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PRODUCING FUNCTIONAL SOY-BASED YOGURT INCUBATED WITH BIFIDOBACTERIUM LONGUM SPM1205 ISOLATED FROM HEALTHY ADULT KOREANS

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ABSTRACT

In the present study Lactobacillus plantarum CBT1209, Pediococcus pentosaceus CBT SL4, and Bifidobacterium longum SPM1205 showed curd formation in soy milk for 8 hours of incubation time in a single-culture experiment. Among these strains, B. longum SPM1205 as a new isolated strain, inhibited the activity of fecal harmful enzymes of rat. Therefore, we investigated the possibility of using a mixed culture containing B. longum SPM1205 for obtaining a curd-type soy-based yogurt with respect to curd formation, proximate composition and sensory attributes. Then we examined the changes in pH, lactic acid, and lactic acid bacteria during cold storage. B. longum SPM1205 was capable of producing moderate sour taste and good overall acceptability in curd-type plain soy-based yogurt for 8 hours of fermentation. Most sensory qualities were not significantly different in the strawberry soy-based yogurt compared to those of commercial milk-based yogurt. The pH of the strawberry soy-based yogurt decreased significantly ($P < 0.05$) during the days of storage (day 0, 5.18; day 1, 4.80), but the decrease slowed down after 3 days of storage. The lactic acid content and lactic acid bacteria population of plain and strawberry soy-based yogurt were not significantly changed over 10 days of storage. The products contained over 8 log of viable lactic acid bacteria. The results provide feasibility that a curd-type soy-based yogurt can be manufactured using B. longum SPM1205. However, more experiments are needed to improve the sensory qualities such as beany or astringent flavor and color.

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Introduction

Yogurt is traditionally manufactured by fermenting starter lactic acid bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in cow's milk (21). The product contains high moisture contents, good quality of protein and calcium, and probiotic bacteria exerting health promoting properties. Additionally, the product is unique in its flavor and aroma. Because of all this, yogurt is widely consumed both in developing and developed countries.

However, there is an increasing demand to developing alternative product(s) to cow's milk due to problems with allergenicity and desire for vegetarian alternatives. Soy-based products have become attractive as a potential alternative of cow's milk (5, 9, 15). Soy-based products can exert health promoting benefits including reduced allergenicity, antiatherogenic, hypolipidemic, and anticholesterolemic activity for people (12, 13). According to Slavin et al. (20), soy is also considered as an important source of isoflavones, proteins, minerals, vitamins, dietary fibers and oligosaccharides. Oligosaccharides as a prebiotic can play a role in promoting the activity of probiotic microorganisms. Consequently, if probiotic bacteria can be added into soy-based yogurt, the product would be an excellent health promoting alternative to cow's yogurt.

On the other hand, *Bifidobacteria* can grow more vigorously in soy than in cow's milk (14). However, there are very wide variations in the growth activities of strains within one and the same species (17), and thus more data are need to further characterize the potential of soy as a substrate to support good growth of *Bifidobacteria.* We previously demonstrated that *B. longum* SPM 1205, a strain isolated from healthy adult Koreans, significantly inhibited the activity of fecal harmful enzymes of rat intestinal microflora such as β-glucosidase, β-glucuronidase, tryptophanase and urease (6). Recently, Kim et al. (11) reported that a mixture of lactic acid bacteria containing *B. longum* SPM 1205 was effective in improving constipation.

The purpose of the present study was first to measure the ability of pure strain *B. longum* SPM1205 from the feces of healthy Koreans to form a curd in a soy-based substrate and then to evaluate the curd-type plain and strawberry soy-based yogurt using a mixed starter culture (*B. longum* SPM1205, *Lactobacillus plantarum* CBT1209 and *Pedicoccus*

pentosaceus CBT SL4) based on its physical (pH and titratable acidity) and microbiological characteristics (lactic acid bacteria) during storage and organoleptic acceptability.

