		3rd month of fermentation	
	Control	Milky miso	Control	Milky miso	Control	Milky miso
Salt% (W/W)	10.8	10.4	10.9	10.7	11.0	10.9
Moisture% (W/W)	40.8	42.7	46.0	46.5	48.4	47.2
pH	5.67	5.98	5.27	5.70	5.22	5.64
Color (Y%)	36.4	48.0	30.2	43.0	26.9	37.1
Total nitrogen	1.60	2.01	1.59	1.59	1.85	2.01
Water soluble nitrogen	0.37	0.38	0.79	0.79	0.90	1.20
Formal nitrogen	0.08	0.09	0.39	0.38	0.38	0.52
Total sugar	25.1	26.2	23.0	24.0	21.9	20.5
Acidity I (mL)	2.7	2.7	6.5	6	7.9	6.5
Acidity II (mL)	2.8	2.9	6.8	8.7	7.7	9.5
Ethanol% (W/W)	0	0	1.65	1.55	2.00	1.90

no acid analysis and the filtrate containing free amino acids were individually subjected automated amino acids analysis using a fully automatic amino acid analyzer (JLC-500/V, Jeol, Ltd., Tokyo, Japan).

2.2.3. *Digestion of skimmed milk*

Koji (5 g) was mixed with 20 ml distilled water, and rotated at 20 rpm for 3 h at room temperature. It was centrifuged at 1700*g* for 15 min at room temperature, and the supernatant was collected. In addition, a 40% skimmed milk solution was prepared and mixed with the *koji* supernatant (1:1).

The mixture of *koji* and skimmed milk was incubated at 30 °C for 0, 1, 3, or 6 h, as shown in the SDS–PAGE profile (Fig. 4). Some of the mixture was centrifuged at 12,000*g* for 20 min at 4 °C, and the supernatant was collected and analyzed by SDS–PAGE. The presence of ACE inhibitory peptides was detected in digested samples. To estimate biologically active peptides, the 6 h sample was analyzed by HPLC to estimate molecular weights.

2.2.4. *SDS–PAGE analysis*

Milk proteins hydrolyzed by enzymes produced during fermentation were separated, and molecular weights were determined by SDS–PAGE at ~20 mA/gel, as described before (Laemmli, 1970), using a gradient slab gel (7.5–20% acrylamide) containing 2-mercaptoethanol. Gels were stained with Coomassie brilliant blue.

2.2.5. *Calcium content determination*

The calcium content [Ca] of MM was determined as described (Kuntz et al., 1983).

2.2.6. *BMD evaluation*

Local neutron analysis was performed before and after treatments (Fig. 2), and results compared with known iliac bone histomorphometric parameters (Simon-Pires, Araujo De Souza, Laitano, & Meyer, 2005). The BMD test, also called a bone densitometry scan (DCS-600EX-III; Hitachi Aloka Medical, Ltd. Tokyo, Japan), is an important diagnostic tool for measuring the amount of calcium in bones and can be used to estimate fracture risk. From feedback provided by patients and specialists, BMD testing is easy, fast, painless, and noninvasive. Different types of machines are used to measure bone density in the hip, spine, or other parts of the body (e.g., heel, finger, or wrist). In this study, we measured the BMD in the legs. The readings of each radiology test were compared with normal values reported by a physician. Dual energy X-ray absorptiometry (DEXA) measured the bone density in the leg (Marie-Pierre et al., 2004). This did not require preparation of the rats and was completed in approximately 15 min. This procedure helps researchers assess rat bone density, and determine the osteoporosis status of experimental animals. We fed rats a normal diet on exam days, and withheld a calcium-treated diet for at least 24 h before the exam. During the procedure, rats were placed on a cushioned table, and kept motionless while the arm of the DEXA machine took measurements. Anesthesia was not used. For total body measurements the machine took measurements of hip bone density by sending a thin, invisible beam of low-dose X-rays through the bones. The amount of radiation is less than one-tenth the dose of a standard chest X-ray. Based on X-ray changes after passing through bones, a picture of the skeleton is generated. Test results were *T* and *Z* scores with *Z* scores showing the

