DEVELOPMENT OF LACTIC ACID FERMENTED BEVERAGE MADE FROM THE EXTRACT OF BROWN RICE AND MUNGBEAN MIXTURE

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ABSTRACT

Cool and hot water extraction procedure were examined for their suitability to yield desirable rice-based beverage for fermentation by two strains of lactic acid bacteria (LAB). Studies revealed that a procedure in which roasted brown rice and mungbean flour were mixed in proportion of 1:1, dispersed in cool water (15% w/v), filtered and added with amylolitic enzyme (0.2 ml of α-amylase and 0.05 of glucoamylase/100 ml) resulted in beverage most desirable for fermentation. The rice-based baverage was then inoculated with 4% culture of *L. bulgaricus or L. acidophillus*. Fermentation was carried out at an ambient temperature for 8, 16, 24, 32 and 40 hours.

It was observed that *L. acidophillus* grew almost the same with those of *L. bulgaricus*. Lactic acid bacteria, titratable acidity and pH of fermented product varied from 20.5 x 10^{11} to 66 x 10^{19} CFU/ml, 0.46%-to-0.55% and 4.00-to-4.62, respectively. On the basis of these experiment, a process for the production of rice-based fermented beverage using *L. acidophillus* and 32-40 hrs of fermentation period was developed. Sensory evaluation showed that the product was well accepted when blended with 15% or 20% syrup.

Key words : lactic acid, fermented beverage, broes rice, mungbean

INTRODUCTION

The protein content of rice is low (6-8%) and it is deficient in lysine. In contrary, le-gume protein is rich in lysine but deficient in cysteine and metionine. Therefore, in order to improve the nutritional value of rice product, legume should be incorporated.

Rice and legume could be processed into several product. However, the develope-ment of lactic acid rice-based fermented beverages seem to be promising. Lactic acid fermentation is probably one of the biological process from which human being has discovered the benefits of fermentation. Some advantages from lactic acid fermentation of food grains included: (1) improvement of nutritional values of food material to the host (Nam and Shon, 1992), (2) reduction in antinutritional factor such as phytic acid and oligosaccharide (Zakaria *et al.*, 1994), (3) formation of characteristic flavour through organic acid and more importantly the reduction of pH to save level (pH 4.0) (Fardiaz *et al.*, 1994) and (4) therapeutic effect in diarrheal diseases (Mitsuoka, 1990). This extremely acidic product, however, might not be tolerated by everybody.

As reported by several authors, lactic acid fermentation of non-dairy beverages has been developed in recent years. A mixture of saccharified rice and soy protein isolate was used by Mok (1992) as substrate for a lactic acid fermented product by using dairy yoghurt, lactic acic bacteria starter. Most of the product are describe as acidic liquids containing particles or solids. Some of them are supplemented with a protein component of either animal or vegetable origin (Collado *et al.*, 1994, Fardiaz *et al.*, 1994, Viet *et al.*, 1992, Nashiru *et al.*, 1992).

Lactic acid is produced by a certain becteria and since becteria behave differently on different sub strates, growth pattern of lactic acid bacteria in rice-based beverages may not be the same in other products. Mok (1992) reported that the quality of lactic fermented rice could be improved by the addition of amylase enzymes.

The experiment was directed to study the effect of fermentation process, of rice-based beverages with lactic bacterial cultures commercially used in dairy industry on its physico-chemical, microbiological and sensory qualities. It was also expected to explore the possibility of palatable rice-based fermented beverages being the alternative of rice product widely consume by the Indonesian.

MATERIALS AND METHODS

A. Rice and Mungbean

Rice (*Oryzae sativa L.*) was obtained from the Research Institute for Rice in Sukamandi, Subang. The varieties used was IR-64. The mungbean grain was purchased from the local market at Sukamandi.

B. Enzymes

The amylolitic enzymes, namely α-amylase (Fungamyl 800L) and glucoamylase (AMG/ amyloglucosidase) were obtained through the NOVO distributor in Jakarta.