Materials and Methods

Preparation of soy milk

The preparation of soy milk is shown in **Fig. 1**. Briefly, soybeans (*Glycine max* Merr) were soaked in water for 3 hours to remove the soybean hulls and drained. The dehulled soybeans were soaked in 0.05% $NaHCO_3$ for 20 minutes at 25 °C, boiled at 100 °C for 5 minutes and rinsed. The blanched soybeans were ground in a Waring blender (Hamilton Beach, Canada) with the addition of enough water to give a water-to-dry-soybeans ratio of 7:1 on a weight basis. The resultant slurry soybeans were filtered through cheese cloth and filtrated with 170-mesh sieve. The filtrated liquid soybeans were boiled for 30 minutes at 80 °C to obtain pasteurized soy milk.

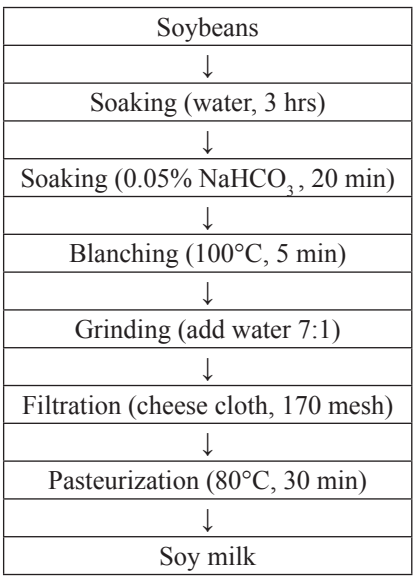

Fig. 1. Flow sheet for the preparation of soy milk.

Bacterial strains

Isolation of *Bifidobacteria* was performed from fecal samples of healthy Koreans (20~30 years old) collected by BBL's anaerobic sample collection and transport system to maintain anaerobic conditions, and were used within 24 hours. Fecal samples were serially diluted 10-fold from 10^{-1} to 10^{-8}, and 100 µl were spread onto selective BL agar containing 5% sheep blood. After 48 hours of incubation in anaerobic conditions (90% N_2, 5% H_2, 5% CO_2) (Bactron Anaerobic Chamber, Sheldon Manufacturing Inc., USA) at 37 °C, brown or reddish-brown colonies 2~3 mm in diameter were selected for further identification (18). A fructose-6-phosphate phosphoketolase (F6PPK) test was performed (1) to ensure that the colonies selected were *Bifidobacteria*. To identify the isolated *Bifidobacterium* spp. at the species level, 16s rRNA sequencing was performed by Bioleaders (Daejeon, Korea).

For a mixed-culture trial, *Bifidobacterium longum* SPM1205, *Lactobacillus plantarum* CBT1209 and *Pedicoccus pentosaceus* CBT SL4 were used as a mixed starter culture (1:1:1) in the soy-based yogurt. They were grown separately at 37 °C in general anaerobic medium (GAM, Nissui Pharm. Co. Ltd., Japan) under anaerobic conditions (90% N_2, 5% H_2, 5% CO_2).

Manufacture and proximate composition of soy-based plain and strawberry yogurt

0.2% agar powder (Yondoo Co. Ltd., Korea) was added into freshly prepared soy milk to stabilize the curd of soy-based yogurt. The soy milk was boiled at 95 °C in water bath (Vision Scientific Co., Korea) for 5 minutes to dissolve the agar powder completely, cooled rapidly at 40 °C and inoculated with 3% (v/v) of single or mixed starter culture (10^7 CFU/ml). The inoculated soy milk was incubated for 8 hours at 40 °C to develop a curd of plain soy-based yogurt, cooled and stored at 4 °C. To the samples for strawberry soy-based yogurt 20% of commercial strawberry syrup were added (Chungwoo Co. Ltd., Korea) and 0.05% of frozen dried diced strawberry (Garunara Co. Ltd., Korea). The proximate composition of the plain and strawberry soy-based yogurt for energy, protein, fat, carbohydrate, total solid, ash, and sodium were carried out in triplicate using standard methods (2).

