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Development of a Yogurt-type Product from Saccharified Rice

Chakamas Wongkhalaung and Malai Boonyaratanakornkit

ABSTRACT

A yogurt-type product from saccharified rice was developed. Jasmine rice was saccharified with amylolytic enzymes at 55 °C. Changes of reducing sugars, sugar components and compositions during hydrolysis were studied to obtain optimum condition and yield of glucose. Rice milk was prepared by fortification of saccharified rice with 3 % casein, 3% soybean oil and 0.4 % calcium lactate to improve both nutritive quality and organoleptic properties.

Mixed cultures of *Lactobacillus acidophilus* and *L. casei* subsp. *rhamnosus* were used for lactic fermentation of rice milk. The effect of beta glycerophosphate, inoculum size and incubation period were investigated. Improvement for a more palatable yogurt was achieved by blending rice yogurt with pectin and strawberry preserve. Resulted rice-based yogurt with strawberry contained 3.05 % protein, 2.67 % fat, 24.4 % glucose, 1.5 % sucrose, 47 mg/100g of calcium, 0.86 % acidity as lactic, pH 3.48 and lactic bacteria count of 7.6×10^7 CFU/g. Sensory evaluation revealed that rice-based yogurt with strawberry was well accepted by panelists when compared with commercial strawberry yogurt. Shelflife of rice-based yogurt stored at 4 °C was at least 20 days.

Key words : rice based yogurt, rice milk, lactic acid fermentation, saccharified rice

INTRODUCTION

Lactic acid fermentation of cereals has been studied extensively in the past few decades. Yogurt-like products have been produced from various kinds of cereals such as liquefied starch (Shin, 1989); prefermented and extruded rice flour (Lee *et al*., 1992); and cooked maize meal mixture (Zulu *et al*., 1997). A product, socalled Risogurt, was produced from mixture of fermented rice and soy protein isolate (Mok *et al.*, 1991). Method for producing a highly concentrated lactic product from rice with improved quality by a secondary enzymatic treatment during fermentation was further developed by Mok *et al*. (1993). Development of lactic beverages from mixture of rice and soybean were also reported by Lee *et al.* (1988).

The cultures starter for lactic fermentation of yogurt-like products from cereals and other non-dairy raw materials were often *Lactobacillus, Streptococcus* and *Leuconostoc* spp. A mixed culture of *Streptococcus thermophilus, Lactobacillus bulgaricus* and *L. plantarum* were used by Shin (1989). Lee *et al.* (1992) compared different kinds of lactic acid bacteria, namely, *Leuconostoc mesenteroides, L.plantarum, L. casei and L. lactis* subsp. *diacetylactis* in fermentation of pre-fermented and extruded rice flour. *S. thermophilus* and *L. mesenteroides* have been used

Institute of Food Research and Product Development, Kasetsart University, Bangkok 10900, Thailand.

as starter for a yogurt-like product from rice flour and soymilk mixture (Collado *et al*., 1994). Tominaga and Sato (1996) reported the production of fermented beverage from rice flour using enzyme hydrolysis followed by lactic fermentation by *L. mesenteroides*. Other lactic bacteria used for developing a fermented rice product was amylolytic Bifidobacterium spp. (Park *et al*., 1997).

This paper reported a method to develop a new yogurt-type product by lactic fermentation of saccharified rice using *Lactobacillus spp.* as starter cultures.

MATERIALS AND METHODS

Rice Jasmine rice (Khao Dok Mali 105) was used in this experiment. It contains 8.17 % protein, 1.1 % fat and 79.5 % starch, of which 17.3 % are amylose (P. Tungtrakool, unpublished data).

Enzyme Heat stable alpha amylase from *Bacillus licheniformis*, Termamyl® 120L, and amyloglucosidase from *Aspergillus niger*, AMG 300L, were obtained from Novo (Novo Nordisk Co., Bagsvaerd, Denmark). According to Novo Nordisk 's Standard Method, one KiloNovo alpha amylase Unit (1 KNU) is the amount of enzyme which breaks down 5.26 g starch per hour at pH 5.6, temperature 37 °C and reaction time 7-20 min. One Novo Amyloglucosidase Unit (AGU) is defined as the amount of enzyme which hydrolyzes 1 micromole maltose per unit at pH 4.3, temperature 25 °C and reaction time 30 min. Both enzymes are food grade products complied with FAO/WHO JECFA and FCC recommendations for food grade enzymes.

