

Physicochemical and Functional Properties of *Lactobacillus* Fermented Soybean Milk

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Abstract. In this research, the physicochemical and functional properties of *Lactobacillus* fermented soybean milk have been studied by testing the pH value, total acidity, viable bacteria count, amino acid nitrogen, and active isoflavones count of single strain and mixed strain fermented soybean milk. The results showed that, the nutritional value of fermented soybean milk was higher than that of unfermented soybean milk in general. At the same time, mixed strain fermented soybean milk showed better results than single strain in the above testing. *Lactobacillus bulgaricus* and *Lactobacillus plantarum* isolated from traditional fermented yogurt in Xinjiang can be used together as starter culture to soybean milk fermentation that can produce more beneficial components.

1 Introduction

Soybean is rich in nutrients. Its protein content is about 40%, which is similar to that of animal food. Soybean also contains soybean phospholipids, soybean oligosaccharides, isoflavones, saponins and other bioactive substances, so soybean has a lot of health benefits [1]. For example, soybean is helpful to reduce the risk of cardiovascular disease by regulating serum cholesterol, improve gastrointestinal function, alleviate constipation, reduce the risk of osteoporosis, prevent the formation of kidney stones, and reduce woman's menopausal symptoms by regulating the sex hormones [2-4]. In 1996, Chinese government put forward the "Soybean Action Plan". This plan aims at improving the dietary habits of people, the production and supply of food resources and the level of consumption. In order to improve the dietary structure, we should make better use of soybean and other high-quality protein from plant food. Soybean milk, as the most popular and commonly used food, is still the main processing and cooking form of soybean intake.

In recent years, probiotic properties of lactic acid bacteria (LAB) have been extensively studied. As a starter culture, LAB are also widely used in food fermentation industry. For example, traditional fermented yogurt in Xinjiang region of China is rich in various LAB strains and embodies very superior function [5]. If they are used in fermented food, it will be a new way to study the functional fermented food.

Fermented soybean milk is a kind of soybean product made by microbial fermentation. It was found that fermentation can decompose macro molecules into small molecules, some insoluble macro molecules into soluble small molecules, also decompose anti-nutritional factors

in soybeans and eliminate the beany flavor of soybeans. At the same time, the auto lysis of microorganisms can produce nutrients that were not found in soybean milk, thus improving the nutritional value of products that makes soybean milk easier to digest, absorb, as well as more nutritional and delicious [6, 7].

Soybean milk contains a variety of bio-active ingredients with health benefits on human body. In the process of microbial fermentation, the changes of the content of these active ingredients in soybean milk are of great significance to improve the nutritional efficacy of fermented soybean milk. In this study, single strain fermentation and mixed strain fermentation were used to ferment soybean milk. The nutritional effects of *Lactobacillus* fermented soybean milk were observed according to the changes of its physical and chemical properties.

2 Materials and methods

2.1 Preparation of Fermented Soybean Milk

High quality soybean produced in Heilongjiang Province were selected. Soybean were soaked in cold water for 12 h, beaten and filtered with gauze, then the soybean milk juice before fermentation was obtained. *Lactobacillus bulgaricus* was purchased from China Industrial Microbial Species Preservation and Management Center (LB). *Lactobacillus plantarum* was isolated from traditional fermented yogurt in Xinjiang, China, that has been evaluated and showed good tolerance to artificial gastric juice and bile salt (LP) [8]. Then LB and LP were inoculated according to 10^5 CFU/mL respectively, also mixed bacterial liquid (LB:LP = 1:1) was inoculated.

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The fermented soybean milk were obtained by fermentation at 37°C for 12 h. According to different inoculated strains, fermented soybean milk were divided into three groups: LBFS (LP fermented soybean milk), LPFS (LP fermented soybean milk), MFS (mixed strain fermented soybean milk).

2.2 Determination of pH and Total Acidity

Fermented soybean milk was taken and titrated with 0.1 mol/L NaOH until pH=8.3. Calculate the total acidity of the sample by following equation:

$$\text{Total acidity} = V_1 \times C \times 0.09 \times 100 / V_2$$

[C: concentration of NaOH (0.1 mol/L), V1: amount of NaOH (mL), V2: amount of sample (mL)].

2.3 Determination of Viable Bacteria

Fermented soybean milk was absorbed at 6 and 12 h after fermentation. After gradient dilution, 10⁶-fold diluted bacterial solution was mixed with MRS medium (Becton, Dickinson and Company, USA) and then poured into a flat plate to determine the colony forming units (CFU/mL) of each group.