Changes in pH, titratable acidity, and lactic acid bacteria during storage

In order to examine any quality changes during storage, both the plain and strawberry soy-based yogurt were stored at 4 °C for 10 days. The pH of soy-based yogurt was directly measured by a pH meter (ThermoOrion, Korea). The titratable acidity of soy-based yogurt was determined by titration with 0.1N NaOH to pH 8.1 and expressed as lactic acid content (%). The number of lactic acid bacteria in soy-based yogurt was examined by the pour plate methods on MRS-agar. Serial dilutions were made with sterile saline solution, 1 ml of the dilute was placed in a Petri dish and the warm MRS-agar was poured and well mixed. The Petri dishes were incubated at 37 °C for 48 hours. The numbers of colony forming units (CFU) are expressed as CFU per gram.

Sensory evaluation of soy-based yogurt

After fermenting for 8 hours, strawberry soy-based yogurt samples were kept at 4 °C in the refrigerator for twelve hours before served. Control yogurt was commercial milk-based strawberry yogurt (Binggre Co. Ltd., Korea). A total of 100 untrained panelists who studied or worked in Sahmyook University evaluated the sensory quality of strawberry soy-based yogurt fermented with a mixed starter culture. The products were evaluated for appearance, overall taste, sourness, color, texture, and overall acceptability using a 5-point scale. The sourness was evaluated as extremely weak [1] to extremely strong [5]. The texture was evaluated as sandy [1] to smooth [5]. The appearance, overall taste, color or overall acceptability was evaluated as dislike extremely [1] to like extremely [5].

TABLE 1

Time for curd formation and sensory evaluation in the soy-based plain yogurt incubated with a single starter culture

Bacterial strain	Time for curd formation (hour)	Sensory evaluation	
		Sour taste[a]	Overall acceptability[b]
Bifidobacterium adolescentis SPM0212	–[c]	–	–
Bifidobacterium adolescentis SPM1005	–	–	–
Bifidobacterium pseudocatenulatum SPM1204	14	2.83	2.67
Bifidobacterium longm SPM1205	14	2.33	2.67
Bifidobacterium longum SPM1207	14	2.66	1.50
Lactobacillus plantarum CBT1209	8	1.83	2.67
Lactobacillus rhamnosus CBT1702	–	–	–
Pediococcus pentosaceus CBT SL4	8	1.67	2.33

[a] Low (1 point), medium (2 points), and strong (3 points);

[b] Poor (1 point), moderate (2 points), and good (3 points);

[c] No curd formation and therefore the product was not available for sensory evaluation

TABLE 2

Proximate composition of the plain and strawberry soy-based yogurt

Sample / Parameter	Energy (Kcal/100g)	Protein (g/100g)	Fat (g/100g)	Carbohydrate (g/100g)	Total solid (%)	Ash (g/100g)	Sodium (mg/100g)
Plain soy-based yogurt	41.02	4.36	2.16	10.4	7.95	0.39	48.11
Strawberry soy-based yogurt	145.65	3.87	1.95	28.15	34.26	0.29	54.89

TABLE 3

Sensory evaluation[a] of the strawberry soy-based yogurt

Sample / Parameter	Appearance[b]	Overall taste[c]	Sourness[d]	Color[e]	Texture[f]	Overall acceptability[g]
Control[h]	3.83±0.12	3.49±0.18	2.60±0.21	3.60±0.16	3.71±0.14	3.68±0.13
Strawberry soy-based yogurt	3.23±0.12	3.22±0.19	1.62±0.16*	2.89±0.18*	3.33±0.24	3.65±0.15

[a] Five-hedonic scale;

[b, c, e, g] Dislike extremely (1 point) to like extremely (5 points);

[d] Extremely weak (1 point) to extremely strong (5 points);

[f] Sandy (1 point) to smooth (5 points);

[h] Commercial strawberry milk-based yogurt;

* $P < 0.05$ statistically significant compared with control

The results of the sensory evaluation were expressed as the means of 25 untrained panelists.

Statistical analysis

Data were analyzed using one-way ANOVA to analyze the differences among storage days using SAS program version 6.11 (SAS Institute Inc., U.S.A.). Differences among storage days, when significant, were compared using Duncan's multiple range test. The values of the sensory parameters were also analyzed using a t-test in SAS program version 6.11 (SAS Institute Inc., U.S.A.). All statistical analyses were considered significantly different at the $P < 0.05$ level.