Microorganisms Lactic acid bacteria (LAB) used in this study were *Lactobacillus acidophilus* (IFRPD 2013) and *L. casei* subsp. *rhamnosus* (IFRPD 2020) from Culture Collections of Institute of Food Research and Product Development, Kasetsart University, Thailand. The strains were cultured and maintained on MRS medium (de Man *et al*., 1960).

Analytical methods Reducing sugars were determined by DNS method (Chaplin and Kennedy, 1986). Sugar composition was analyzed by HPLC (Sugars Analyzer I, Waters Associates, Milford, MA, USA) using Sugar Pak column. The temperature of column was 90 °C with 50 mM EDTA-deionized water at flow rate of 0.2 ml/min and 20 µl injection volume. Differential Refractometer Detector, Waters 410, was used. Titratable acidity as lactic acid was determined by the method of Frazier *et al*. (1968). Proximate composition and calcium content were determined by the methods of AOAC (1990). Color was determined by Spectraflash SF 600 Plus (Data Color International, USA) and viscosity by Brookfield Viscometer (Model LV, Brookfield Engineering Laboratories, USA).

Total viable count, yeast, mold and coliforms were determined according to AOAC (1990). LAB was detected by plating method on MRS Medium supplemented with 0.5 % $CaCO_3$ and counting the colonies surrounding with clear zones.

Saccharification process Rice was washed and soaked in water at 1:2.5 ratio, wt by wt, for about 30 min. It was adjusted to pH 6.5, heated up to 90-95 ° and 0.2 % Termamyl® was added. The rice mixture was held at this temperature for 2 hr. Saccharification process was carried out using 0.2 % of AMG at 55 °C, pH 5.0 for about 24 hr. Suspension obtained was filtered through 350-mesh nylon (Monyl 140T) to remove coarse solids. It was then heated at 90 °C for 30 min to sterilize and inactivate the enzymes.

Preparation of rice milk Saccharified rices was adjusted to 18 °Brix before blending with 3 % casein, 3 % soybean oil and 0.4 % calcium lactate. The mixture was homogenized for 3 min at high speed (Ultra-Turax, Janke u. Kunkel KG, Staufen, I Breisgar) and pasteurized at 90 °C for 15 min. The rice milk was instantaneously subjected to lactic acid fermentation.

Lactic acid fermentation Two strains of Lactobacillus, *L. acidophilus* 2013 and *L. casei* 2020, were used as starter in this study. They were propagated separately in MRS medium for 24 hr at 37 °C to get viable cell counts in the range of 10^8 to 10^9 CFU/g. Beta glycerophosphate was added at different concentrations to enhance growth of LAB. Various inoculum sized at 2, 3 and 4 % as well as incubation periods of 18, 24 and 36 hr were investigated.

Preparation of rice-based yogurt Improvement of texture and flavor of rice-based yogurt obtained after lactic fermentation was made by addition of pectin and strawberry preserve. The mixture was blended with 1 % pectin and 20 % fruit preserve in a mixer (Bamix, Model M 133, Switzerland). Strawberry flavor (F 11720 Universal Flavors, 0.1 %) and color (Winner's Ponceur 4 R , 0.03 %) were also added to disguise the fermented rice odor and simulate strawberry dairy yogurt. The product was kept chilling at 4 °C for 1-2 hr before sensory testing.

Sensory evaluation Sensory evaluation of rice-based yogurt was performed in comparison with commercial dairy yogurt of the same flavor. They were scored for color, odor, flavor, texture/ consistency and overall acceptability by 20 expereinced panels, using 9-point hedonic scale rating from dislike very much (1) to like extremely (9).

Keeping quality and safety Strawberry rice-based yogurt was kept at 4 °C for 20 days. Samples were taken during storage to examine for titratable acidity, pH, LAB and non-LAB counts, yeast, mold and coliforms.

