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Bioconversion of Soybean Curd Residues into Functional Ingredients with Probiotics

Soo-Myung Oh, Chan-Shick Kim[1] and Sam-Pin Lee[†]

Department of Food Science and Technology, Keimyung University, Deagu 704-701, Korea
[1]Faculty of Horticultural Life Science, Cheju National University, Jeju 690-756, Korea

Abstract

Soybean curd residues (SCR) obtained from hot and cold manufacturing processes were fermented by indigenous microorganisms, *Lactobacillus rhamnosus* LS and *Bacillus firmus* NA-1 for 15 h at 37°C. The pH, acidity, viable cell counts, and tyrosine content were evaluated in samples with variations in sugar, starter and type of SCR. The raw Doowon SCR (D-SCR, cold-processed) fermented by indigenous microorganism had a 0.9% acidity and 6.7×10^7 CFU/g viable cell counts, compared with the 0.11% acidity and 6.7×10^6 CFU/g viable cell counts of raw fermented Pulmuwon SCR (P-SCR, hot-processed). After fermentation of raw P-SCR with 1% glucose and 1% *L. rhamnosus* LS starter, the viable cell counts, tyrosine content and acidity were 4.7×10^8 CFU/g, 16.3 mg% and 0.9%, respectively. In addition, the raw P-SCR fermented with *Bacillus firmus* NA-1 as co-starter had a 0.45% acidity, 2.4×10^8 CFU/g lactic acid bacteria, and 3.3×10^6 CFU/g *Bacillus* sp. In particular, the tyrosine content was increased 5 fold. The drying of fermented SCR was completed by hot-air drying (50°C) within 12 h; the dried P-SCR and D-SCR had 1.8×10^7 CFU/g and 5.3×10^6 CFU/g viable cell counts, respectively. The concentrate of methanol extract from fermented D-SCR inhibited the initial cell growth *of E. coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* in liquid culture.

Key words: soybean curd residue, lactic acid bacteria, *Bacillus*, peptide

INTRODUCTION

Soybean curd residue, known as Biji, is a by-product from tofu and soymilk industries (1). Dehydration of the soybean curd residue (SCR) has been attempted to prevent the spoilage of SCR, which has a high moisture content. as well as to improve its utilization as a food ingredient (1,2). Although powdered SCR has been used as a food ingredient for bread making (3), most of the SCR produced has been utilized as animal feed or agricultural fertilizer (4). It has been reported that SCR is rich in valuable nutrients including water soluble protein and isoflavones (4,5). In particular, SCR is rich in dietary fiber, an important component of healthy foods, therefore fortification with SCR could improve the nutritional quality of biscuits and snacks (6,7).

A typical SCR is a potentially useful substrate for microbial fermentation (1). It has been reported that SCR was used for the production of seasonings and tempeh (8). SCR fermented by *Aspergillus oryzae* or *Neurospora intermedia* (ontjom) had some beneficial functional properties, such as reducing cholesterol level in humans and counteracting dietary free radicals (7). A lipopeptide antibiotic was produced from SCR used as a solid-state substrate for *Bacillus subtilis* (1). Despite efforts to utilize SCR by traditional fermentation using *Bacillus* strains (7,9), a practical method for commercial utilization of SCR has not been developed. Since various indigenous microflora are present in SCR, it is necessary to carefully control the fermentation conditions. Baek et al. (10) reported that lactic acid fermentation of raw SCR was successfully carried out by controlling the fermentation temperature with indigenous lactic acid bacteria (LAB). The same group also isolated a *Lactobacillus* strain from SCR that was suitable for lactic acid fermentation of SCR (11). Therefore, lactic acid fermentation of raw SCR converted a perishable by-product into valuable functional ingredient with probiotic properties. Generally, traditional fermented soybean foods produced by alkaline fermentation have excellent nutritional and functional properties (12,13). The natural fermentation of SCR is predominantly performed by alkaline fermentation using *Bacillus* strains (9). Seo and Lee (12) reported that a valuable *Bacillus firmus* was isolated from traditional Japanese fermented soybean food and applied to the alkaline fermentation of soybean.

