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Fermentation properties of rice-added yogurt using two types of blended lactic acid bacteria as a starter

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Abstract

These days, different types of yogurt are being manufactured by adding various starters and functional ingredients for health. The purpose of this study was to prepare yogurt added with rice followed by fermentation with two types of starters and to examine its attributes. Ten percent of skim milk powder and 0, 2.5, 5.0, 7.5, or 10% rice were mixed in water (w/v) and then inoculated with two types of starter: 1) Type A, *Streptococcus thermophiles* and *Lactobacillus delbrueckii ssp. bulgaricus* as starter; and 2) Type B, *Streptococcus thermophiles, Lactobacillus acidophilus*, and *Bifidobacteium animalis ssp. lactis* as starter. The pH of B type yogurt was lower ($p < 0.05$) than that of A type yogurt from 6 hours to 14 hours after fermentation. The number of microorganisms in all fermented milk showed maximum increases at 2 and 6 hours of fermentation ($p < 0.05$). The number of microorganisms in fermented milk peaked at 6 hours after fermentation and maintained this level thereafter. There was no effect of rice addition on microbial growth or acidity of the fermented milk. Sensory attributes of yogurt samples with and without added rice were not significantly different. This experiment showed that the production efficiency of yogurt with added rice was not different when two different types of starters were used to manufacture yogurt.

Keywords: lactic acid bacteria, rice, starter, yogurt

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Introduction

Nowadays, it is becoming increasingly important for effects of food on the maintenance of health and counteracting diseases besides the basic role of nutrition. As one of such foods, yogurt has attracted attention because it contains live cultures of probiotics with beneficial effects on immunity, cardiovascular health, and metabolic health of the host (Astrup, 2014; El-Abbadi et al., 2014; Gijsbers et al., 2016; Park et al., 2016). Among starter culture microorganisms, *Streptococcus thermophiles* and *Lactobacillus delbrueckii spp bulgaricus* as well as *Bifidobacterium* and *Lactobacillus* strains have been added to yogurt due to their probiotic properties (Kok and Hutkins, 2018). FAO/WHO has defined probiotics as “Live microorganisms which when administered in adequate amounts confer a health benefit on the host” (FAO, 2001).

Streptococcus thermophiles is a very important starter culture for the production of yogurt in the dairy industry (Marco et al., 2017). Most fermented foods are produced using mixed cultures containing different microbial species that can interact with each other. There is an cooperation between *Streptococcus thermophilie* and *Lactobacillus delbrueckii spp. bulgaricus* during milk fermentation that can improve the growth rate and size of each other (Arioli et al., 2017). *Lactobacillus acidophilus* is associated with decreased symptoms of lactose maldigestion (Montes et al., 1995). *Bifidobacterium animalis spp. lactis* is effective in preventing diarrhea (Salminen et al., 2005). Yogurt containing *Lactobacillus acidophilus* and *Bifidobacterium animalis spp. lactis* has been developed. There is an increasing interest in yogurt containing specific bacterial species with potential health benefits (Portier et al., 1993).

Rice is the staple food for more than half of the population in the world (Steiger et al., 2014). Rice production and consumption are the highest in Asian populations. High consumption of rice in Latin America, Caribbean countries, and Africa has also been reported. Recently, rice is also consumed in North America and Europe in a small amount (Muthayya et al., 2014). People around the world are getting familiar with the taste of rice. As a commodity in the dairy product market, yogurt has been improved to have more functionality and taste for consumer choice. The manufacture of yogurt with various fruits has also been reported (Kailaspathy et al., 2008; Salama et al., 2019; Sengupta et al., 2019).

In order to increase rice consumption due to reduced amount of rice consumed annually in Korea and to produce yogurt containing more diverse lactic acid bacteria, two types of starters were used to make yogurt with rice added in this experiment. Therefore, the purpose of this experiment is to prepare yogurt with rice added using two different types of starters, and to investigate the characteristics of yogurt according to each starter and the amount of rice added.