RESULTS AND DISCUSSIONS

Saccharified rice

Rice was saccharified in closed container at 55 °C for 24 hr, during which samples were drawn for analysis of reducing sugars and sugar composition. Reducing sugar at 0 hr in cooked, liquefied solution before adding AMG was 9.36 %. During the first few hours of saccharification, the rate of hydrolysis was markedly high and sugar produced was about double (18.9%) of the original amount as shown in Figure 1. After that the rate was gradually increased until about 20-21 hr when it reached the maximum content (23.8 %). Prolonged incubation to 24 hr did not give higher yield of reducing sugars.

Sugar composition by HPLC revealed that at 0 hr, major sugars in the solution were higher oligosaccharides (Degree of polymerization [DP] = 3 and DP > 3) and maltose (DP =2) with only small amount of glucose i.e. 15.74, 6.5 and 2.86 %, respectively. However, after 30 min, glucose increased to 8.67 while higher oligosaccharides decreased to 5.72 % (Figure 2). As saccharification proceeded, glucose increased somewhat linearly after 1, 2, 3 and 4 hrs of incubation. Maltose liberated was eventually broken down to glucose and remained at low concentration in the solution. At 18^{th} hr, 16.92 % glucose was obtained with higher oligosaccharides and maltose comprised for about 2 %. Saccharification process was considered completed after 20-21 hr when maximum amount of glucose (23.8 %) was produced with only 0.5 % higher oligosaccharides and 0 % maltose left . The yield of hydrolysis (100 x g glucose produced / expected glucose in starch, dry basis) was 81 % and the yield of glucose was 89.1 g per 100-g rice starch. Glucose yield was calculated from the real amount obtained in the saccharified solution excluding glucose that remained in the spent solid removed by filtration.

Preparation of rice milk

Saccharified rice when adjusted to 18 ° Brix, contained 17.25 % reducing sugars. After fortification with 3 % casein, 3 % soybean oil and 0.4 % calcium lactate and homogenized, the resulted suspension obtained was called rice milk due to its milky color similar to that of cow's milk (Table 1). The lightness was a little lower but it was less greenish and yellowish than that of cow's milk. Casein comprised for higher protein content and soybean oil increased fat content as well as provided smooth texture of the product. Calcium lactate is a salt of lactic acid which is very soluble in water and has a high percentage of calcium and the lactate ion is the L(+) isomer, the same as the naturally present isomer in human. Fortification of calcium lactate to

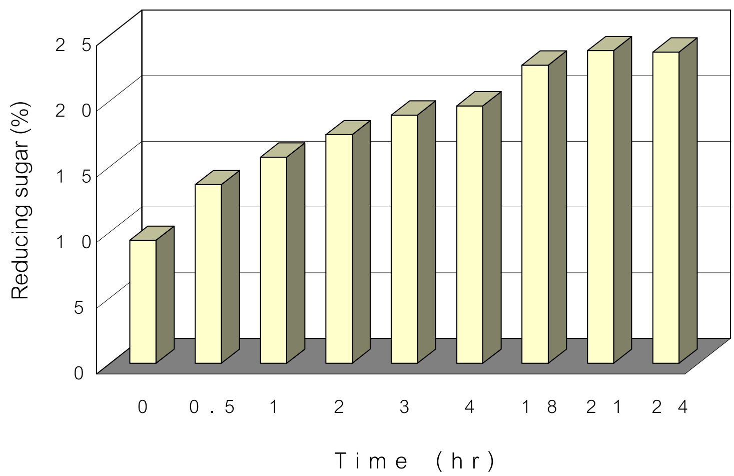

Figure 1 Changes of reducing sugars during saccharification of rice by amylolytic enzymes at 55 °C.

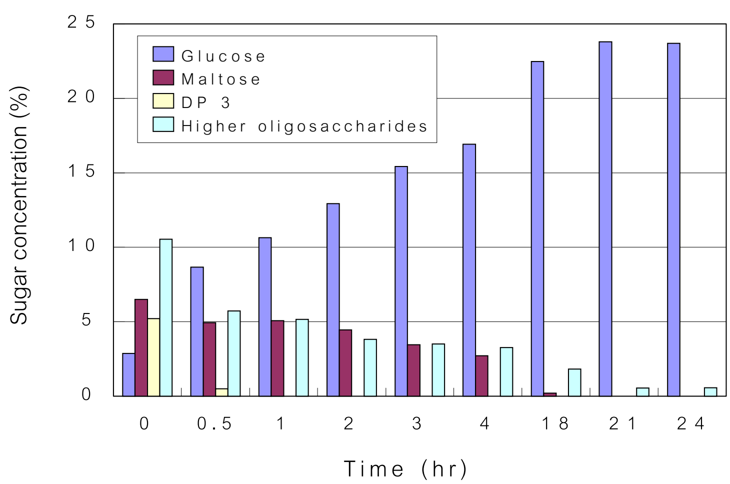

Figure 2 Changes of sugars compositions during saccharification process of rice by amylolytic enzymes at 55 °C.

