Antioxidant activity and phytochemical analysis from black rice bran

Amirotul Muniroh¹, Slamet Budijanto¹³, Bambang Pontjo Priosoeryanto²

¹Food Science and Technology Department, Faculty of Agricultural Technology, Bogor Agricultural University, Bogor 16680, Indonesia e-mail: amirotul.muniroh@gmail.com
²Division of Pathology; Department of Veterinary Clinics, Reproduction and Pathology (CRP), Faculty of Veterinary Medicine, Bogor Agricultural University, Bogor 16680
³Center for South East Asia Food and Agricultural Science and Technology (Seafast Center), Bogor Agricultural University, Bogor 16680, Indonesia

Abstract. Black rice bran is a one of potential antioxidant component source. Black rice bran bioactivity is better than white rice bran. However, the high bioactivity of black rice bran is not accompanied by its utilization as a functional food ingredient. The ability of R. oryzae to degrade lignocellulose and polysaccharide matrix through its enzyme activity is proven to increase antioxidant activity on rice bran variety of white rice. The capability of these molds on the black rice bran matrix is expected to maximize the potential functional properties of antioxidants. This study aims to determine the content of phytochemicals and antioxidants from black rice bran and the comparison between before and after being given fermentation treatment with addition of Rhizopus oryzae. In this study, black rice bran was given two different treatment that is non-fermentation and fermentation using Rhizopus oryzae for 96 hours. After the cultivation period, each sample was extracted with methanol solvent. Further phytochemicals including flavonoid, alkaloid, tannin, saponin, quinone, steroid and triterpenoids were evaluated by qualitative phytochemical tests while the antioxidant capacity was evaluated by DPPH assay. The results showed that the fermented black rice bran samples contained flavonoids, saponins and triterpenoids while non-fermented black rice bran sample contained flavonoids, tannins and steroids. Antioxidant activity of fermented black rice bran (67.37%) was more effective to inhibit free radicals than non fermented black rice bran (43.93%). According to the result, studies are needed to produce a black rice bran functional food product that can be used as a source of food containing bioactive compounds and antioxidants.

Keywords: Black Rice Bran, Rhizopus oryzae, Phytochemical, DPPH

1. Introduction

Black rice bran is a one of potential antioxidant component source. Black rice bran bioactivity is better than white rice bran. However, the high bioactivity of black rice bran is not accompanied by its utilization as a functional food ingredient. Fermentation process have been studied for many decades. Simple technique for the production of bioactive compounds was solid state fermentation. It is helps reduce the environmental impact of their disposal and economically viable due to the use of agro-industrial residues [8,11]. Degradation of structural components of the cell wall during fermentation is proven to be able to free phenolic bound and increase free phenolic components. The high antioxidant capacity in fermented black rice bran samples is thought to be a consequence of the increase in free...
phenolic components such as ferulic acid, anthocyanin, and oryzanol. According to [6,9], ferulic acid as the main phenolic compound in black rice bran have been reported to act as radical scavengers against free radical. The ability of R. oryzae to degrade lignocellulose and polysaccharide matrix through its enzyme activity is proven to increase antioxidant activity on rice bran variety of white rice. The capability of these molds on the black rice bran matrix is expected to maximize the potential functional properties of antioxidants. This study aims to determine the content of phytochemicals and antioxidants from black rice bran and the comparison between before and after being given fermentation treatment with addition of Rhyzopus oryzae.

2. Material and Methods
2.1. Fermentation of the rice bran
2.1.1. Preparation of the rice bran. Black rice bran (cempo ireng variety) as the main ingredient, was obtained in the milled dry grain from Cigudeg farmers, Bogor, Indonesia. The grain was peeled by milling it with huller machine and then put on a Satake polisher machine for 1.5 minutes every 200 grams. From this process, two outputs will be obtained rice and mixture of rice bran. The mixture of rice bran was sieved with a 20 mesh sieve. The obtained black rice bran is ready to proceed to the next stage.

2.1.2. Preparation of Inoculum. The fungus R. oryzae from IPB culture collection were cultured in PDA (potato dextrose agar) media. R. oryzae culture used in the fermentation process is a culture that is 7 days old and has entered the sporulation phase so that the mold isolate must be refreshed before use. The fungus culture refresher technique is one ose of each mold culture from PDA media as long as it is scratched on to oblique the new PDA media. The new PDA media was incubated for 7 days at 30 °C. After 7 days, the mold inoculum is ready to be used as a spore suspension at the stage of the fermentation process. The spore were spread by adding 10 mL of an sterile distilled water then the spores counted to reach 106 spores/mL. In the fermentation process, the suspension solution is ready to be inoculated into the black rice bran substrate [10].

2.1.3. Fermentation process. The fermentation technique used in this study is solid media fermentation. The fermentation process begins with a petri dish which is entered by 50 grams of black rice bran and added with 25 mL of water (50% of the weight of the substrate) [10]. Bran substrate was sterilized at 121°C for 15 minutes using autoclave. Furthermore, the substrate is cooled at 30°C and the substrate is ready for the inoculation stage. Fifteen percent of spores (106 spores/mL) from each liquid R. oryzae culture were inoculated into petri dishes containing sterile rice bran then stirred evenly with a sterile spatula. The inoculated rice bran was incubated at 30°C for 4 days (96 hours) [12].