Results and Discussion

Development of functional soy-based yogurts fermented with *Bifidobacterium longum* SPM1205

Choi et al. (6) reported that *Bifidobacterium longum* SPM1205, a strain isolated from healthy adult Korean feces, may be a

potent probiotic strain for human use because feeding rats with this strain for four weeks had no adverse effects on their general health status, growth, blood chemistry, and histology. The authors also demonstrated that *B. longum* SPM1205 played a key role in the inhibition of fecal harmful enzymes (β-glucosidase, β-glucuronidase, tryptophanase and urease) of rat intestinal microflora. Moreover, Kim et al. (11) reported that the mixture of lactic acid bacteria containing *B. longum* SPM1205 can improve constipation in humans

However, we needed to know whether *Bifidobacterium longum* SPM1205 would form a curd in a soy-based substrate. Therefore, in the present study, we investigated the possibility of a starter culture of *Bifidobacterium* isolated from healthy Korean feces to produce curd-type soy-based yogurt with respect to curd formation and sensory attributes. As shown in **Table 1**, there was no curd formation in the presence of *B. adolescentis* SPM0212, *B. adolescentis* SPM1005, or *L. rhamnosus* CBT1702 in the soy milk within 24 hours of incubation time. *B. psedocatenulatum* SPM1204, *B. longum* SPM1205, *B. longum* SPM1207, *Lactobacillus plantarum* CBT1209, and *Pediococcus pentosaceus* CBT SL4 appeared capable of producing curd in soy milk for the incubation time of 14 h, 14 h, 14 h, 8 h, and 8 h, respectively. Especially, *B. longum* SPM1205 appeared to be capable of producing moderate sour taste (2.33) and good overall acceptability (2.67) in curd-type plain soy-based yogurt. This fact may indicate that *B. longum* SPM1205 could be a potent starter culture for the manufacture of curd-type soy-based yogurt.

On the other hand, Murti et al. (14) reported that *B. longum*, *B. infantis*, *B. bifidum*, and *B. adolescentis* are regarded as promising starter cultures for good tasting fermented soy milk products. In general, *Bifidobacteria* play a role in the production of acetate and lactate during the carbohydrate catabolism through the bifidus pathway (3). In a total of eight lactic acid bacteria tested for the curd formation, both *L. plantarum* CBT1209 and *P. pentosaceus* CBT SL4 also showed curd formation in soy milk for 8 hours of incubation and good overall acceptability.

On the basis of these results, in the second series of studies *B. longum* SPM1205, *L. plantarum* CBT1209 and *P. pentosaceus* CBT SL4 were used as a mixed starter culture (1:1:1) in the manufacture of plain and strawberry soy-based yogurt. When incubated for over 8 hours, the yogurt was more sour (data not shown). This would indicate that acid production was more vigorous in the soy-based yogurt incubated due to the longer incubation time. When incubated for less than 8 hours, the overall acceptability of the yogurt was somewhat bad (data not shown). This may be associated with a beany flavor present in the soy-based yogurts incubated for less than 8 h because there was not enough fermentation (data not shown). Usually, acid production by fermentation may function as a tool to reduce beany flavor in the soy-based yogurt for long incubation time.

Proximate composition and sensory evaluation of functional soy-based yogurt fermented with *Bifidobacterium longum* SPM1205

