Effects of Different Lactic Acid Bacteria on the Quality of Soy Yogurt

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Abstract [**Objectives**] This study was conducted to solve the problems of heavy beany smell and serious whey precipitation of soy yogurt products. [**Methods**] Soybeans were selected as the raw material, which was soaked and ground, and different strains were used to ferment soy yogurt. The effects of single-strain and mixed-strain fermentation on the quality of soy yogurt were compared, and the optimal process conditions for fermenting soy yogurt were optimized. [**Results**] The quality of soy yogurt fermented by the mixed strain of *Bifidobacterium bifidum* and *Lactobacillus casei* was better than other single-strain fermentation and mixed-strain fermentation, and the best fermentation process adopted following parameters: 2% of inoculum ratio, 8% of carbon source including sucrose, lactose and glucose in equal proportion, and fermentation time 10 h. Under these conditions, the soy yogurt had a higher sensory score, the best stability, low beany smell and the mildest flavor. [**Conclusions**] This study provides a theoretical basis for developing high-quality soy yogurt.

Key words Soy yogurt; Fermentation; Bacterial strain; *Bifidobacterium bifidum*; *Lactobacillus casei*

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With the improvement of people's living conditions, people's demand for food nutrition and health is increasing[1]. In addition, due to lactose intolerance, flexible dietary choices, and environmental and health problems, people increasingly prefer plant foods[2]. Soybeans are an important food crop for human beings and an important source of nutrients such as protein, oil and vitamins. In addition, soybeans have strong antioxidant properties, which can prevent obesity and reduce the possibility of cardiovascular disease, chronic inflammation, diabetes and cancer[4]. Soybean protein is the only whole plant protein comparable to animal protein[5].

Soybean milk contains isoflavones, oligosaccharides and saponins, which can provide a variety of health benefits. It is reported that lactic acid bacteria (LAB) fermentation can increase the amino acid content of soybean yogurt[6], and transform bound soybean isoflavones into corresponding aglycones, so that they can be absorbed and utilized by the body more easily[7]. Lactic acid bacteria fermentation can transform glycosides, malonyl glycosides and acetyl glycoside isoflavones into aglycones by producing β-glucosidase, thus exerting their biological activities[8]. A study shows that compared with non-fermented bean products or raw beans, fermented bean products have significant benefits to human body[9], including coronary heart disease, antioxidants, prevention of cancer, diabetes and obesity[10]. Therefore, vegetable soy yogurt fermented by different strains plays an important role in the beverage market because of its good nutritional value[11].

As commercial starter cultures for fermented milk, *Lactobacillus delbrueckii*, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* have been studied for decades[12]. In addition, they are also considered to have the function of improving the flavor, taste and texture of food[13]. *S. thermophilus* is effective in improving the taste, viscosity and texture of yogurt. As we all know, soybean products fermented by strains such as *Bifidobacterium bifidum*, *Lactobacillus rhamnosus* and *Lactobacillus casei* show significant changes in conjugated linoleic acid content and pharmacological ability. *Lactobacillus plantarum* is a beneficial probiotic, and its potential probiotic characteristics have been extensively studied[15]. *L. plantarum* not only improved the sensory characteristics of fermented milk, but also improved the nutritional value of fermented milk[16]. It is reported that lactic acid bacteria have many beneficial characteristics[17], including reducing irritable bowel syndrome, inhibiting acute cadmium toxicity and regulating immune response[18]. At present, most of the research is on the use of mixed strains for fermenting yogurt, and there is little introduction about fermented yogurt.

Therefore, in this study, fermented soy yogurt was prepared using soybeans as the main raw material and *L. plantarum*, *B. bifidum*, *L. casei* and *L. rhamnosus* as starter cultures[19], and a starter culture with better fermentation performance was screened out through comparative analysis on sensory characteristics, rheological properties and flavor characteristics of the products, hoping to further optimize the production techniques of soy yogurt and provide better vegetable protein drinks to meet different market demands.