2.4 Determination of Amino Acid Nitrogen

Determination of amino acid nitrogen by Formaldehyde Value Method [9].

2.5 Determination of Active Isoflavones

Kromasil C18 column (4.6 mm × 25 mm, 5 um); mobile phase: 40% methanol; flow rate: 1.0 mL/min; detector sensitivity: 0.02 AUFS; detection wavelength: 260 nm; column temperature: 50°C; injection volume: 20 mL were used to determine the chromatographic conditions. Daidzein and genistein control standard (Sigma, USA) were dissolved in 80% (V/V) ethanol to prepare standard solutions of 0.002-0.020 g/L. Then standard solution 20 mL was injected into the six-way valve to determine the peak area. The regression equation was obtained and the standard curve was obtained by the regression equation. The supernatant was obtained by centrifugation at 4000r/min after 100 mL of fermented soybean milk sample was absorbed at 6 and 12 h of fermentation and dried in the oven at 80°C. The dried matter was extracted by reflux for 2 h with 80% (V/V) ethanol of 200 mL in a constant temperature water bath for twice. Then the extract was combined and steam-dried under vacuum. The absolute ethanol was used as the constant volume to 10.00 mL. The isoflavones content of extract was determined by comparing with the standard curve.

2.6 Data Statistics

SPSS 19.0 software (SPSS Inc., Chicago, IL, USA) was used to analyze the statistical differences among the data

of three repeated experiments by one-way ANOVA test ($p < 0.05$).

3 Results and discussion

3.1 pH and Total Acidity

Lactic acid and acetic acid produced by LAB fermentation can reduce the pH value and redox potential of food to provide acidic conditions for food. Generally, the optimal growth pH of bacteria is 6-7. The acidic environment produced by LAB fermentation can reduce the growth rate of some bacteria. At the same time, the metabolites produced during fermentation can stimulate the catalase thiocyanate system. It can inhibit and eliminate harmful bacteria such as Gram-negative bacteria and catalase-positive bacteria in food [10]. It is also important for the activity of endogenous enzymes in fermented food [11]. Table 1 showed that unfermented soybean milk was nearly neutral. After 6 h of fermentation, the pH value of the three kinds of fermented soybean milk were lower than that of unfermented soybean milk with significant difference ($p < 0.05$). Because a certain amount of organic acid was produced in the fermentation process. However, with the progress of fermentation, the accumulation of other metabolites and the production of nitrogen-containing substances such as ammonia and amines slowed down the change of pH value. It is also related to the change of total acid content.

Table 1. pH and total acidity of fermented soybean milk.

Index	Fermented time (h)	LBFS	LPFS	MFS
	0	7.18±0.01	7.18±0.01	7.18±0.01
pH	6	5.62 ^b ±0.01	5.12 ^a ±0.01	5.12 ^a ±0.01
	12	4.87 ^c ±0.01	4.73 ^b ±0.01	4.70 ^a ±0.01
	0	/	/	/
Total acidity (%)	6	1.43 ^a ±0.12	1.56 ^b ±0.12	1.57 ^b ±0.12
	12	1.57 ^a ±0.12	1.64 ^b ±0.12	1.79 ^c ±0.12

Note: Significant differences were found between groups with different letter representations ($p < 0.05$).

3.2 Viable Bacteria Count

After the fermentation of soybean milk, nutrients in soybean milk were released due to osmotic pressure, which was making a good growth environment of LAB strains and effectively inhibited the growth of spoilage bacteria in the fermentation process. At the same time, nutrients in fermented soybean milk were rapidly utilized to promote the growth of LAB strains [12, 13]. As shown in Table 2, the viable bacteria count of

fermented soybean milk increased in the fermentation process. And more viable bacteria count was shown in mixed strains fermented soybean milk than single strain fermented soybean milk. This suggested that, mixed strains fermentation can make up for the deficiency of single strain fermentation, shorten the fermentation time, give full play to the advantages of multi-strain fermentation, and obtain more satisfactory fermentation products. But in the fermentation process, we still need to consider the antagonism between different strains [14].

Table 2. The viable bacteria count of LAB in fermented soybean milk.

Index	Fermented time (h)	LBFS	LPFS	MFS
Viable count (CFU/m L)	0	/	/	/
	6	1.09×10^{8a}	1.40×10^{8b}	1.47×10^{8c}
	12	1.98×10^{8a}	2.15×10^{8b}	2.33×10^{8c}

Note: Significant differences were found between groups with different letter representations ($p < 0.05$).