The proximate composition of plain and strawberry soy-based yogurt is presented in **Table 2**. The energy contained in the strawberry soy-based yogurt (145.65 kcal/100 g) was approximately 3.5-fold more than that in the plain soy-based yogurt (41.02 kcal/100 g). The carbohydrate content was approximately 27.5-fold more in the strawberry soy-based yogurt (28.15 g/100 g) than in the plain soy-based yogurt (1.04 g/100 g). These analyses directly indicate that the strawberry soy-based yogurt contained 20% strawberry syrup mainly manufactured with refined sugar and fructo-oligosaccharide. Furthermore, the total solid was approximately 4.3-fold higher in the strawberry soy-based yogurt (7.95%) than in the plain soy-based yogurt (34.26%). The sugar converted to glucose is metabolized to pyruvate through the Embdem-Meyerhof-Parnas pathway. The pyruvate is later converted to lactic acid by lactate dehydrogenase (22). The content of frozen dried diced strawberry and the high sugar content of the syrup added to the strawberry soy-based yogurt might have contributed to the higher total solid recorded. The protein in the plain and strawberry soy-based yogurt was 4.36 and 3.87 g/100 g, respectively. These values for soy-based yogurt were comparable to those reported by Osundahunsi et al. (16) for plain and strawberry soy-based yogurt containing 3.75 and 3.95%, respectively. Osundahunsi et al. (16) also noted that fermentation can lead to increased protein content compared to soy milk.

When compared to plain soy-based yogurt, strawberry soy-based yogurt was more preferred in our preliminary test. Therefore, the sensory properties were evaluated and the strawberry milk-based yogurt (commercial product) was compared with soy-based yogurt (our product) in order to determine the potential commercial acceptability of the new product, a curd-type soy-based yogurt, as a non-dairy fermented yogurt, in Korea. As shown in **Table 3**, there were no significantly different values in the appearance, overall taste, texture, and overall acceptability between the strawberry soy-based yogurt and the control (strawberry milk-based yogurt) ($P > 0.05$). However, the sourness values for the strawberry soy-based yogurt (1.62) were significantly ($P < 0.05$) lower than those for the control (2.60). It could be suggested that the beany flavor slightly present in the strawberry soy-based yogurt can mask the sourness. Color values for the strawberry soy-based yogurt (2.89) were also significantly ($P < 0.05$) lower than those for the control (3.60). Because the color of the strawberry soy-based yogurt was a combination of brown related to soybean and red related to strawberry or strawberry syrup, panelists may have liked the bright color of the control. Based on these results, we will further attempt to improve the color and develop acidity of the strawberry soy-based yogurt for the consumer acceptance in the non-dairy food industry.

Changes in pH, lactic acid, and lactic acid bacteria during cold storage

Changes in pH and lactic acid contents during the cold storage are shown in **Fig. 2** and **Fig. 3**, respectively. The pH of plain soy-based yogurt was slightly decreased over 10 days of storage (day 0, 5.36; day 10, 5.13) although the pH of plain soy-based yogurt was not significantly ($P > 0.05$) different over the storage days at 4 °C. The pH of the strawberry soy-based yogurt was significantly ($P < 0.05$) decreased stepwise during the days of storage (day 0, 5.18; day 1, 4.80), but the decreasing slowed down after 3 days of storage (day 3, 4.64; day 7, 4.55; day 10, 4.50). These pH values were comparable to those in the study of Donkor et al. (8), who demonstrated that the pH of the soy-based yogurt at the end of fermentation was 4.50. The pH of commercial milk-based yogurt distributed in Korea was 3.17~4.19 (10).

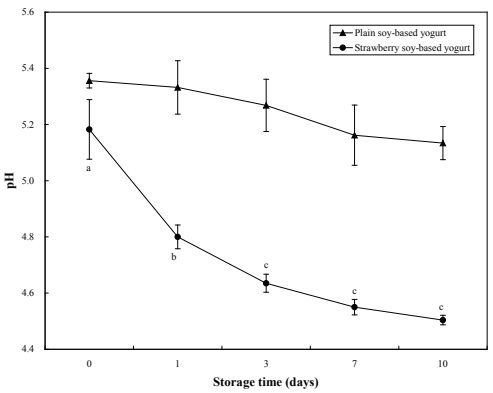

Fig. 2. Changes of pH in the plain and strawberry soy-based yogurt during cold storage.