Materials and Methods

Main materials and equipment

Soybeans produced in Anhui; *L. plantarum* and *B. bifidum*

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powder (Shaanxi Yuzhou Biotechnology Co., Ltd.); *L. casei* and *L. rhamnosus* powder (Xi'an Mixianer Biotechnology Co., Ltd.); MRS culture medium (Hangzhou Baisi Biotechnology Co., Ltd.); yellow slurry water (produced in the laboratory); electric furnace (FuXING1000W, Huxing Electric Heating Appliance Factory); integrated pulp boiling machine (Beijing Kangdeli Intelligent Technology Co., Ltd.); homogenizer; electrothermal constant-temperature incubator (DPH series, Supa).

Experimental methods

Preparation of starter cultures *L. plantarum* (*B. bifidum*, *L. casei*, *L. rhamnosus*) powder was mixed into MRS culture medium according to the proportion of 1%, and cultured at 37 ℃ for 24 h. Next, it was inoculated into a yellow slurry water medium according to the inoculum ratio of 1%, and cultured at 37 ℃ for 24 h. Subsequently, it was again inoculated into a new yellow slurry water medium according to the inoculum ratio of 1%, and cultured at a constant temperature of 37 ℃ for 24 h. After plate counting, it was confirmed that the number of live bacteria in the activated bacterial liquid was $10^7 - 10^8$ CFU/ml, and the culture was stored in a refrigerator at 4 ℃ for later use.

Preparation of fermented soy yogurt Full grains of high-quality soybeans free of pests and diseases and deterioration were selected and soaked for 8 – 10 h. Next, the soybeans were ground to get soybean slurry, which was boiled and homogenized with a homogenizer of 40 MPa. A suitable carbon source (sucrose, lactose, glucose) was added, and the mixture was stirred for dissolution[20]. After sterilizing at 85 ℃ for 15 min, cooling to 43 ℃ and inoculating, the fermentation system was put in a constant-temperature incubator for culture at 37 ℃ for 10 h. Finally, the fermented soybean milk was stored at 4 ℃ for 12 h to obtain mature soy yogurt.

Experimental design In this study, the effects of different carbon sources, different lactic acid bacteria, different inoculum ratio and different fermentation time on the quality of soy yogurt were investigated. Main influencing factors and suitable levels were selected to make a four-factor three-level orthogonal experimental design.

Determination of pH pH of samples were determined with a pH meter[21].

Sensory evaluation After the soy yogurt matured, 10 professionals were invited to rate the experimental products from five aspects: appearance, aroma, mouthfeel, taste and overall preference. The products were scored by a 100-point system, and an average value was taken as the final score of each product[22].

Table 1 Sensory evaluation form of soy yogurt

Item	Score	Sensory index
Appearance (10 points)	7 – 10	Uniform, without other different colors, with obvious luster
	3 – 6	Slightly different color, and slightly shiny
	0 – 3	Uneven color, dull luster
Aroma (20 points)	14 – 20	Strong fragrance, no other peculiar smell
	5 – 13	General fragrance, no other peculiar smell
	0 – 4	Without obvious fragrance, with strange odor
Mouthfeel (20 points)	14 – 20	Rich and full, no granular sensation
	5 – 13	Relatively rich, with sensation of residual granules
	0 – 4	Not rich, with obvious granular sensation
Tissue (10 points)	8 – 10	Good coagulability, smooth and uniform surface
	5 – 7	Relatively good coagulability, relatively good smooth and uniform surface
	1 – 4	Poor coagulability, rough surface
Taste (20 points)	14 – 20	Moderate, no beany smell
	5 – 13	Slightly sweet or slightly sour, with light beany smell
	0 – 4	Too sweet or too sour, obvious beany smell
Overall preference (20 points)	0 – 20	

Rheological characteristics of yogurt gel The rheological properties of yogurt were determined by a rotational rheometer (DHR-2, Waters Corporation, Milford, MA, USA). Yogurt (5 g) was put on the bench and balanced for 5 min, and then rheological analysis was carried out. A parallel geometric plate with a diameter of 40 mm was installed on the rheometer for testing. The storage modulus (G'), loss modulus (g''), (loss tangent) tanδ and viscosity of dough samples were recorded in the linear viscoelasticity region. The test was carried out in the linear viscoelasticity region of 1% strain while keeping the temperature at 25 ℃. The frequency range was 0.1 – 10 Hz, and the shear rate increased from 0 to 500 s^{-1}.