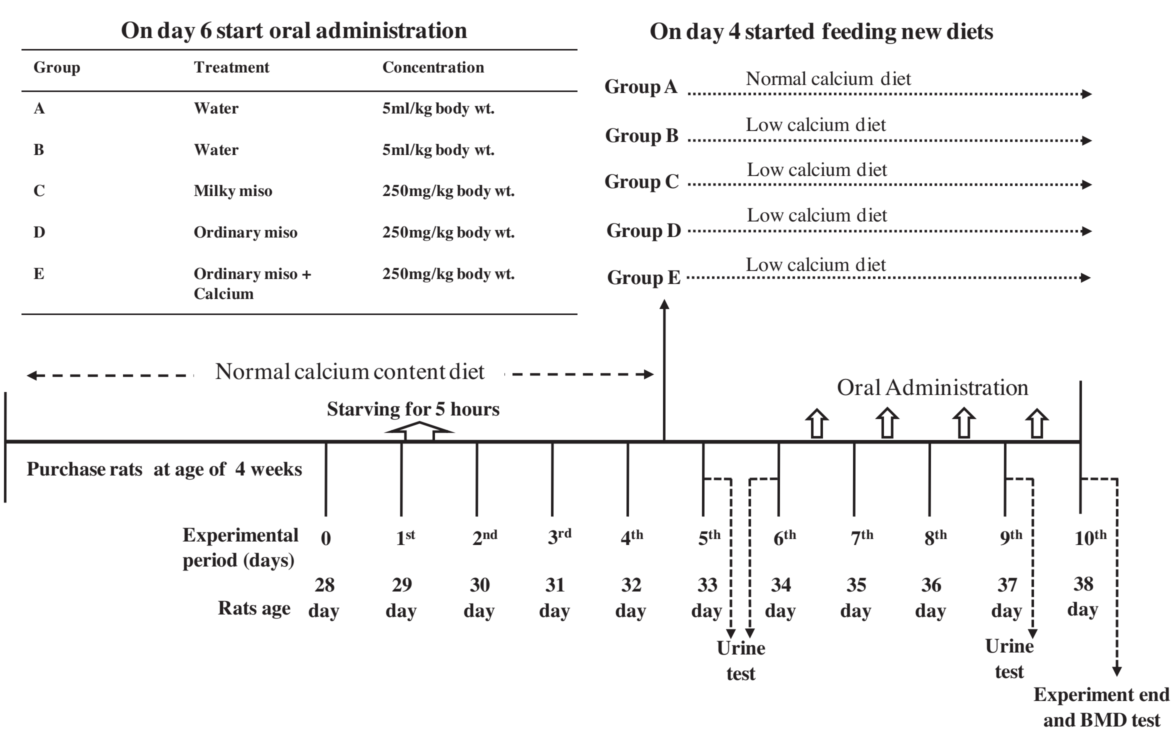

Fig. 2 – Animal experiment design. Normal diet contained 0.5% calcium and 0.035% phosphorous; added calcium for group E was 1.847 mg/g of miso.

amount of bone compared to other rats of the same age, gender, and size.

2.2.7. Pyridinoline analysis

Urine samples were collected from rats on days 5, 6 and 9, with volumes recorded. Samples were stored at −80 °C. Pyridinoline in the urine was hydrolyzed by adding 6 M-HCl and measured using kits from Mistubishi, BCL (Metra Total DPD EIA Kit, Mitsubishi Chemical Co., Ltd. Tokyo, Japan). Generally, Metra DPD kits are used in a urinary assay that provides a quantitative measure of the excretion of deoxypyridinoline (DPD) crosslinks as an indicator of bone resorption. Increased levels of urinary DPD indicate elevated bone resorption in individuals.

2.2.8. Organoleptic property tests

A group of 20 trained panelists (12 men and 8 women) with previous experience in sensory evaluation of dairy and miso products evaluated differences in flavor intensity and expressed preferences in terms of product palatability and acceptability. Evaluation was carried out at lunch time for realistic test conditions and tests were performed in a standard testing chamber. Mineral water was also provided for washing out the mouth, so panelists could accurately analyze the product and perceive the texture and flavor (combination of taste and small). Samples were approximately 50–70 ml and were served in traditional Japanese style, as warm soup in a bowl.