2.2. Phytochemical Analysis
2.2.1. Flavonoid. Black rice bran was added distilled water, then heated at 100°C for 3-5 minutes. After cold, the solution is filtered. Ethanol was added to the filtrate, Mg powder, amyl alcohol and concentrated HCl. the filtrate mixture is shaken homogeneously. A positive test for flavonoids is indicated by the orange color in the solution.

2.2.2. Alkaloid. Black rice bran added NH3 and NHCl3, then shaken homogeneously and filtered. The filtrate is added with H2SO4 2 M, then shaken homogeneously. The acid layer (the top) is pipetted in another test tube. Alkaloid positive test is indicated by the formation of precipitated orange to brown red after penetrated by Dragendorf reagent, white precipitate after Meyer reagent drops or deposition of light brown to yellowish after Wagner reagent drops. The standard used is Tapak Dara leaves.

2.2.3. Tannin. Black rice bran was added distilled water, then heated at 100°C for 3-5 minutes. After cold, the solution is filtered. The resulting filtrate was added with FeCl3 10%. Blackish green solution indicates tannins.
2.2.4. Saponin. Black rice bran was added distilled water, then heated at 100°C for 3-5 minutes. After cold, the solution is filtered. The filtrate is shaken vigorously. The positive saponin test is indicated by the formation of a stable foam for 30 seconds.

2.2.5. Quinon. Black rice bran was added metanol, then heated at 100°C for 3-5 minutes. After cold, the solution is filtered. The filtrate was added NaOH 10%. The positive quinon test is indicated by the red color in solution. Steroid and triterpenoid. Black rice bran was added etanol, then heated at 100°C for 3-5 minutes. After cold, the solution is filtered. The filtrate is heated until the extract is produced. The extract was added diethyl eter and shake homogeneously. The solution is poured into pourcelain, was added H2SO4 and CH3COOH anhydrous. A positive test for steroid is shown when it color was blue or green. While purple or orange color was positive result for triterpenoid.

2.3. Antioxidant activity of black rice bran

2.3.1. Extraction process. The extraction techniques used is maceration. Black rice bran were place in an erlenmeyer and added methanol solvents. During extraction process, erlenmeyer was covered by aluninium foil. This process was carried out for 3 hours with orbital platform shaker innova 2300 (New Brunswick Scientific, USA) at room temperature. After that, for 15 minutes, black rice bran were resuspend in an ultrasonic bath (Branson 8510) with distilled water. Furthermore, the extract obtained was filtered through filter paper (Whatman no. 1). After centrifugation (10 m, 1791 g, room temperature), filtrate was separated and put into a 100mL flask. The extract has been evaporated with Rotavapor (R-300 BUCHI, Switzerland) at temperature 50°C until all solvent is evaporated.

2.3.2. DPPH assay. The antioxidant activity of black rice bran was measured by the reduction of free radicals 1,1-diphenyl-2-pikrihidrazil (DPPH) according to the method described [1]. The DPPH radical scavenging capacity of samples was defined as Trolox Equivalent Antioxidant Capacity (mg TEAC per g dry weight samples). The DPPH radical scavenging capacity assay was performed by 96 well plate method. Each sample (20 µL) were transferred into 96 well plate and added working solution DPPH (180 µL). Furthermore, the sample incubated at the dark room temperature for 40 min. The absorbance was measure by Epoch microplate spectrophotometer (BioTek, USA) at 515 nm. The percentage of DPPH radical scavenging was measured by following equation:

\[
\% \text{Scavenging} = \left(1 - \frac{A_{\text{sample}}}{A_{\text{control}} - A_{\text{blank}}} \right) \times 100
\]

This method begin with is based on the transfer of electrons from one antioxidant substance to a free radical, DPPH, which loses its purple color upon reduction, becoming yellow.

2.4. Statistical

One way Analysis of Variance (ANOVA) test was used to determine significant differences between variable. Differences with a probability value of <0.05 were considered significant and all data were reported as mean ± sd.

3. Result and discussion

3.1. Phytochemical Analysis

Phytochemical compounds are natural bioactive compounds produced by secondary metabolites found in plants that act as nutrients and natural fibers that can prevent disease. Phytochemical compounds commonly found in plants, namely alkaloids, flavonoids, quinones, tannins, polyphenols, saponins, steroids and triterpenoids [5]. In this study, analysis of black rice bran phytochemicals was carried out based on [5]. Identification carried out is the test of alkaloids, flavonoids, saponins, tannins, quinones, steroids and triterpenoids. Fermented black rice bran is shown positive result to flavonoid, saponin and
triterpenoid compounds. Meanwhile unfermented black rice bran is shown positive result to flavonoid, tannin, and steroid compounds. Flavonoids are commonly found in plants, bound to sugar as glycosides and aglycone flavonoids (sugar-bound flavonoids) are present various forms of structure [7]. It is known that antioxidant activity from plants is due to their presence phenol compounds. The antioxidant effect of this compound is caused by capture free radicals through donor hydrogen atoms from flavonoid hydroxyl groups. Antioxidants are compounds that protect cells against damage caused reactive oxygen. The imbalance between antioxidants and reactive oxygen resulting in oxidative stress, which causes cell damage [3].

