

Fermentation of Soy Milk by Lactic Acid Bacteria. A Review

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ABSTRACT

Fermentation of soy milk with lactic acid bacteria offers a means of preserving soy milk and the possibility of modifying the characteristic flavor and texture to make it more acceptable to Western taste. It is possible to make soy milk yogurt-like products with acceptable texture and clean acid flavor. The choice of fermenting organisms is limited to those that can ferment the sugars typical of soy milk, i.e. stachyose, raffinose or sucrose, unless sugars fermented by the desired culture(s) are added to the soy milk. Utilization of stachyose and raffinose in soy milk should decrease its tendency to produce flatulence in the intestinal tract and therefore improve the digestibility and acceptability. Further research is needed regarding activity of the lactic organisms in soy milks.

Soy milk is a milk-like product obtained by aqueous extraction of soybeans. Since the protein content of soy milk is similar to that of cow's milk, it can contribute to infant and child nutrition in areas of the world where cow's milk is in short supply or too expensive for the general population. Soy milk is widely used in parts of East Asia, including Thailand, Taiwan, Hong Kong, and Singapore. A soy milk satisfactory to 6th grade Filipino school children was developed by Steinkraus et al. (47). However, in other parts of the world soy milk to date has been used primarily in feeding infants allergic to human or cow's milk.

Development of a beany flavor during manufacture of soy milk has limited its use in populations other than those accustomed to soybean foods. Thus much effort has been directed toward elimination of beany flavor in processing of soy milk. One approach has been to heat the soybeans either before or during initial processing to inactivate lipoxigenase to minimize development of undesirable flavors due to degradation or oxidation of lipids (31,32,54). A second approach has been complete extraction of lipids to remove the substrate which leads to development of off-flavors (43). A possible third approach is the use of fermentation to modify and improve flavor. This approach has been quite successful with fungal fermentations. Fungi such as *Rhizopus oligosporus* (23,44,45) *Neurospora sitophila* (46,48), and *Aspergillus oryzae* (51) and bacteria such as *Bacillus natto* (17) have been successfully used to prepare fermented soybean foods. Their preparation and the action of these organisms on soybean constituents has

been studied in detail (5,6,9,10,33,40,50,52). Recently Gray (14) reviewed the use of these organisms in food fermentations. Use of lactic acid bacteria in preparation of fermented soybean foods has received increased attention recently. The information on the behavior of these organisms in soy milk is in scattered form. This review summarizes the pertinent literature on the growth and activity of lactic organisms in soy milk.

FERMENTED PRODUCTS

Kellogg (19) was the first to prepare a fermented product by using a lactic culture in soy milk. A butter-like product was obtained by using *Lactobacillus acidophilus*. Later on Gehrke and Weiser (11,12) found that soy milk is a satisfactory medium for growth of lactic acid bacteria. However, they also found that lactic cultures produced less acid in soy milk than in cow's milk. *Streptococcus citrovorus* (*Leuconostoc cremoris*) *Streptococcus paracitrovorus* (*Leuconostoc dextranicum*), and *Streptococcus lactis* produced about half as much acid in soy milk as in cow's milk. In contrast to this, no significant differences in the volatile acidity measurements were observed in either of the milks.

Ariyama (7) developed a process for manufacture of a synthetic yogurt from soybeans with protein (9.8%) and mineral contents higher than those of cow's milk yogurt. The soy milk was supplemented with 15% sucrose and fermented with *Lactobacillus bulgaricus* or cow's milk yogurt cultures to obtain a desired product. Studies conducted on fermentation of carbohydrates by the Subcommittee on Taxonomy of Lactobacilli (49) and other workers (27,38) have clearly established that *L. bulgaricus* does not use sucrose. The fermentation reported by Ariyama (7) must have been obtained by using cow's milk yogurt as it contains both *Streptococcus thermophilus* and *L. bulgaricus*. *S. thermophilus* ferments sucrose (27) and hydrolysis products of sucrose are utilized by *L. bulgaricus*. It appears that acid production in the product may, therefore, have been due to the activity of cow's milk yogurt organisms rather than of *L. bulgaricus* alone as stipulated by Ariyama (7). Moreover, in view of the very high solids content, this product would be much different in appearance and consistency from that of dairy yogurt.

Streptococcus faecalis has been used to prepare a cheese-like product from soybeans (20). Soy milk was

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supplemented with casein, glucose, milkfat and vegetable oil. The mixture was treated with *S. faecalis*, rennet extract and calcium chloride. The resulting curd was processed according to the conventional method of cheesemaking.