3. Results and discussion

3.1. Chemical analysis of milky miso at different fermentation stages

Table 1 shows the chemical composition of MM subjected to three different fermentation times. The salt and moisture contents remained unchanged and were stable at acceptable target levels in both control (rats fed ordinary miso) and MM samples under the three conditions tested. The pH values of MM were higher than ordinary (control) miso, possibly because of lactic acid production and the hydrolysis of milk proteins to peptides and amino acids. The amounts of water-soluble nitrogen in the second and third fermentation times were higher than that in the first period. This indicated that as fermentation progressed, the free amino acid content increased. Ethanol percentage was elevated by the fermentation process, and was found to improve the flavor. The data also suggested that skimmed milk was a good additive for improving the nutrient content of miso.

The results suggested that MM samples that were either fermented normally or under different fermentation conditions were acceptable (nutritional profile), and use of the traditional miso production system could contribute to a healthy diet. Proteins are a nutritionally important source of both nitrogen and essential amino acids (Liu & Guo, 2008). The modification of soy and skimmed milk proteins by fermentation usually results in structural changes and results in loss of protein solubility and increased viscosity and essential amino acid content.

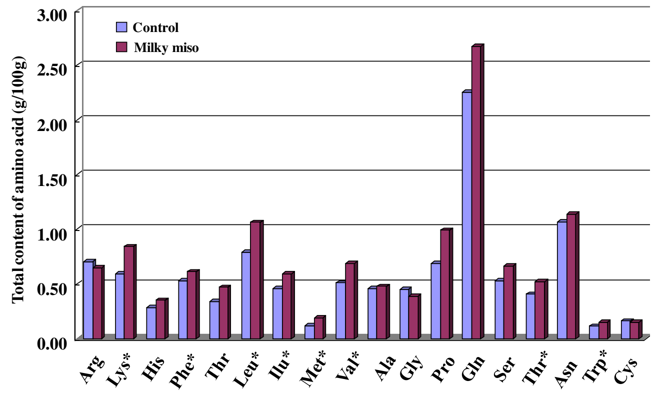

Fig. 3 – Contents of total and free amino acids in milky miso (g/100 g). *Essential amino acid.

3.2. Total and free amino acid content (g/100 g)

Fig. 3 shows the total amino acid contents of ordinary (control) miso and MM. Starred amino acids are essential. MM appeared to combine the benefits of plant and animal products. For example, the total amino acid content was elevated in MM, especially essential amino acids, which had a value higher than in ordinary miso samples. The free amino acid content was also higher in MM than in ordinary (control) miso. These data suggested that the amino acid balance increased in MM, indicating that the nutrient content of this product had improved. Proteolytic activity releases bioactive peptides of specific amino acid sequences from parent proteins that can have physiological benefits (Gobbetti, Ferranti, Smacchi, Goffredi, & Addeo, 2000; Pihlanto-Leppala, Rokka, & Korhonen, 1998; Yoshikawa et al., 2000). The rate of hydrolysis/degradation of bioactive peptides in cultures over 8 weeks of storage probably depends on the type of peptide (amino acid sequence), since some peptides were hydrolyzed faster than others. The final results indicated that some peptides of interest might disappear and new ones might appear during prolonged storage (Donkor et al., 2007). This phenomenon (protein degradation) might occur because of calcium in the milk, because calcium influences protein activity. Calcium is required to control the flow of information in cellular processes such as proliferation, functioning, and cell death. These activities are regulated by extracellular calcium and intracellular calcium stores. However, in dead cells, calcium might be involved in limiting the function of certain proteins so that some amino acids are released, resulting in elevation of their levels compared to ordinary miso.

3.3. Digestion of skimmed milk proteins by barley koji extract

Little information is available on how the constituents of skimmed milk and soybeans interact with each other during fermentation. We estimated the degradation of proteins in MM. *Koji* extract, which is believed to contain digestive enzymes that result from the fermentation process, was mixed with skimmed milk and incubated at 30 °C for four different time periods.

