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Effects of Prefermentation and Extrusion Cooking on the Lactic Fermentation of Rice-Soybean Based Beverage

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Abstract

The enhancement of the growth of lactic bateria in rice-based beverage was achieved by the prefermentation of cereals with a mixed culture of *Bacillus* and yeast followed by extrusion cooking. The rice-soybean milk blend was inoculated with a mixed culture of *Bacillus laevolactis* and *Saccaromyces cerevisiae,* and fermented in solid state at 45℃. It was extruded in an autogenous single screw extruder for sterilization as well as for partial digestion, and subjected to lactic fermentation in liquid state. The combined prefermentation and extrusion cooking increased the content of water soluble solid. It stimulated the growth of lactic bacteria as well as the acid production and increased dispersion stability and sensory acceptability.

Key words: lactic fermentation, rice-soybean beverage, extrusion-cooking

Introduction

Considering the difficulties in the attack of the rigid tissue matrix of cereals by *Lactobacillus* in contrast to milk constituents, pretreatment to break down the tissue structure and macromolecules of cereals are needed for a new fermentation process. A new fermentation process including prefermentation with proteolytic and amylolytic bacteria and yeast, and extrusion cooking followed by a lactic fermentation was suggested in a previous study[1].

The favorable effect of yeast mixed culture on the lactic fermentation of soybean milk was demonstrated by Yu *et al.*[2]. It was assumed that *Sac. cerevisiae* could produce some nutrients required for the growth of *Lactobacillus* in plant substrates. However, the use of yeast in the conventional lactic fermentation products causes the development of objectional smell in the cereal beverages.

The microbial make-up of *Sikhae,* a Korean traditional lactic fermented fish product, was studied in detail and the microflora useful for the present research were isolated and identified[3]. Several strains of *Bacillus* and yeast isolated from *Sikhae* and its ingredients had the ability to grow at low pH(< 4.0), a temperature 10-45℃ and under anaerobic conditions. They were used in the initial prefermentation step for the partial proteolytic and amylolytic breakdown of raw rice and defatted soybean meal. The extrusion cooking of prefermented substrate was applied for further breakdown of the plant tissue, sterilization of the substrate for the subsequent lactic fermentation and elimination of undesirable volatiles. The effect of these pretreatments was evaluated by measuring the sensory quality and physical properties of the lactic fermented cereal beverage.

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Materials and methods

Materials

Japonica type sticky rice, Milyang 23, was obtained in a market in Seoul, Korea. The rice was ground into powder(18-80 mesh) in a pin mill. Defatted soybean meal (DSM) (NSI < 30) was obtained from a soybean oil extraction company in korea (DongBang Oil Co.).

Microbial inoculum

For the prefermentation of rice or rice-DSM blend, a mixed culture of *Bacillus laevolacticus* and *Saccharomyces cerevisiae*, isolated from *Sikhae* were used. The *B. laevolacticus* isolated from *Sikhae* was characterized to produce strong protease and able to hydrolize uncooked starch into acid production. The inoculum was prepared in a sterile media contataining 5g rice flour and 20g DSM in 1*l* of water and incubated at 37℃ for 48hrs. The culture obtained was diluted 10 times with a sterilized salt solution containing 16g $(NH_4)_2SO_4$, 1.6g $MgSO_4$ and 4.5g KH_2PO_4 in 1 liter of water and used as the inoculum for the solid state prefermentation.

A mixed culture of *Lactobacillus plantarim* and *Leuconostoc mesenteroides* was prepared according to Han *et al.*[4], and used for the inoculum for the lactic fermentation of the pretreated cereals.

Extrusion-cooking

An autogeneous single screw extruder(ϕ = 53mm) constructed in this laboratory was used. The extrusion condition was established from the results of the preliminary studies on the effect of extrusion conditions on the physico-chemical properties of cereals, as reported in a previous paper[5]. The screw L/D ratio was 11, screw compression ratio 1.5, screw rotational speed 300 rpm and feed rate 200g/min. The moisture content of rice flour was adjusted to 25% and that of rice-DSM blend to 19.5%. The barrel temperature was 120℃.

