Total phenolic contents and antioxidant activity of rice bran fermented with lactic acid bacteria

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ABSTRACT

Rice bran, a by-product of the rice milling process, is rich source of bioactive compounds which have potential nutraceutical effect. Its dietary fiber and phenolic compounds are reported enhancing the functionalities of foodstuffs related to their beneficial properties as antioxidant. The aim of this research is to evaluate the antioxidant activity and total phenolic contents of fermented rice bran using two lactic acid bacteria (LAB), Lactobacillus lactic and Lactobacillus plantarum. Rice bran was fermented in a solid state with ratio of single culture LAB were 5%, 7.5%, and 10%. The antioxidant activities were found to be highest in 10% ratio of Lactobacillus plantarum as well as its total phenolic contents. The functional properties of rice bran were also investigated at varied time of fermentation (24 h, 36 h, and 48 h). Antioxidant activity of rice bran fermented with Lactobacillus plantarum for 48 h showed a almost two-fold increase compared to non-fermented counterpart. The results indicated that the phenolic contents and antioxidant activity of rice bran showed significant improvement upon fermentation with LAB.

Keywords: Rice bran, fermentation, lactic acid bacteria, total phenolic contents, antioxidant activity

1. Introduction

Rice bran, the outer husk of paddy, is one of the most by-products of rice mils production which rich in vitamins, proteins, oil and fiber. It becomes the most abundant agricultural wastes in Indonesia due to the highly demand of rice in this country. Rice bran extract contains several types of polyphenolic compounds as sources of antioxidants, such as phytic acid, ferulic acid and oryzanols [1]. Fermentation process is commonly used for production of value-added products and can be applied in the food, health and cosmetics industries [2]. In the agricultural wastes handling, fermentation using lactic acid bacteria could improve the chemical composition and bioactivity of the substrates by produces enzymes that degrade the cell wall of plants. A number of fermentation studies have been
performed on rice bran throughout the years. Fermentation of rice bran produced biologically active metabolites such as lipid, protein, vitamin B, and essential amino acids, phenolic acid, ferulic acid, vanillic acid, protocatechuic acid, γ-oryzanol, phytic acid, and inositol [3]. Several features that beneficially alter the bioactivity to promote health have been evaluated in fermented rice bran with bacterial or fungal agents through previous studies. Fermentation of rice bran with combination of *Lactobacillus rhamnosus* and *Saccharomyces cerevisiae* was reported considerably reduced the melanin synthesis of the resulting extract to B16F1 melanoma cells [4]. Numerous plant phenols, such as ferulic acid, are often biologically unavailable after intake, thus fermentation could assist improvement of the antioxidant potency [5]. This study aimed to investigate the phenolic contents and antioxidant capacity of rice bran fermented with *Lactobacillus plantarum* and *Lactobacillus lactis*.

2. Materials and Methods

2.1. Collection of plant materials
The rice bran was collected from local rice milling company, located in Yogyakarta, Indonesia. Rice bran was packed and stored at room temperature with silica gel until used in the fermentation process.

2.2. Preparation of lactic acid bacteria
For the starter culture, *Lactobacillus plantarum* (FNCC 0027) and *Lactobacillus lactis* (FNCC 0080) were cultured in MRS broth medium (Merck, Darmstadt, Germany) at 37 °C for 24 h and kept in the microbiology laboratory.

2.3. Fermentation of rice bran using lactic acid bacteria
Fermentation of rice bran was carried out using *Lactobacillus plantarum* and *Lactobacillus lactis* in MRS broth medium at static condition. Approximately, 30 g of rice bran in Erlenmeyer flask was suspended with 50 mL of distilled water and sterilized at 121 °C for 15 min. After cooling to the room temperature, the starter bacteria were added into each flask at varied ratio 2.5%, 5%, 7.5%, and 10% of total medium, mixed, and incubated at 37 °C for 24 h. Each experiment was performed in triplicate. Non fermented rice bran was used as a control. For time-controlled fermentation experiment, the same procedure was carried out to other rice bran samples. The rice bran sample was inoculated with 10% starter. The fermentation time was varied from 24 h, 36 h, and 48 h [6].

2.4. Extraction of the fermented rice bran
The incubated samples were harvested and dried at 60 °C for 24 h. Dried samples (5 g) were exhaustively extracted with methanol in an ultrasonic bath for 3 x 60 min at room temperature. The supernatant was filtrated and evaporated for analysis.

2.5. Determination of the total phenolic contents
The total phenolic content of fermented rice bran extracts was determined by a Folin-Ciocalteu reaction according to our previous method. An aliquot of 0.5 mL samples from the stock solution was mixed with 7.5 ml distilled water and 0.5 mL of Folin-Ciocalteu reagent. After 8 min of incubation, 1.5 mL of 20% Na₂CO₃ solution was added and further incubated for 2 hours. The absorbance was measured at 765 nm using a UV-Vis spectrophotometer (UV Vis Spectrophotometer Hitachi HALO RB-10) against blank sample. The results were expressed as gallic acid equivalents (mg GAE/g dw) of dry weight and the experiment was conducted in triplicate analysis [7].