Use of *S. thermophilus* in preparation of a cheese-like product from soy milk has been investigated by Hang and Jackson (15,16). They concluded that satisfactory cheese could be prepared by lactic fermentation of soy milk. The cheese thus obtained was superior in body and texture and contained less moisture than the product obtained using acetic acid or salt precipitation. Incorporation of skim milk and rennet extract along with lactic culture improved the flavor of the product. Skim milk stimulated growth of the organisms because of supplementation of soy milk carbohydrates with lactose. The soy milk constituents did not show any change except for the carbohydrates which were presumably used for acid production.

Obara (34) suggested that no satisfactory product can be made from soy milk by the conventional cheesemaking process. He, therefore, treated the curd obtained by salt precipitation with proteolytic enzymes before inoculating with *Streptococcus cremoris* and *S. lactis*. Among the single enzymes tested, papain gave the best results with respect to flavor and texture. Trypsin and molisin were found unsatisfactory because of poor digestibility and inferior flavor of the product. A combination of papain, biopraxe and pronase exhibited a higher rate of ripening as well as more desirable flavor and texture than other enzyme combinations or single enzymes tested.

A combination of *S. thermophilus* and *L. bulgaricus* has been used by Yamanaka et al. (55) in preparation of a sour milk beverage from soybean protein, cow's milk and sucrose. They also added certain amino acid mixtures to mask the characteristic flavor of soybean protein. An amino acid mixture containing alanine, arginine, aspartic acid, sodium glutamate, lysine, methionine and glycine appreciably masked soy flavor. Replacement of this mixture with proline or a mixture of proline and alanine gave equally good results.

ACID PRODUCTION IN SOY MILK

S. thermophilus produces remarkably greater amounts of acid in soy milk than other lactic cultures. Matsuoka et al. (24) found that *S. thermophilus* produced a greater amount of acid in soy milk than *S. lactis* and *L. bulgaricus*. However, the cheese-like product made from soy milk using *S. thermophilus* darkened significantly during ripening. Kim and Shin (21) also described greater acid production by *S. thermophilus* than *S. cremoris* and *L. bulgaricus*. However, contrary to earlier findings (15), they observed that the amount of acid produced by *S. lactis* subsp. *diacetylactis* was comparable with that of *S. thermophilus*. They also prepared a cheese-like product using *S. thermophilus* and smeared the surfaces with *Penicillium caseolyticum* and sodium

chloride. The proteolysis observed during ripening was due to action of the mold on soybean protein.

Acid production by a number of lactic cultures in soy and soy-skim milk combinations as compared to skim milk alone has been studied by Yamanaka and Purukawa (56). Acid production by *S. thermophilus*, *S. faecalis*, *L. acidophilus*, *L. bulgaricus* and *Lactobacillus casei* was higher in soy-skim milk combinations containing up to 70% soy milk than in skim milk alone. They also observed that supplementation of soy-skim milk combinations with glucose enhanced acid production by all cultures whereas addition of sucrose increased acid production by *L. acidophilus* only. They concluded that curd texture became harder with increasing proportions of soy milk.

Angeles and Marth (1) reported that acid production in soy milk was not always directly related to the growth rates of the organisms. They found that *S. thermophilus*, *Lactobacillus delbrueckii*, *Lactobacillus plantarum* and *L. dextranicum* produced greater amounts of acid in soy milk than did other species of lactic acid bacteria tested because of their ability to utilize the sugars. They did not study use of individual sugars by the organisms. Increased acid production by *S. lactis*, *S. cremoris*, *S. lactis* subsp. *diacetylactis*, *L. casei* and *Lactobacillus helveticus* in soy milk on supplementation with glucose, whey powder, or lactose showed that either the carbohydrates present in soy milk are a limiting factor or the lactic cultures tested do not fully utilize them.

Acid production in the medium depends upon growth of the organisms and their ability to ferment the available carbohydrates. The fermentable carbohydrates in soybean and soybean products are water soluble, low molecular weight oligosaccharides such as sucrose (5.0%), raffinose (1.1%) and stachyose (3.8%) (18). Mital et al. (27) tested a number of lactic acid bacteria for their ability to utilize soybean oligosaccharides for acid production. They found that organisms such as *S. thermophilus*, *L. acidophilus*, *Lactobacillus cellobiosus* and *L. plantarum*, which utilized sucrose, the major fermentable sugar in soybeans, exhibited significant growth and produced substantial amounts of acid in soy milk. Although *Lactobacillus buchneri* ferments sucrose, it exhibited a rather slow rate of growth and produced less acid in soy milk than those organisms. The poor growth and acid production in soy milk by *L. bulgaricus* was explained by the authors on the basis of inability of this organism to ferment sucrose and other soybean carbohydrates.