| Parameter   | Fermented Black Rice Bran | Non-fermented Black Rice Bran |
|-------------|---------------------------|-------------------------------|
| Flavonoid   | Positive                  | Positive                      |
| Alkaloid    | Wagner Negative           | Negative                      |
| Mayer       | Negative                  | Negative                      |
| Drugendorf  | Negative                  | Negative                      |
| Tannin      | Negative                  | Positive                      |
| Saponin     | Positive                  | Negative                      |
| Quinon      | Negative                  | Negative                      |
| Steroid     | Negative                  | Positive                      |
| Triterpenoid| Positive                  | Negative                      |

Phytochemical compounds play an important role in maintaining health. These compounds complement each other in the mechanism of action that occurs in the body. Phytochemical compounds have antioxidant activity, detoxification by enzymes, stimulation of the immune system, hormone metabolism and antibacterial and antiviral. Several studies have shown that phytochemicals are contained in fruits, vegetables and nuts. These bioactive components can inhibit the process of premature aging and reduce the risk of various diseases, such as cancer, liver disease, stroke, high blood pressure, cataracts, osteoporosis and gastrointestinal infections [4].

3.2. Antioxidant Activity

The antioxidant activity of the black rice bran was evaluated based on [1] method. Primary antioxidant or "chain-breaking antioxidant" acts as a free radical scavenger through termination of a chain oxidation reaction by donating H atoms to free radical compounds to produce more stable products[2].
Antioxidant activity of fermented black rice bran (1) was more effective to inhibit free radicals than non-fermented black rice bran (2). During fermentation, degradation of structural components of the cell wall is proven to be able to free phenolic bound and increase free phenolic components. The high antioxidant capacity in fermented black rice bran (67.37%) is thought to be a consequence of the increase in free phenolic components such as ferulic acid, oryzanol, and anthocyanin. Phenolic components such as ferulic acid (the main phenolic compound in black rice bran) have been reported to act as radical scavengers against radical DPPH [6,9].

4. Conclusion

Fermented black rice bran contained flavonoids, saponins and triterpenoid while non-fermented black rice bran sample contained flavonoids, tannins and steroids. Antioxidant activity of fermented black rice bran was more effective to inhibit free radicals than non fermented black rice bran.

References

[1] Bobo-Garcia G, Daidov-Pardo G, Arroqui C, Virseda P, Marin-Arroyo M R and Navarro M 2014 Intra-laboratory validation of microplate methods for total phenolic content and antioxidant activity on polyphenolic extracts, and comparison with conventional spectrophotometric methods Journal Science Food Agriculture 95(1) 204-209

[2] Craft B C, Kerrihard A L, Amarowicz R and Pegg R B 2012 Phenol-based antioxidants and the in vitro methods used for their assessment Compr Rev Food Sci Food Saf 11 148-173

[3] Cristobal M and Donald R 2000 Aktivitas Antioksidan Flavonoid (Oregon: Oregon State University Pr.)

[4] Hamburger M and Hostettmaun K 1991 Bioactivity in plants: the link between phytochemistry and medicine Phytochemical 30(12) 3864-3874.

[5] Harborne J B 1987 Metode Fitokimia (Bandung: Institut Teknologi Bandung)

[6] Jun H I, Shin J W, Song G S and Kim Y S 2015 Isolation and identification of phenolic antioxidants in black rice bran Journal of Food Science 80(2) C262-C268

[7] Markham K R 1988 Techniques of Flavonoid Identification (London: Academic Pr.)

[8] Oliveira M S, Kupski L, Feddern V, Cipolatti E, Badiale-Furlong E, and Souza-Soares L A 2010 Physico-chemical characterization of fermented rice bran biomass CyTa - Journal of Food 8 236–269
[9] Razak D L A, Rashid N Y A R, Jamaluddin A, Sharifudin S A and Long K 2015 Enhancement of phenolic acid content and antioxidant activity of rice bran fermented with Rhizopus oligosporus and Monascus purpureus Biocatal Agric Biotechnol 4 33-38.

[10] Schmidt C G, Goncalves L M, Prietto L, Hackbart H S and Furlong E B 2014 Antioxidant activity and enzyme inhibition of phenolic acids from fermented rice bran with fungus Rizhopus oryzae Food Chemistry 146 371-377

[11] Schmidt C G, and Furlong E B 2012 Effect of particle size and ammonium sulfate concentration on rice bran fermentation with the fungus Rhizopus oryzae Bioresource Technology 123 36-41.

[12] Zulfafamy K E, Ardiansyah and Budijanto S 2018 Antioxidative properties and cytotoxic activity against colon cancer cell widr of Rhizopus Oryzae and Rhizopus Oligosporus-fermented black rice bran extract Curr Res Nutr Food Sci 6(1)

Acknowledgments
Author thank to Direktorat Jenderal Pendidikan Tinggi RI for financial support.