[†]Corresponding author. E-mail: splee@kmu.ac.kr
Phone: +82-53-580-5554, Fax: +82-53-580-5554

Therefore, bioconversion of raw SCR by lactic acid bacteria and/or *Bacillus* strains might contribute to the utilization of the thousand of tons of SCR produced as a by-product of tofu-processing. This study was carried out to optimize the production of organic acids and peptides by lactic acid and alkaline fermentations of SCR as well as the production of functional food ingredients with probiotic properties.

MATERIALS AND METHODS

Materials

Soybean curd residue (SCR) was obtained from Pulmuwon Co. (Kyungsangnamdo, Korea) and Doowon Co. (Kimcheon, Korea), designated as P-SCR and D-SCR, respectively, divided into 1 kg samples, and stored at -20°C. Micronized full-fat soy flour (MFS) was purchased from Perican Co. (Japan). Trichloroacetic acid and sodium hydroxide were purchased from Sigma Chemical Company (St. Louis, MO, USA). Phenol reagent for tyrosine assay was purchased from Junsei Chemical Company (Japan).

Microorganisms and medium

The lactic acid fermentation of SCR was performed by *L. rhamnosus* LS that was previously isolated from SCR of Pulmuwon (11). The *L. rhamnosus* LS strain was cultured in MRS agar. The culture medium for seed starter was prepared by mixing four parts of 5% MFS and one part of 5% soy/skim milk, and sterilized at 121°C for 15 min. *L. rhamnosus* LS as a seed starter was cultured in 5% MFS/soymilk (4:1) at 37°C for 24 h. *B. firmus* NA-1 isolated from a Japanese traditional fermented food (Natto) was used for alkaline fermentation (12). Pathogenic microorganisms such as *E. coli*, O-157 ATCC 43888, *Staphylococcus aureus* KCTC 1927, *Pseudomonas aeruginosa* KCTC 1930 were used for determining antimicrobial activity of the methanol extract of fermented SCR.

Fermentation of SCR

Frozen SCR was rapidly thawed in a microwave oven (Amanda, USA). The raw SCR was fortified with 0~2% glucose. SCR was fermented by an indigenous microorganism, *L. rhamnosus* LS or by inoculation with *B. firmus* NA-1. The LAB starter cultured as curd was diluted 10 fold with MFS / skim milk (4:1) and then was inoculated into the SCR at a 1.0% level. The SCR mixed with a seed starter was fermented at 37°C. As a co-starter, the seed culture of *B. firmus* NA-1 was prepared with 5% MFS milk and was transferred into the SCR at a 1% level.

Physicochemical properties of fermented SCR

Twenty grams of the fermented SCR was mixed with 80 mL of distilled water, and homogenized in a homogenizer (AM, Nihonseiki Kaisha Ltd., Japan), and then passed through a filter paper (No. 2, Whatman, England). The pH of the filtrate was determined with a pH meter (420A, Thermo Orion, USA). For determining titratable acidity (% lactic acid), 20 mL of the filtrate was titrated with 0.1 N NaOH to reach pH 8.3 (14). The tyrosine content in the filtrate was determined by reacting with Folin reagent at 37°C for 30 min (10). The color developed was measured at 660 nm by using a spectrophotometer (UVICON, Kontron Instruments, France). Viable cell counts were determined by plating serially diluted sample on MRS agar.

Drying of fermented SCR

The fermented SCR was dried in a convection drying oven (HS, Hwashin, Korea) at 50°C. The moisture content of the SCR during the drying process was determined by an AOAC method (15). The dried SCR was powdered using a mill (F-kurt Retsch ZM100, Germany). The fermented SCR powder (1.5 g) was suspended in 50 mL of distilled water and then its acidity and viable cell counts were determined.

Antimicrobial activity of SCR extract

The fermented SCR powder (10 g) was extracted with 400 mL of 50% methanol by shaking at 150 rpm for 24 h. The filtrate was obtained by centrifugation at 12,000 × g for 30 min and then passed through filter paper (No. 2, Whatman, England). The filtrate (400 mL) was concentrated by vacuum evaporation at 48°C. The concentrate was adjusted to pH 6.8 by adding 5 N NaOH and then re-concentrated to a final volume of about 2 mL.

Pathogenic microorganisms such as *E. coli* O-157, *S. aureus*, *P. aeruginosa* were grown in 5 mL of nutrient broth at 37°C, with shaking at 200 rpm for 24 h. The nutrient broth (5 mL) was fortified with 1% concentrate of fermented D-SCR, and then was inoculated with 1% seed starter that was grown in nutrient broth. The absorbance of culture broth was measured at 660 nm during the initial growth period.