Materials and Methods

Starter cultures

Streptococcus thermophiles, Lactobacillus delbrueckii spp. bulgaricus, Lactobacillus acidophilus, and Bifidobacteium animalis ssp. lactis were purchased from Korean Culture Center of Microorganisms (Seoul, Korea). All microorganisms were inoculated into 50 mL De Man, Rosa and Shape agar (MRS) broth followed by incubation at 40℃ for 24 h. All cultures were adjusted to 10^8 CFU·g^{-1} and stored at -80℃.

Yogurt preparation

Rice was pulverized. Its powder was filtered through 80 mesh. A series of concentrations of rice powder (0, 2.5, 5.0, 7.5, and 10%) and 10% skim milk were mixed in water (w/v). The suspension solution was heated at 92℃ for 10 minutes while maintaining the homogeneity of the solution with a magnetic stirrer. The microorganisms were inoculated after cooling at room temperature while maintaining a homogeneous state. Two types of starter were used for making yogurt: type A starter, *Streptococcus thermophiles* and *Lactobacillus delbrueckii spp. Bulgaricus;* and type B starter, *Streptococcus thermophiles, Lactobacillus acidophilus*, and *Bifidobacteium animalis ssp. lactis*. Bacteria of each starter were mixed at the same ratio (w/w). Each starter was inoculated into the mixed solution of rice and skim milk at 2% (w/v) followed by incubation at 40℃ for 14 h.

Measurement of yogurt pH

The pH was determined using a pH meter (Mettler Delta 345, Greifensee, Swiss) with a standardized glass electrode. Reference pH 4.0 and 7.0 buffer solutions were used for calibration. Three consecutive measurements were made for each yogurt sample at a temperature of 21±1℃.

Number of colonies of microorganisms in yogurt

Fermented milk was diluted decimally with sterilized water and plated onto Lactobacilli MRS agar (Becton, Dickinson Co., Sparks, USA). Plates were then placed into an anaerobic jar and incubated for 48 hours at 42℃. After the incubation, the number of colonies was counted.

Sensory analysis

Sensory measurements of yogurt samples were carried out by four trained panelists using a sensory scale of 1 - 5 (poor-excellent) for all attributes including color, sourness, sweetness, texture, flavor, and overall acceptability.

Statistical analysis

All statistical analyses including Student's t-test and Duncan's multiple range tests were carried out using SAS statistical package 9.4 (SAS Institute, Cary, NC, USA) (SAS, 2003). Values of $p < 0.05$ indicated significant differences.

Results and discussion

Manufacture of rice-added yogurt

Rice is one of the most important and stable food crops for human as a major source of carbohydrates and protein as well as other essential nutrients (Parengam et al., 2010). During rice polishing the fat and micronutrient-rich bran layers are removed to produce the commonly consumed starch-rich white rice. Because of rich starch, white rice powder increases the viscosity when heated. Paik et al. (2004) improved the flavor and texture by increasing the viscosity added 4% of rice powder to yogurt production. Rice powder had a lower solubility and an increase in viscosity by applying heat to form a lump. The homogeneity of the solution was maintained by using a magnetic stirrer to suppress the formation of solutes during heating. The skim milk powder was dissolved in sterile distilled water, and was added rice powder sufficiently to homogenize. It was possible to improve the agglomeration of rice-added yogurt by keeping the skim milk powder and rice powder mixed solution homogenized until the microbial inoculation.