Processing procedure

Fig. 1 shows the flow diagram of the new fermentation process comprising prefermentation and extrusion cooking prior to the lactic fermentation. Three parts of prefermentation inoculum was mixed with 7 parts of rice flour or rice-DSM blend for the solid state fermentation at 45℃ for 18 hrs. It was dried at 55℃ in hot air drier in order to grind into powder of less than 0.1mm diameter. It was made into 6% water suspension, sterilized and inoculated with lactic bacteria.

Measurements

The total viable cell count was determined on yeast glucose lemco-agar medium[6]. *B. laevolacticus* was isolated on yeast glucose lemco agar and identified according to the characters described in Bergey's manual[7]. Yeast was isolated on malt extract agar and identified as described by Barnett et al.[8]. The acidity was measured by titration method[9] and reducing sugar by Schoorl method[10]. The water soluble solid was measured by AOAC method of determination solids in canned vegetables, modified by using water at 30℃ instead of hot water for souble extraction. The dispersion stability was measured by gravitational sedimentation method[4].

The sensory quality of lactic fermented beverage was made by 6 members of trained panel selected from the graduate students of Korea University. Color, flavor, taste, mouthfeel and overall likeness were evaluated by scalar scoring test (1: very poor, 2: poor, 3: good, 4: very good, 5: excellent).

Results and discussion

Changes during prefermentation

Table 1 shows the growth of *B. laevolacticus* and yeast *Saccaromyces* during the solid state fermentation of raw rice or rice-DSM blend. The number of total viable cell increased from 10^4 to 10^{10} in one day and it was mostly contributed by the growth of *B. laevolacticus*. The number of *B. laevolacticus* increased 5×10^5 times in 24 hrs on rice substrate, whereas 5×10^3 times on rice-DSM blend. The number of yeast increased 10^3 times on rice, but 3×10^4 times on rice-DSM substrate for 24 hrs of solid state fermentation.

Fig. 2 shows that the pH of rice substrate decreased from 5.8 to 4.6 in 12 hrs of fermentation and further to 4.2 in 18 hrs. Whereas, that of rice -DSM remained to a level of pH 5.6 after 24 hrs of fermentation. It was mainly due to the buffering

