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Optimized Lactic Acid Fermentation of Soybean Curd Residue (Biji)

Joseph Baek, Chan-Shick Kim* and Sam-Pin Lee†

Dept. of Food Science and Technology, Keimyung University, Daegu 704-701, Korea
**Faculty of Horticultural Life Science, Cheju National University, Cheju 690-756, Korea*

Abstract

Soybean curd residue (SCR) was fermented by lactic acid bacteria, *Lactobacillus rhamnosus* LS and *Entercoccus faecium* LL, isolated from SCR. The pH, titratable acidity and viable cell counts were determined from the fermented SCR to evaluate the lactic acid production and growth of lactic acid bacteria. Optimal amounts of protease enzyme and glucose, and ideal fermentation time for SCR fermentation were estimated by response surface methodology (RSM). Raw SCR fermented by indigenous microorganisms had 0.78% titratable acidity. The acid production in SCR fermented by *L. rhamnosus* LS was greatly enhanced by the addition of glucose and lactose. However only glucose increased acid production by *Ent. faecium* LL. The proof test of SCR fermentation demonstrated that similar results for titratable acidity, tyrosine content and viable cell counts to that predicted could be obtained by the at optimized fermentation conditions. In the presence of 0.029% (w/w) protease enzyme and 0.9% (w/w) glucose, the SCR fermented by *Ent. faecium* LL showed 1.07% (w/v) of titratable acidity, 1.02 mg% tyrosine content and 2×10^9 (cfu/g) of viable cell counts. With the SCR fortified with 0.033% protease enzyme and 1.7% glucose, *L. rhamnosus* LS showed 1.8% (w/v) of titratable acidity, 0.92 mg% of tyrosine content and 2×10^9 (cfu/g) of viable cell counts.

Key words: soybean curd residue, lactic acid bacteria, response surface method, lactic acid

INTRODUCTION

Soybean curd residue, known as Biji, is produced as a by-product of the tofu and soymilk industries (1). Much of soybean curd residue (SCR) produced by the tofu manufacturing industry has been used as animal feed or agricultural fertilizer (2). However, SCR still contains about 27% protein with high nutritive quality (3) and dietary fiber (1), suggesting that it has potential food ingredient. Pectic polysaccharide, which differs from citrus pectin, has also been extracted and purified from soybean curd residue (4).

Because typical SCR is rich in various ingredients such as protein, fat, starch, sugar, fiber and ash, it is also a potentially useful media for microbial fermentation (1). In general, raw SCR is easily putrefied by indigenous microorganisms and quickly spoiled. Dehydration of SCR has been used to improve shelf life but the process is too costly for SCR, which has little market value. SCR powder has been used to partially replace wheat flour for bread baking and for soy flour in tofu production (5,6). SCR can also be used as a fermentation stock for the production of seasonings, spices and tempeh (3). SCR fermented with *Aspergillus oryzae* (koji), *Neurospora intermedia* (ontjom) and *Rhizopus oligosporus* (tempeh) reduces cholesterol concentrations in humans and contains substances that counteract dietary free radicals (7). Furthermore, soybean curd residue has been used as the solid-state substrate in antibiotic production by *Bacillus subtilis* (1). Generally, alkaline fermentation of SCR has been used for the application of SCR as a food ingredient in traditionally fermented foods. The natural fermentation of SCR is predominantly performed by indigenous *Bacillus* strains (8). Although there has been considerable research on the fermentation of SCR, a practical method for commercial utilization of SCR has not yet been developed. Because of the variety of microorganisms present in SCR, the natural fermentation of raw SCR could be greatly affected by various factors such as temperature and media composition. During storage at room temperature, raw SCR is easily putrefied, resulting in acidification and an unpleasant flavor. Recently, lactic acid bacteria were isolated from raw SCR and partially characterized (9).

Therefore, lactic acid fermentation of raw SCR could produce a perishable by-product with an enhanced shelf life and functional properties due to various metabolites and probiotics. Bioconversion of raw SCR by lactic acid bacteria might contribute to the utilization of the thousand tons of SCR produced as a by-product of the tofu-processing industry. The present study was conducted to evaluate

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

the lactic acid fermentation of SCR as well as to establish its optimization by the response surface method.