Measurements of pH in fermented milk over time

In order to investigate the fermentation degree of rice-added yogurt, pH values of all fermented milk samples were measured at 0, 2, 6, 10, and 14 hours. Type A yogurt used *Streptococcus thermophiles* and *Lactobacillus delbrueckii spp. Bulgaricus* as the starter and type B yogurt used *Streptococcus thermophiles, Lactobacillus acidophilus, and Bifidobacteium animalis ssp. lactis* as a starter. Both types of fermented milk showed decreased pH values over time, reaching approximately pH 4.5 after 6 hours of fermentation ($p < 0.05$; Table 1). The decrease in pH over time was due to lactic acid produced by lactic acid bacteria (LAB). Lactic acid is a metabolite that is synthesized in amounts reaching over 1% after LAB fermentation (Marco et al., 2017). At 2 hours after fermentation, the pH of type A yogurt was lower than that of type B yogurt. However, the pH of type B yogurt was lower than that of type A yogurt from 6 hours to 14 hours after fermentation (Fig. 1; Table 1). *Streptococcus thermophiles* is used as an important starter in dairy products because it can ferment milk very quickly. When *Streptococcus thermophiles* and *Lactobacillus delbrueckii spp. bulgaricus* are used together as a starter, there is a synergistic effect through interaction with each other (Kermiche et al., 2018; Rodriguez-Serrano et al., 2018). *Streptococcus thermophiles, Lactobacillus acidophilus, and Bifidobacteium animalis ssp. lactis* used in a starter culture have been reported to be less proteolytic than *Streptococcus thermophiles* and *Lactobacillus delbrueckii ssp. bulgaricus* as a starter (Shihata and Shah, 2000). For this reason, *Lactobacillus acidophilus,* and *Bifidobacteium animalis ssp. lactis* used in a starter in milk could lead to a slower growth rate and a smaller population size. However, recommended probiotic bacteria used in dairy products are mainly members of *Lactobacillus* and *Bifidobacterium* (Gomes and Malcata, 1999; Saarela et al., 2002; Penner et al., 2005). In this study, after 2 hours of fermentation, the pH was lower in the starter containing *Lactobacillus acidophilus, and Bifidobacteium animalis ssp. Lactis,* different from results of previous studies (Arioli et al., 2017). Such results might be because *Lactobacillus acidophilus* grows readily at rather low pH values (Bâati et al., 2000). In addition, nutrients favored by these two bacteria might be abundant in rice. Moreover, rice might act a buffer against environmental stress. Further research is needed in the future to clarify this.

Number of microorganisms in fermented milk over time

After 14 hours of fermentation, numbers of microorganisms in fermented milk were counted (Table 2). The number of microorganisms in fermented milk increased with the passage of fermentation time. The number of microorganisms in fermented milk showed the maximum increase at 2 and 6 hours of fermentation ($p < 0.05$; Table 2), peaking at 6 hours after fermentation. At that time, the colony number of microorganisms in all the fermented milk was more than 108 CFU·mL^{-1}. For yogurt to confer health benefits, it was recommended that yogurt contained 107 - 108 CFU·g^{-1} of live bacteria (Lourens-Hattingh and Viljoin, 2001). The LAB number of the fermented milk showed a slight decrease or increase after that, but it did not deviate significantly and was maintained. The growth of lactic acid bacteria peaks through the logarithmic growth period and then reaches a plateau. There might be a slight decrease or increase in the number of the microorganisms during the stagnation phase (Bae et al., 2004). In this experiment, it can be seen that the peak of the number of microorganisms in fermented milk coincides with the time for the pH of fermented milk reaching 4.5 (Table 1 and Table 2). After 6 hours of fermentation, the growth of microorganisms in the fermented milk became stagnate but the pH of the fermented milk gradually decreased. This was considered to be due to acid generated and accumulated in the fermented milk. There was no effected of rice addition on microbial growth or acidity of the fermented milk (Table 1 and Table 2).