C. Lactic Acid Bacteria (LAB) and Culture Method

Lactobacillus bulgaricus (FNCC 0041) and *Lactobacillus acidophillus* (FNCC 0051) were used for the lactic acid fermentation of rice-based beverage. They were

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obtained from the Food and Nutrition Culture Collection (FNCC) of the Gadjah Mada University, Yogyakarta.

Pure culture were adapted to grow in a medium consisting of skim milk (10%) by transfering twice at 24-h interval. Stock culture were held in medium consisting of rice-based beverage at 24-h interval and once transfer (as inoculum) after 24-h incubation.

D. Preparation of Rice-based Beverage

Brown rice and dehulled mungbean were pregelatinized and ground into powder in a coffee mill. The pre-gelatinized brown rice and mungbean powder were mixed together in proportion of 1:1. Cool and hot water extraction procedures were examined for their suitability to yield desirable extract. Pre-gelatinized of rice-mungbean flour mixture was dispersed in water, and blended in a waring blender at high speed for 3 minutes, and then filtered through muslin cloth. The filtrate was boiled for 5 minutes, cooled down to 50°C and the amylolitic enzyme were added to it for a saccharification to provide sugar for the initial growth of the LAB. The saccharified rice-based beverage was bottled and pasteurized for 20 minutes and stored at 5°C until used for fermentation.

E. Fermentation Procedure

Rice-based beverage (100 ml) in 250 ml shaking flask adjusted to 37°C was inoculated with 4ml of a 24-h culture of *L. bulgaricus* and *L. acidophillus*, respectively. Fermentation was carried out at 37°C. Analysis were performed after various periods of fermentation during 40-h periods. Based on the optimum condition, the initial up-scaling of the fermentation process was developed. It was carried out in a 2 litre of fermenting vessel.

The acceptability of the product was improved by the addition of commercial vanilla syrup from 0-to-20% (v/v).

F. Analysis Methods

Chemical composition of rice-based beverage were determined by AOAC, (1984) method. Glucose content of rice-based beverage was measured by Anthron method, while the total soluble solids (TSS) was evaluated by using an oven method.

Population of LAB was determined by serially diluting fermented product in steril water and plating on MRS Agar. Colonies were counted after incubating for 48-h at an ambient temperature.

Titratable acidity of the fermented product was measured by titrating fermented product with 0.1 N of NaOH using 1% phenolphtalein as an indicator. Titratable acidity was calculated and expressed as percent of lactic acid. The pH was also monitored during the course of fermentation.

The samples (chilled product) were subjected to a scoring different test of sensory attribute, namely color (1=brown, 2=light brown, 3=cream, 4=yellow cream, 5=yellow white), beany flavor (1=intense beany, 2=beany, 3=slightly beany, 4=trace of beany, 5=not perceptible) and astringency (1=intense, 2=moderate, 3=not perceptible). Preference ranking test was applied to evaluate the sweet-sour taste of sample. The best taste was ranked as first and the least as fifth. The rank were converted to scores according to the method of Fisher and Yates (1942) in Larmond (1977). The reseacher of Research Institute of Rice served as panelits. Evaluation was done according to the method of Larmond, (1977).

RESULTS AND DISCUSSION

A. Preparation of Rice-based Beverage

The rice mungbean extrach was produced the first step, we produced the rice-mungbean extract. It was prepared to stimulate the protein of non-dairy milk Extraction was carried out using 10, 15 and 20 percent of pre-gelatinized rice-mungbean powder with cool or hot water. In this case, roasting method was applied to pre-gelatinized the brown rice and mungbean.

The result showed that the protein content and TSS of extract from the cool or hot water process were almost the same. Dispersing 10% of pregelatinized rice-mungbean powder resulted low of protein content. However, the significant differences in protein content of extract produced by dispersing 10% and 20% of the powder was not observed (Figure 1). Significant different in term of TSS was observed among the extract which was produced by dispersing 10, 15 and 20 percent of pre-gelatinized powder, respectively (Figure 2).