MATERIALS AND METHODS

Materials

Skim milk powder and micronized full-fat soy flour (MFS) were purchased from Seoul Dairy Company (Korea) and Perican Co. (Japan), respectively. Rochelle salt, phenol, sodium metabisulfite, trichloroacetic acid and sodium hydroxide were purchased from Sigma Chemical Company (St. Louis, MO, USA). Sodium carbonate and dinitrosalicilic acid were obtained from Yakuri Chemical Company (Japan). Phenol reagent for the tyrosine assay was purchased from Junsei Chemical Company (Japan). Protease (Kojizyme^{TM} 500MG) used was a commercial product containing exo-peptidase, endo-protease and amylase supplied by Novo Nordisk A/S (Bagsvaerd, Denmark). Soybean curd residue (Biji) was obtained from Pulmuwon Co. (Korea), and stored at 20°C after dividing into vinyl bags with 1 kg each.

Microorganism and medium

The microorganisms used for SCR fermentation were *Lactobacillus rhamnosus* LS and *Entercoccus faecium* LL that were isolated from raw SCR. Lactic acid bacteria were cultured in Man-Rogosa-Sharpe (MRS) broth or MRS agar, and then activated with 5% MFS and skim milk mixture (4:1) on an agar plate. Culture broth for starter was mixed with four parts of 5% MFS milk and one part of 5% skim milk, and then autoclaved at 121°C for 15 min (9).

Fermentation

Frozen SCR was thawed for 5 min using a microwave oven (Amana, USA). A seed starter culture was prepared with 100 mL of MFS and skim milk mixture (4:1) by fermentation at 37°C for 15 hr. The curd formed was diluted with 10 times with MFS/skim milk (4:1) mixture. A 0.25% (v/w) of diluted seed starter was mixed with 20 g of sterilized Biji in 250 mL glass bottle, and then solid-state fermentation was performed by incubation at 37°C for 15 hr. The SCR fortified with protease and/or carbon source was fermented under the same conditions.

Analytical methods

Moisture content, crude ash, crude protein and crude fat of raw SCR were determined by AOAC methods (10). Crude fiber and reducing sugar were determined by the Henneberg-Stohmann method (11) and DNS method (12), respectively. The pH of fermented MFS/skim milk mixture was determined with a pH meter (Digital pH meter 110, Wheaton, USA). For determining titratable acidity (%, lactic acid), a 10 mL aliquot of fermented MFS/skim milk mixture was mixed with 20 mL of distilled water and then titrated with 0.1 N NaOH to reach pH 8.3 (13). Viable cell counts were determined by plating diluted culture broth on MRS agar. Fermented SCR was mixed with four volumes of sterilized water and then analyzed by the same methods described above.

Tyrosine content

Raw SCR and fermented SCR were both diluted with distilled water and passed through a filter paper (No2, Whatman, England). One part of filtrate was mixed with one part of trichloroacetic acid (30%) and the mixture incubated at 37°C for 30 min. After centrifugation of the reaction mixture, the supernatant was reacted with Folin reagent and sodium carbonate at 37°C for 30 min. Color development from the reaction was determined at 660 nm using a spectrophotometer (UVICON, Kontron Instruments, France).

Experimental design for optimizing lactic acid fermentation of SCR

Optimization of SCR fermentation was designed by the response surface methodology (RSM) (14). Enzyme concentration (0~0.4%, X_1), glucose content (0~0.4%, X_2) and fermentation time (10~40 hr, X_3) were selected as independent variables for a central composite design (15). The sixteen experimental groups were evaluated by fermentation by *L. rhamnosus* LS and *Ent. faecium* LL. The titratable acidity (Y_1), tyrosine content (Y_2) and viable cell counts (Y_3), were the dependent variables and determinants of fermented SCR. The data was analyzed using SAS (SAS, Cary, NC) program (16) for multiple regression analysis. An optimal point was obtained by a ridge analysis when a critical point is a saddle point beyond a maximum or a minimum point. The optimum condition for lactic acid fermentation was selected from the variable values of overlapped region obtained by superimposing contour maps for titratable acidity, tyrosine content and viable cell counts. An optimum condition selected was then confirmed by performing practical experiments.

RESULTS AND DISCUSSION

Composition of soybean curd residue

Raw soybean curd residue (SCR) contained 78.2% moisture, 1.0% crude ash, 6.9% crude protein, 0.9% crude fat, 3.5% crude fiber, 0.58% reducing sugar and 0.15% (w/w) titratable acidity. Microorganisms detected were primarily lactic acid bacteria with 2×10^7 viable cells per g SCR; while *Bacillus* sp. and other contaminants were present in very low numbers (9). The large number of viable lactic acid bacteria was unexpected in the raw SCR since it

v as originally sterilized at temperatures above 80°C be-
f re the separation of soymilk and the insoluble residue
(Biji). The moisture content of raw SCR can vary due
t different processing conditions, such as the separation
c f soymilk. It was observed that Pulmuwon SCR, pro-
c ced by the filter pressing method, contained relatively
l w moisture content.