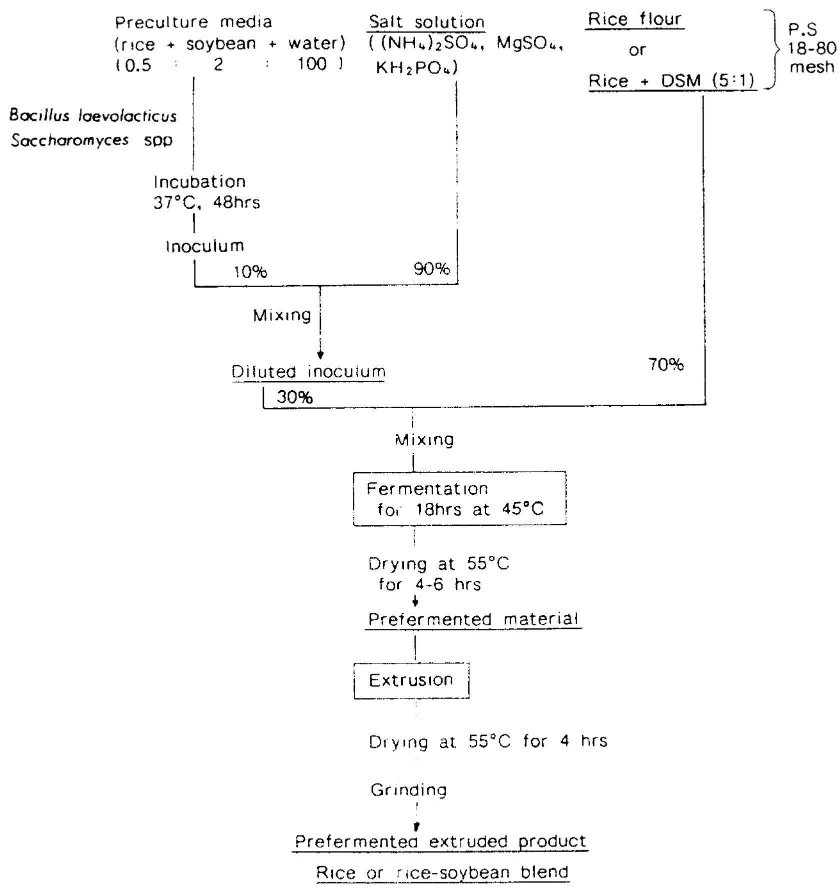

Fig. 1. Flow diagram for the pretreatment of rice or rice-DSM blend used for lactic fermentation. DSM: Defatted soybean meal.

Table 1. Growth of *Bacillus laevolacticus* and *Saccharomyces spp.* on Rice-soybean blend in solid state culture at 45°C (viable cell count/g of product)

Substrates	Microorganisms	Fermentation time				
		0 hour	6 hours	12 hours	18 hours	24 hours
Rice flour	Total count	1.9×10^4	1.4×10^6	2.8×10^8	1.5×10^{10}	1.3×10^{10}
	Bacillus laevolacticus	3×10^3	3×10^5	2.8×10^8	1.5×10^8	1.3×10^8
	Saccharomyces spp	1×10^3	3×10^5	7×10^5	10^6	10^6
Rice-DSM	Total count	2.2×10^5	1.2×10^7	1.6×10^9	3×10^9	8.8×10^8
	Bacillus laevolaticus	1.6×10^5	2.4×10^6	5.6×10^8	3×10^9	8.8×10^8
	Saccharomyces spp	1×10^3	3×10^5	6×10^7	1×10^7	3×10^7

action of protein from DSM, since the acidities of both substrates increased significantly, as shown in the same Figure.

Fig. 3 shows that the content of reducing sugar increased rapidly during the solid state fermentation. In case of rice-DSM substrate the content of reducing sugar decreased after 12 hrs of fermentation. This may due to the rapid growth of yeast, which converts sugar into alcohol.

Effect of extrusion cooking

Table 2 shows the contents of water soluble solids of raw rice, rice-DSM blend, prefermented and/or extruded samples. The extrusion-cooking increased the water soluble solid significantly from 20-30% level to 60-70%, while prefermentation did not influence on it. Addition of DSM prior or after extrusion did not appear to change the soluble, solid content.

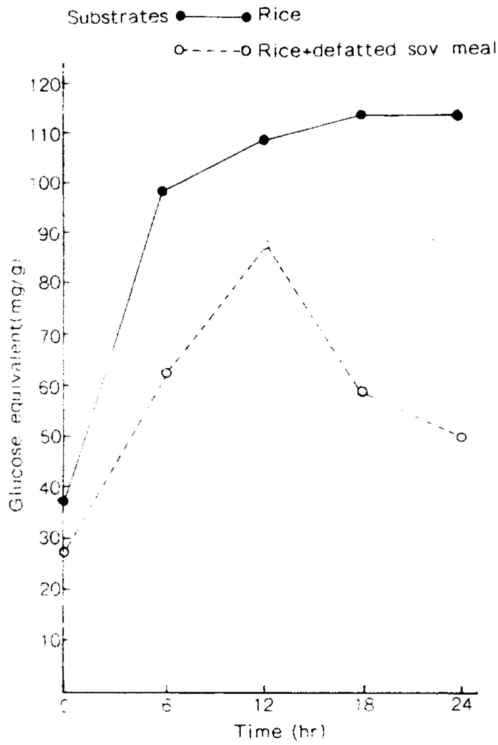

Fig. 3 Changes in the content of reducing sugars during the solid state prefermentation of rice or rice-DSM blend with a mixed culture of *B. laevolacticus* and *Sac. cerevisiae*.