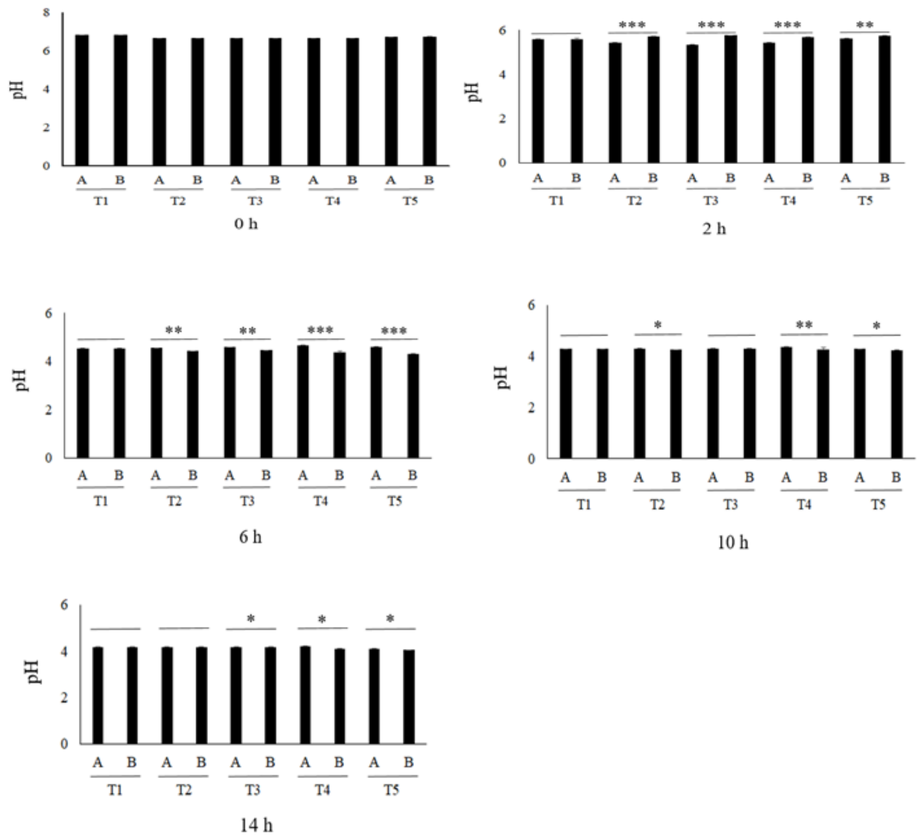

Fig. 1. Comparison of pH between two types of fermented milk over time. T1, no rice added to the yogurt; T2, T3, T4, and T5: 2.5, 5.0, 7.5, and 10.0% rice added to the yogurt, respectively. A, yogurt fermented with *Streptococcus thermophilus* and *Lactobacillus delbrueckii spp. bulgaricus* as a starter. B, yogurt fermented *Streptococcus thermophiles, Lactobacillus acidophilus and Bifidobacterium animalis spp. lactis* as a starter. Values are presented as mean ± standard error. Each sample was repeated three times. Asterisk means significant difference. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table 1. pH changes of rice-added yogurt during fermentation.

Samples	0 h	2 h	6 h	10 h	14 h
T1A	6.81 ± 0.01a	5.58 ± 0.01b	4.53 ± 0.01e	4.28 ± 0.01d	4.15 ± 0.01c
T2A	6.63 ± 0.01d	5.44 ± 0.01c	4.55 ± 0.01d	4.30 ± 0.01b	4.17 ± 0.01b
T3A	6.64 ± 0.01c	5.35 ± 0.01d	4.58 ± 0.01c	4.29 ± 0.01bc	4.16 ± 0.01bc
T4A	6.63 ± 0.01d	5.44 ± 0.01c	4.65 ± 0.01a	4.35 ± 0.01a	4.20 ± 0.01a
T5A	6.71 ± 0.01b	5.62 ± 0.01a	4.61 ± 0.01b	4.28 ± 0.01cd	4.09 ± 0.01d
T1B	6.81 ± 0.01a	5.58 ± 0.01d	4.53 ± 0.01a	4.28 ± 0.01b	4.15 ± 0.01b
T2B	6.63 ± 0.01d	5.70 ± 0.04b	4.42 ± 0.01c	4.25 ± 0.01c	4.17 ± 0.01a
T3B	6.64 ± 0.02c	5.76 ± 0.01a	4.45 ± 0.00b	4.29 ± 0.01a	4.14 ± 0.02b
T4B	6.63 ± 0.02d	5.68 ± 0.01c	4.36 ± 0.04d	4.27 ± 0.01b	4.10 ± 0.01c
T5B	6.71 ± 0.02b	5.75 ± 0.02a	4.31 ± 0.02e	4.24 ± 0.01c	4.04 ± 0.00d

Values are presented as mean ± standard error. Each sample was repeated 3 times.

