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Defatting and Fermentation Treatment on The Bioactive Compounds of Rice Bran

Febiana Putri Ramadhan[1], Slamet Budijanto[2], and Ardiansyah[1]*

[1]*Department of Food Technology, Universitas Bakrie, Jl.H.R.Rasuna Said Kav. C-22 Kuningan, Jakarta 12920, Indonesia*

[2]*Department of Food Science and Technology, Faculty of Agricultural Engineering and Technology, IPB University, Jalan Raya Dermaga, Bogor 16680, Indonesia*

**Corresponding author's E-mail address: ardiansyah.michwan@bakrie.ac.id*

Abstract— Rice bran is a by-product of brown rice milling process. The aim of this study was to determine the effect of fermentation on the changes of total phenolic content, antioxidant activity, and bioactive components in rice bran with the combination of defatting treatment. Coloured rice bran Inpari 30 (white), Inpari 24 (red), and Cempo Ireng (black) were used in this study. Total phenolic content (TPC) and antioxidant activity were analysed with Folin-Ciocalteau and DPPH radical scavenging activity (RSA) method, respectively. Bioactive component of rice bran was analysed by HPLC. The highest TPC and RSA were shown on defatted and fermented white rice bran (288.18±2.52 mg GAE/100 g DB and 67.95±0.75%, respectively). The highest γ-oryzanol and ferulic acid were shown in non-defatted fermented black rice bran and defatted fermented black rice bran with 24.83 mg/g and 1.45 mg/g sampel, respectively. The results of this study indicated that the fermentation treatment could increase the bioactive component of rice bran. Furthermore, combination of defatting and fermentation was only effective on white rice bran.

Keywords— **antioxidant activity, bioactive components, fermentation, rice bran, total phenolic content.**

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INTRODUCTION

Rice bran is containing 10% of total weight of rice from milling process and mostly used as animal feed ingredient. However, lipid content of rice bran can be extracted to produce rice bran oil (Gul et al., 2015). Rice bran can be utilized for human health by producing rice bran oil (Issara and Rawdkuen, 2016). Rice bran has perishable properties which are mostly caused by the oxidation process. Whole rice bran contains more than 20% lipid. Oxidation might occur during milling process and storage due to the activity of lipase and lipoxygenase enzymes. Lipase can hydrolyse triglycerides as primary lipids into glycerol and free fatty acids. The increase of free fatty acids reduces pH and increase the acidity which caused rancidity. Lipoxygenase oxidizes free fatty acids into peroxides to support peroxidation which leads to rancidity (Patil et al., 2016).

Process or method were carried-out to stabilized rice bran to inactivate lipase and lipoxygenase in rice bran such as heating and fat removal (defatting). Fat removal is usually done as soon as possible on the rice bran to prevent oxidation which results in rancidity (Wang et al., 1999). Defatted rice bran contains protein, vitamins, minerals, and more than 120 types of antioxidants (Geetha et al., 2015). The high content of antioxidants in defatted rice bran makes it has a good antioxidant activity. Enhancement of antioxidant properties in whole rice bran can be achieved by fermentation (Ardiansyah et al., 2019). The diverse nutritional content, mainly carbohydrates and polysaccharides, makes rice bran a good substrate for microbial growth. Defatted fermented rice bran was reported to increase the growth of good bacteria in the digestive system (Geetha et al., 2015).

Solid-state fermentation (SSF) was reported as the most optimal method of rice bran fermentation in increasing the nutritional content in rice bran (Schmidt et al., 2014; Rashid et al., 2015; Oliveira et al., 2010; and Oliveira et al., 2012). *Rhizopus* sp. is a type of fungus that is most used in solid-state fermentation to produce various products. It was reported that the total phenolic content and antioxidant activity in fermented white rice bran with *Rhizopus oligosporus* species increased significantly (Razak et al., 2015). There still no studies that show the changes in total phenolic content, antioxidant activity, and bioactive components in white, red, and black rice bran given the combination of defatting and fermentation treatment. Therefore, this study aims to determine the effect of rice

bran types, fermentation treatment with *Rhizopus oligosporus*, and defatting on the changes in total phenolic content (TPC), antioxidant activity, and bioactive components in white, red, and black rice bran.