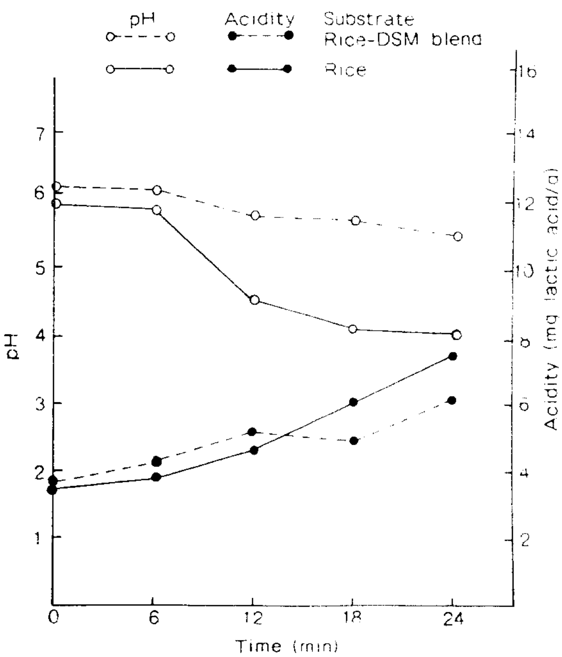

Fig. 2. Changes in pH and acidity during solid state prefermentation of rice or rice-DSM blend with a mixed culture of *B. laevolacticus* and *Sac. cerevisiae*.

Table 2. The conent of water soluble solids in pretreated rice-DSM blends

Treatments	Water soluble solids(% of total solids)	
	DSM was added after extrusion	DSM was added before prefermentation
Raw rice+DSM	26.8	34.5
Prefermentation	19.3	34.5
Extrusion	62.8	68.0
Prefermentation and extrusion	70.7	68.5

The expansion ratio of extrudates varied from 3.2 to 3.8 as shown in Table 3. It was reduced by prefermentation, and thus the bulk density of extrudate increased. The relatively large expansion ratio of rice-DSM blend was partly caused by

Table 3. Expansion ratio and bulk density of the extrudates

Feed material	Feed moisture content (%)	Expansion ratio	Bulk density (g/cm^3)
Raw rice	25	3.8	0.28
Prefermented rice	25	3.2	0.33
Prefermented rice+DSM	19	3.5	0.21

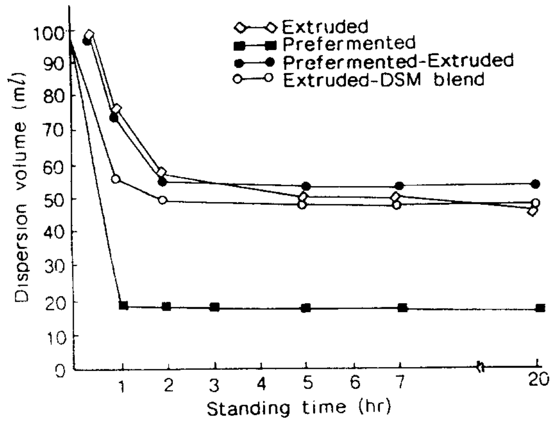

Fig. 4. Effect of prefermentation and extrusion-cooking on dispersion stability of rice flour in water.

the low feed moisture content.

Fig. 4 shows that extrusion-cooking increased the dispersion stability significantly. The prefermented rice sedimented rapidly in one hour to the level of 20% bed volume, while extruded rice suspension was quite stable for an hour and remained to 50% level of bed volume for 20 hrs. Addition of DSM slightly reduced the physical stability of the suspension.

The state of lactic fermentataion

Table 4 shows the growth of total viable cells in the lactic fermentation of rice flour dispersion. It was assumed that the viable cells were those of inoculated *Lac. plantarum* and *Leu. mesenteroides*.

Prefermentation promoted the growth of lactic bacteria significantly. This effect was reflected to the production of acid and thus lowering of pH during the fermentation, as shown in the Fig. 5 and 6. The lactic fermentation of rice and extruded rice, both added with DSM, could increase the number of cells 5-10 times in 12 hours and the pH was lowered from 6.0 to the level of 4.5. The prefermented substrates could increase the cell number by 10^3 times and the pH was lowered to below 4.0. Extrusion cooking did not influence much to the bacterial growth and acid production.