Lactic acid fermentation of raw SCR

Because of the heterogeneous population of microor-
g anisms in raw SCR, fermentation temperature is a crit-
i al factor for controlling the fermentation type in SCR.
I reviously, lactic acid bacteria in raw SCR exhibited
(ptimal growth at 37°C resulting in the highest acid pro-
(uction. On the other hand, alkaline fermentation of raw
S CR was successfully performed at higher temperatures
(42°C), and in this case, *Bacillus* sp. was a more important
l acterium in the fermentation (11). Shin et al. reported
t at *Acinetobactor calcoaceticus* var. *anitrat*, *Klebsiella*
p neumoniae sub. *pneumoniae*, and *Acenitobacter calco-*
(ceticus var. *anitra* were isolated from a putrefied tofu
(17). Therefore, to effectively inhibit the growth of *Ba-*
(illus sp. and other contaminants present in raw SCR,
i was necessary to drop the pH quickly after fermentation
v as initiated. As shown in Fig. 1, the lactic acid fermen-
t ation of SCR was effectively carried out without addi-
t onal carbon sources at 37°C for 20 hrs, resulting in
(.78% acidity from an initial 0.14% acidity. No bacterial
(ontaminants, including *Bacillus* sp., were detected in the
f rmented SCR, which implied that the lactic acid fermen-
t ation effectively inhibited the growth of indigenous
b acteria, except for lactic acid bacteria. Previously, lactic
a cid fermentation of SCR for 15 hrs at 37°C reached

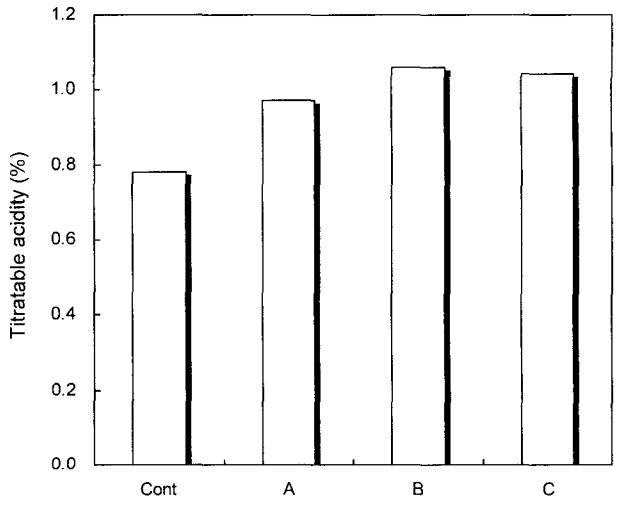


Fig. 1. Effects of carbon sources and protease on the titratable acidity of raw SCR fermented at 37°C for 20 hr.
Cont: raw SCR, A: raw SCR with 2% lactose, B: raw SCR with 2% glucose, C: raw SCR with 0.025% protease.

0.72% acidity, but the acidity decreased with longer incubation (40 hrs, unpublished results).

Acid production in fermented SCR may also be sensitive to carbon sources and hydrolyzing enzymes. Either 2% glucose or 2% lactose and 0.025% crude protease were added to raw SCR and fermented at 37°C for 20 hrs. The addition of either glucose or lactose resulted in higher acid production than in SCR fermented without additional carbon sources with titratable acidities of 1.06% and 0.97%, respectively (Fig. 1). Furthermore, the addition of crude protease, containing amylase, increased acid production (1.05% acidity) to a similar degree as additional sugar. Cha et al. reported that *L. acidophilus* in soy yoghurt fermentation produced higher acidity with the addition of glucose, galactose and lactose, but not sucrose (18). Crude protease may increase acid production by releasing additional fermentable sugars and protein hydrolysates derived from the hydrolysis of protease or amylase enzymes.

Previously, indigenous lactic acid bacteria in raw SCR were isolated and identified as *L. rhamnosus* LS and *Ent. faecium* LL by a biochemical analysis (9). Audisio et al. reported that *Ent. faecium* CRL 1385 showed inhibitory activity against *Salmonella pullorum* (19). Also, swine fed with *Ent. faecium* Cernelle 68 had a reduced incidence of diarrhea (20). In addition, *L. rhamnosus* GG (ATCC 53103) showed an inhibitory effect against *Streptococcus sorbrinus* at below pH 5.0 (21). Gopal et al. reported that *L. rhanmosus* DR20 inhibited *E. coli* growth on TSB plate (22). Furthermore, Vanhaverbeke et al. reported that extracellular polysaccharide was produced by *L. rhamnosus* strain C83 (23). These results suggest that *L. rhamnosus* LS and *Ent. faecium* LL isolated from raw SCR could be utilized for the preservation of raw SCR as well as a source of probiotics.