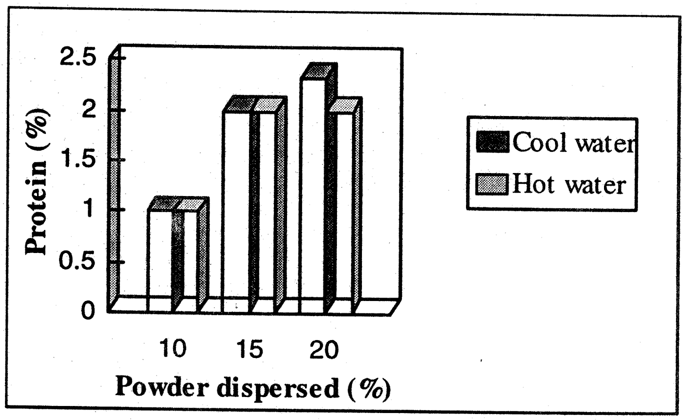

Figure 1. Protein content of rice-mungbean extract

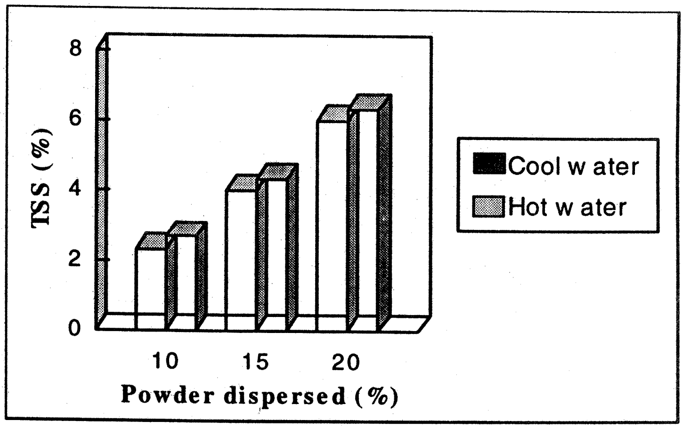

Figure 2. TSS content of rice-mungbean extract

Considering the difficulties in the attack of the rigid tissue matrix of cereal by LAB in contrast to milk constituents, pretreatment to break down the tissue culture and macromolecules of cereals are needed. A new fermentation process including prefermentation with proteolytic and amylolitic bacteria and yeast and extrusion cooking followed by a lactic acid was suggested by Lee and Chee, (1992). In this study, pre-treatment was done by roasting both of brown rice and mungbean or puffing of brown rice (puffing was not suitable for maungbean grain).

In yoghurt, glucose which is a degradation product of lactose, is an important metabolic precursor for acetaldehyde synthesis through pyruvate and acetyl-CoA inter-mediates of glycolysis (Lees and Jago, 1976 cited by Lee and Beuchat, 1991). Amino acid such as threonine and metionine may also be direct precursor of acetaldehyde. LAB convert threonine into acetaldehyde and glycine by threonine aldolase, where as metionine is metabolically converted to threonine. In this study, glucose was provided by hydrolysis of macromolecules (starch) by the addition of amylolitic enzymes.

Table 1 shows the chemical composition of the rice-based beverages for the lactic fermentation. Pre-treatment of brown rice (by roasting and puffing methods) and amylolitic pre-treatment did not show any noticeable improving effect on the chemical composition. However, rice-based beverage which was prepared by roasting of the rice grain and included 0.2 ml of α-amylase and 0.05 ml of AMG per 100 ml of rice-based beverage showed the highest stability. After 3 weeks, storage at refrigerator temperature did not produce the excessive whey on the top of the bottle, while the others did.

