Physicochemical properties of fermented rice bran in optimal lactic acid bacteria growth

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Abstract. This work aimed to characterize the physicochemical properties of fermented rice bran in optimal incubation time with two lactic acid bacteria. Fermented rice bran was prepared by inoculation of rice bran using Lactococcus lactis and Lactobacillus plantarum (6.0-7.5 x 10⁴ CFU/mL) at 37°C for 96 h in a solid state condition. Physicochemical composition of fermented rice bran was analyzed by proximate, color test, reducing sugar, solubility, phenolic content and morphology image. Fermentation of rice bran with lactic acid bacteria had increased its protein content and phenolic content (25-70%). In contrast, fat content, carbohydrate, and reducing sugar of rice bran were decreasing during fermentation. SEM analysis indicated changes in rice bran surface structure after enzymatic process compared to unfermented rice bran.

1. Introduction
More than half of people in the world are having dietary demands on rice. As a staple food, rice is growing abundantly in China, India, Indonesia, Japan, Thailand, Philippines, Bangladesh, Vietnam, and Burma. Rice bran, outer layer of rice, is a by-product of rice milling industry which commonly used as animal feed. Since it is a good source of protein, mineral, fatty acids, and dietary fibre, public started to give more attention and generated a wide range of research activities [1]. Nowadays, dietary fiber sources become highly and popular demand to the development of functional foods. Moreover, rice bran is the subject of different researches interesting due to its abundance bioactive compounds that reveals great potential to be used in biotechnological processes. Several active compounds such as oryzanols, tocopherols, tocotrienols, phytosterols, and nucleotides have been identified in rice bran which is widely used to enhance the functionalities of some food stuffs and improve their capacity against chronic diseases [2-3]. The fermentative process implies employing microorganisms to get resultant transformations of their metabolic activity as well as increasing the availability of nutrients in raw materials. Biochemical changes associated with microbial metabolism and enzyme actions during fermentation are the main factors affecting the nutritional and textural properties of the fermented product. Many rice bran fermentation processes have been studied during the years. Several active metabolites are produced during fermentation of rice bran like γ-oryzanol, inositol, ferulic acid, vanillic acid, protocatechuic acid, and phytic acid [4-5].

Several investigations of bioactivity effect on fermented rice bran for promoting health have been performed previously using bacterial or fungal agents This work was performed to study the influence
of lactic acid bacteria (LAB) in physicochemical properties of modified rice bran by a solid-state fermentation treatment.

2. Materials and Methods

2.1. Materials
The materials were purchased from a rice processing company located in Yogyakarta, Indonesia. The by-product of rice milling i.e rice bran was re-sorted by sifting into powder form using a standard sieve (60 mesh). Starter bacteria which used in this work were Lactobacillus lactis FNCC 0080 and Lactobacillus plantarum FNCC 0027.

2.2. Lactic acid fermentation of rice bran
Rice bran fermentation was performed with L. lactis and L. plantarum in static condition. Before used, rice bran (30g) was sterilized (121°C; 15 min) by adding of 50 mL distilled water in flasks. The sterile rice bran powder was cooled. Each LAB was poured into the flask in 6% ratio of total volume. The mixture was then incubated for 96 h at 37°C. After 96 h (4 days), the fermented rice bran was harvested and dried for 60°C.

2.3. Physicochemical characterization of fermented rice bran
The dried fermented rice bran was characterized for their physicochemical properties. The water content, ash content, protein, and lipid were examined based on AOAC (1984) method [6]. The crude protein was expressed as total nitrogen and multiplied in a factor 6.25 by Kjeldahl method [7]. The sample was dried at 105°C until obtained a constant weight to determine its water content. Rice bran sample (1 g) was incinerated in a muffle furnace (550°C; 5h) to measure the ash content. Crude lipid was analyzed by extracting samples (5 g) using petroleum ether in a Soxhlet apparatus. The pH value was measured in pH meter (Eutech PC700, NY, US). Reducing sugar was determined by Nelson Somogyi method [8]. The physical characteristics of fermented products were also identified by Scanning Electron Microscopy (Hitachi SU3500).

2.4. Total phenolic contents determination
Dried samples (10 g) were extracted with methanol by ultra-sonication at room temperature. Total phenolic content (TPC) of fermented rice bran extracts was determined by Folin-Ciocalteu reaction method. A stock solution (0.5 mL), distilled water (7.5 mL), and Folin-Ciocalteu reagent (0.5 mL) were mixed and continued by incubation. After 8 min, 20% Na₂CO₃ solution (1.5 mL) was added and re-incubated for 2h. The absorbance of the mixture was read using a UV-Vis spectrophotometer (Hitachi HALO RB-10) at 765 nm. TPC value was expressed as gallic acid equivalents of dry weight (mg GAE/g dw) [9].

3. Results and discussion

3.1. Physicochemical characterization
Lactic acid fermentation needs nutrients and medium for LAB to grow which can be provided by rice bran. Therefore, the effectiveness of fermentation depends on the starter used. Many bioactive compounds could release through bio-synthesis process which structural breakdown rice bran cell walls stimulated by fermentation [10]. The physicochemical properties of fermented rice bran are presented in table 1.

The moisture content of unfermented rice bran was lower than that of fermented rice bran. It might be caused fermentation process involved LAB media contained amount of water although it was solid state condition. In contrast, the ash content decreased due to the structure breakdown of lignocelluloses caused by enzymatic process. Amount of fat tended to be reduced after incubation, but the protein content was not significantly changed. Microbial fermentation can produce some
proteolytic enzymes, which can stimulate the degradation of peptides and amino acids, hence support the other microorganisms to grow [11].