RESULTS AND DISCUSSION

Characteristics of raw SCR

The P-SCR used for fermentation was obtained from the hot processed tofu (Pulmuwon Co.) which was subjected to heat treatment before separation of soymilk. On the other hand, D-SCR was obtained from the cold process (Doowon Co.), which allowed separation of soymilk without heat treatment. The raw P-SCR had a 72.0% moisture content, pH 7.5, and 0.05% reducing sugar. The raw D-SCR had a 80.7% moisture content, pH

6.1, 0.45% reducing sugar. The raw P-SCR and D-SCR had 0.1% (w/w), and 0.2% (w/w) acidities, respectively. To evaluate indigenous microorganism between P-SCR and D-SCR, raw SCRs were diluted with sterile water and then inoculated on MRS agar. The numbers of lactic acid bacteria from raw P-SCR and D-SCR were 6.5×10^6 CFU/g and 6.0×10^7 CFU/g, respectively. Also, Bacilli were detected from both P-SCR and D-SCR. As we expected, the raw D-SCR had a higher number of LAB than raw P-SCR. It may be due to the absence of heat-treatment of D-SCR. Because of the difference in heat-treatment of the raw SCR, the fermentation of two types of raw SCR could be distinguished by indigenous microorganisms and fermentation condition. It has been reported that LAB such as *L. rhamnosus* LS and *Enterococcus faecium* were isolated from the SCR and lactic acid fermentation of SCR was optimized (10).

Effect of sugar

To determine the effect of sugar on the lactic acid fermentation of SCR, 40% glucose was added to sterile P-SCR to a concentration of 0~2.0%. *L. rhamnosus* LS starter was inoculated at 1% and then fermented at 37°C for 15 h. Previously, the optimum fermentation time for P-SCR was determined to be 15~18 h at 37°C. As shown in Fig. 1, fermented P-SCR without added glucose showed 0.1% acidity, but its acidity was greatly increased by the addition of 1% glucose, reaching 1.0% acidity and pH 4.5, and then did not change with the addition of more glucose. This implies that the acid production in SCR by *L. rhamnosus* LS could be restricted by limiting fermentable sugar. Therefore, for maximal LAB fermentation of SCR, it is necessary to fortify SCR with 1% glucose. It was previously reported that the acid production by *L. rhamnosus* LS was optimized by the addition of 1% glucose or 1% lactose (11). In particular, viable cell counts from P-SCR with 1% glucose was about 5.5×10^8 CFU/g after fermentation for 15 h. Considering the initial inoculum size with 8.5×10^6 CFU/g viable cells, the viable cell counts in P-SCR fermented was increased by 65 fold.

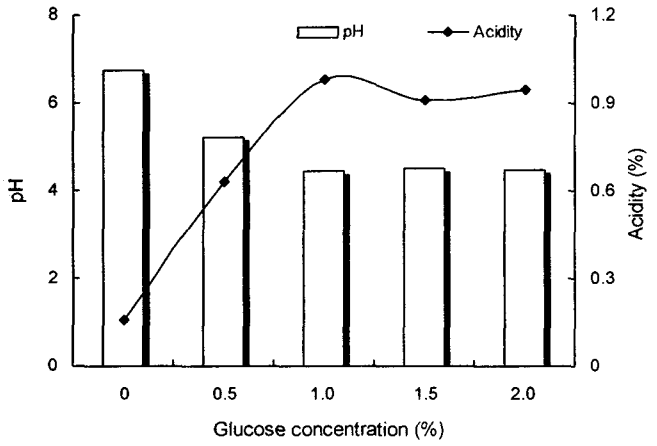

Fig. 1. Changes in pH and titrable acidity from P-SCR fermented according to glucose concentration. P-SCR: Pulmuone SCR.

