

# Prospects of Fermented Soymilk as an Expected Plant-based Food

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## Abstract:

Fermented soymilk is receiving more concern worldwide as consumers have pursued a healthy and low-carbon lifestyle in recent decades. Fermented soymilk has not only an inexpensive cost and plentiful nutrition including high content of proteins, low in cholesterol, and lactose-free but also can produce many bioactive substances after fermentation by various microorganisms. The bioavailability of nutrients such as the digestion of proteins and the reduction of antinutritional factors are improved because of the hydrolyzation of different enzymes generated from the growth of microorganisms in soymilk. Furthermore, the contents of bioactive compounds including the bioactive peptides and the free isoflavones are promoted significantly. These bioactive compounds are associated with some health benefits such as antihypertensive, antioxidant, antidiabetic, anticancer, and hypocholesterolemic effects. The sensory qualities are changed as well after fermentation. This shows that fermented soymilk can be expected as a novel nutritional and functional food in the food markets. This review covers the changes in main nutrients, bioactive compositions, and flavor compounds after the fermentation of soymilk to provide more information for the development of fermented soy milk as one of the burgeoning soy products.

**Keywords:** Fermented soymilk; plant-based food; lactic acid bacteria.

## 1. Introduction

Soymilk is a well-known soy product in China and Southeastern Asian countries, which is an aqueous extract of soy through soaking, grinding, heating, and filtering. Soy is a rich source of many nutrients including proteins, lipids, carbohydrates, dietary fiber, and other functional compounds such as isoflavones,

minerals, vitamins, and phenolic acids. Soymilk is a popular food in many Chinese breakfast dishes because it is excellent with low in fat, economical protein, and other plentiful nutrients. However, its global acceptance is limited due to its unpleasant beany flavor, antinutritional factor, and indigestible oligosaccharides [1].

Fermentation is an old and effective process technol-

ogy for foods. The fermentation process not only prolongs the preservation, modifies the flavor and texture, but also produces many bioactive substances for the beneficial health of human beings. Moreover, it is well known that the microorganisms themselves, for instance, probiotics and bifidobacterial, have many benefits for humans. The fermented soy products have been consumed for several centuries in Eastern Asia. Natto, miso, douchi, stinky tofu, soy sauce, etc. which are fermented by different microorganisms, are the representative traditional foods in different Asian diets. These foods provide or improve kinds of nutritional values and healthy benefits through their fermentation process with their special microorganism, including bacteria, fungus, and yeast [2].

Consequently, it is expected that soymilk can lower its shortcomings through fermentation by microorganisms. In recent decades, food researchers have conducted many studies on fermented soymilk to meet consumers' demands worldwide. Numerous studies showed that soymilk can be mainly fermented well with bacteria, fungi, and yeast which are thought to be safe and beneficial microorganisms in traditional fermented foods. By employing these various microorganisms, fermentation may induce to get the more desirable final products. It is well known that foods fermented through lactic acid bacteria (LAB), similar to fermented animal-based milk, not only improve the sensory properties of foods but also provide many benefits for human health. Fermented soymilk is also looked forward to being popular through using various strains for fermentation and controlling fermentation conditions.

Up to now, many studies have reported that the nutrients, functional ingredients, and sensory properties of soymilk can be changed or generated after the fermentation, and the consumption of fermented soymilk might protect human beings from cancer, diabetes, hyperlipidemia [3]. In addition, soymilk is low in cholesterol and barely lactose. Fermented soymilk has received notice as a potential substitute for fermented animal-based milk, such as yogurt and its derivative products. However, the mature final products of fermented soymilk are not so widespread in the food markets due to the kinds of reasons.

In this article, our main aim is to summarize the recent studies on the changes in bioavailability of nutrients and bioactive substances in fermented soymilk, to provide more information for the development of fermented soy milk as one of the burgeoning soy products.

## 2. Changes of Main Ingredients in Fer-

## mented Soymilk

### 2.1 Improvement of Bioavailability of Nutrients

As known, soy protein is one of the most important components accounting for about 40% of the soybean. Due to their high quality and low cost, soybean is seen as the best source of protein for vegetarians, lactose-intolerant consumers, and those living in countries and regions with a shortage of animal-based protein. However, soy proteins not only have a large molecular weight but also contain some antinutritional substances (such as trypsin inhibitors), which can reduce the digestion and adsorption of soy proteins in the human body. The low bioavailability of proteins limits and affects the application of soy products [4].

Soy protein is mainly made of 7 S ( $\beta$ -soybean companion globulin) and 11 S (soybean globulin) globulins, which together occupy over 70% of the total protein contents. These proteins are not easily degraded or converse into simple compounds in the human digestive system due to the complexity of structure. Many researchers found that the fermentation process for soymilk can change effectively these large-molecular-weight into small peptides and even free amino acids [5]. These changes should be attributed to the various proteases generated during the growth and production of the different LAB and other microorganisms since these produced enzymes can hydrolyze these macro-molecular proteins. The significant increase of smaller molecular weight peptides and free amino acids in soymilk after fermentation has been observed in many studies.