Electronic nose analysis A 5 g of sample was weighed, and added in a 30 ml headspace bottle. After balancing at 30 ℃ for 5 min, it was measured with electronic nose equipment. The parameters were set as follows: sampling interval 1.0 s, cleaning time 120 s, pre-sampling time 10 s, detection time 120 s, sensor room flow rate 300 ml/min, and sample flow rate 300 ml/min.

Statistical analysis of data

All measurements were carried out in three independent replicates, and the final result was expressed as mean ± standard deviation of multiple samples. SPSS21 software was employed for significance analysis of data, and ANOVA was used for Duncan's multiple difference analysis ($P < 0.05$). According to Duncan significance test, the same letter indicates no significant difference ($P > 0.05$), while lowercase letters (a, b, c) or uppercase letters (A, B, C) indicate significant differences ($P < 0.05$). Origin 2021 was used for for data processing and drawing.

Results and Analysis

Effects of different carbon sources and proportions on soy yogurt

Different kinds and proportions of carbon sources were added to soybean milk, and different lactic acid bacteria such as *L. plantarum*, *L. rhamnosus*, *L. casei* and *B. bifidum* were inoculated for fermentation at an inoculum ratio of 2%, and the material was fermented at a temperature of 37 ℃ for 12 h. After the fermentation, the sensory evaluation and pH value of soy yogurt were determined. The results are shown in Fig. 1. The soybean milk fermented with lactose, glucose and sucrose as a compound carbon source showed the smoothest surface and best appearance, and the sensory score was highest. The pH value ranged from 3.95 to 4.15. Under this compound carbon source, the sensory score of yogurt fermented by *L. casei* was the highest.

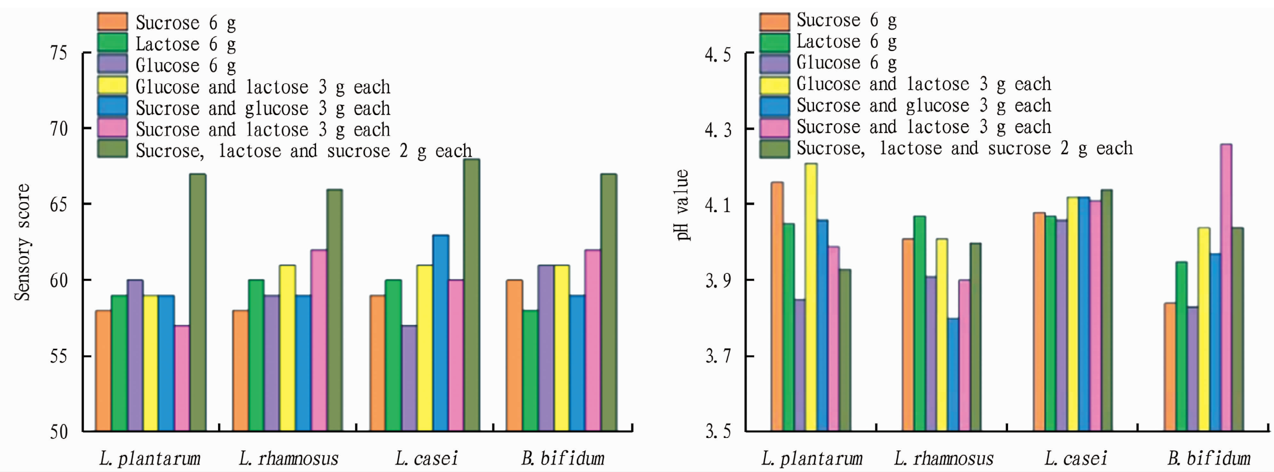


Fig. 1 Effects of different carbon sources and different strains on sensory evaluation (A) and pH (B)