Sensory quality of lactic beverage

Table 5 summarizes the sensory preference test results of the variously treated lactic beverages. The best scores were obtained by extruded rice in color, prefermented and extruded (Rice-DSM) blend in flavor, prefermented and extruded rice in taste, extruded or prefermented rice in mouth feel

Table 4. Growth of the micorflora during the fermentation of liquid culture of *Lactobacillus plantarum* and *Leuconostoc mesenteroides* (Total viable cell count)

composition of substrate	Fermentation time (hr)			
	0	6	12	16
Raw rice + Raw soybean	$3x10^7$	$6x10^7$	$1.4x10^8$	$8.6x10^7$
Extruded rice-soybean blend	$2.1x10^7$	$5.8x10^7$	$1.8x10^8$	$1x10^8$
Prefermented rice-soybean blend	$2.8x10^7$	$5.2x10^8$	$7.5x10^{10}$	$3.6x10^{10}$
Prefermented and extruded rice-soybean blend	$2.5x10^7$	$3.7x10^8$	$8.7x10^9$	$8x10^9$

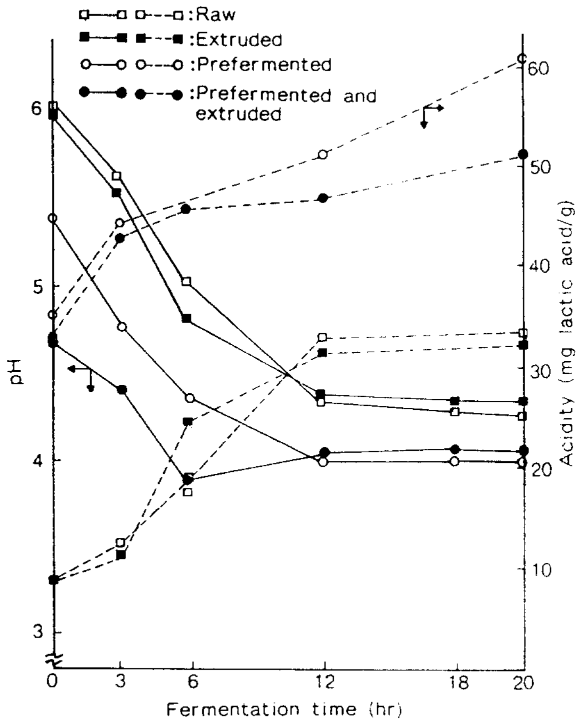

Fig. 5. Effect of rice prefermention and extrusion on the pH change and acidity during lactic fermentation.
Note; The substrates were enriched with DSM before lactic fermentation.

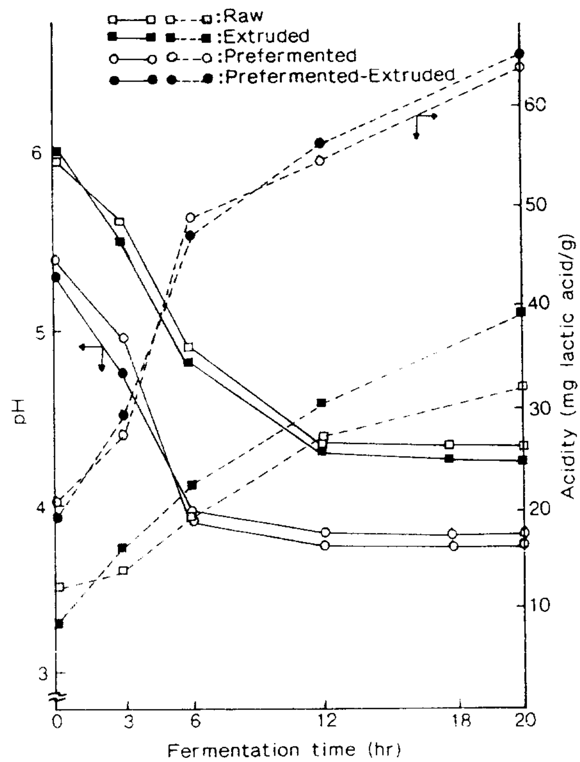

Fig. 6. Effects of rice-DSM blend pretreatment on the pH change and acidity during lactic fermentation.