Table 1. Chemical composation of rice-based beverages prepared by various methods of rice grain pregelatinization and amount of added enzyme

Rice grain pre-gelatinization method	Added enzyme per 100 ml beverages	Chemical component				
		Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Glucose (g./ml)
Roasting	0.02 ml of α-amylase and 0.05 ml of AMG	94.24	0.38	0.19	1.88	0.12
Roasting	0.2 ml of α-amylase and 0.05 ml of AMG	94.43	0.42	0.20	1.90	0.11
Puffing	0.02 ml of α-amylase and 0.05 ml of AMG	93.68	0.37	0.05	1.66	0.15
Puffing	0.2 ml of α-amylase and 0.05 ml of AMG	93.58	0.35	0.03	1.69	0.17

B. Starter Characteristics

Lactobacillus bulgaricus and *Lactobacillus acidophillus* adapted to the medium which consisted of rice-based beverage. Viable cell count and pH value of starter were about 10^9-10^{10} CFU/ml and 3.7-3.8, respectively. We noted that after several trasfer in the rice-based beverage maintenance medium, *L. bulgaricus* and *L. acidophillus* appeared to have a shorter lag time once inoculated into rice-based beverage substrate. This adaptation period may also be required by other lactics and should be considered by reseachers exploring the area of cereal-legume extract fermentation.

L. bulgaricus and *L. acidophillus* are well known as lactic bacterial culture commercially used in dairy industry. Moreover, L. *acidophillus* are able to implant in the intestine. However strain variability in adherence capacity in the intestine needs to be studied. Some reseachers reported that cells of *L. acidophillus* were effective in the treatment of different types of diarrhea in humans and in gnotobiotic chikens colonized with pathogenic *E. coli* (Nam and Shon, 1992).

C. Rice-based Lactic Acid Fermented Product

Single culture of strain of *L. bulgaricus* and *L. acidophillus* was tested for the lactic acid fermentation of rice based beverage. Studied revealed that *L. acidophillus* grew almost the same with those of *L. bulgaricus* (Figure 3). At 8 hr of fermentation period, the viable cell count of *L. bulgaricus* and *L. acidophillus* were about 20.5×10^{10} CFU/ml and 17.5×10^{10} CFU/ml, respectively. After 40 hr of fermentation period the population of *L. bulgaricus* increased to 50.8×10^{16} CFU/ml, while those of *L. acidophillus* increased to 66×10^{18} CFU/ml.

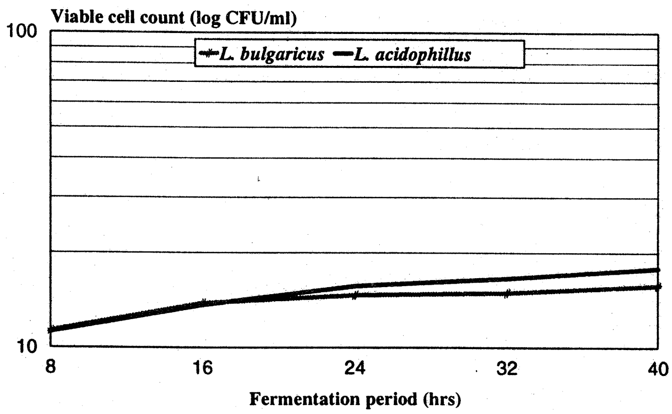

Figure 3. Changes in viable cell populations of rice-based beverage fermented with *L. bulgaricus* and *L. acidophillus*.

The lactic acid produced by *L. acidophillus* was higher than those produce by *L. bulgaricus* (Figure 4). Lactic acid produced by *L. acidophillus* after 8 hr-to-40 hr of fermentation period had a range of 0.46-to-0.66%. *L. bulgaricus*, however, showed the acidity of 0.53-0.55%. These result was lower than those of acidity of commercial dairy yoghurt (0.8-to-1.0%) as stated by Yukuchi *et al.*, (1992).

Figure 5 shows the changes in pH during lactic acid fermentation of rice-based beverage. The pH of rice-based beverage fermented with *L. acidophillus* decreased more rapidly compared to the pH of rice-based beverage inoculated with *L. bulgaricus*. The pH value of rice-based beverage fermented with *L. bulgaricus* and *L. acidophillus* did not reach that of yoghurt made from cow's milk, which is about 3.27-to-4.10 (Salji and Ismail, 1983).