Lactic acid fermentation of sterilized SCR

To determine acid production in sterilized SCR by a single starter of lactic acid bacteria previously isolated from raw SCR, sterilized SCR was fermented by *Ent. faecium* LL or *L. rhamnosus* LS starter with the amounts of 0.13%, 0.25%, and 0.5%. As shown in Fig. 2 the acid production in fermented SCR was enhanced by adding 0.13% of *Ent. faecium* LL starter, showing 0.55% titratable acidity, and gradually increased with the addition of higher concentrations of starter. However, *L. rhamnosus* LS starter only produced 0.25% acidity with the addition of 0.13% starter, and increasing the concentration of starter did not affect the acid production. Inoculations with 0.25% *Ent. faecium* LL and *L. rhamnosus* LS starters produced 0.59% and 0.22% of titratable acidity, respectively. Yu et al. reported that soymilk fermented by *L. acidophilus* showed 0.25% of titratable acidity (24). It turned

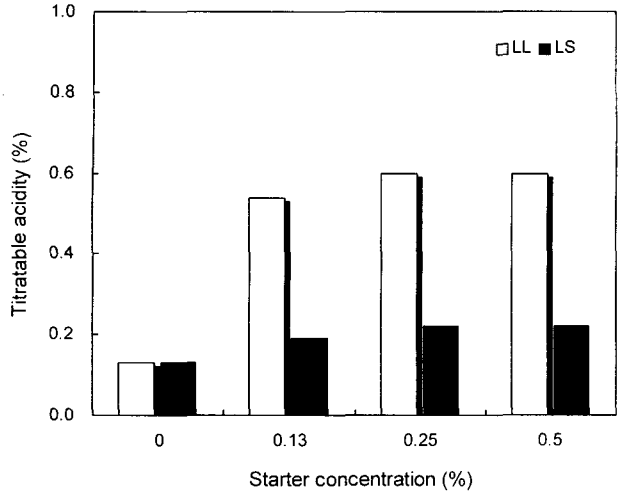


Fig. 2. Effects of starter concentration on the titratable acidity of a sterilized SCR fermented by lactic acid bacteria. LL: *Ent. faecium* LL, LS: *L. rhamnosus* LS.

out that *Ent. faecium* LL effectively utilized the fermentable sugars in SCR, but SCR contained limited amounts of sugars that are fermentable by *L. rhamnosus* LS. Based on the acid production, according to the amounts of starter inoculated, the optimal concentration of starter was determined to be 0.25% (w/v) for lactic acid fermentation of SCR.

Carbon source

In the experiments above, acid production was enhanced by the addition of glucose or lactose in raw SCR. The effect of carbon source on the acid production in sterilized SCR is shown in Fig. 3. Addition of *Ent. faecium* LL and *L. rhamnosus* LS starter enhanced the acid production in the sterilized SCR with added glucose or lactose, compared with the 0.13% titratable acidity in sterilized

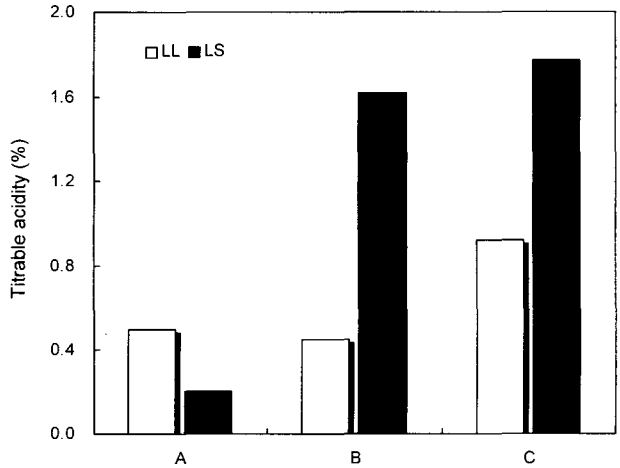


Fig. 3. Changes in titratable acidity of the autoclaved SCR fermented by lactic acid bacteria according to the addition of sugar. A: no sugar added, B: 2% lactose, C: 2% glucose. LL: *Ent. fecium* LL. LS: *L. rhamnosus* LS.