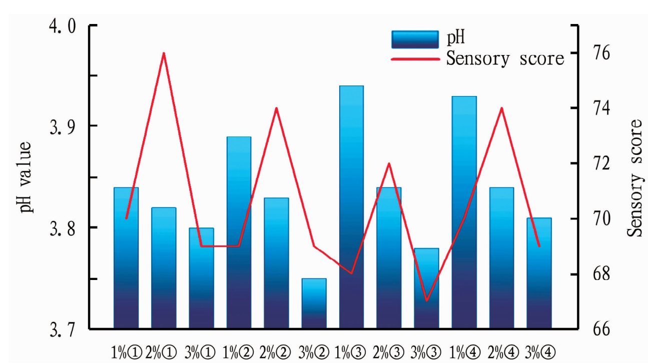
On the basis of above research result, sucrose, lactose and glucose were added in equal proportion as a compound carbon source, and the effects of different addition amounts on soy yogurt were studied. *L. casei* was inoculated at an inoculum ratio of 2% to fermented soy yogurt. The sensory scores are shown in Table 2. When the addition amount of the complex carbohydrate was 8%, the sensory score of soybean milk was (76 ± 2.0) points, significantly higher than the products with other addition amounts.

Table 2 Effects of different carbon source additions on sensory evaluation

Addition amount of carbon source//%	Sensory score
6	68 ± 1.6 bc
7	70 ± 1.8 b
8	76 ± 2.0 a
9	71 ± 1.5 b
10	65 ± 2.2 c

Effects of different inoculum ratios on the quality of soy yogurt

B. bifidum, *L. rhamnosus*, *L. plantarum* and *L. casei* were inoculated into soybean milk with 8% of compound carbohydrate (glucose 2.66%, lactose 2.66% and sucrose 3%) as the carbon source at the inoculum ratios of 1%, 2% and 3% respectively, and then, the materials were fermented in an incubator at a constant temperature of 37 ℃ for 12 h. Next, the pH value was determined, and sensory evaluation was carried out. It can be seen from Fig. 2 that the pH value of soy yogurt with the inoculum ratio of 2% was in the middle level, and the sensory score was the highest among the products fermented with the same strain. Comparing the sensory evaluation of the four single bacteria, it was found that the sensory scores of soy yogurt fermented by *B. bifidum* and *L. casei* alone were higher, at (76 ± 1.1) and (74 ± 1.3) points, respectively.

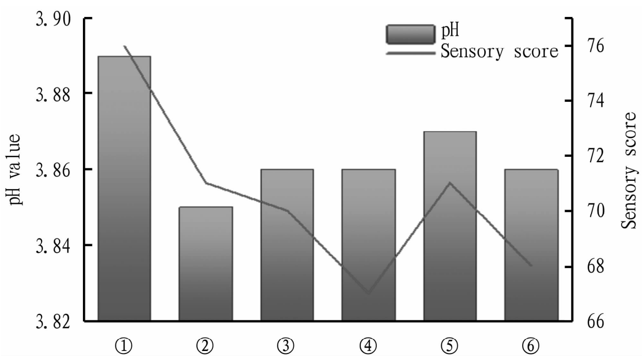


① *B. bifidum*; ② *L. rhamnosus*; ③ *L. plantarum*; ④ *L. casei*.

Fig. 2 Effects of different strains and different inoculum ratios on pH and sensory evaluation

Optimization experiment on the effects of compound strains on the quality of soy yogurt

Two kinds of bacteria were added in the same amount as compound strains, and the inoculum ratio was 2% (1% for each strain). They were inoculated into soybean milk with 8% of complex carbohydrate (glucose 2.66%, lactose 2.66% and sucrose 2.66%) as the carbon source, and then, the materials were fermented in an incubator at a constant temperature of 37 ℃ for 12 h. Next, the pH value was measured, and sensory evaluation was carried out. The results are shown in Fig. 3. From the sensory evaluation, it could be concluded that the sensory score of soybean milk fermented with *B. bifidum* and *L. casei* as a compound strain was the highest, and the texture, color, mouthfeel and taste of soy yogurt reached a satisfactory level. Specifically, the final score reached (84 ± 1.3) points, and the pH value was at the middle level. Compared with the effects of soy yogurt fermented by single strains (as shown in Fig. 2), the sensory score of soy yogurt fermented by the combination of *B. bifidum* and *L. casei* was higher than those of soy yogurt fermented by *B. bifidum* and *L. casei* alone, indicating that there was a certain synergistic effect between *B. bifidum* and *L. casei*, which could promote each other's growth and improve the fermentation effect.