Effect of SCR type on fermentation

To distinguish the fermentation characteristics between P-SCR and D-SCR, both raw SCRs were fermented with the addition of glucose or/and *L. rhamnosus* LS starter. As shown in Table 1, among indigenous microorganisms in naturally fermented raw SCR, LAB like bacteria were counted and found to be 6.7×10^6 CFU/g and 6.7×10^7 CFU/g viable cell counts in P-SCR and D-SCR, respectively. In addition, *Bacillus* like strains from fermented P-SCR were counted to be 3.3×10^6 CFU/g viable cell counts. In natural fermentation, indigenous LAB in D-SCR produced high quantities of acid compared with P-SCR, resulting in the 0.9% acidity. This suggests that the LAB in D-SCR is able to produce lactic acid efficiently. On the other hand, LAB in P-SCR produced acid weakly, showing 0.2% acidity, suggesting that the higher acid production in D-SCR was caused by the higher content of reducing sugar with 0.45% compared with 0.05% in P-SCR. The acid production in both SCRs was increased by the addition of 1% glucose. In P-SCR, the acid production was enhanced by the addition of *L. rhamnosus* LS starter. The viable cell counts of LAB were gradually increased by the addition glucose and LS starter. On the other hand, the acid production in D-SCR was affected by the addition of glucose, but was little changed by *L. rhamnosus* LS. From these results, we concluded that the lactic acid fermentation of P-SCR could be enhanced by the addition of *L. rhamnosus* LS starter and glucose. But, indigenous LAB in D-SCR is

Table 1. Changes in pH, acidity, tyrosine content and viable cell counts of raw and fortified SCRs

	Type	SCR[1]	SG[2]	SGL[3]
pH	P[4]	7.3	5.5	4.6
	D[5]	4.8	4.3	4.2
Acidity (%)	P	0.2	0.4	0.9
	D	0.9	1.4	1.5
Tyrosine content (mg%)	P	49.3	24.7	16.3
	D	28.2	30.1	35.5
Viable cell count[6] (CFU/g)	P	6.7×10^6	3.8×10^8	4.7×10^8
	D	6.7×10^7	7.2×10^7	7.7×10^7

[1]SCR: Soybean curd residue.
[2]SG: SCR with 1% glucose.
[3]SGL: SCR with 1% glucose and 1% *L. rhamnosus*.
[4]P: Pulmuone SCR (P-SCR).
[5]D: Doowon SCR (D-SCR).
[6]Lactic acid bacteria.

able to produce the acid efficiently compared with LAB in P-SCR.

It can be concluded that lactic acid fermentation of SCR is dominantly affected by indigenous LAB. It is necessary to isolate and characterize LAB present in D-SCR. The tyrosine content of both fermented SCRs was about 28~50 mg%. This suggests that the production of peptides from fermented SCR is limited by the fermentation by lactic acid bacteria and indigenous microorganism.

Effect of co-starter on SCR fermentation

To increase the tyrosine content of fermented SCR, the raw P-SCR fortified with 1% glucose was fermented by *L. rhamnosus* LS or/and *B. firmus* NA-1 at the 1% level. To facilitate the growth of *L. rhamnosus* LS, the fermentation was carried out at 37°C for 15 h. As shown in Fig. 2, the acidity of P-SCR fermented by *L. rhamnosus* LS was 1.0%, and the tyrosine content was 24 mg%. The pH of the diluted SCR was 4.6. On the other hand, the P-SCR fermented with *B. firmus* NA-1 as a co-starter had a higher tyrosine content at 187 mg%, but a decreased acid production with 0.5% acidity. The lower acid production of *L. rhamnosus* LS may be due to the shortage of fermentable sugar because *B. firmus* NA-1 consumed glucose as the carbon source in the fermentation. Conclusively, co-culture with *B. firmus* NA-1 greatly enhanced the peptide content compared with the fermentation by *L. rhamnous* LS as a single starter.

It can be concluded that the alkaline fermentation of raw P-SCR is possible at 37°C, a lower temperature than the 42°C used in some studies. Without the addition of glucose, the raw P-SCR fermented by *L. rhamnosus* LS and *B. firmus* NA-1 also had higher tyrosine content with 183 mg% (w/w) and 0.4% (w/w) acidity. Therefore, co-culture with *L. rhamonsus* LS and *B. firmus* NA-1 facilitates the production of lactic acid, which functions as a preservative. as well as increasing the amounts of peptides released during fermentation of P-SCR at 37°C for 15 h.