2.6. Assay for antioxidant activity
The fermented rice bran extracts were homogeneously dissolved in methanol solvent at a series of concentrations (0.1, 0.2, 0.4, 0.6, and 1.0 mg/mL). The sample extracts (3.2 mL) were added to 1.2 mL of methanolic DPPH (Merck, Darmstadt, Germany) solution (0.2 mM). The reactions were
performed in the dark at room temperature for 30 min. Absolute methanol was used as a blank and gallic acid (Sigma Aldrich, USA) as a positive control. The absorbance was measured at 517 nm and the scavenging activities were expressed as the percent inhibition of DPPH radicals calculated using the formula:

\[
\text{Percentage Inhibition} \% = \left[ \frac{A_0 - A_1}{A_0} \right] \times 100 \%
\]

where \( A_0 \) is the absorbance of the control (absence of sample), and \( A_1 \) is the absorbance of the sample [7].

3. Results and Discussion

3.1. Total Phenolic Content

The fermentation of rice bran in this study was performed using two starter lactic acid bacteria, which are \( L. \text{lactic} \) and \( L. \text{plantarum} \). Fermentation process on rice bran might stimulate the structural breakdown of cell walls, causing the releasing or bio-synthesis of various bioactive compounds [8]. The efficiency of the fermentation process could be studied by its phenolic compounds evaluation. Total phenolic content was determined according to fermentation parameters such as the amount of lactic acid bacteria during the fermentation (LAB ratio) and the fermentation time. The phenolic contents were expressed as gallic acid equivalents (% GAE) of dry weight. The LAB ratios used in this fermentation were 2.5%, 5%, 7.5%, and 10% as shown in the Table 1 below.

| LAB Ratio | Total Phenolic Contents (mg GAE/g dw) |  |
|-----------|------------------------------------|---|
|           | \( L. \text{lactic} \) | \( L. \text{plantarum} \) |
| Control   | 24.922                             | 24.922 |
| 2.5%      | 25.789                             | 25.789 |
| 5%        | 26.222                             | 26.020 |
| 7.5%      | 27.464                             | 29.081 |
| 10%       | 28.966                             | 30.410 |

Control = non fermented rice bran

The effectiveness of fermentation depends on the starter used. The optimized ratio of the starter varied according to the each bacteria strains. Lactic acid bacteria require media and nutrients that are quite complex to grow in which rice bran can provide them. Microbial activity in LAB fermented bran will increase the amount of organic acids which each LAB has different capabilities in producing organic acids. The higher phenolic content were demonstrated by the increasing of LAB ratio that used in the fermentation of rice bran. However, on the fermented rice bran involving \( L. \text{lactic} \) was not particularly different among each LAB ratio compared by non fermented rice bran (control). Both of \( L. \text{lactic} \) and \( L. \text{plantarum} \) gave the highest total phenolic content of rice bran at the ratio of 10% as 28.966 mg GAE/g dw and 30.410 mg GAE/g dw, respectively. While the phenolic content of fermented rice bran by \( L. \text{plantarum} \) was slightly higher than that of fermented rice bran by \( L. \text{lactic} \). The effectiveness of fermentation process was not only evaluated based on the starter used, but also the fermentation time of rice bran as shown in the table 2. The optimum fermentation time obtained in this experiment would be the basic of fermentation of rice bran by each starter.
The higher of phenolic content were exhibited by the increasing of fermentation time of rice bran. There is a different capability between \textit{L. lactic} and \textit{L. plantarum} to breakdown the substrate by fermentation process. The higher ability to breakdown the substrate was shown by \textit{L. lactic} than \textit{L. plantarum}. In the fermented rice bran involving \textit{L. plantarum}, the phenolic content was not particularly different during fermentation process in 24 h and 36 h. However, in the 48 h, the fermentation achieved the optimum process, by giving the total phenolic value of 50.416 mg GAE/g dw. In contrast, the fermentation of rice bran significantly increased by addition of fermentation time. The optimum phenolic content was reached in the 48 h of fermentation as 73.144 mg GAE/g dw.

During fermentation, microorganism synthesizes such enzymes which able to break ester links and effect in the releasing bound phenolic acids. Consequently, it may improve the nutraceutical value of cereals and increase the bioavailability due to the improvement of free phenolic acids [9]. An improvement of phenolic contents in plants commonly relates to the action of enzymes produced by microorganism such as β-glucosidase, α-amylase and laccase, together with other enzymes [10]. The increasing of ferulic acid in fermented samples inoculated with \textit{P. acidilactici}, may be due to production of ferulic acid esterase enzyme by \textit{P. acidilactici} [11]. Fermentation process would cleave the bonds between phenolic compounds with other substance and release the monomers of phenolic compounds or antioxidants.