MATERIALS AND METHODS

Rice Bran Preparation

Brown rice of Inpari 30 variety (white), Inpari 24 variety (red), and Cempo Ireng variety (black) were used from Indonesian Center for Rice Research, Indonesian Agency for Agricultural Research and Development, Ministry of Agriculture, Subang, West Java, Indonesia. To obtain rice bran, 100 g of the brown rice was put into the milling machine (Satake, Japan) for 1 minute. The bran is then collected and separated for two different treatments, defatting and non-defatting or whole bran.

Defatting

The defatting process was performed according to the method reported by (Sirikul, et al. 2009) with some modifications. White, red, and black rice bran were defatted by n-hexane 1:10 (w/v) for 2 hours using an orbital shaker, then macerated for 22 hours at room temperature. After that, the samples were filtered using a Buchner vacuum and dried at room temperature for 24 hours. Defatted white, red, and black rice bran were then sterilized using an autoclave at 121˚C for 15 minutes. Sterilized defatted rice bran then dried by freeze drying method for 24 hours and the results were stored at -18˚C.

Inoculum Preparation and Fermentation

R. oligosporus inoculum was prepared a week before the fermentation was carried out based on previous study (Ardiansyah et al., 2019). Whole and defatted rice bran was weighed by 20 grams using petri dish. Each sample added by 20% distilled water. After that, the sample was stirred and sterilized using autoclave at 121˚C for 15 minutes. The sample was stirred so that the inoculum spread evenly, then the sample was incubated at 30˚C with 72 hours of incubation time. To ensure the absence of contamination, the spores form that grew on the rice bran was observed using a microscope at 100 times magnification and using pure *R. oligosporus* culture as a comparison. The fermented and defatted fermented rice bran was then dried by freeze drying method for 24 hours and the results were stored at -18˚C.

Sample Extraction

The extraction process for the analysis of TPC and antioxidant activity was carried out by Razak et al. (2015) method with some modifications. A total of 1 g of sample was dissolved with 10 ml of distilled water and boiled for 15 minutes in ultrasonic bath. After that the sample was centrifuged at 10,000 rpm for 10 minutes. The supernatant was then filtered with Whatman paper No.1. For analysis of bioactive components, 500 mg white, red, and black rice bran was extracted by 10 ml of methanol each, then sonicated at room temperature for 60 minutes. After that, the methanol extract was filtered on 0.45 µm membrane filtration (Sabir et al., 2017).

Total Phenolic Content and Antioxidant Analyses

TPC analysis was conducted by Iqbal et al. (2005) method with some modifications. The sample extract (0.2 ml) was added with 0.8 ml of Folin-Ciocalteu solution, then stored at room temperature for 15 minutes. The solution was added with 2 ml of 7.5% sodium carbonate and stored again for 15 minutes. The solution was diluted to 7 ml using distilled water and placed in a dark room for 2 hours. The solution was then measured using a spectrophotometer at 765 nm. The standard used is gallic acid (60–360 mg/l) and the results were expressed with milligrams of gallic acid equivalent (GAE) per gram of dry sample (mg GAE / 100g sample).

Antioxidant activity was analysed based on the method used by Webber et al. (2014). A total of 100 µl of rice bran extract was added with 3.9 ml of DPPH methanolic solution (1,1-Diphenyl-2-picrylhydrazyl) 25 mg/L, then homogenized with Vortex shaker for 10 seconds and incubated in dark conditions for 30 minutes. The sample absorbance was measured by UV-VIS spectrophotometer at 515 nm and compared with the ascorbic acid standard (20-100 mg/ L). DPPH radical scavenging activity was determined by the following equation.

$$\text{DPPH (\%)} = \frac{Abs\ blank - Abs\ sample}{Abs\ blank} \times 100\%$$

Abs blank= DPPH solution absorbance
Abs sample= DPPH solution absorbance + sample extract

Bioactive Components Analysis

The bioactive components were analyzed based on the method used by Sabir et al. (2017). High performance liquid chromatography (HPLC) was used to detect bioactive components in rice bran, ferulic acid and γ-oryzanol. The 10 mg standard of ferulic acid and γ-oryzanol were each dissolved in 10 ml of methanol, then diluted to 100 ppm. The standard and rice bran extract were injected 20 µL into a C-18 reverse-phase column with 1 ml/minute flow rate. The mobile phase used were methanol (A) and acetonitrile with a gradient system of 35-65% A for 0-60 minutes. The separation process was carried out by UV detection at 325 nm.

Statistical Analyses

The data in this study were processed using variance analysis or ANOVA with the SPSS 16.0 program. If the test has significant differences at the confidence level of α=0.05, then a further test would be carried out with Duncan's different test.