Singh et al. reported that soy proteins are hydrolyzed by the proteinases produced by an indigenous *Lactobacillus plantarum* resulting in an increase of the peptide contents after the fermentation of soymilk [6]. They found the protein fraction of 10KDa has the highest peptide contents compared to 3KDa and 5KDa fractions and exhibited the strongest antioxidant. These peptides were proven from the hydrolysis of two major proteins glycinin and  $\beta$ -conglycinin. Zheng et al. indicated that all the 7 S protein and 11 S acidic protein were degraded in soymilk fermented by a new LAB spore isolated from Chinese fermented vegetables, while the proteins with the lower molecular weight of less than 25KDa increased in intensity [7]. These findings showed that soy protein can be degraded into smaller peptides and free amino acids through the fermentation of LAB.

Additionally, other researchers studied the mechanism of the proteases from LAB for soy proteins. They found that some hydrolyzation of proteases can change the spatial structure, such as destroying the secondary structure

and reducing the  $\beta$ -fold content. Some researchers also indicated that altering the composition of the secondary was related to an improvement in the digestibility of soy proteins. They found that LAB fermentation altered the microstructure and conformation of soy protein, and introduced the changes of the digestibility of soy protein [8]. Consequently, the changes in the spatial structure or degradation of macro-molecular soy protein after fermentation also lead to the alteration of allergenic proteins in soymilk. Some studies already observed the structure of proteins having antigenicity and potential allergenicity in soymilk was destroyed after fermentation. Lu et al. investigated the allergenicity of major allergenic proteins in soymilk fermented by *Lactobacillus* [9]. They found that the advanced structure of proteins including secondary and tertiary structure was destroyed and the antigenicity of protein was also significantly reduced in fermented soymilk. Furthermore, it was also proven that the antigenicity significantly became weak in vitro simulated gastrointestinal digestion.

Antinutritional factors in soy are harmful substances that interfere with nutrient absorption and decrease the nutrients' bioavailability. Trypsin inhibitors, phytate, and lipoxigenase are the main antinutritional compositions in soymilk. Trypsin inhibitor is a protein-based anti-nutritional factor in soybean, which influences protein digestion by inhibiting trypsin activity. The changes or degradation of trypsin inhibitors was crucial to enhance the digestion property of soybean products. Phytate generally chelates minerals such as  $\text{Ca}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Fe}^{2+}$  and bonds with amino acids resulting in the decrease of digestion of the nutrients. Lipoxigenase can oxidize unsaturated fatty acids to produce an unpleasant taste in soymilk. Many studies found that fermentation can reduce the accounts of these anti-nutritional compounds in soy products and improve the digestion and absorption of nutrients [10]. Madjirebaye et al. reported the contents of the phytic acids and trypsin, and the activity of lipoxigenase in soymilk fermented by *Lactobacillus plantarum* and *Streptococcus thermophilus* were notably decreased respectively than those in unfermented soymilk [11].

Raffinose and stachyose are the major oligosaccharides in soymilk, which can cause flatulence after the intake of soybeans by human beings. The reason is that these oligosaccharides are just utilized by the putrefactive bacteria in the large intestine leading to produce the gases because there is no specific enzyme to hydrolysis raffinose and stachyose in the small intestine of humans. However, LAB and other microorganisms can produce  $\alpha$ -galactosidases which can hydrolyze stachyose or raffinose during fermenting the soymilk. Singh et al. assessed the abilities of producing the  $\alpha$ -galactosidases and reducing oligosac-

charides in soymilk fermented by six *Lactobacillus*. Results demonstrated that all of the used *Lactobacillus* have the potency to generate the amount of  $\alpha$ -galactosidase and breakdown oligosaccharides although their abilities are various [12].

These results suggested that the fermentation can produce abundant and various enzymes to hydrolyze or transform the complex compounds resulting in the increase of the bioavailability and the digestibility of nutrients in soymilk.

## 2.2 Increasing Bioactive Compounds

Isoflavones are a significant bioactive constituent in soy. Most soy isoflavones exist in glucosides form which is conjugated with free isoflavone moieties and sugar moieties through the  $\beta$ -glycosidic bond, which have no bioactivities. Free isoflavones, as the aglycon moieties of glucosides, exhibited many kinds of bioactivities after hydrolyzation of the glucosides, which are associated with the antioxidant, immunomodulatory, anticancer, and antidiabetic capabilities, enhanced blood lipid levels, and insulin levels, and controlled blood pressure [13]. The activities of  $\beta$ -glucosidase produced from microbial fermentation are the most important factor because they can break down the  $\beta$ -glycosidic bond of glucosides into bioactive free isoflavones. Therefore, many studies are focused on investigating the ability to produce  $\beta$ -glucosidase in various microbial strains.