### 3.2. DPPH radical scavenging assay

Figure 3 shows the inhibition percentages of the DPPH radical by fermented rice bran using \textit{L. lactic} and \textit{L. plantarum}. An antioxidant reduces the DPPH radical to diphenylpicrylhydrazine (a yellow-colored compound), and the level of discoloration is depended on the hydrogen-donating capacity of
its antioxidant. The antioxidant activity was investigated in the varied of LAB ratio used in fermentation process and fermentation time. By increasing ratio of \( L. \) \textit{lactic} used as starter in fermentation process caused significant improvement of antioxidant capacity compared with the non-fermented rice bran. However, there is no different between 5%, 7.5%, and 10% ratio of \( L. \) \textit{plantarum} handling in the fermentation medium.

![Figure 3. Antioxidant activity of fermented rice bran based on LAB ratio](image)

The highest antioxidant activity of fermented rice bran extract was demonstrated by addition of 10% both \( L. \) \textit{lactic} and \( L. \) \textit{plantarum} in the fermentation process with shows 42.36 ± 1.08% and 39.94 ± 2.25% of inhibition of DPPH radicals at 1 mg/mL, respectively. The low of activity may be partly due to the fermentation process was conducted inadequate inoculation time.

![Figure 4. Antioxidant activity of fermented rice bran at concentration 1 mg/mL](image)

| LAB ratio | Antioxidant activity (1 mg/mL) |
|-----------|-------------------------------|
|           | \( L. \) \textit{lactic} | \( L. \) \textit{plantarum} |
| Control   | 24.43 ± 0.47                   | 24.43 ± 0.47                   |
| 10%       | 42.36 ± 1.08                   | 39.94 ± 2.25                   |
| 7.5%      | 38.35 ± 0.55                   | 39.38 ± 0.33                   |
| 5%        | 29.03 ± 0.52                   | 40.06 ± 1.83                   |

![Table 3. Antioxidant activity of fermented rice bran based on LAB ratio](image)
The optimized fermentation process was determined by observation of antioxidant activity within varied fermentation time, which is 24 h, 36 h, and 48 h. The scavenging effect of the DPPH radical in all fermented extracts samples improved through increasing fermentation time. The rice bran fermented with *L. plantarum* evenly showed the higher antioxidant activity than rice bran fermented with *L. Lactic*. This result indicated that *L. plantarum* has ability to breakdown the ester bonds composed in rice bran liberating free phenolic compounds. Phenolic, the major secondary metabolites presence in plant, are commonly established in conjugated forms of the hydroxyl group. Phenolic compounds could act as antioxidants through several different mechanisms, such as free radical scavenging capacity, chelating of metal ions and the inhibitory activity of pro-oxidant enzymes [12]. The increasing of antioxidant activity is related to the phenolic contents produced in fermented rice bran which suggest that it might be attributed to hydrolytic enzymes. These enzymes work through the substrate and turn up the free hydroxyl groups on the phenolic structure, consequently the antioxidant activity of the substrate is increased due to the presence of the content of free phenolic [13]. The obvious difference of antioxidant activity in non fermented and fermented rice bran extracts may be due to the high antioxidant activity of bioactive compounds released during fermentation of rice bran, such as ferulic acid, α-tocopherol and β-oryzanol [14].

![Figure 5. Antioxidant activity of time-controlled fermented rice bran.](image)

The strongest scavenging effects on the DPPH radical were found in rice bran after 48 h fermentation with both starters. The highest scavenging effect at 1 mg/mL was reached by rice bran fermented with *L. Plantarum* (Table 4). Fermentation with LAB had favourable effect on its antioxidant capacity, which is showed by [6], who observed increase of bioactive compound in rice bran resulting in an improved antioxidant activity of rice bran after fermentation with *P. acidilactici, P. pentoseous and L. lactis*.

| Fermentation time (h) | Antioxidant activity (1 mg/mL) |
|-----------------------|--------------------------------|
|                       | *L. lactic*                     | *L. plantarum*                  |
| Control               | 24.43 ± 0.47                    | 24.43 ± 0.47                    |
| 24 h                  | 16.19 ± 0.74                    | 29.80 ± 1.35                    |
| 36 h                  | 30.05 ± 0.76                    | 40.31 ± 0.92                    |
| 48 h                  | 32.96 ± 0.48                    | 40.08 ± 0.23                    |
Figure 6. Antioxidant activity of time-controlled fermented rice bran at concentration 1 mg/mL.

Bioprocessing methods through microbial fermentation involving enzymatic reaction have been widely applied to release the chemical bounds, which can improve its free forms and bioavailability [15].

4. Conclusion

This study demonstrates the increasing of the total phenolic contents subsequently the antioxidant capacity of rice bran through microbial fermentation with lactic acid bacteria. The highest total phenolic content of fermented rice bran extract achieved after 48 h fermentation process with 10% of lactic acid bacteria used in medium. The strongest scavenging effects on the DPPH radical were found in rice bran after 48 h fermentation with both starters. The highest scavenging effect at 1 mg/mL was reached by rice bran fermented with *L. Plantarum*. The antioxidant activity of fermented rice bran extracts were positively correlated with their total phenolic contents. It suggested that fermentation of rice bran with lactic acid bacteria, could improve its functional properties. As the result, further study is required to determine the active phytochemicals in the extract of fermented rice bran.

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