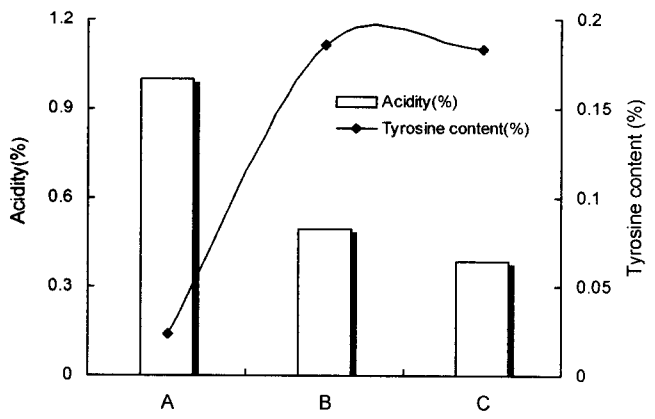

Fig. 2. Changes in titrable acidity and tyrosine content of raw P-SCR fermented by co-starter at 37°C for 15 h.
A: Addition of 1% glucose and 1% *L. rhamnosus*.
B: Addition of 1% glucose, 1% *L. rhamnosus* and 1% *B. firmus*.
C: Addition of 1% *L. rhamnosus* and 1% *B. firmus*.

With the addition of 1% glucose and 1% *L. rhamnosus* LS, the viable cell counts of fermented raw P-SCR was 6.3×10^8 CFU/g of LAB. Co-culture with *B. firmus* NA-1 and 1% glucose resulted in viable cell counts in fermented raw P-SCR of 2.4×10^8 CFU/g LAB and 3.3×10^6 CFU/g *Bacillus* sp. Under the same conditions, but without the addition of glucose, viable cell counts were 2.1×10^8 CFU/g for LAB and 6.6×10^6 CFU/g for *Bacillus* sp. Therefore, by co-fermentation of raw P-SCR with *L rhamnosus* LS and *B. firmus* NA-1, the viable cell count of *L. rhamnosus* LS reached 2×10^8 CFU/g, along with *B. firmus* NA-1, effectively hydrolyzed the soy protein resulting in the higher production of peptides. Interestingly, as shown in Fig. 2, raw P-SCR fermented only by *L. rhamnosus* LS was more acidic than those fermented with the co-culture. In addition, *Bacillus* like strains were not detected even if indigenous *Bacillus* sp. were present in the raw P-SCR. This suggests that the growth of indigenous *Bacillus* sp. present in raw SCR may be inhibited by the lactic acid which is produced by *L. rhamnosus* LS. In contrast, the alkaline fermentation of raw D-SCR was not completed under the same condition (unpublished result) perhaps due to the rapid acid production by indigenous LAB present in D-SCR. These results conclusively demonstrate that under the optimum conditions with co-starter, the lactic acid fermentation of raw P-SCR can be successfully achieved, resulting in a moderate production of lactic acid. At the same time, the *Bacillus* strain was able to grow and produce peptides, resulting in the increase of tyrosine content. However, it may be expected that the alkaline fermentation by *Bacillus* strain will be dominant, resulting in the reduction of acid production if the fermentation temperature increases to 42°C. But, to produce the functional ingredients with LAB probiotics and peptides, raw P-SCR is needed to be fermented by co-starter at moderate temperatures. Generally, *Bacillus* sp. has been used for soybean fermentation to produce biologically active compounds such as mucilage, peptides, fibrinolytic enzyme etc. (16,17). In particular, because *B. firmus* NA-1 isolated from Natto has the ability to produce fibrinolytic enzyme, it will be possible to produce the fibrinolytic enzyme from SCR fermented with co-starter.

Effect of hot-air drying on the viable cell counts

To facilitate the utilization of fermented SCR, both fermented SCRs were dried at 50°C and then powdered.

The moisture content, acidity and viable cell counts were determined after varied drying times. As shown in Table 2, after drying for 12 h, the moisture contents of powdered P-SCR and D-SCR were 7.3% and 11.7%, respectively. The acidities of powdered P-SCR and D-SCR were 3.3% and 4.4%, respectively, and the viable cell count of LAB in the powdered P-SCR was 1.8×10^7 CFU/g and 5.3×10^6 CFU/g in powdered D-SCR, demonstrating less than a 10% reduction of viable cell counts. However, after drying for 22 h at 50°C, the viable cell counts were greatly decreased. Therefore, the drying time is a very critical factor for preserving the viability of LAB. Furthermore, the moisture content of powdered SCR may affect the viability of LAB. After fermentation at 37°C followed by hot-air drying, the indigenous *Bacillus* sp. initially present in P-SCR also appeared in powdered P-SCR. But *Bacillus* sp. was not detected in the powdered D-SCR.