other products such as orange juice, calcium-enriched beverages and drinking yogurt was recommended at 0.3 - 1.5 % level resulting in calcium enrichment of 40-200 mg per 100 g product (Anonymous, 1993). Concentration of calcium lactate higher than 0.4 % resulted in precipitation of calcium salt in the rice milk.

Lactic fermentation of rice milk

From preliminary study of Chakamas *et al*. (1992), *L casei 2020* and *L acidophilus 2013* were selected for lactic fermentation of rice hydrolysed by koji prepared from *Amylomyces rouxii*. Since both strains proved to grow well and produced up to 0.6 % lactic acid in hydrolyzed rice, they were also used in this experiment as single inoculum and as the mixed cultures for lactic fermentation. The effect of beta glycerophosphate(BG) is shown in Figure 3. BG seemed to enhance acid production, especially when mixed culture (1 % each) was used as starter. BG at 0.5 % level helped increasing the acidity up to 0.8 % compared to 0.67 % of the control. Sodium salt of beta glycerophosphate was widely used in fermented dairy products, particularly yogurt, drinking yogurt and cheese since it was effective as buffering agent suitable for growth of LAB and increased lactic acid production (Rebecchi *et al*., 1993).

The incubation period of 24 hr gave the product with 0.8 % acidity and flavor and odor was

Table 1 Color measurement of rice milk in comparison with fresh cow's milk.

Factors	Rice milk	Cow's milk
Lightness (L*)	90.25	93.50
a* (+ : red; - : green)	-0.89	-1.93
b* (+ :yellow; - : blue)	2.86	16.25
C* (Chroma)	3.00	6.54
H* (arctan)	107.28	107.13

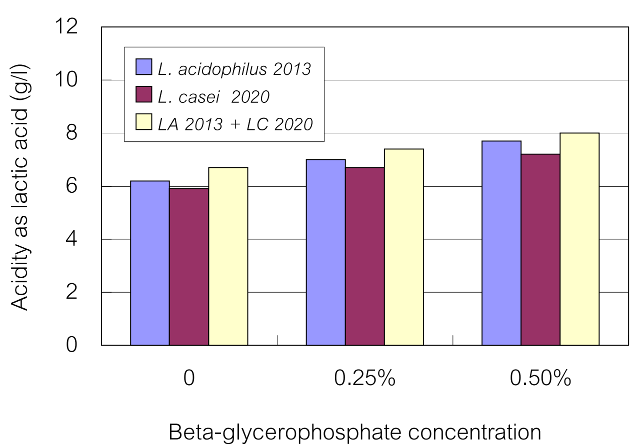

Figure 3 Effect of beta-glycerophosphate on acidity of rice milk fermented with 2 % LAB at 37 °C for 24 hr.

found to be more palatable than the longer period. After 36-hr incubation, either using as single or mixed cultures, the sour taste of the saccharified rices was too profound (Figure 4). Inoculum size higher than 2 % gave no advantage for lactic fermentation both when the strains were used separately and as mixed culture (Figure 5). *L. acidophilus* 2013 could grow well and produce high lactic acid during the first stage of incubation but it also gave strong acidic smell. *L. casei* 2020 produced slower rate of lactic acid but the odor was much more favorable. Combination of the two strains, at 1 % each, resulted in the product with appropriate acidity and improved flavor than when separately used. Therefore, it has been chosen for the fermentation of saccharified rice fortified with 0.5 % beta glycerophosphate for 24 hr at 37 °C. Resulted rice-based yogurt contained 0.8% lactic acid at pH 3.58 and lactic bacteria count of 1.0×10^8 CFU/g.