### Table 1. Physicochemical properties of unfermented rice bran (URB), 96 h fermented with *L. plantarum* (RBLP) and *L. lactic* (RBLL).

| Physicochemical properties | URB       | RBLP      | RBLL      |
|----------------------------|-----------|-----------|-----------|
| Moisture content           | 5.27% ± 0.07 | 8.60% ± 0.07 | 8.72% ± 0.02 |
| Ash content                | 11.50% ± 0.07 | 8.46% ± 0.03 | 10.31% ± 0.02 |
| Protein                    | 11.78% ± 0.06 | 11.46% ± 0.03 | 11.89% ± 0.28 |
| Lipid                      | 15.79% ± 0.08 | 14.88% ± 0.08 | 14.83% ± 0.12 |
| Carbohydrate               | 55.67% ± 0.10 | 56.64% ± 0.11 | 54.25% ± 0.16 |
| Reducing Sugar             | 1.16% ± 0.01 | 0.74% ± 0.001 | 0.63% ± 0.004 |
| pH                         | 6.41       | 4.55       | 4.57       |
| Solubility                 | 0.151%     | 0.130%     | 0.136%     |
| Total Phenolic Content     | 43.68 mg GAE/g | 77.98 mg GAE/g | 54.77 mg GAE/g |

During 96 h incubation, the pH of rice bran significantly decreased from 6.4 to 4.5. The amount of organic acids will be increased by microbial activity of LAB in fermented rice bran which each LAB has different capabilities in producing organic acids. Homolactic fermentation emerged to be the process which generates lactic acid as the main product yet detects of very low ethanol levels in the broth [12]. Consequently, pH of products was decreased since all the available carbohydrate was consumed and organic acids would be produced [13]. Glucose is the main sugar released during thermal/enzymatic cellulose hydrolysis. A significant decrease in glucose levels after 96 h incubation was presented by fermented rice bran using *L. plantarum* and *L. lactic* compared with unfermented rice bran. During fermentation, LAB hydrolyzed the substrate carbohydrates into glucose, which is used as its nutrients [14].

The physicochemical properties of fermented rice bran were also studied by evaluation of phenolic contents. The phenolic content of rice bran was enhanced during fermentation. Fermented rice bran with *L. plantarum* gave the higher phenolic content than fermented rice bran with *L. lactic*. Activity of enzymes produced by microorganism such as β-glucosidase, α-amylase and laccase, together with other enzymes affected in the improvement of phenolic contents in plants. Furthermore, cleavage of the bonds between phenolic compounds with other substances during the fermentation process releasing monomers of phenolic compounds [15].

Based on the light performance, food products can be categorized as opaque, transparent/translucent. Rice bran powder might be categorized as opaque foods since it is seen wholly by reflected light. Color pattern and stability is important indicator of the quality of food product.

### Table 2. Color test of unfermented rice bran (URB), 96 h fermented with *L. plantarum* (RBLP) and *L. lactic* (RBLL).

| Parameter | URB       | RBLP      | RBLL      |
|-----------|-----------|-----------|-----------|
| L*        | 67.5 ± 0.6 | 62.8 ± 0.2 | 62.9 ± 0.2 |
| a*        | 3.6 ± 0.3  | 5.2 ± 0.1  | 5.4 ± 0.2  |
| b*        | 20.6 ± 0.4 | 23.5 ± 0.2 | 23.6 ± 0.4 |

Table 2 reveals changes in the lightness (L*), yellowness (b*) and redness (a*) values of rice bran after and before fermentation. The lightness value of rice bran decreased after 96 h incubation with LAB. In contrast, the redness and yellowness level significantly increased. The release of some compounds by enzymatic process might effect to the color of rice bran product. Changes in a* and b* values after fermentation was correlated with changes in the rice bran pigments such as carotenoids and polyphenols (anthocyanin) [16]. The surface morphology images of fermented rice bran were
carried out using SEM on voltage acceleration 5 kV and 5.4 to 5.5 mm working distance as shown in figure 1.

![SEM images of rice bran morphologies](image)

**Figure 1.** The morphology images of unfermented rice bran (top), fermented rice bran with *L. plantarum* (middle) and fermented rice bran with *L. lactic* (bottom).

SEM images revealed changes on surface structure of rice bran after enzymatic process compared by unfermented rice bran. The surface morphology of unfermented rice bran exhibited as a compact and well-organized surface, whereas the fermentation product appeared more hollow and cracked morphology. Cracks on rice bran surface structure might be affected by enzymes activity in degradation of rice bran cell wall such as cellulase and xylanase. Xylan is major component of hemicelluloses in rice bran which found between lignin and lower cellulose fibers compounds. A number of monomers like xylose, xylooligosaccharide, and arabinose is generated by the hydrolysis of xylan involving the activity of xylanase. Several studies reported that hydrolysis enzymes such as cellulase, xylanase, and those both combinations on rice stalk caused cracks on the surface structure of rice bran [17-18].
4. Conclusion
This study revealed the altering of the physicochemical properties on the fermentation of rice bran using LAB for 96 h. Fermented rice bran had significantly increased protein content, solubility, and phenolic content. While, fat content, carbohydrate, and reducing sugar of rice bran were decreasing during fermentation. It suggested that the utilization of cellulose and hemicellulose enzymes produced by LAB during fermentation could improve the nutrition value of rice bran.

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