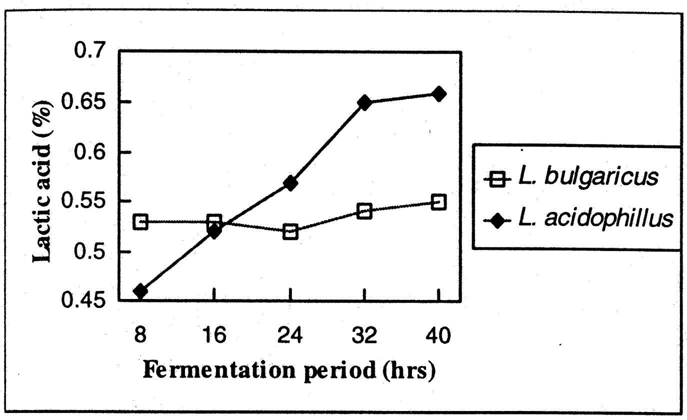

Figure 4. Lactic acid of rice-based beverage fermented with *L. bulgaricus* and *L. acidophillus.*

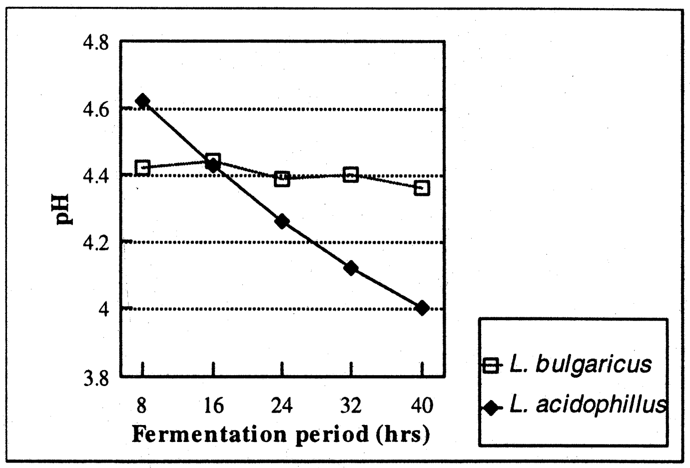

Figure 5. Changes in pH of rice-based beverage fermented with *L. bulgaricus and L. acidophillus.*

Ingestion of foods fermented by LAB may alter the pH of the intestine and change the intestine environment which may influence matabolic activities. Based on a gnotobiotic study, Coates (1975) cited by Nam and Son, (1992) suggested that a lower pH influences the solubility of some minerals, the efficientcy of digestives enzymes, and the oxidation state of iron. Others metabolic activities also influenced by the ingestion of LAB include the alteration of lipids and steroids, and dehydrogenization of unsturated fatty acid.

The results of the current study deviated from previous findings in other reports. Beucahat and Nail (1978) reported that acidity and pH of peanut milk fermented with *L.acidophillus* or *L. bulgaricus* ranged from 0.38-to-0.53% and 4.76-to-4.43, respectively. Other reseachers have reported variations in the ability of lactic acid bacteria to ferment vegetable milk (Bucker *et al.*, 1979; Cheng *et al.*, 1990; Lee and Beuchat, 1991; Collado *et al.*, 1994; Kusumaningrum *et al.*, 1996). In general, acid production was related to ability of strain to utilize the major fermentable sugar.

Based on the highest acidity and lowest pH value, rice-based beverage fermented with *L. acidophillus* culture for 32-40 hr was adopted. In the other hand, *L. acidophillus* could reach the human intestine in a living state.

The initial up-scaling of the process was carried out in our laboratory. The uses of a conventional fermentor is obviously too costly. Therefore a 2 litre of fermenting vessel was developed. The process was modified and improved on a number of points, and it can now be carried out smoothly. The most critical unit operation in this case been: fermentation. The titratable acidity (lactic acid) and pH value of fermented rice-based prepared in fermenting vessel was about 0.48% and 3.6, respectively. Despite the initial succesful, it soon became evident that there was a lack of understanding, why the process sometimes resulted in a good taste and sometimes in a bad tasting beverage.

D. Sensory Characteristics

The sour product required the addition of sugar (syrup) for optimum sweet-sour blend to improve sensory acceptability of the product. Sweet-sour blending refers to the optimum combination of perceived acidity and sweetness which contribute to the who-lesomeness of the product. Commercial vanilla syrup were added to enhance the flavor of the fer-mented product and the resultant chilled product were evaluated for the sensory-characteristics. Sensory score are presented in Table 2.