Hati et al. investigated the  $\beta$ -glucosidase activities in soymilk fermented by six probiotic *Lactobacillus* cultures respectively [14]. They gained the highest  $\beta$ -glucosidase activity and isoflavone bioconversion rates after fermentation for 12 hours. The increase of free isoflavone was thought to contribute to the enhancement of the biological functionality of soymilk. Yuksekdag et al. indicated that  $\beta$ -glucosidase activity depends on the strains of culture, growth medium, and growth conditions [15]. They found the  $\beta$ -glucosidase-specific enzyme activity in soymilk fermented by *Lactobacillus* and *Bifidobacterium* strains is higher than those by *Propionibacterium* strains after screening 54 microorganisms. These studies showed that the fermentation of soymilk by microorganisms was effective in increasing the bioactive isoflavones.

Bioactive peptides from the degradation of protein are another significant bioactive compound in soymilk. Numerous studies indicated that small-molecular peptides from the hydrolyzed proteins during fermentation of soymilk not just can improve the digestibility of soy proteins, but also may have bioactive functions (antioxidative, antihypertensive, hypocholesterolemic, anticancer, immunomodulatory, etc.) [13].

Antioxidant and angiotensin-converting enzyme (ACE) inhibitory properties are the representative bioactivities for the short-chain peptides in fermented soymilk. Free radicals produced from oxidation can accelerate or induce some diseases such as cardiovascular disease, neurodegenerative disorders, cancer, etc., because free radicals destroy DNA, proteins, and lipids, leading to damage the cell function. ACE has the capability of increasing blood pressure. Singh and Vij reported that they identified 18 antioxidant peptides, 16 ACE inhibitory peptides, and 17 peptides having both antioxidant and ACE inhibitory activities in fermented soymilk [6]. Hasan et al. reported that the bioactive peptides generated from soymilk fermented by different LAB strains were remarkably increased in quantity and exhibited antioxidant and ACE-inhibitory activity [16].

Plentiful studies have proven that it is affirmative that microbial fermentation increases significantly the bioactive compounds in fermented soymilk.

### 2.3 Improvement of the Flavor

As known, the unpleasant beany-flavor greatly limits the soymilk to be accepted by consumers all over the world, although soymilk is beneficial for human health. Previous studies found alcohols and aldehydes are important off-flavor volatile compounds. These compounds are mainly generated from the oxidation of unsaturated fatty acids and amino acids in soy [17]. To reduce or change the unpleasant flavor through different strains and fermentation conditions is always one of the most important works for many researchers. Zheng et al. revealed the synergistic fermentation of soymilk with prebiotics and probiotics may improve its flavor properties [18]. They found that some ketone and acetoin modified significantly the flavor of soymilk. The volatile compounds related to the beany flavor, such as 1-octen-3-ol and hexanal, were not detected in their fermented soymilk.

Philippe et al. indicated the different fermentation conditions with two strain starters decreased significantly the content of aldehydes such as hexanal which is responsible for the characteristic beany flavor in soymilk. Besides, a high level of abundance of 2-butanone, acetoin, and acetic acids was observed in fermented soymilk, which improved the overall sensory acceptability of fermented soymilk [11].

Zhang et al. studied the key compounds of spoiled odors which are thermally generated in the sterilization process and hurt the sensory quality of fermented soymilk. They found the spoiled intensity is different with the process conditions and the strongest odors were perceived at the sterilization of 90°C while the titratable acidity was 5.4 g/

kg. They indicated 2-methyltetrahydrothiophen-3-one was the key compound causing the spoiled odor for fermented soymilk among 44 volatile compounds identified [19].

These studies clarified that the fermentation strains and process are crucial to changing the sensory quality of the products of fermented soymilk.

### 3. Conclusion

Besides the abovementioned substances, changes in other nutritional compositions are also reported, such as the increase of B group vitamins, total soluble iron, polyphenol, and tocopherol, etc. The increase of these compounds is based on the hydrolysis of various enzymes and the metabolites generated from the microorganisms.

Fermented soymilk, as a novel plant-based functional food for consumers, showed huge attraction in the market. The microorganism with the ability to produce specific hydrolytic enzymes for fermenting soymilk is undisputedly the most crucial factor in the process of fermented soymilk. The different species of microorganisms produce a lot of various enzymes, such as protease,  $\alpha$ -galactosidases, and  $\beta$ -glucosidase, and meanwhile generate many different metabolites. Part of them enrich the nutritional value, some ameliorate the flavor and others may modify the texture. Researchers are expecting that the specific starters for fermentation can not only increase the functional ingredients but also impart good sensory properties. Consequently, many researchers are devoting the challenge to selecting suitable strains using advanced knowledge and technologies. The deeper works include the commercialization of starters, the stability of products, and even the production of new functional ingredients. Predictably, the screening the excellent combined starters is the most important for the fermentation of soymilk.

Besides the deeper study of the fermented strains, the stable and mature fermentation process is also important for the industrial production of fermented soymilk. Sensory acceptance is the decisive factor for consumers in the food markets. The improvement of flavor, texture, mouthfeel, and stability is the next major work followed by the selection of strains. A systematic study is necessary on the fermentation conditions and the kinds of additives that can support the success of the final products. Fermented soymilk is expected as a popular soy product all over the world.

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