RESULTS AND DISCUSSIONS

Total Phenolic Content

The results of the analysis of (TPC) were shown in Table 1. Defatting and fermentation increased the TPC of white rice bran significantly, whereas red and black rice bran were not significant. White rice bran, the highest TPC was observed on defatted fermented white rice bran, 288.18 ± 2.52 mg GAE/100 g of DB. The highest TPC in red rice bran and black rice bran were observed on non-defatting and

fermentation treatment and control (294.63±5.19 mg GAE/100 g DB and 306.12±5.03 mg GAE/100 g DB, respectively).

As shown in Figure 1, TPC of black rice bran was higher than white and red rice bran. The results also showed that there was influence of rice bran varieties on the TPC as reported by Moongngarm et al. (2012). Rice bran varieties significantly affected the TPC of control samples and those treated with defatting and non-fermentation process. The results of the study by Razak et al. (2015) showed that there was an increase on the TPC of white rice bran fermented with *R. oligosporus*. This occurred due to the activity of mold in rice bran which was capable in enzymes production such as β-glucosidase, α-amylase, xylanase, protease, and lipase. These enzymes helped to break the phenolic compounds that were bound to the constituent components of cell walls such as cellulose, fat, and protein. It was reported that β-glucosidase was the main enzyme produced by the *R. oligosporus*. This enzyme was capable to hydrolyze the glycosidic bonds between carbohydrates or between carbohydrates and non-carbohydrates, such as the binding of carbohydrates and phenolic compounds in rice bran, so that phenolic compounds were released and their availability in rice bran increased.

Table 1. Total phenolic content of rice bran

Treatment combinations	Total phenolic content (mg GAE/100 g DB)		
	Rice bran types		
	White	Red	Black
No defatting, no fermentation (control)	188.51±1,17[a]	282.19±5.15	306.12±5.03
Defatting, no fermentation	207.97±0,62[b]	272.61±9.50	296.56±5.24
No defatting, fermentation	273.76±3,86[c]	294.63±5.19	298.2±1.71
Defatting, fermentation	288.18±2,52[d]	289.41±2.45	296.07±1.12

Each value is expressed as the mean±SD. The values in each column with the same letter are not significantly different at the level of 0.05 (p>0.05). DB (dry basis).

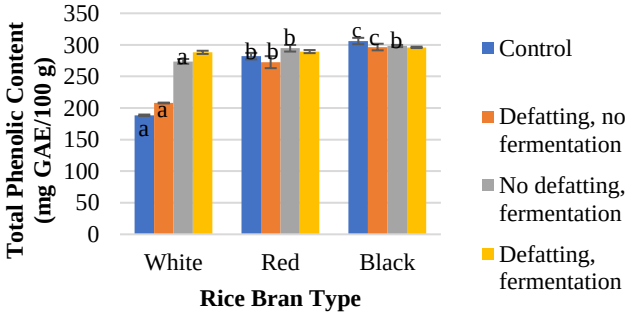

Figure 1. Effect of rice bran type to the total phenolic content. Data shown as mean±SD

In this study the TPC was not significantly different in red and black rice bran. Based on the results of the physical characteristic, there were indications that the growth of fungi in red and black rice bran was not as good as in white rice bran. This could be caused by the high content of anthocyanin and anthocyanidin which have antimicrobial properties (Suket et al., 2012) so that the growth of fungi was less optimal, and the amount of β-glucosidase did not help enough in releasing phenolic compounds from the bonds with the matrix. To optimize the content of phenolic compounds in red and black rice bran, fermentation can be carried out with other microbes that support the activity of other enzymes that are higher than β-glucosidase. The results also showed that red and black rice bran given defatting treatment had lower TPC compared to fermented rice bran and control. From these results it was shown that the defatting treatment was not suitable to increase the TPC of red and black rice bran. This occurred because of the differences in the composition of the cell wall between white, red, and black rice bran.

Antioxidant Activity

Radical scavenging activity (RSA) of white rice bran increased in each treatment, with the highest value 149.88±2.77% in the defatting and fermentation treatment.

Table 2. The enhancement of antioxidant activity of rice bran

Treatment combinations	Enhancement of antioxidant activity (%)		
	Rice bran types		
	White	Red	Black
Defatting, no fermentation	27.38±21.78	6.26±14.07	15.05±6.77
No defatting, fermentation	58.68±14.18	11.88±21.11	2.74±0.19
Defatting, fermentation	149.88±2.77[b]	-17.17±36.77[a]	-5.20±14.12[a]

Each value is expressed as the mean±SD. The values in each column with the same letter are not significantly different at the level of 0.05 (p > 0.05).