L. acidophilus and *L. casei* are considered to be probiotics and they are natural inhabitants of

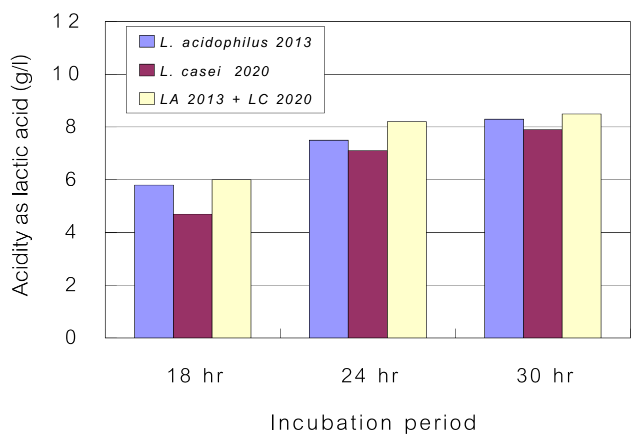

Figure 4 Effect of incubation period on acidity of rice milk fermented with 2% LAB at 37 °C.

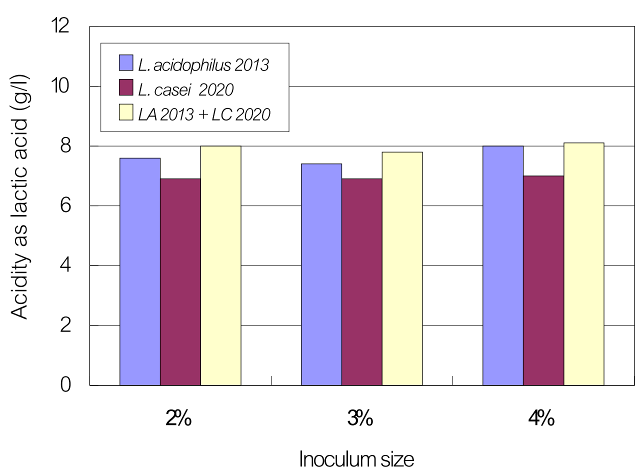

Figure 5 Effect of inoculum size on acidity of rice milk fermented at 37 °C for 24 hr.

the intestinal tracts (Saloff-Coste, 1997). *L. casei*, Shirota has been used in Yakult, which is a well-recognized drinking yogurt-like product. *L. casei* subsp. *rhamnosus*, a human origin strain, was reported to be used in fruit-flavored drink and yogurt from whey (Salminen *et al.*, 1991), and as a direct-to-vat inoculum for fermented milk (Russell, 1996).

Rice-based yogurt preparation

Rice-based yogurt obtained after 24-hr lactic fermentation contained 0.8 % as lactic acid, pH 3.48 and viable cell count of LAB of 1.0×10^8 CFU/g. The curd was well set, yet relatively soft but without separation of the liquid on the surface of curd. Texture and consistency of the yogurt could be improved by addition of 1 % pectin. Moreover, in order to enhance acceptability, 20 % strawberry preserve was employed instead of sugar. The preserve used which contains 65 % total sugar, consists of of 57 % glucose and 8 % sucrose. This would result in about 11 % glucose and 1.6 % sucrose in the resulted rice yogurt. The rice curd was blended with the fruit and pectin to make custard-type or swiss-style yogurt (Sellars and Babel, 1970). Strawberry flavor and color were also added to disguise the fermented rice odor and simulate strawberry dairy yogurt. After chilling at 4 °C for 1-2 hr, texture of the curd became smooth and firmer with a light pink color and strawberry odor. Some physicochemical properties of rice-based yogurt in comparison with commercial dairy yogurt are shown in Table 2. Despite protein content of rice-based yogurt was a little lower than dairy yogurt, fat content was also lower and can be furtherly adjusted to give a low-fat and cholesterol-free product. The major component of sugars was glucose with small amount of sucrose derived from the preserve. Acidity was 0.8 % as lactic acid and 0.14 % as citric acid from the preserve.

Sensory evaluation

The overall acceptability of strawberry rice-based yogurt with 0.86 % acidity as lactic, pH 3.58 and LAB count of 7.6×10^7 was rated by the panelists as like moderately (6.85) which was comparable with commercial strawberry dairy yogurt (6.98) as shown in Table 3. Commercial

Table 2 Physico-chemical properties of strawberry added rice-based yogurt in comparison with commercial dairy yogurt with strawberry.

