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Lactic Acid Fermentation of Rice and Quality Improvement by Amylolytic Enzyme Treatment during Fermentation

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Abstract

A palatable paste-type lactic fermented rice (LFR) was prepared by lactic acid fermentation after liquefaction and saccharification of cooked rice. A mixed culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (1 : 1) produced the LFR of the best quality. A great improvement in quality of the LFR was achieved by 0.02% each α-amylase and glucoamylase treatment during the fermentation (simultaneous saccharification and fermentation), which resulted from the increased sourness and sweetness and the decreased size of solid particles contained in the LFR. The resulted LFR was superior in quality. Physical and chemical properties of the LFR were evaluated.

Key words: rice, lactic acid fermentation, simultaneous saccharification and fermentation

Introduction

Rice is one of the leading crops of the world and is the staple food of over half the world's population. In Korea, rice production has increased markedly with the aid of the improvement of breeding technique and agricultural technology. In contrast, the consumption of rice has decreased year by year; from 135.6 kg/capita/yr in 1979 to 119.6 kg/capita/yr in 1990.[1] The decrease in rice consumption was due to the diversification of food intake pattern and the preference tendency of animal-originated foods such as meat, poulty and dairy products. The increased production and the decreased consumption of rice have given rise to a great accumulation of rice. The surplus of rice led to a great need to develop new applications of rice.[2]

Lactic acid fermentation has been widely used as the methods of the preservation and the enhancement of flavor and nutrition for dairy and vegetable products for a long time. *Kimchi* is the most typical and popular lactic acid fermented vegetable food in Korea and has become an international food. Yoghurt is one of the most popular and the fastest growing foods in Korea. The production of yoghurt in Korea in 1989 was almost tripled compared with that in 1982.[3] The popularity regarding the types of the yoghurt is being changed from a drink-type to a paste-type.[4] In contrast with the vegetable and dairy foods, rice has not been used so popularly for the lactic acid fermentation. Shin[5] tried to make a drink-type yoghurt-like rice product, and Lee et al.[6] attempted a lactic acid fermentation of rice-soybean blend.

Rice has the lowest protein content among cereals and the rice protein is deficient in lysine.[7] The nutrition of rice was found to be improved by lactic acid fermentation increasing relative nutritive value and available lysine content as reported by Hammad and Fields[8] and by Tongnual and Fields.[9]

This research was instituted to explore a possibility of palatable rice-based paste-type lactic fermented product in order to exploit a new area of the utilization of rice and to boost rice consumption, thereby to lessen the burden of the stock of the accumulated rice of Korea.

Materials and Methods

Rice

A variety of Japonica x Indica type medium grain rice, Suwon 294, harvested and milled in Soonchang, Chonbuk, in 1989 was used for this study.

Enzymes

Amylolytic enzymes, α-amylase and glucoamylase (amyloglucosidase) were purchased from Sigma Che-

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mical Co. (St. Louis, MO, U.S.A.). The activities of the α-amylase and the glucoamylase were 90 units /mg and 8,400 units/g, respectively. One unit of the α-amylase was defined as the amount of enzyme liberating 1.0 mg of maltose from starch in 3 min at pH 6.9 at 20℃ while that of the glucoamylase producing 1.0 mg of glucose from starch in 3 min at pH 4.5 at 55℃.

Lactic acid bacteria (LAB)

Lactobacillus acidophilus ATCC 11506, *L. bulgaricus* KCTC 2179, or *L. plantarum* ATCC 8014 was used alone or together with *Streptococcus thermophilus* KCTC 2185 for the lactic acdi fermentation of rice. The LAB was propagated separately in the filtrate of saccharified rice for 24 hr at 37℃.

Prepareation of lactic fermentation rice (LFR)

Polished rice was soaked in water (rice:water=1: 1.25) and warmed for 15 min. The rice was cooked for 30 min in an electric rice cooker. The cooked rice (100 g) was transferred to a 250 m*l* autoclavable media bottle and 75 m*l* of enzyme solution (0.1% each α-amyalse and glucoamylase) was added to it, then incubated for 1 hr at 55℃ for a saccharification to provide sugars for the initial growth of the LAB. The saccharified rice was heated to 95℃ and kept for 30 min for the sterilization and the inactivationn of the enzymes. The material was cooled to room temperature and the propagated LAB inoculum was added at 2% level to the material. For mixed cultures of *Lactobacillus* sp. and *S. thermophilus*, 1% each strain was inoculated to the material. The material was incubated at 37℃ for a predetermined optimum fermentation time (18 hr). The fermented material was homogenized on a homogenizer (Nissei AM-8, Nippon Scientific Inc., Japan) at 10,000 rpm for 5 min.