Lipolytic and proteolytic activities of lactic cultures in soy milk have also been studied (3,4). Most of the cultures tested did not hydrolyze soy lipids. However, *L. casei*, *L. delbrueckii* and *S. thermophilus* liberated some free fatty acids in soy milk and in MRS broth fortified with 2% soy lipids, *L. delbrueckii* and *S. thermophilus* also exhibited proteolytic activity in soy milk. Both were, however only weakly proteolytic as compared to *Bacillus cereus* and *Micrococcus conglomeratus*.

Attempts have been made to improve the flavor and texture of soy-cheese by incorporating skim milk and by mold ripening (39). The addition of skim milk was insufficient to mask the dominating beany flavor or appreciably change the texture of the finished cheese because of the effect imparted by the fibrous soybean matter. Mold ripening brought about desirable changes in the texture of the product but the improvements were off-set by the development of bitter flavors, presumably resulting from proteolysis.

PROCESSING SOY MILK

Various processing conditions and methods of preparation of soy milk affect acid production by lactic cultures in soy milk. During soaking of soybeans, carbohydrates leach into the soak water which is discarded. This practice reduces the carbohydrate concentration in the resulting soy milk medium and thus decreases acid production by lactic cultures. Lo et al. (22) found that as the soaking time for soybeans increased, larger quantities of water-soluble solids leached out in the soak water and were lost during the manufacturing process. Analysis of dry solids in soak water showed that mainly carbohydrates were lost. Badenhop and Hackler (8) found that soaking soybeans in 0.05 N sodium hydroxide yielded milk with a pH of 7.37. Such an alkaline pH will not favor the growth of lactic cultures. Mital et al. (27) observed that all lactic cultures exhibited higher growth and acid production in soy milk prepared by a hot grind method than in soy milk prepared from dehulled defatted soybeans. They concluded that loss of some of the growth factors during solvent extraction of the defatted beans explains the difference in the behavior of the organisms in the two milks.

The effect of heating on acid production in soy milk has been studied by Angeles and Marth (2). They found that heat treatment of soy milk (60 C for 15 min) enhanced acid production, whereas heating of soy milk to 80 C for 5-60 min greatly reduced acid production as a result of marked increases in the concentrations of inhibitory substances such as sulfhydryls and toxic volatile sulfides. More severe heating at 100 or 120 C progressively improved the quality of soy milk as a substrate. The beneficial effect of severe heating on acid production was attributed by the authors to expulsion of toxic compounds and a decrease in the oxidation-reduction potential of the medium.

Soybean oligosaccharides such as raffinose and stachyose contain $\alpha(1 \rightarrow 6)$ galactoside linkages. Since the human gastrointestinal tract does not possess an α -galactosidase (13), the metabolic fate of these oligo-saccharides is uncertain. Some investigators (35,36, 37,41,42) have suggested that raffinose and stachyose are responsible for flatulence often experienced following consumption of soy products. With a view to removing these oligosaccharides by lactic fermentation, Mital et al. (26) tested a number of lactic cultures for α -galactosidase activity. They found that the enzyme is constitutive in *L.*

buchneri, *L. brevis*, *L. cellobiosus*, *Lactobacillus fermentum* and *Lactobacillus salivarius* subsp. *salivarius* and present in the soluble fraction of the cell. However, it could also be induced in *L. plantarum* (25). Fermentation of soy milk with lactic cultures possessing α -galactosidase activity reduces raffinose and stachyose contents (28). However, rapid utilization of sucrose resulted in a pH low enough to inhibit further use of higher saccharides and thus proved to be a limiting factor in their complete removal.

The changes in pH of soy milk fermented with *L. acidophilus* and *L. bulgaricus* have been compared by Wang et al. (53). *L. acidophilus* significantly lowered the pH of soy milk, indicating its ability to use soy milk carbohydrates for acid production. In conformity with the findings of Mital et al. (27), they also found that *L. bulgaricus* did not change the pH of soy milk, reflecting the inability of this organism to ferment sucrose, the major soy milk carbohydrate. They also observed that beany flavor was masked by fermentation of soy milk with *L. acidophilus*.