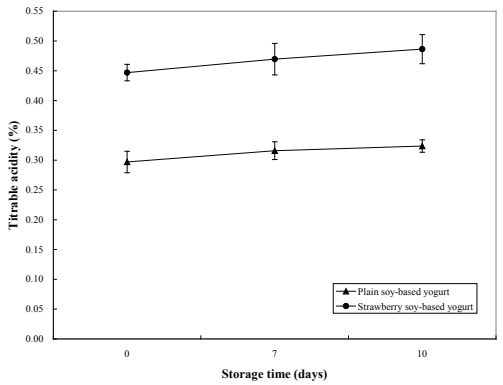

Fig. 3. Changes of lactic acid contents in the plain and strawberry soy-based yogurt during cold storage.

The lactic acid contents of plain (day 0, 0.30%; day 10, 0.32%) and strawberry (day 0, 0.45%; day 10, 0.49%) soy-based yogurt was slightly increased over 10 days of storage, although the acidity of the plain and strawberry soy-based yogurt was not significantly ($P > 0.05$) different over the storage days at 4 °C.

The initial pH of soy milk is 7.1 (8). The acid production by fermentation resulted in a decrease in pH of the soy-based yogurts. The increase in lactic acid contents was concomitant with decrease in pH values. As shown in **Fig. 2** and **Fig. 3**, the strawberry soy-based yogurt had more lactic acid contents and thus was more acidic when compared to the plain soy-based yogurt at the initial time of storage (day 0). This fact may suggest that strawberries and the syrup containing high sugar contents in soy milk can help fermentation.

The changes in lactic acid bacteria counts during the cold storage are shown in **Fig. 4**. The number of viable lactic acid bacteria of plain (day 0, 8.67 log; day 10, 8.99 log) and strawberry (day 0, 8.82 log; day 10, 8.95 log) soy-based yogurt was slightly increased over 10 days of storage although the viable cells in the plain and strawberry soy-based yogurt were not significantly ($P > 0.05$) different over the storage days at 4 °C. During the days of storage, both plain and strawberry soy-based yogurt may have a probiotic health effect because these products contained over 8 log (CFU/g) populations of viable lactic acid bacteria. For a probiotic health effect, viable bacteria must be available in a minimum concentration of 10^{-6} CFU/g of a product (4, 7, 19).

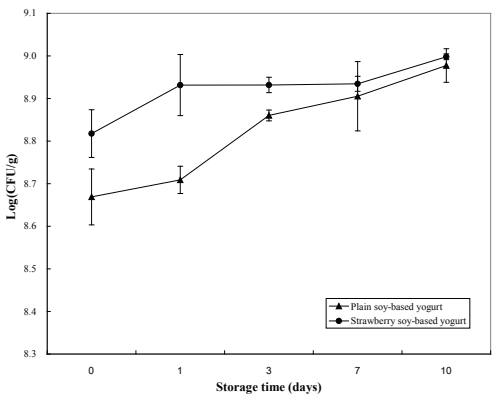

Fig. 4. Changes of lactic acid bacterial counts in the plain and strawberry soy-based yogurt during cold storage.

After two weeks of storage at 4 °C, there were some pink spots on the surface of both plain and strawberry soy-based yogurt (data not shown). These pink spots may have been molds. Based on the above chemical and microbiological analysis and visual observations, the quality of the soy-based yogurt was acceptable until 10 days of storage at 4 °C.

B. longum SPM1205 appeared to be capable of producing moderate sour taste and good overall acceptability in curd-type plain soy-based yogurt for 8 hours of fermentation. Most sensory qualities were not significantly different in the strawberry soy-based yogurt fermented with a mixed starter culture (*B. longum* SPM1205, *L. plantarum* CBT1209 and *P. pentosaceus* CBT SL4) compared to those of commercial milk-based yogurt. The pH of the plain and strawberry soy-based yogurt was decreased over 10 days of cold storage. The contents of lactic acid and the populations of lactic acid

bacteria in the plain and strawberry soy-based yogurt were slightly increased over 10 days of cold storage. Especially, both the plain and the strawberry soy-based yogurt may have a probiotic health effect because these products contained over 10^8 CFU/g populations of viable lactic acid bacteria.

Conclusions

The results of this study may have an important indication that there could be feasibility in developing curd-type soy-based yogurt using *B. longm* SPM1205. However, further experiments are needed to improve the sensory qualities such as beany or astringent flavor and color.

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