Interestingly, the free water from fermented SCR was easily removed by hot-air drying at 50°C, possibly due to the presence of a large amount of dietary fiber in SCR. Therefore, the fermented SCR with about 10% moisture content could be obtained by hot-air drying of 50°C for a short time period and could be utilized as functional ingredient for food and as a biomaterial by industry. Chung et al. (2) reported that dehydration of soybean residue was performed within 95 min at 120°C using hot-air in conjunction with filter pressing. The fermented SCR powder was successfully used as an ingredient in bread-baking at a 5% level, without the deterioration of taste and quality (unpublished results). Therefore, it is considered to be a promising ingredient contributing dietary fiber and probiotics in the food industry.

Antimicrobial activity of fermented SCR

To evaluate the antimicrobial activity of fermented SCR, 50% methanol extract was obtained from the powdered P-SCR and D-SCR and then concentrated. Antimicrobial activity of each concentrate was examined against pathogenic microorganisms. Only the concentrate from fermented D-SCR inhibited bacterial growth as evaluated by the disk plate method, with no inhibition by fermented P-SCR against the pathogenic microorganisms (unpublished results). As shown in Fig. 3, in the presence of fermented D-SCR concentrate at the 1% level, the culture broth showed the inhibition of initial cell growth for *E. coli*, *P. aeruginosa* and *S. aureus*. In particular, the fermented D-SCR concentrate strongly inhibited *P. aeruginosa*. Its antimicrobial activity may be due to the presence of a bacteriocin-like metabolite from LAB. Because the pH of the concentrate was neutralized, the role of acid as a preservative could be discounted. LAB have played an important role as functional starters for the food fermentation (18). It has been well known that LAB produce a heterogeneous group of peptide inhibitors such as bacteriocins (19). Antimicrobial substances from LAB have been widely used as food preservatives (20). To confirm the antimicrobial activity and facilitate the SCR fermentation, it is necessary to purify and characterize the antimicrobial compounds.

Table 2. Changes in the moisture content, acidity, and viable cell counts of different raw fermented SCR according to the drying time

Time (h)		0	12	22
Moisture content (%)	P[1]	72.5	7.3	6.4
	D[2]	81.0	11.7	8.6
Viable cell count[3] (CFU/g)	P	4.7×10^8	1.8×10^7	3.3×10^3
	D	7.7×10^7	5.3×10^6	3.3×10^3
Acidity (%)	P	0.8	3.3	4.8
	D	1.2	4.4	4.8

[1]P: Pulmuone SCR, [2]D: Doowon SCR.
[3]Lactic acid bacteria.

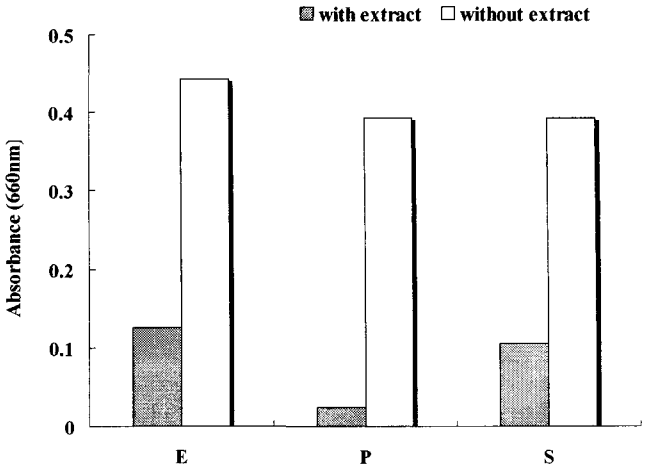

Fig. 3. Inhibitory effect of fermented D-SCR extract on the growth of pathogenic bacteria. E: *E. coli*, P: *Pseudomonas aeruginosa*, S: *Staphylococcus aureus*. The culture broth was used after fermentation for 4 h.