Table 2. Mean score of sensory evaluation of the rice-based beverage fermented with *L. acidophillus*

Syrup (% v/v)	Score of sensory attribute			
	Color[a]	Astringent[b]	Beany flavor[c]	Taste
0	2.1 a	1.1 a	1.5 a	-0.95 a
5	2.4 a	1.9 b	2.7 b	-0.45 b
10	2.1 a	2.4 c	3.5 c	0.05 c
15	2.6 a	2.7 c	3.9 c	0.74 d
20	2.5 a	2.6 c	3.5 c	0.83 d

[a] 1:brown, 2: light brown, 3: cream, 4: yellow-cream, 5: yellow white
[b] 1: intense, 2: moderate, 3: nor perceptible
[c] 1: intense beany, 2: beany, 3: slightly beany, 4: trace of beany, 5: nor perceptible

With the addition of syrup from 0 to 20% (v/v) there was no significant in perceive color. Astringency, a common problem for cereal-based lactic acid fermented foods, has been attributed to tannins, phenolic acid and flavonoids (Kim, 1992). In the samples, the astringent aftertaste was masked by sweetness. As the syrup concentration in the samples increased, the perception of the astringent aftertaste diminished. With addition of syrup from 10 to 20%, there was also minimal perception of beany flavor since there was no significant difference among mean score. Addition of 15% or 20% resulted samples which was best like as first in term of taste by panelists.

Yakult (a commercial fermented product made from dairy milk) contained 0.77% of protein, 0.74% of lactic acid and 18.89 of TSS, respectively. Syrup addition did not change the protein content and titratable acidity (lactic acid) of the fermented product (Table 3). Protein content of rice-based fermented beverage was higher than those of commercial product marketed in Indonesia.

TSS was the important characteristic which related to the mouthfeel. The extremely high or low TSS value was not desirable. Purwani *et al.*, (1996) reported that the accep-table soybean milk consisted about 15-20% of TSS. The TSS of the fermented product prepared using 15% of syrup reached that of commercial yakult, which is about 18%. Based on the above mentioned result, a fairly acceptable product can be obtained from fermented rice-based beverage with 15% of syrup (vanilla syrup).

Table 4. Protein, lactic acid and TSS of rice-based beverage fermented with *L. acidophillus*

Commercial syrup (% v/v)	Protein (%)	Lactic acid (%)	TSS (%)
0	1,60	0,48	5,30
5	1,53	0,48	9,30
10	1,10	0,45	13,73
15	1,13	0,43	17,70
20	1,13	0,42	21,99

From an improved-utilization point of view, processing of lactic acid fermented of rice-based beverage is much relevant developement. This is particularly so in developing country such as Indonesia where the hitherto overdependence on imported food products had become not only economically unreasonable but also wholesomely inferior to what had been passed on from ages. Lactic fermented product form the basis of the diets of most countries in the developing world and over the years, a comparatively slow but gradual improvements on the processing (mostly in the area of upgrading indigenous technology) had been approach with vigour. Most of the so-called independent nations, however, soon lost their local values and research effort are mostly undeveloped. It is hoped that these studies would have significant impact on the lives of the people when developed.

CONCLUSION

- Rice based-beverage made from the water extract of pre-gelatinized brown rice-mungbean powder mixture with amylolitic enzyme could be fermented by both of ***L. bulgaricus*** or ***Lactobacillus acidophillus***. However, the more, higher acidity and lower pH product was obtained from the rice-based beverage inoculated with ***L. acidophillus***.
- Rice-based beverage inoculated with ***L. acidophillus*** for 32-to-40 hours produced an acceptable product when blended with 15% of syrup (vanilla).

FUTURE STUDIES

- Further experiments to improve the process and the quality of the product yielded are necessary before scale-up studies.
- Storage stability of the fermented product needs to be determined.
- It would be also necessary to investigate on the health effect of the rice based beverage fermented with ***L. acidophillus***.

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