Treatment of enzyme during fermentation

One milliliter of aseptically filtered enzyme solution containing corresponding amounts of α-amylase and glucoamylase was added to achieve a simultaneous saccharification and fermentation. The addtion of the enzymes was done after the inoculation of LAB and prior to the incubation.

Microstructure of LFR

Microstructure of the LFR was observed under a stereo-type microscope. The size (diameter) of discrete particles in the LFR was mesured 20 times using a micrometer-type size measurement apparatus attached to the microscope.

Sensory evaluation

Sensory evaluation was done by 6 trained panels in triplicate. The LFR was evaluated for its color, flavor, taste, smoothness, consistency (body), and overall eating quality. The sensory properties were rated as dislike very much (1) to like extremely (5).

Viable cell count

The number of viable cell of the LFR was enumerate by a plating method[10] on a plate count agar containing 0.004% bromophenol blue as an indicator.

Physical properties

Rheological properties of the product were determined at 25℃ on a viscometer (Viscotron Model-80 24, Brabender OHG, Germany). The shear rate vs. shear stress relationship was analyzed by the power law model[11], $\tau = K\gamma^n$, where τ=shear stress (Pa), γ =shear rate (s^{-1}), K=consistency coefficient ($Pa \cdot s^n$), and n=flow behavior index. Color was analyzed on a color difference meter (Yasuda, Model 600-IV, Yasuda Seiki Seisakusho Ltd., Osaka, Japan) in Judd-Hunter system.[12]

Chemical analysis

Chemical composition of the product was determined by AOAC methods.[13] Total amino acid and lysine contents were determined on an amino acid analyzer (LKB 4151 Alpha Plus, LKB Biochrom Ltd., England). All analyses were done in duplicate.

Results and Discussion

Single culture of the three strains of Lactobacilli and *S. thermophilus* was tested for the lactic acid fermentation of rice. The mixed culture of *Lactobacillus* strains and *S. thermophilus* was also tested. The titratable acidity of the LFR with respect to LAB and amylolytic enzyme treatment is shown in Fig. 1.

For the lactic fermentation of saccharified rice with no enzyme treatment during fermentation, all the tested LAB except *L. platnarum* showed a great fermentability. The acidity after 18 hr fermentation had a range of 0.44-0.53%. *L. plantarum,* however, showed the acidity of 0.19% which was much lower than

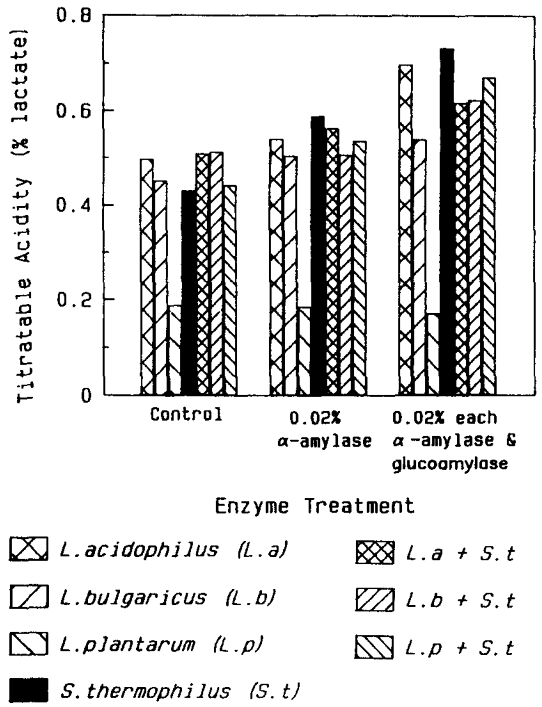

Fig. 1. Effects of lactic acid bacteria and enzymes treated during fermentation on titratable acidity of LFR