In conclusion, under optimal conditions, SCR by-products can be converted into valuable functional ingredients rich in peptides and probiotics. In particular, the co-culture with *L. rhamnosus* LS and *B. firmus* NA-1 of raw P-SCR at moderate temperatures efficiently produced peptides as well as lactic acid as a preservative. The probiotics and peptides in LAB-fermented SCR together with other additional bioactive compounds, which may be present in SCR fermented by *B. firmus* NA-1, demonstrate that fermented SCR has the potential to be a valuable functional ingredient in the food industry.

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REFERENCES

1. Ohno A, Ano T, Shoda M. 1996. Use of soybean curd residue, Okara, for the solid state substrate in the production of a lipopeptide antibiotic, Iturin A, by *Bacillus subtilis* NB22. *Process Biochemistry* 31: 801-806.
2. Chung SS, Chang HN, Park MY. 1978. Dehydration of soybean residue by hot-air in conjunction with filter pressing. *Korean J Food Sci Technol* 10: 1-5.
3. Kurokochi K, Matsuhashi T, Nakuzawa M, Nakazawa A. 1977. Use of okara powder for bread. *New Food Ind* 19: 49-53.
4. Ma CY, Liu WS, Kwok KC, Kwok F. 1997. Isolation and characterization of proteins from soymilk residue (okara). *Food Res Int* 29: 799-805.
5. Jackson CC, Dini JP, Lavandier C, Rupasinghe HPV, Faulkner H, Poysa V, Buzzell D, DeGrandis S. 2002. Effects of processing on the content and composition of isoflavones during manufacturing of soy beverage and tofu. *Process Biochemisty* 37: 1117-1123.
6. Jimenez-Escrig A, Sanchez-Muniz FJ. 2000. Dietary fibre from edible seaweeds: chemical structure, physicochemical properties and effects on cholesterol metabolism. *Nutr Res* 20: 585-598.
7. O'Toole DK. 1999. Characteristics and use of okara, the soybean residue from soymilk production; a review. *J Agric Food Chem* 47: 363-371.
8. Wang HL, Cavins JF. 1989. Yield and amino acid composition of fractions obtained during tofu production. *Cereal Chem* 66: 359-361.
9. Lee MS. 1987. Studies on the isolation, characterization of microorganism and compositional change during natural fermentation of soybean curd residue. *MD Dissertation.* Korea University.
10. Baek J, Kim CS, Lee SP. 2002. Optimized lactic acid fermentation of soybean curd residue (Biji). *Nutraceut Food* 7: 397-404.
11. Baek J, Lee IS, Lee SP. 2002. Characterization and fermentation characteristics of lactic acid bacteria isolated from soybean curd residue. *J Korean Soc Food Sci Nutr* 31: 583-588.
12. Seo JH, Lee SP. 2004. Optimization of the production of fibrinolytic enzyme for *Bacillus firmus* NA-1 in fermented soybeans. *J Food Sci Nutr* 9: 14-20.
13. Larroche C, Besson I, Gros JB. 1999. High pyrazine production by *Bacillus subtilis* in solid substrate fermentation on ground soybeans. *Process Biochemistry* 34: 667-674.
14. Amerine MA, Ough CS. 1980. *Methods for analysis of musts and wines.* A Wiley-Interscience Publication, New York. p 46-48.
15. AOAC. 2000. *Official methods of analysis.* 17th ed. Association of official analytical chemists, Washington DC. Chapter 4, p 1-40.
16. Shih IL, Van YT. 2001. The production of poly-(γ -glutamic acid) from microorganisms and its various applications. *Bioresource Technology* 79: 207-225.
17. Urano T, Ihara H, Umemura K, Suzuki Y, Oike M, Akita S, Tsukamoto Y, Suzuki I, Takada A. 2001. The profibrinolytic enzyme subtilisin NAT purified from *Bacillus subtilis* cleaves and inactivates plasminogen activator inhibitor type I. *J Biological Chem* 276: 24690-24696.
18. Leroy F, Vuyst LD. 2004. Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends in Food Science Technol* 15: 67-78.
19. Parente E, Ricciardi A. 1999. Production, recovery and purification of bacteriocins from lactic acid bacteria. *Appl Microbiol Biotechnol* 52: 628-638.
20. Daeschel MA. 1989. Antimicrobial substances from lactic acid bacteria for use as food preservatives. *Food Technol* 43: 164-167.

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