SCR. However, addition of *Ent. faecium* LL starter only increased titratable acidity to 0.45% in SCR with 2% lactose, but with 2% glucose it increase to 0.92% (Fig. 3). Thus the effect of lactose on acid production was not significant, considering the similar acidity with SCR fermented without additional carbon. However, the addition of glucose resulted in a two-fold increase of titratable acidity (Fig. 3). It previously reported that the titratable acidity in MFS/skim milk fermented by *Ent. faecium* LL was not increased by the addition of lactose (9). On the other hand, with *L. rhamnosus* LS starter acidity dramatically increased to 1.62% and 1.77% by the addition of 2% glucose and 2% lactose, respectively, suggesting that *L. rhamnosus* LS can utilize both glucose and lactose efficiently as a fermentable carbon source.

SCR fermentation by RSM

The data thus far obtained indicated that the critical factors for optimal fermentation of SCR are: concentration of glucoses, enzyme activity, and fermentation time. As shown in Table 1, the titratable acidity, viable cell counts and tyrosine content were obtained from SCR fermented according to a central composite design. Polynomial equations of titratable acidity, tyrosine content and viable cell

Table 1. Effects of critical factors on titratable acidity, tyrosine content and viable cell counts under different conditions based on central composite design for response surface methodology

Enzyme (%, X_1)	Glucose (%, X_2)	Time (h, X_3)	Y_1	Y_2	Y_3
0.01(-1)	1(-1)	15(-1)	0.95	0.83	9.27
0.01(-1)	1(-1)	25(1)	1.13	0.82	9.13
0.01(-1)	3(1)	15(-1)	0.96	0.73	9.24
0.01(-1)	3(1)	25(1)	1.15	0.79	9.11
0.03(1)	3(1)	25(-1)	1.09	0.93	9.20
0.03(1)	3(1)	25(1)	1.19	1.01	9.24
0.03(1)	1(-1)	15(-1)	1.25	0.95	9.25
0.03(1)	1(-1)	25(1)	1.27	1.06	9.16
0.02(0)	2(0)	20(0)	1.19	0.92	9.26
0.02(0)	2(0)	20(0)	1.17	0.87	9.21
0.00(-2)	2(0)	20(0)	0.87	0.54	9.02
0.04(2)	2(0)	20(0)	1.20	1.15	9.14
0.02(0)	0(-2)	20(0)	0.78	0.88	9.05
0.02(0)	4(2)	20(0)	1.14	0.87	9.25
0.02(0)	2(0)	10(-2)	0.97	0.81	9.23
0.02(0)	2(0)	30(2)	1.17	0.95	9.22

Ent. faecium LL starter was used for SCR fermentation.

*Y_1: titratable acidity (%), Y_2: tyrosine (mg%), Y_3: viable cell counts (log cfu/g)

$Y_1 = -0.312688+4.078125X_1+0.272313X_2+0.061713X_3-3.4375X_1^2-0.35125X_2X_1-0.054375X_2^2-0.06075X_3X_1+0.002425X_3X_2-0.00108X_3^2$

$Y_2 = 1.234563+1.936875X_1-0.051688X_2+0.008062X_3-2.7625X_1^2+0.16125X_2X_1-0.011625X_2^2+0.06675X_3X_1+0.001925X_3X_2-0.0003X_3^2$

$Y_3 = 9.37325+0.43125X_1+0.0205X_2-0.019275X_3-3.825X_1^2+0.1075X_2X_1-0.020125X_2^2+0.055X_3X_1+0.0031X_3X_2-0.000055X_3^2$

Table 2. Maximal response outcomes of titratable acididty, tyrosine content, and viable cell counts predicted by ridge analysis following SCR fermentation

Response	R^2	Prob>F	Enzyme (%)	Glucose (%)	Time (h)	Maximum	Morphology
Acidity (%)	0.7670	0.1754	0.039	1.57	19	1.23	maximum
Tyrosine (mg%)	0.9375	0.0055	0.037	2.08	25	1.14	saddle point
VCC[1)] (log cfu/g)	0.7409	0.2228	0.016	1.80	10	9.29	saddle point

Ent. faecium LL starter was used for SCR fermentation.
[1)]VCC: viable cell counts.

counts were derived from the response surface method. Table 2 shows the predicted maximal levels of acidity, tyrosine content and viable cell counts in SCR fermented by *Ent. faecium* LL. The level of independent variables for maximum acidity predicted were: 0.039% enzyme, 1.57% glucose and 19 hrs fermentation. Titratable acidity was 1.23% (w/v) at the predicted stationary point as a maximum point. The predicted maximum tyrosine content was obtained at 0.037% enzyme, 2.08% glucose and fermentation time of 25 hrs. The tyrosine content was estimated as 1.14 mg% through a ridge analysis since the stationary point was a saddle point. A polynomial equation for tyrosine content indicated a high regression coefficient (R^2=0.9375) with a 5% level of significance. Because the predicted stationary point of viable cell counts was a saddle point, A 1.9×10^9 (cfu/g) of viable cell counts was also estimated through a ridge analysis. The maximum response of viable cell counts was obtained at 0.016% enzyme, 1.8% glucose, and fermentation time of 10 hrs.