FLAVOR OF SOY MILK

Generally, the flavor of soy milks is unacceptable in the Western World. Usually soy milks have compared to other soy milks to determine if a flavor improvement has been achieved. Mital and Steinkraus (29) compared the flavor acceptability of soy milks prepared by a hot-grind process and an extraction process utilizing defatted soybean flour to that of fresh homogenized cow's milk, a well known, highly acceptable flavor standard. Hot-grind soy milk was significantly different and distinctly inferior to cow's milk in flavor. Soy milk prepared from defatted soybean flour by aqueous extraction and addition of 2.5% refined soy oil and 2% sucrose was rated slightly inferior to fresh cow's milk in flavor. Soy milk prepared from defatted flour was also lactic-fermented to prepare a yogurt-like product whose flavor acceptability was compared with fermented cow's milk. The fermented soy milk had a satisfactory gelatinous curd and an acceptable flavor. Reduced acid production in the fermented soy milks was an impediment to higher acceptability. Later Mital et al. (30) found that acid production in fermented soy milks could be enhanced by enrichment of soy milk with sucrose, glucose or lactose and further increased by using selected lactic cultures.

CONCLUSION

Use of lactic cultures in improving the acceptability of soy products holds great promise. However, more information is needed regarding activity of these organisms in soy milk. Use of soybean oligosaccharides essential for acid production needs further investigation if lactic cultures are to be used in preparation of fermented soy products. Use of oligosaccharides in soy products for acid production will also make the products less flatulent. Also, information on proteolytic and lipolytic activities of lactic cultures needed for prepara-

tion of products such as cheese is still insufficient. Investigations along these lines will be of great help in formulating acceptable soy foods for human consumption in the future.

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Report of The Journal Management Committee

The Journal Management Committee met on August 12, 1979 and discussed the actions taken on previous recommendations as well as the current status of the Journal. As in the past, the Committee decided to reaffirm a few previous recommendations that had not been acted upon by the Executive Board and to make new recommendations for consideration by the Board as follows:

1. The Committee recommends that a feasibility study be conducted of splitting the *Journal* into two monthly publications with contents as follows:
 - (a) New Journal
 - (1) Articles and speeches of interest to the practicing sanitarian and fieldman
 - (2) News and Events
 - (3) Association Affairs
 - (4) Program-Annual Meeting
 - (5) 3A Standards
 - (6) E-3A Standards
 - (7) Letters to the Editor
 - (8) Abstracts of papers published in the *Journal of Food Protection*
 - (9) Advertisements
 - (10) Coming Events
 - (11) Sustaining Membership
 - (b) *Journal of Food Protection*
 - (1) Scientific Papers
 - (2) Advertisements
 - (3) Sustaining Membership

2. The Committee recommends that the Assistant Managing Editor of the *Journal* review other publications to identify and obtain material of direct interest to the practicing sanitarian for reprinting in the *Journal*. (Repeat from 1978)
3. The Committee recommends that the Instructions to Authors material be expanded by adding a section specifically for the writing of articles for the practicing sanitarian. (Repeat from 1978)
4. The Committee commends the printing of information from *Morbidity and Mortality Reports* of CDC and recommends that this kind of coverage be extended to the *Federal Register*.
5. The Committee recommends that the source of the material printed as filler in the main section of the *Journal* and in the News and Events section be identified at the end of each article. (Repeat from 1978)
6. The Committee recommends that a Directory of Members of IAMFES be published.
7. The Committee recommends that a Committee be appointed to seek sustaining members from the food industry.
8. The Committee recommends that the *Journal* name, volume and data be published on each page of the *Journal* that is used for scientific papers.
9. The Committee recommends that the page charge for scientific articles be

increased to \$30.00. It further recommends that a \$35.00 page charge be placed into effect if the added revenue is needed for initial support of the increased costs of publishing two journals.

10. The Committee recommends that the IFT and Am. Dietetic Assoc. meetings be attended and a suitable display be prepared to call attention to the IAMFES and the *Journal*.
11. The Committee recommends that the Asst. Managing Editor appoint a committee of knowledgeable sanitarians and a committee of dairy fieldmen whose responsibilities would be to develop lists of subjects of current concern that should be developed and published in the *Journal* as well as to identify people who could prepare these articles. (Repeat from 1978)
12. The committee recommends that the size of type used for abstracts, titles of figures and tables, and column headings in tables be increased.

R. B. Read, Jr., Ph.D.
Chairman