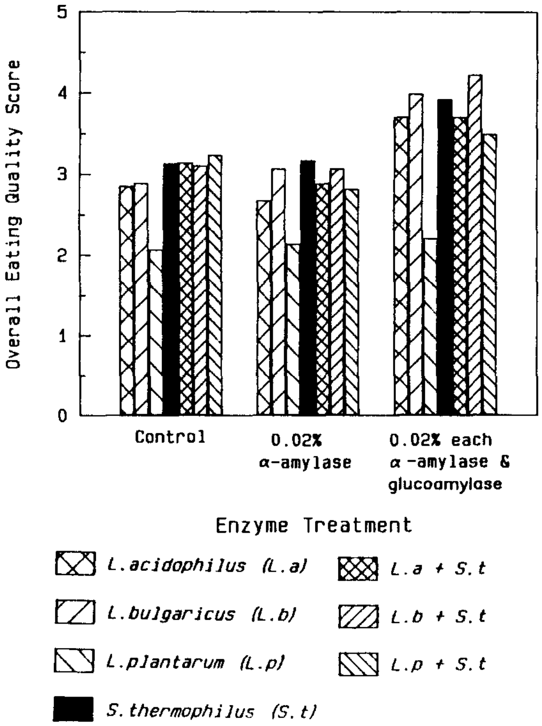

Fig. 2. Effects of lactic acid bacteria and enzymes treated during fermentation on overall eating quality of LFR

other tested LAB. This result was opposite to that of Shin,[5] where *L. plantarum* showed acidity of 0.38 % after 18 hr fermentation whereas *S. thermophilus* showed only 0.17%. The variation of the results might be attributed to the different types of strains used. The acidity of the LFR increased with the amylolytic enzyme treatment during the fermentation. With 0.02% α-amylase treatment, the acidity increased to 0.51-0.57%, and with 0.02% each of α-amylase and glucoamylase treatment, to 0.53-0.74%. However, the fermentability of *L. plantarum* was not improved even with the amylolytic enzyme treatment. The treatment of the amylolytic enzymes during fermentation provided a simultaneous saccharifiction and fermentation system, where a fast and continuous production of glucose and lactic acid was achieved, thereby resulted in a higher acidity.

Sensory data of the LFR were shown in Fig. 2. The overall eating quality scores of the LFR with no amylolytic enzyme treatment during fermentation (control) were in the range of 2.0-3.3. The α-amylase treatment did not show any noticeable improving effect on the overall eating quality at 0.02% level. However, the overall eating quality of the LFR was greatly improved by 0.02% each α-amylase and glucoamylase treatment during the fermentation, showing the scores of 2.2-4.3. The increased glucose concentration by the actions of α-amylase and glucoamylase contributed to the enhanced sweetness and also to the continuous supply of the substrates for lactic acid production, thereby produced a sweeter and sourer LFR. The LFR fermented with the mixed cultures of *L. bulgaricus* and *S. thermophilus* and treated with 0.02 % each of α-amylase and glucoamylase during the fermentation showed the highest overall eating quality score. Regarding LAB strains, the selection of this work; a mixture of two strains of *S. thermophilus* and *L. bulgaricus*, coincided with the universal selection in dairy yogurt production.[14~16] The superiority of the mixture of the two LAB strains for the LFR production might be attributed to their symbiotic relationship as previously demonstrated in the lactic acid fermentation of milk.[14,15,17]

Figure 3 shows the microstructure of the LFR. The LFR fermented with no amylolytic enzymes had particles over 20 μm in size. The LFR fermented with 0.02% α-amylase treatment contained particles of