To determine the optimum fermentation parameters for raw SCR by *L. rhamnosus*, LS dependent variables such as titratable acidity, tyrosine content and viable cell counts were determined according to the concentration of enzyme and glucose established by a central composite design (Table 3). Polynomial equations for titratable acidity, tyrosine content and viable cell counts were derived. Table 4 shows the maximum response of acidity, tyrosine content and viable cell counts in SCR fermented by *L. rhamnosus* LS. The predicted stationary point of acidity, indicated as a maximal point, and a predicted maximum acidity (2.49%) were attained by fermentation for 25 hrs in the presence of 0.034% enzyme and 2.99% glucose. The polynomial equation has a regression coefficient (R^2 =0.9137) with a 5% significance level. The polynomial equation for tyrosine content showed a high regression coefficient (R^2=0.9672) with a 5% significance level. Because the predicted stationary point of tyrosine content was a saddle point, the maximum tyrosine content (1.09 mg%) was predicted by a ridge analysis, with optimal conditions determined to be 0.034% enzyme, 1.39% glucose and fermentation time of 14 hrs. The polynomial

Table 3. Effects of critical factors on titratable acidity, tyrosine content and viable cell counts under different conditions based on central composite design for response surface methodology

Enzyme (%, X_1)	Glucose (%, X_2)	Time (h, X_3)	Y_1	Y_2	Y_3
0.01(-1)	1(-1)	15(-1)	1.55	0.73	9.61
0.01(-1)	1(-1)	25(1)	1.48	0.66	9.70
0.01(-1)	3(1)	15(-1)	1.58	0.74	9.48
0.01(-1)	3(1)	25(1)	2.06	0.66	9.51
0.03(1)	3(1)	25(-1)	1.95	1.06	9.60
0.03(1)	3(1)	25(1)	2.27	0.92	9.65
0.03(1)	1(-1)	15(-1)	1.70	1.04	9.55
0.03(1)	1(-1)	25(1)	1.78	0.95	9.50
0.02(0)	2(0)	20(0)	2.03	0.80	9.77
0.02(0)	2(0)	20(0)	2.05	0.79	9.72
0.00(-2)	2(0)	20(0)	1.68	0.35	9.47
0.04(2)	2(0)	20(0)	2.24	1.02	9.67
0.02(0)	0(-2)	20(0)	0.81	0.95	9.42
0.02(0)	4(2)	20(0)	2.06	0.81	9.45
0.02(0)	2(0)	10(-2)	1.03	0.90	9.39
0.02(0)	2(0)	30(2)	2.14	0.81	9.82

L. rhamnosus starter was used for SCR fermentation.
*Y_1: titratable acidity (%), Y_2: tyrosine(mg%), Y_3: viable cell counts (cfu/g)

$Y_1 = -1.108687+1.9475X_1+0.41975X_2+0.180225X_3-2.1X_1^2+0.16X_1X_2-0.151625X_2^2-0.004X_1X_3+0.01975X_2X_3-0.004530X_3^2$

$Y_2 = 1.5525+6.225625X_1-0.158312X_2-0.052787X_3-5.3625X_1^2-0.051250X_1X_2+0.043625X_2^2-0.04175X_1X_3-0.002125X_2X_3+0.001285X_3^2$

$Y_3 = 8.597313+1.266875X_1+0.159563X_2+0.071337-4.35X_1^2+0.62875X_2X_1-0.07675X_2^2-0.02675X_3X_1+0.000875X_3X_2-0.00139X_3^2$

Table 4. Predicted level of SCR fermentation for the maximum response of titratable acidity, tyrosine content, viable cell counts by the ridge analysis

Response	R^2	Prob>F	Enzyme (%)	Glucose (%)	Time (h)	Maximum	Morphology
Acidity (%)	0.91	0.0136	0.034	2.99	25	2.49	maximum
Tyrosine (mg%)	0.97	0.0009	0.034	1.39	14	1.09	saddle point
VCC[1)] (log cfu/g)	0.80	0.1251	0.021	2.07	30	9.72	saddle point

L. rhamnosus LS starter was used for SCR fermentation.
[1)]VCC: viable cell counts.

equation for viable cell counts had a regression coefficient (R^2=0.7983) and a lower level of significance. Because the predicted stationary point was a maximal point, the viable cell counts were predicted with 5.2×10^9 (cfu/g) under the following conditions: 0.021% enzyme, 2.07% glucose and fermentation time of 30 hrs.