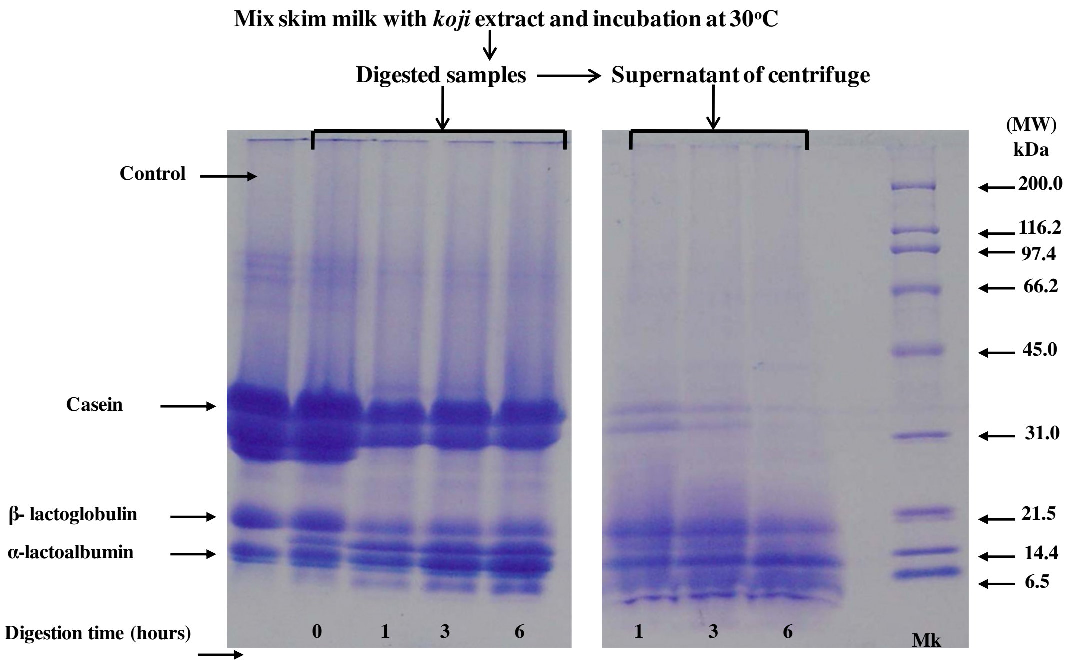

Fig. 4 – SDS–PAGE of skimmed milk proteins digested by barley *koji* extract.

For control digested samples, both casein and β-lactoglobulin were degraded after 1, 3, and 6 h. The bands of these proteins on SDS–PAGE sheet were diminished and their size was reduced (Fig. 4). In the control sample, band sizes decreased in a stepwise manner during the digestion time course. Digestibility of proteins from animal and plant products appeared to depend strongly on the fermentation process. To verify this, samples from the original digested samples were centrifuged, and the molecular weights of active peptides in supernatants were determined by SDS–PAGE. These samples showed a slight decrease in size. However, we observed some differences in band density. Samples digested for 6 h had almost no density compared to samples digested for 1 h. The biological protein activities of both centrifuged and non-centrifuged samples did not differ, and degradation had occurred to the same extent. Regardless of calcium, the results suggested that the increase in both essential and free amino acids partly occurred from digestion of milk proteins. These results suggested that skimmed milk proteins were degraded to smaller molecular species and some newly generated peptides might be present in the product; these peptides might have potential health benefits.

To further determine the molecular weight range of proteins in the skimmed milk powder digested with *koji* extract for 6 h, we centrifuged 6-h digestion samples for HPLC analysis. Low molecular-weight fractions were observed (data not shown). These data support our hypothesis that new peptides are generated during fermentation. Thus, we explored the hypothesis that biological modification of soybeans by fermentation results in hydrolysis of milk proteins, which leads to the generation of peptides and essential amino acids.