① *B. bifidum* + *L. casei*; ② *B. bifidum* + *L. plantarum*; ③ *B. bifidum* + *L. rhamnosus*; ④ *L. plantarum* + *L. rhamnosus*; ⑤ *L. plantarum* + *L. casei*; ⑥ *L. rhamnosus* + *L. casei*.

Fig. 3 Effects of different bacterial strains on pH and sensory evaluation

Optimization experiment on the effects of different fermentation time on the quality of soy yogurt

The inoculation strain was *B. bifidum* + *L. casei*, and the inoculum ratio was 2%. The addition amount of compound carbon source was 8%, and the fermentation was carried out at 37 ℃. The sensory score and pH value of soy yogurt after fermentation are shown in Fig. 4. With the extension of fermentation time, the pH of soy yogurt showed a downward trend. It was because lactic acid bacteria produced lactic acid and other organic acids by using sugars in soybean milk during fermentation, which made the pH of soy yogurt drop. When the fermentation time reached 10 h, the sensory score of soy yogurt was the highest at (89 ± 1.5) points.

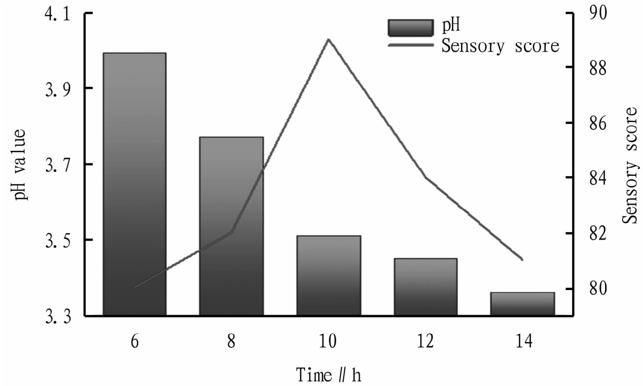


Fig. 4 Effects of different fermentation time on the acidity and sensory evaluation of soy yogurt

Rheological properties of soy yogurt

The rheological properties of soy yogurt are shown in Table 3. The storage modulus (G′) reflected the elastic property of soy yogurt. The higher the value, the better the elasticity of the material. According to the data, the soy yogurt fermented by the mixed strain of *B. bifidum* and *L. casei* had the best elasticity, while the soybean milk fermented by the mixture of *L. plantarum* and *L. casei* showed the lowest elasticity. The loss modulus (G″) reflected the stickiness property of soy yogurt. The higher the value, the greater the stickiness of the material. The stickiness of soy yogurt fermented by *B. bifidum* was the strongest, while the stickiness of soy yogurt fermented by the mixture of *B. bifidum* and *L. rhamnosus* was the lowest. The loss tangent (tanδ) reflected the relationship between fluidity and elasticity of the material. A lower value indicates that the material was more elastic, while the higher the value, the more sticky the material. The soy yogurt fermented by the mixture of *B. bifidum* and *L. rhamnosus* had the best elasticity, while the soy yogurt fermented by the mixed strain of *L. plantarum* and *L. casei* had the highest stickiness. Viscosity (η *) reflected the overall flow resistance of the material. The higher the value, the greater the stickiness and the worse the fluidity. The soy yogurt fermented by the mixed strain of *B. bifidum* and *L. casei* (optimal) exhibited the highest stickiness and the best coagulability.

Table 3 Rheological properties of fermented soy yogurt

Strain	Storage modulus (G′) // Pa	Loss modulus (G″) // Pa	Loss tangent (tanδ)	Viscosity (η *) // Pa. s
B. bifidum	410.466	85.531 6	0.208 377	11.878 3
L. rhamnosus	392.283	85.282 7	0.217 401	11.821 6
L. plantarum	404.864	83.938 9	0.207 326	11.711 4
L. casei	411.170	84.441 6	0.205 369	11.446 4
B. bifidum + *L. rhamnosus*	415.213	82.123 8	0.197 787	11.289 3
B. bifidum + *L. plantarum*	413.329	84.418 4	0.204 240	11.648 2
L. rhamnosus + *L. plantarum*	411.170	84.441 6	0.205 369	11.711 4
L. rhamnosus + *L. casei*	404.864	83.938 9	0.207 326	11.821 6
L. plantarum + *L. casei*	392.283	85.282 7	0.217 401	12.382 5
B. bifidum + *L. casei*	421.620	83.444 5	0.197 914	108.414 0