Optimization of lactic acid fermentation by superimposing contour maps

It was previously proven that lactic acid fermentation of SCR for 15 hrs resulted in higher acidity. Therefore, the contour maps for titratable acidity, tyrosine content and viable cell counts were analyzed at a fixed fermentation time (15 hr) with differing concentrations of enzyme and glucose. The optimum condition of fermentation was determined by superimposing each contour map for titratable acidity, tyrosine content and viable cell counts. Fig. 4 shows the superimposed contour maps of titratable acidity, tyrosine content and viable cell counts of SCR fermented by *Ent. faecium* LL for 15 hrs, The analysis of contour maps indicated that acidity was not only increased by higher enzyme concentration, but also by the addition of 1~2% of glucose. Tyrosine content was also increased by higher concentrations of enzyme, but was not affected by the addition of glucose. Based on the contour map for viable cell counts in SCR fermented by *Ent. faecium* LL, the viable cell counts were higher value with 0.025% enzyme and 2% glucose.

The superimposed contour maps of titratable acidity, tyrosine content and viable cell counts in SCR fermented by *L. rhamnosus* LS are shown in Fig. 5. The tyrosine content was increased by adding higher concentrations of enzyme, but was not changed by the addition of higher

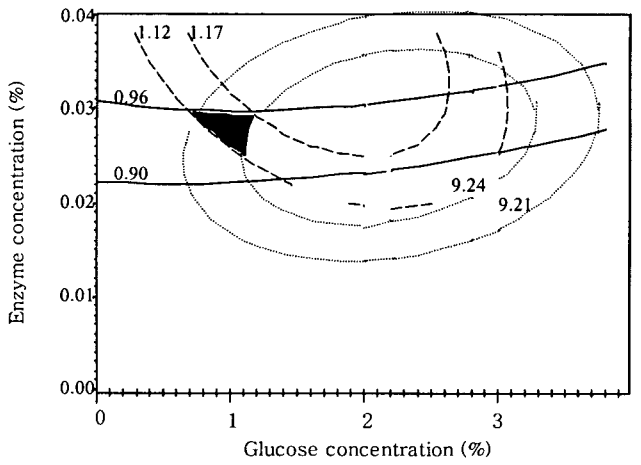


Fig. 4. Superimposed contour maps of optimized conditions for titratable acidity, tyrosine content, and viable cell counts of SCR fermented by *Ent. faecium* LL.
--- : titratable acidity (%), — : peptide content (mg%), ··· : viable cell counts (log cfu/g).

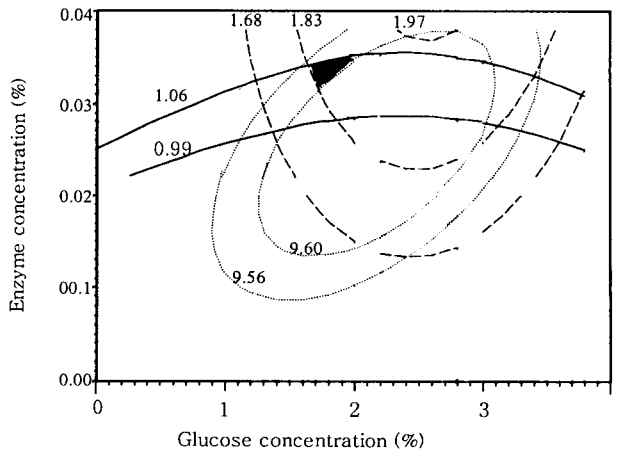
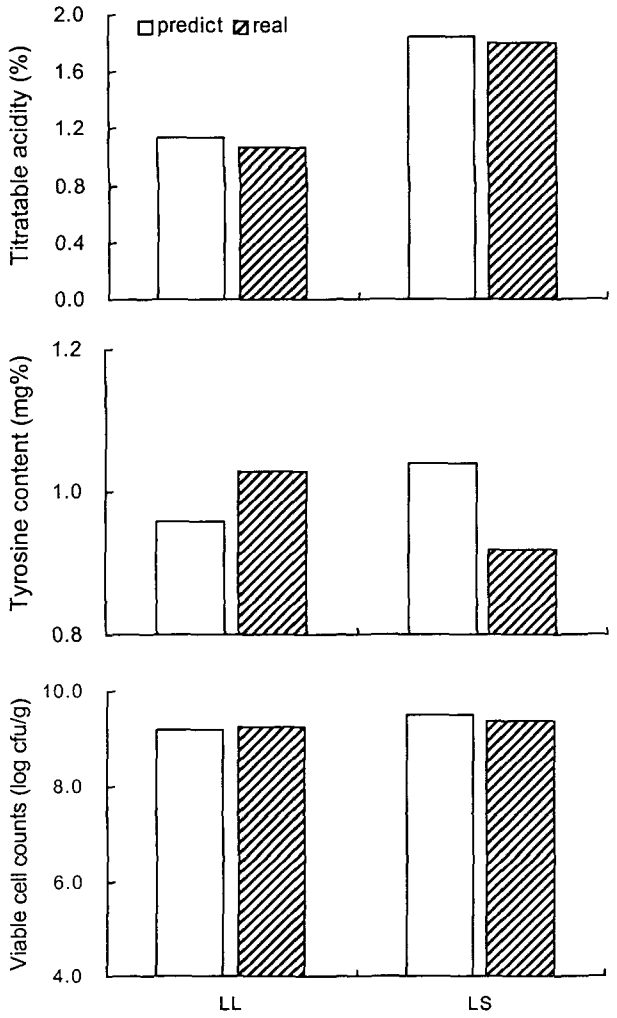