3.4. *Calcium content of milky miso (mg/100 g)*

Understanding interactions between soy proteins and milk calcium could provide insights into ways of improving the nutritional properties of products such as miso shiru. Fig. 5 shows how calcium content increased in MM during fermentation over 3 months. The data indicated that the calcium content in MM was five-fold higher than in control samples. This is a considerable increase in an element that is considered to be crucial for human function. In general, daily consumption of a certain amount of calcium-containing foods such as vegetables, cereals, and non-fat milk products is recommended. The greater the consumption of such foods, the healthier the diet, allowing the body to obtain sufficient amounts of calcium. Therefore, we propose that MM is healthy for elderly women, especially for women whose diet

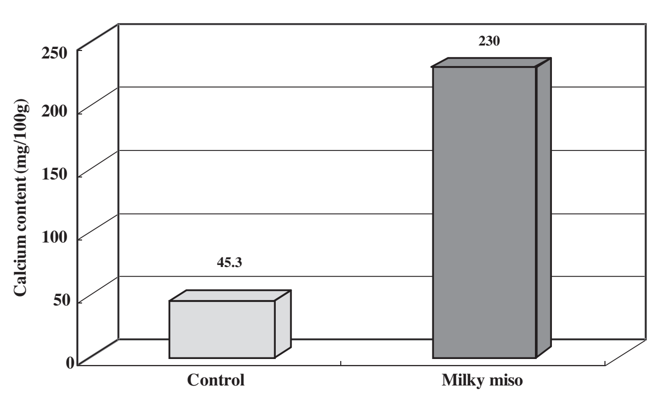

Fig. 5 – Total calcium content in milky miso (mg/100 g).

lacks calcium. Scientists have long proposed that calcium deficiencies can lead to osteoporosis, a major disease among elderly people and women. Beyond a certain age, the bone calcium and collagen structure becomes weak and fragile. There is a little information on how the constituents of skimmed milk and miso beans interact with each other, and how fermentation affects this process, especially in terms of calcium concentration and peptide yield. This study revealed the apparently higher essential amino acid and calcium contents of MM and its development as a model functional food. In this new product, the calcium content of skimmed milk complemented the lack of calcium in soybeans. The data presented in this study provide suggestive evidence that mixing legumes and dairy products may be important in developing functional foods with potential health benefits.

Such a product might help in preventing osteoporosis or minimizing its risks. For people who consume large amounts of soybeans, the data suggested that mixing skimmed milk prior to fermentation is beneficial to overcoming the lack of nutritional elements in both foods. It might also reduce the incidence of low-density bones or bone loss and the risk of osteoporosis, all of which are major problems caused by low calcium intake over an individual's lifetime. In general, interaction might influence calcium concentrations and peptide yields. Skimmed milk likely improves some nutritional properties and could be used to enhance the value of functional foods.

3.5. *BMD analysis of Wistar rats*

We measured the effects of MM on the BMD of Wistar rats (Fig. 6). Rats were fed a diet with a normal calcium content (0.5% calcium and 0.035% phosphorous) for 4 days, then subjected to diets and oral administrations that differed in Ca content and the presence or absence of miso (Fig. 2). Group A had a diet with a normal Ca content; the BMD values of this group were quite good in terms of bone improvement. Group B had a diet with a low Ca content; the BMD values of this group were significantly lower than group A ($P < 0.05$). Group C had a low Ca diet with MM, and this diet was found to be beneficial, because the BMD in this group increased gradually manner compared to groups B, D and E ($P < 0.05$). Group D had a diet with normal miso and Group E had normal miso to which Ca was added. The BMD values of both groups were the same as group B. Thus, the results indicated that MM resulted in significantly higher BMD values ($P < 0.05$) than in all groups except group A. We note that the values of group E were low even when samples were mixed with Ca.

The experimental data suggested that MM improved BMD because significant changes were observed in the BMD of Wistar rats fed MM. The fermentation process might help to improve the nutritional profile. Thus, if soybean-based food mixtures are fermented with milk products, beneficial health effects might be observed even if the essential amino acid and calcium contents do not increase. Foods that are produced through biotechnological processes (such as fermentation) might prevent or at least minimize the risk of lifestyle-related diseases.