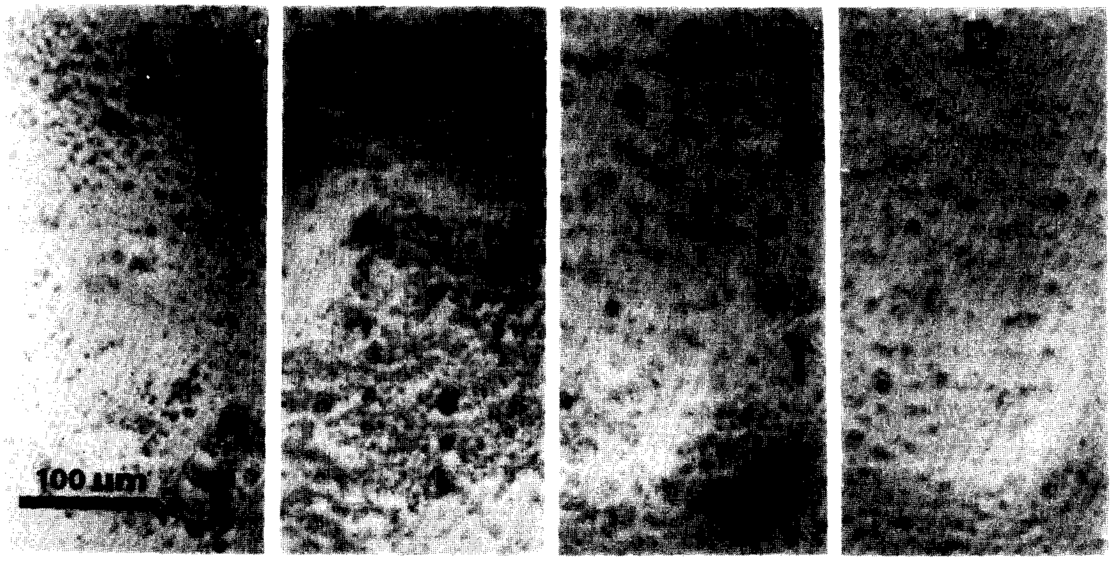

Fig. 3. Effect of amylolytic enzymes treated during fermentation on microstructure of LFR (A: control, B: 0.02 % α-amylase, C: 0.02% each α-amylase and glucoamylase, D: 0.06% each α-amylase and glucoamylase treated)

Table 1. Effect of amylolytic enzyme treatment during fermentation on size of solid particles in LFR

Inoculum[a]	Particle Size (µm)		
	Control	α-amylase[b]	α-amylase[b] + glucoamylase[b]
L.a	27.0±4.1	11.8±3.8	8.2±1.8
L.b.	17.4±4.3	14.5±4.9	8.9±1.6
L.p.	23.1±1.3	21.0±3.9	12±1.6
S.t	20.6±6.4	8.8±1.8	6.9±1.2
L.a.+S.t.	22.7±3.4	10.5±2.5	12.5±1.6
L.b.+S.t.	24.2±5.9	13.5±2.5	9.5±3.9
L.p.+S.t.	19.8±2.3	10.3±1.4	8.1±1.7

[a]*L.a.*=*L. acidophilus, L.b.*=*L. bulgaricus, L.p.*=*L. plantarum, S.t.*=*S. thermophilus*
[b]The level of treated enzyme was 0.02%

smaller size than the LFR with no enzyme treatment. The size of particles decreased with the treatment of amylolytic enzymes during the fermentation. The size of particles in the LFR fermented with different LAB strains are listed in Table 1. The particles contained in the LFR fermented with no amylolytic enzymes had a range of 17.4-27.0 µm in size. The LFR treated with 0.02% α-amylase during the fermentation showed particle size of 8.8-21.0 µm. It is generally recognized that the particles of the size smaller than 20 µm are not perceptible in mouth. Hough *et al*%.[18] reported that sensory threshold perception of

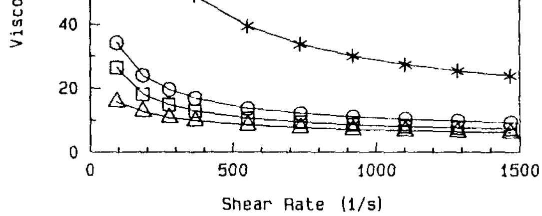

Fig. 4. Effect of levels of α-amylase and glucoamylase treated during fermentation on viscosity of LFR

Table 2. Rheological parameters of LFR treated with α-amylase and glucoamylase of varying concentration during fermentation

Amylase level (% each)	Flow behavior index	Consistency coefficient ($Pa \cdot s^n$)
0	0.47	1.162
0.02	0.52	0.284
0.06	0.54	0.204
0.10	0.65	0.077

Table 3. Sensory scores of LFR treated with amylolytic enzymes during fermentation

Sample[a]	Sensory Items[b]				
	Flavor	Taste	Smoothness	Consistency	Overall
Control	3.21a	3.29bc	3.68a	3.34a	2.82c
A	3.42a	2.92c	2.68b	3.03a	3.32bc
B	3.55a	3.95ab	3.66a	2.82a	3.79ab
C	3.50a	4.25a	4.00a	2.67a	4.25a

[a]Sample A: 0.02% α-amylase treated, Sample B: 0.02% each α-amylase and glucoamylase treated, Sample C: 0.06% each α-amylase and glucoamylase treated
[b]Values of each column with same letters are not significantly different at 0.05 level