Fig. 5. Superimposed contour maps of optimized conditions for titratable acidity, tyrosine content, viable cell counts of SCR fermented by *L. rhamnosus* LS.
--- : titratable acidity (%), — : peptide content (mg%), ··· : viable cell counts (log cfu/g).

concentrations of glucose. Titratable acidity was increased by higher concentrations of enzyme and glucose; higher glucose concentrations reduced the amount of enzyme needed. The 1.83% of titratable acidity was obtained by fermentation with 2.5% glucose and 0.024% enzyme.

As shown in Fig. 4 and 5, the combined concentrations of enzyme and glucose as dependent variables that optimized titratable acidity, tyrosine content and viable cell counts in fermented SCR are indicated as the marked area. The optimum conditions for *Ent. faecium* LL fermentation were 0.025~0.030% enzyme and 1.7~1.25% glucose, respectively. With *L. rhamnosus* LS the optimum conditions were 0.03~0.04% enzyme and 1.7~2.0% glucose. Among the concentrations of glucose and enzyme that optimized titiratable acidity, tyrosine content and viable cell counts, an optimum condition was selected to compare with the real value of practical fermentation. Fermentation for 15 hrs with 0.029% enzyme and 0.9% glucose were selected for *Ent. faecium* LL, 15 hrs with 0.033% enzyme and 1.7% glucose were selected for *L. rhamnosus* LS, and practical fermentation was performed, and the real values of titratable acidity, tyrosine content and viable cell counts were determined. Fig. 6 shows the real values of titratable acidity (1.8%), tyrosine content (0.92 mg%) and viable cell counts (2.5×10^9 cfu/g) in SCR fermented by *L. rhamnosus* LS. SCR fermentation by *Ent. faecium* LL resulted in 1.07% of titratable acidity, 1.03 mg% of tyrosine content and 1.8×10^9 of viable cell counts (Fig. 6). The real values of titratable acidity, tyrosine content and viable cell counts were similar to the values predicted by RSM. In particular, the titratable acidity of SCR fermented by *L. rhamnosus* LS was higher than that of SCR fermented by *Ent. faecium* LL. The tyrosine content and viable cell counts were similar between SCR fermented



ig. 6. Comparison of predicted levels with the real value of CR fermented under optimal condition predicted.
L: *Ent. faecium* LL, LS: *L. rhamnosus* LS.

y *L. rhamnosus* LS and *Ent. faecium* LL. The initial tyrsine content (0.65 mg%) in sterilized SCR was increased n fermented SCR to 1.03 mg% by *Ent. faecium* LL and .92 mg% by *L. rhamnosus* LS, suggesting that the added rotease played a role in the production of peptides. In ddition, it was expected that some peptides could be erived from the proteolytic activity of lactic acid bacria. Delcour et al. reported that a stronger proteolytic ctivity was found in thermophilic lactic acid bacteria hat were traditionally used for the manufacture of yogurt 25). The fermented SCR contained viable lactic acid bacria at counts of 2.5×10^9 (cfu/g). Cha et al. reported hat viable cell counts in the soy yogurt fermented by *L. cidophilus* were in the range of 0.4×10^9 to 3.2×10^9 (cfu/nL) (18). Comparing with 3.1×10^8 (cfu/mL) of viable ell counts in soymilk fermented by *L. acidophilus* (23), he SCR fermented by *L. rhamnosus* LS or *Ent. faecium* L had superior numbers of viable cell counts. Therefore, actic acid fermentation of SCR by *L. rhamnosus* LS or *Ent. faecium* LL is a promising means for utilizing SCR in the production of probiotics.

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