Evaluation of yogurt by electronic nose

Fermented soy yogurt generally had a certain beany smell, and the electronic nose reading of *L. rhamnosus* was the highest (2.8), indicating that the soy yogurt fermented by it had the strongest volatile flavor substances. The readings of *B. bifidum* and *L. plantarum* were both 2.5, indicating that their flavor characteristics were similar and mild. The combination of *B. bifidum* and *L. casei* produced the lowest electronic nose reading (1.4), which might indicate that the soy yogurt fermented by this combination had the mildest or most delicate flavor. The combination of *L. rhamnosus* and *L. plantarum* got a reading of 2.0, indicating that the combination might have produced a relatively balanced flavor characteristics.

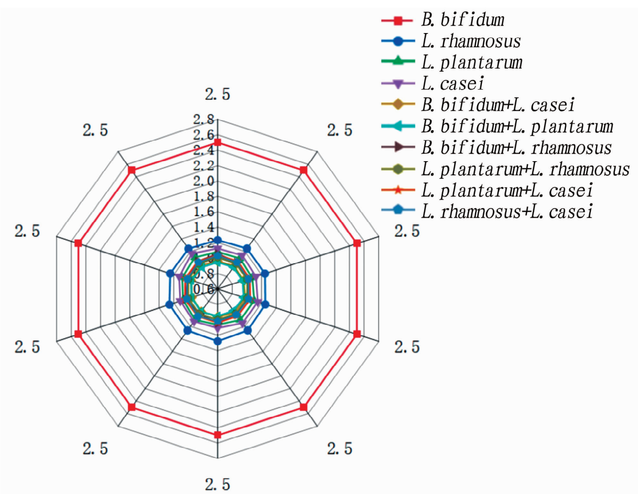


Fig. 5 Effects of fermentation with different strains on the odor of soy yogurt

Conclusions and Discussion

In this study, the effects of different carbon sources, inoculum ratios, fermentation time and strains on the quality of fermented soy yogurt were discussed. The results showed that after fermentation at 37 °C with 8% of complex carbohydrate (glucose 2.66%, lactose 2.66% and sucrose 2.66%) as the carbon source and mixed strain of *B. bifidum* and *L. casei* (inoculum ratio 2%) as the starter for 10 h, the quality of fermented soy yogurt was the best. Under these conditions, the comprehensive score of the fermented soy yogurt obtained in sensory evaluation was 89 points, showing good texture and flavor. Fermentation time and compound strains are the most important factors affecting the sensory quality of fermented soy yogurt. The effects of single-strain and mixed-strain fermentation were compared, and it was found that the mixed strain of *B. bifidum* and *L. casei* had certain advantages in improving the quality of fermented soy yogurt. The results of rheological properties showed that the stability of soy yogurt fermented by the mixed strain of *B. bifidum* and *L. casei* was the best. The flavor of soy yogurt was analyzed by the electronic nose technology. The results showed that the soy yogurt fermented by the mixed strain of *B. bifidum* and *L. casei* had the mildest flavor characteristics, which might be related to metabolites produced in the fermentation process. To sum up, this study not only optimized the production process of fermented soy yogurt, but also will provide important theoretical support and technical guidance for the development of plant-based fermented milk. Future research can further explore the influence mechanism of different strain combinations on the flavor of soy yogurt.

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analysis such as chromatography and mass spectrometry, and there is a lack of talents familiar with product characteristics in food science, agricultural product storage and processing, *etc*. They cannot accurately grasp the quality indicators and evaluation of fruits and vegetables. Therefore, on the basis of existing testing personnel, relevant fruit and vegetable quality testing and evaluation personnel should be recruited or trained to carry out the laboratory work of fruit and vegetable quality evaluation. In addition, according to the implementation requirements of fruit and vegetable quality grading work, relying on technical personnel of agricultural quality inspection stations in various provinces, cities and counties, a group of on-site graders for agricultural product quality evaluation in the field should be trained to establish a special team of graders for fruit and vegetable quality evaluation.

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