Table 4. Physical and chemical properties of lactic fermented rice

Titratable acidity (%)	0.53
pH	2.95
Sacch. Reading (° Brix)	24.0
Moisture (%)	76.1
Protein (%)	2.0
Amino acid (mg%)	
Total	1504.0
Lysine	62.5
Color	
L	61.7
a	−0.9
b	3.7
Apparant viscosity (mPa · s)	
At 110 s^{-1}	31.7
220 s^{-1}	21.2
Viable cell count (CFU/ml)	1.54×10^{8}
Sensory Score[a]	
Color	3.72
Flavor	3.77
Taste	4.18
Smoothness	3.71
Consistency	2.66
Overall	3.98

[a]Rated as dislike extremely (1) to like extremely (5)

sandiness of Dulce de Leche, a typical Argentine dairy product, was 15 μm. The particles in the LFR could be reduced to a size below the perceptual threshold of tongue with 0.02% each α-amylase and glucoamylase treatment showing 8.1-12.8 μm. This result indicated that the treatment of α-amylase and glucoamylase improved the texture of the LFR by decreasing the size of contained particles as well as the taste of the LFR by enhancing the sweetness and sourness.

The amylolytic enzyme treatment, however, decreased the viscosity of the LFR as shown in Fig. 4. The LFR had pseudoplasticity showing decreasing viscosity as shear rate increased. The rheological properties of the LFR with respect to the treated amylolytic enzyme levels are listed in Table 2. The LFR with no enzyme treatment had the flow behavior index of 0.47. The flow behavior index increased with the treated enzyme level, indicating that the fluid became less pseudoplastic and closer to the Newtonian fluid. The consistency coefficient decreased markedly with the enzyme treatment and the degree of decrease was proportional to th treated enzyme levels.

The sensory scores of the LFR with respect to the treated enzyme levels are shown in Table 3. The enzyme treatment had no influence on the flavor of the LFR. The taste of the LFR was improved by the treatment of both α-amylase and glucoamylase, whereas was deteriorated by the treatment of α-amylase alone. The smoothness of the LFR increased with the α-amylase and glucoamylase treatment during fermentation although there were no significant differences, while decreased with the treatment of α-amylase alone. The consistency score decreased with the enzyme treatment although no significant difference was observed. Overall preference score of the LFR increased with the amylolytic enzyme treatment. The simultaneous treatment of α-amylase and gucoamylase was more effective in improving the quality of the LFR than the treatment of α-amylase alone. No significant difference in overall preference score existed between the 0.02% each treated and the 0.06% each treated ones. Therefore, it was concluded that the optimum levels of the enzymes treated during the fermentation for the production of the LFR were 0.02% each α-amylase and glucoamylase.

Table 4 shows the physical and chemical data of the finalized LFR. The titratable acidity and pH of the LFR were 0.53% and 2.95, respectively. The saccharometer reading, which was mostly attributed to glucose produced from rice starch, was 24.0° Brix. The LFR contained 76.1% moisture and 2.0% protein. Total amino acid content of the LFR was 1.50%. The content of lysine, a limiting amino acid of rice, was 62.5 mg%. The color values were 61.7, −0.9 and 3.7 for the lightness, redness and yellowness, respectivley. The apparent viscosities were 31.7 and 21.2 mPa s at shear rates of 110 and 220 s^{-1}, respectively. The viable cell count of the LFR was 1.54×10^8 CFU/ml. The LFR was considerably superior in all sensory quality showing scores above 3.5 except the consistency. The consistency of the LFR was, however, still acceptable showing the score of 2.66.

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쌀의 젖산발효 및 발효중 전분가수분해효소 처리에 의한 품질 향상

목철균 · 한진숙 · 김영진 · 김남수 · 권대영 · 남영중

한국식품개발연구원

호화시킨 쌀을 액 · 당화한 후 젖산발효하고 균질화하여 호상 쌀젖산발효물을 제조하였다. 발효시 α-아밀라제와 글루코아밀라제를 각각 0.02% 수준으로 처리하여 2차 당화와 동시에 발효를 진행함으로써 쌀젖산발효물의 품질을 크게 향상시킬 수 있었다. 이들 효소처리에 의하여 쌀젖산발효물의 신맛과 단맛이 증대되었으며 꺼끌꺼끌한 촉감을 주는 불용성 고형물 입자의 크기가 감소하였다. 쌀젖산 발효물의 관능적 품질은 매우 양호하였으며 기호도가 높았다. 최적화된 공정에 의거하여 제조된 쌀젖산발효물의 이화학적 특성을 조사하였다.