T1A, T2A, T3A, T4A, and T5A: 0.0, 2.5, 5.0, 7.5, and 10.0% rice, respectively, added to yogurt fermented with *Streptococcus thermophilus* and *Lactobacillus delbrueckii spp. bulgaricus* as a startery.

T1B, T2B, T3B, T4B, and T5B: 0.0, 2.5, 5.0, 7.5, and 10.0% rice, respectively, added to yogurt fermented with *Streptococcus thermophiles*, *Lactobacillus acidophilus and Bifidobacterium animalis spp. lactis* as a starter.

a - e: Different superscripts in the same row differ significantly at $p < 0.05$.

Table 2. Changes in count of viable microorganisms during fermentation of rice-added yogurt.

(Unit: CFU · mL^{-1})

Samples	Incubation Time (h)				
	0	2	6	10	14
T1A	5.3×10^6c	3.8×10^7b	5.2×10^8a	4.7×10^8a	4.4×10^8a
T2A	5.4×10^6c	3.1×10^7b	3.9×10^8ab	3.6×10^8a	5.4×10^8a
T3A	6.1×10^6c	4.4×10^7b	3.9×10^8ab	3.3×10^8a	2.9×10^8a
T4A	5.8×10^6c	5.5×10^7b	4.5×10^8a	4.1×10^8a	4.2×10^8ab
T5A	5.5×10^6c	3.4×10^7b	4.0×10^8a	3.3×10^8a	3.8×10^8a
T1B	5.9×10^6c	5.2×10^7b	4.0×10^8ab	4.2×10^8a	3.7×10^8a
T2B	5.3×10^6c	3.7×10^7b	3.6×10^8a	4.0×10^8a	4.8×10^8a
T3B	5.5×10^6c	4.5×10^7bc	4.1×10^8ab	3.6×10^8a	3.9×10^8ab
T4B	4.9×10^6b	4.9×10^7b	4.0×10^8a	3.5×10^8a	3.1×10^8a
T5B	4.2×10^6b	3.2×10^7b	3.8×10^8a	3.2×10^8a	2.6×10^8a

Values are presented as means. Each sample was repeated 4 times.

T1A, T2A, T3A, T4A, and T5A: 0.0, 2.5, 5.0, 7.5, and 10.0% rice, respectively, added to yogurt fermented with *Streptococcus thermophilus* and *Lactobacillus delbrueckii spp. bulgaricus* as a starter.

T1B, T2B, T3B, T4B, and T5B: 0.0%, 2.5%, 5.0%, 7.5%, and 10.0% rice, respectively, added to yogurt fermented with *Streptococcus thermophiles, Lactobacillus acidophilus and Bifidobacterium animalis spp. lactis* as a starter.

a - c: Different superscripts in the same row differ significantly at $p < 0.05$.

Sensory attributes of yogurt

To analyze sensory attributes of two types of yogurt, sensory attributes such as color, sourness, sweetness, texture, flavor, and overall acceptability of yogurt fermented for 14 hours were evaluated. Results are shown in Table 3. There was no clear difference in sensory attributes between the two types of yogurt. From this experiment, it could be seen that the use of different starters and the amount of rice added did not affect sensory attributes of yogurt.

Table 3. Sensory attributes of two types of yogurt.