	Rice-based yogurt	Commercial dairy yogurt
Protein (%)	3.05	4.0
Fat (%)	2.67	2.8
Ash (%)	0.26	
Carbohydrate	25.1	27.0
Glucose (%)	24.4	16.8
Sucrose (%)	1.5	9.9
Calcium (mg/100 g)	47	117
Acidity (% as lactic acid)	0.86	1.02
pH	3.48	4.2
Viscosity (cps)	3,900	4,200
Viable LAB count (CFU/g)	7.6×10^7	3.4×10^9

yogurt was slightly preferred in all categories tested but the differences were not significant at 95 % confidential level.

Shelf-life study of Rice-based yogurt

Shelf-life study revealed that during 6 day-storage at 4 °C, pH of rice-based yogurt decreased slightly from 3.58 to 3.40 and remained unchanged. Acidity gradually increased throughout 20-day storage while number of LAB increased from 7.6×10^7 CFU/g (day 1) to 4.5×10^8 at day 6 and decreased thereafter to 10^3 at day 20 (Table 4). Coliforms, yeasts, mold and bacteria other than LAB were not detected during storage up to 20 days. LAB counts of two commercial dairy yogurts after 10 day-storage were none and 8×10^4 CFU/g even though both contained about 1 % acidity.

CONCLUSION

Saccharification of Jasmine rice with amylolytic enzymes at 55 °C yielded the highest amount of reducing sugar after 21 hr. Sugar composition of saccharified rice by HPLC revealed that 98 % was glucose and only 2 % were higher oligosaccharides (DP>3). Saccharified rice at 18 ° Brix when fortified with 3% casein, 3% soybean oil and 0.4% calcium lactate, homogenized and pasteurized, resulted in rice milk for lactic acid fermentation.

Mixed culture of *L. acidophilus* and *L. casei* subsp. *rhamnosus*, 1 % each, were selected for lactic fermentation of rice milk. Optimum conditions for lactic fermentation were as follows: rice milk added with 0.5 % beta glycerophosphate

Table 3 Sensory evaluation of rice-based yogurt and commercial dairy yogurt with strawberry.

	Color	Odor	Flavor	Texture	Acceptability
Commercial dairy yogurt	7.35[a]	6.87 [a]	7.10 [a]	7.18 [a]	6.98 [a]
Rice-based yogurt	6.70 [a]	6.73 [a]	6.75 [a]	7.05 [a]	6.85 [a]

* Values of each column with the same letter are not significantly different at 0.05 level.

Table 4 Shelf-life study of Rice-based yogurt with strawberry**.**

Day	LAB count (CFU/g)	Lactic acid (%)	pH
1	7.6×10^7	0.86	3.58
2	9.8×10^7	0.94	3.52
4	1.3×10^8	1.10	3.45
6	4.5×10^8	1.13	3.40
8	1.5×10^7	1.20	3.40
10	5.6×10^6	1.18	3.40
13	1.9×10^4	1.25	3.40
16	1.0×10^4	1.26	3.40
20	9.8×10^3	1.26	3.50

as substrate; 2% inoculum and 37°C incubation for 24 hr. Resulted product contained 0.8% lactic acid, pH 3.58 and lactic bacteria count of 1.0×10^8 CFU/g. This rice yogurt was used as base to prepare swiss-style type yogurt by blending with 20% strawberry preserve, 1 % pectin and flavoring and coloring agents.

Rice-based yogurt with strawberry consisted of 3.05 % protein, 2.68 % fat, 24.4 % glucose, 1.5 % sucrose, 47 mg/100g of calcium, 0.86 % acidity as lactic, pH 3.58 and lactic bacteria count of 7.6×10^7 CFU/g. The viscosity was 3,900 centipoises and could be kept at 4 °C for at least 20 days without deterioration and separation of liquid. No contamination from other microflora was dectected throughout the storage period and at day 20, 9.8×10^3 CFU/g of viable lactic bacteria remained in the product. Sensory evaluation indicated that rice-based yogurt with strawberry was accepted by panelists when compared with commercial strawberry yogurt with no significant difference at 0.05 % level.

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