This study on the role of milk calcium demonstrates the importance of calcium in human health and defines its many complex roles in dead cells or other postharvest animal/plant product processes. Calcium, which is the most abundant mineral in the body and essential for many processes, accounts for 1–2% of body weight. Although, introducing nutritional features into food compositions is difficult, fermenting soybeans with skimmed milk might provide considerable health benefits. Korhonen (2009) reported that bioactive milk peptides possess potential health benefits for bone and digestive health. The results of this study show that essential amino acids were significantly increased, and the BMD data also showed major improvements especially group C. Consump-

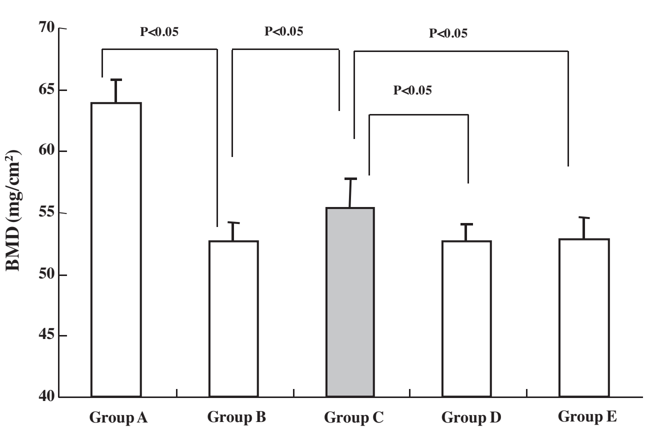

Fig. 6 – Nutritional effects of milky miso on bone mass density of Wistar rats. Results are mean ± standard error for five independent experiments, compared by Student's *t*-test.

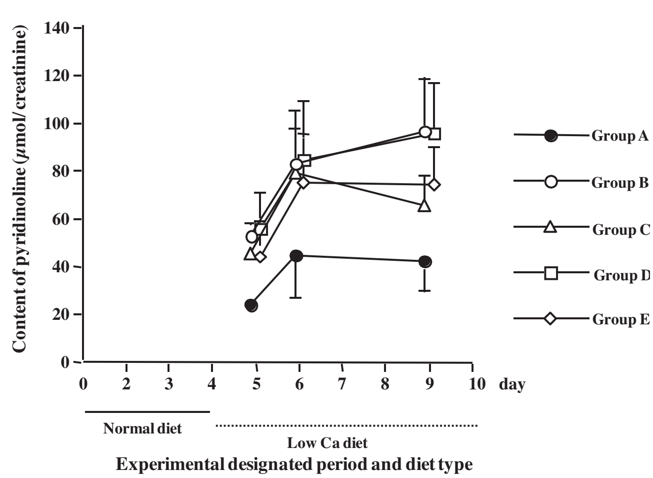

Fig. 7 – Pyridinoline content in urine of Wistar rats. Treatment was: group A, normal Ca diet with water treatment; group B, low Ca diet with water treatment; group C, low Ca diet with milky miso treatment; group D, low Ca diet with ordinary miso treatment; group E, low Ca diet with ordinary miso and added Ca treatment. Results are mean ± standard error for three independent experiments, compared by Student's *t*-test.

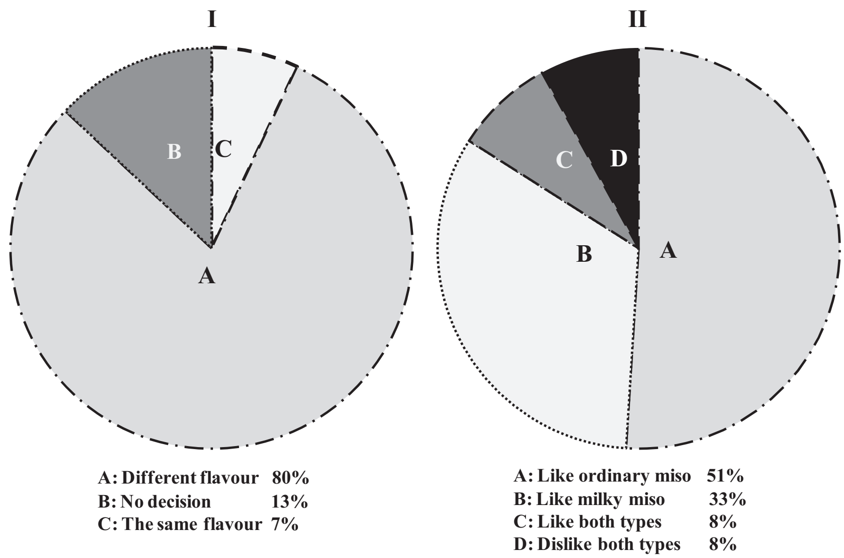

Fig. 8 – Palatability and organoleptic evaluation of 10% solutions of milky miso and miso soup. The two questions were whether the panelist could differentiate between the soups and the panelist's preferred soup.

tion of MM might enhance the individual nutritional health. This product might be consumed as a functional food. In summary, MM had increased essential amino acid and calcium contents, both of which are considered to have crucial, positive effects on human health.