3.3 Amino Acid Nitrogen

Amino acid nitrogen is an important factor reflecting the sensory quality of fermented soybean milk. The kinds and contents of amino acids can not only affect the quality of fermented food, but also play an important role in the taste of fermented food. For example, the main flavor amino acids in food, aspartic acid and glutamic acid can combine with Na^+ to form flavor substances. And it has been reported that protein can be decomposed into amino acids more easily through microbial fermentation, which can improve the efficiency of protein utilization in human body [15]. The content of amino acid nitrogen in soybean milk fermented by LAB was higher than that of unfermented soybean milk. And the content of amino acid nitrogen in 12 h fermented soybean milk was higher than that in 6 h fermented soybean milk (see Table 3).

Table 3. The amino acid nitrogen content of fermented soybean milk.

Index	Fermented time (h)	LBFS	LPFS	MFS
Amino acid nitrogen (%)	0	0.105 ± 0.01	0.105 ± 0.01	0.105 ± 0.01
	6	0.189 ± 0.01	0.219 ± 0.04	0.233 ± 0.04
	12	0.214 ± 0.02	0.249 ± 0.02	0.271 ± 0.05

Note: Significant differences were found between groups with different letter representations ($p < 0.05$).

3.4 Active Isoflavones

Soybean isoflavones are secondary metabolites formed during the growth of soybean. The content of isoflavones in soybean is about 0.1%-0.5%. Its structure is similar to that of mammalian estrogen 17-beta-estradiol. This structural similarity determines that isoflavones have certain estrogenic activity [16]. It has been reported that, isoflavones can bind to endogenous estrogen receptors, thus exhibiting estrogenic-like physiological effects, such as anti-cancer, cardiovascular disease protection, alleviation of menopausal syndrome, etc [17-21]. At present, there are 12 isoflavones isolated and identified from soybean, three of which are free aglycone structures (2%-3% of soybean isoflavones), and the other nine are glycoside structures (97%-98% of soybean isoflavones). Isoflavones in fermented soybean products are mainly free isoflavones. Glycosidic isoflavones are hydrolyzed into free isoflavones under the action of beta-glucosidase. The main components of glycosidic isoflavones are daidzein and genistein. Studies have shown that free isoflavones have stronger biological activity than glycoside isoflavones [22].

As shown in Table 4, during the first 6 h of fermentation, the content of daidzein in soybean milk fermented by LAB strains increased rapidly, especially in mixed strain fermented soybean milk. During 6-12 h fermentation, the content of daidzein in three kinds of fermented soybean milk increased slowly with the fermentation proceeding, but the increasing range decreased significantly compared with the initial 6 h fermentation. The content of genistein showed the same trend as daidzein.

Table 4. The content of daidzein and genistein in fermented soybean milk

Isoflavones	Fermented time (h)	LBFS	LPFS	MFS
Daidzein (ug/mL)	0	3.98 ± 0.61	3.98 ± 0.61	3.98 ± 0.61
	6	44.55 ± 2.12	46.18 ± 2.49	50.12 ± 3.11
	12	46.17 ± 3.12	49.73 ± 2.65	50.70 ± 2.81
Genistein (ug/mL)	0	7.19 ± 0.90	7.19 ± 0.90	7.19 ± 0.90
	6	28.46 ± 2.01	28.88 ± 2.34	29.07 ± 2.55
	12	29.32 ± 1.12	34.94 ± 1.71	36.91 ± 2.04

Note: Significant differences were found between groups with different letter representations ($p < 0.05$).

The above results showed that the nutritional value of LAB strains fermented soybean milk have been improved to a certain extent, the mixed strain fermented soybean milk showed higher nutritional value than single strain. *Lactobacillus bulgaricus* and *Lactobacillus plantarum* isolated from traditional fermented yogurt in Xinjiang showed synergistic effect in fermentation.

These results suggested that reasonable mixing proportion of strains can strengthen the interaction between strains and produce more beneficial components for fermentation.

4 Conclusion

This study confirmed the physicochemical and functional properties of *Lactobacillus* strains fermented soybean milk. According to the results of the pH value, total acidity, viable bacteria count, amino acid nitrogen, and active isoflavones count of single strain and mixed strain fermented soybean milk, *Lactobacillus bulgaricus* and *Lactobacillus plantarum* isolated from traditional fermented yogurt in Xinjiang mixed strains showed the highest nutritional value in general. Therefore, these two kinds of LAB strains can be used together as starter culture to soybean milk fermentation that can produce more beneficial components for soybean milk. It also laid a theoretical foundation for the follow-up study on this mixed strain and fermented soybean milk.

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