Table 5. Sensory preference test results of the fermented lactic beverages

Composition		Color	Flavor	Taste	Mouth feel (griffiness)	Likeness
Raw rice + DSM		3.5	3.0	2.6	2.2	2.2
Extruded rice + DSM		3.9	2.7	1.3	3.0	3.0
Prefermenced rice + DSM		3.2	2.3	1.3	3.0	3.0
Prefermented extruded rice + DSM		3.8	3.7	2.7	2.4	2.4
Raw (rice + DSM), blend		3.0	2.6	2.7	2.0	2.0
Extruded (rice + DSM) blend		3.3	3.3	1.7	2.9	2.9
Prefermented (rice + DSM) blend		3.3	2.3	1.8	2.6	2.6
Prefermented extruded (rice + DSM) blend		1.8	4.0	1.4	2.6	2.6
Prefermented extruded rice		3.8	3.7	2.7	2.4	2.4
Best score products	1st	Extruded	Prefermented	Prefermented Extruded	Extruded Prefermented	Extruded Prefermented
	2nd	Prefermented Extruded	Extruded Raw	Raw	-	-
	3rd	Raw material	-	Extruded	Raw	Prefermented Extruded

Notation: 1, very bad; 2, bad; 3, good; 4, very good; 5, excellent.

Table 6. A summary of the effects of prefermentation and extrusion cooking on the quality of lactic fermented rice beverage

Treatment	Advantage	Disadvantage
Prefermentation	Increase reducing sugar Reduce fermentation time	Improve acceptability Decrease solubility and dispersion stability if D.S.M. added without neutralization
Extrusion	Increase solubility Improve flavor Improve dispersion stability	Reduce acceptability if soybean added prior to to extrusion
Prefermentation- -Extrusion	Reduce fermentation time Increase solubility Improve dispersion stability Improve sensory acceptance	Reduce sensory acceptability if DSM added prior to extrusion (taste, color)

and also in overall likeness.

Conclusion

The prefermentation and extrusion-cooking of cereal ingredients exerted advantageous effect on the lactic fermentation of rice. However, addition of defatted soybean meal prior to extrusion was detrimental for the color and taste of lactic beverage. Table 6 summarizes the advantage and disadvantage of the pretreatments of rice befor lactic fermentation.

The optimum condition was suggested to be prefermentation and extrusion-cooking of rice, and then addition of DSM prior to lactic fermention.

Acknowledgement

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예비발효 및 압출조리 전처리가 쌀-대두분 혼합액의 유산균 발효에 미치는 영향

이철호·무사수안네·류기형

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쌀을 기질로 하는 유산균 음료 발효에서 *Bacillus* 와 효모의 혼합배양을 이용한 고체상태 예비발효와 extruder를 이용한 압출조리 전처리가 유산균의 생육을 증진하는 효과에 대하여 검토하였다. *Bacillus laevolacticus* 와 *Saccharomyces cerevisiae* 의 혼합배양을 쌀과 탈지 대두분의 혼합물에 접종하여 고체 상태로 45℃에서 배양한 후 자가발열형 단일축 압출성형기를 통하여 처리함으로서 살균과 조직의 변화를 도모하였다. 이렇게 처리된 물질을 분산액으로 만들어 *Lactobacillus plantarum* 과 *Leuconostoc mesenteroides* 혼합배양을 접종하여 유산발효시켰다. 예비발효와 압출조리에 의하여 유산균의 증식속도와 산생성이 증가하였으며 가용성 고형분의 함량이 크게 증가하였고 분산안정성이 향상되었고 관능적 기호도도 증가하였다.