3.6. *Determination of deoxypyridinoline*

Deoxypyridinoline is a crosslink of type I collagen present in bone that is excreted unmetabolized in urine and is a specific biomarker of bone resorption. It is also called D-pyrilinks and is determined though pyridinoline level tests. It was measured in the urine of rats to evaluate osteoporosis after MM treatment. The results indicated that rats on a normal Ca diet had the lowest pyridinoline level, meaning that the BMD was high in group A rats. The pyridinoline level in group B and D rats was non-significantly higher than the other groups, except for group A ($P < 0.05$). However, pyridinoline was decreased in rats treated with MM (group C), and group E had slightly lower levels than groups B and D. Therefore, pyridinoline, a biomarker that represents osteoporosis status (bone frailty) in Wistar rats, was different in rats given different oral administrations including MM. The MM (group C) showed an impact on BMD in rats, as cross-linked type I collagen decreased as indicated by urine pyridinoline content (Fig. 7). Results suggest that, fermenting a mixture of plant and animal products is associated with an improvement in nutritional value and is correlated with health benefits that have a crucial impact on lifestyle-related diseases.

3.7. *Evaluation of ordinary miso and milky miso palatability*

To compare the organoleptic properties of MM and ordinary miso, we prepared miso soup with each product at a concentration of 10% (w/v). The soups were presented to a panel of 12 men and 8 women. To determine their preferences, they were asked two questions. The first was if they could differentiate between the soups. The second was the preferred soup. The responses to the first question are shown in Fig. 8-I: 80% found that the soups tasted different, 13% had no response, and 7% found that the taste was the same. The responses to the second question are shown in Fig. 8-II: 51% responded that they liked the ordinary miso soup, 33% preferred the soup with milky miso, 8% liked both, and 8% did not like either preparation. Flavor perception occurs through a complex crossmodal system in which taste, aroma, and texture interact to form perception (Gonzalez-Tomas, Bayarri, Taylor, & Costell, 2008). Some panelists noted that miso has been used for about a thousand years, whereas MM is a new and unique type of miso, and may require some time before it gains general acceptance. Our results found 33% positive responses and 8% who said that they liked MM. Thus, 41% accepted the product. This is a considerable figure, indicating that the product is promising even though it is new and has not yet been presented commercially.

The palatability of satsuma (Japanese sweet potato) soup made with: (I) MM and pieces of satsuma; or (II) ordinary miso were also evaluated. The same questions were asked to the 20 panelists; 0% said the soup tasted the same and 100% responded that the taste was different. To the second question, 53% responded that they liked the soup made with ordinary miso and 20% liked the soup made with MM. Those who liked both preparations were 20% and those who disliked both were 7%. Therefore, we proposed that the product was acceptable in terms of taste and flavor, but its palatability was slightly less than that of ordinary miso. However, we again note that ordinary miso has been used for many years whereas MM is still an experimental product. We believe that this product is promising and may be useful as a functional food in the near future. Fermented foods are of great importance because they provide and preserve vast nutritious food in a wide diversity of flavors, aromas and textures that enrich the human diet (Steinkraus, 1994).

4. Conclusions

The potential use of fermentation to prepare mixtures of indigenous foods and dairy products for use as functional foods is an effective technique for improving the nutritional value. In such new products, the functional and organoleptic properties can be maximized, while the lack of certain nutrients can be minimized. The results of this study suggest that MM may be beneficial for young rats to develop denser BMD when rats are exposed to low calcium diet but supplemented with MM. Subsequently, fermenting soybeans with skimmed milk can substantially improve bone condition of young people and increase the nutritional value of the food.

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