Samples	Color	Sourness	Sweetness	Texture	Flavor	Overall acceptability
T1A	1.62 ± 0.25	4.00 ± 0.27a	1.25 ± 0.50	2.75 ± 0.50	2.50 ± 1.29	2.75 ± 0.50
T2A	1.62 ± 0.25	4.37 ± 0.47a	1.62 ± 0.75	2.00 ± 0.70	2.62 ± 0.75	2.50 ± 0.40
T3A	1.62 ± 0.25	3.37 ± 0.25b	1.25 ± 0.50	3.00 ± 0.70	2.75 ± 0.64	3.00 ± 0.70
T4A	1.62 ± 0.25	4.25 ± 0.28b	1.25 ± 0.50	2.87 ± 0.62	2.62 ± 0.75	2.75 ± 0.50
T5A	1.37 ± 0.47	3.25 ± 0.28b	1.50 ± 0.57	3.00 ± 0.70	2.75 ± 0.64	3.00 ± 0.70
T1B	1.62 ± 0.25	4.00 ± 0.05a	1.25 ± 0.50	2.75 ± 0.50	2.50 ± 1.29	2.75 ± 0.05
T2B	1.62 ± 0.25	4.12 ± 0.25a	1.50 ± 0.57	2.62 ± 0.47	2.87 ± 0.85	3.00 ± 0.40
T3B	1.62 ± 0.25	4.12 ± 0.47a	1.62 ± 0.75	2.75 ± 0.64	2.62 ± 0.62	2.75 ± 0.64
T4B	1.62 ± 0.25	3.62 ± 0.47ab	1.50 ± 0.57	2.62 ± 0.75	3.12 ± 0.62	3.12 ± 0.47
T5B	1.62 ± 0.25	3.12 ± 0.25b	1.62 ± 0.75	2.87 ± 0.62	3.25 ± 0.50	3.12 ± 0.25

Values are presented as means ± standard error. Each sample was repeated 4 times.

T1A, T2A, T3A, T4A, and T5A: 0.0, 2.5, 5.0, 7.5, and 10.0% rice, respectively, added to yogurt fermented with *Streptococcus thermophilus* and *Lactobacillus delbrueckii spp. bulgaricus* as a starter.

T1B, T2B, T3B, T4B, and T5B: 0.0, 2.5, 5.0, 7.5, and 10.0% rice, respectively added to yogurt fermented with *Streptococcus thermophiles*, *Lactobacillus acidophilus and Bifidobacterium animalis spp. lactis* as a starter.

a, b: Different superscripts in the same row differ significantly at $p < 0.05$.

Conclusion

In order to produce yogurt containing various microorganisms as a probiotic source and promote rice consumption in Korea, two types of starters (*Streptococcus thermophiles* and *Lactobacillus delbrueckii ssp. bulgaricus* as the starter for type A yogurt; *Streptococcus thermophiles, Lactobacillus acidophilus, and Bifidobacteium animalis ssp. lactis* as the starter for type B yogurt) were used to prepare yogurt with rice added. At 2 hours after fermentation, the pH of type A yogurt was lower than that of type B yogurt. After that, the pH of type B yogurt was lower. The number of microorganisms in the fermented milk increased up to 6 hours after fermentation. After 6 hours, there was no change in the number of microorganisms. There was no difference in sensory attributes by rice addition or starter.

This experiment showed that the production efficiency of yogurt with rice added was not different when two different types of starters were used to manufacture yogurt. When making rice-added yogurt with *Streptococcus thermophiles, Lactobacillus acidophilus and Bifidobacteium animalis ssp. lactis* as a starter, the same efficiency could be obtained using *Streptococcus thermophiles* and *Lactobacillus delbrueckii ssp. bulgaricus* as a starter. In addition, the characteristics of yogurt prepared with rice were not different from that of yogurt without rice. Therefore, those show that it is possible to manufacture various yogurt using different types of starters and rice.

Conflict of Interests

No potential conflict of interest relevant to this article was reported.

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