This was different from red and black rice bran which antioxidant activity have decreased in defatting and fermentation treatments. The decreased in antioxidant activity could be caused by less optimality of the fungi used in breaking antioxidant compounds during fermentation and due to the antioxidant compounds damage during defatting process. It was also shown in Table 2 that the antioxidant activity of the rice bran increased by the fermentation process.

Correlation coefficient between antioxidant activity and TPC in white, red, and black rice bran was 0.44 (Figure 2A). This value was so small that it could be said that phenolic compounds were not the dominant compounds in increasing the antioxidant activity in rice bran. The correlation coefficient for white rice bran was 0.80 (Figure 2B). The correlation value between antioxidant activity and TPC was higher compared to the results obtained by Razak et al. (2015). This shown that most of the phenolic compounds

contained in white rice bran contribute to their antioxidant activity.

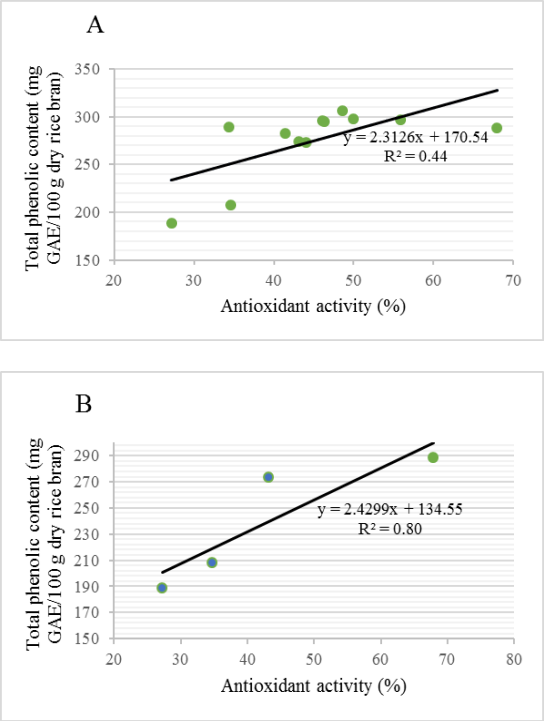

Figure 2. A. Correlation between antioxidant activity and TPC. B. Correlation between antioxidant activity and TPC of white rice bran. Values are shown as the mean±SD. The coefficient of correlation (R2) values, used to determine the relationships between two variables

Hydrolytic enzymes produced by fungi during fermentation such as β-glucosidase, protease, lipase and phenolic esterase could increase the availability of free hydroxyl groups on phenolic structures so that the content of phenolic compounds in rice bran increases. The hydroxyl group of the phenolic compound becomes a hydrogen donor for DPPH free radicals so that the more phenolic compounds existed, the more free radicals were captured, the stronger the antioxidant activity (Bhanja et al., 2009; Razak et al., 2015).

Bioactive Compounds

The chromatogram (Figure 3) shown that the standard γ-oryzanol has four peaks that shows its constituents, which are ferulate cycloartenol, ferulate cyclobranol, ferulate campesterol, and ferulate β-sitosterol (Sabir et al., 2017). The γ-oryzanol content can be calculated by summing the levels of each of the constituent compounds. Table 3 shows that the treatment of fermentation without defatting could increase significantly γ-oryzanol content in all types of rice bran ($P<0.05$). The fermentation process increased the release of the constituent compounds by the mechanism of enzyme hydrolysis which was like the process of increasing the antioxidant activity. This indicated an association between γ-oryzanol levels and antioxidant activity of rice bran.

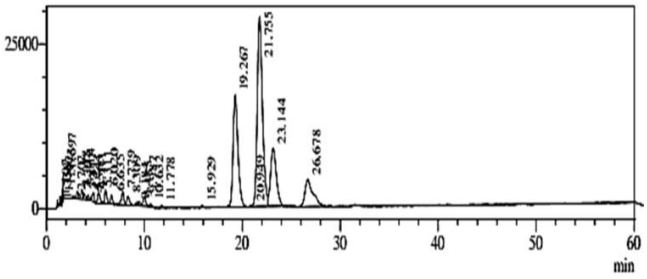

Figure 3. Standard γ-oryzanol chromatogram

In Table 3 it is shown that the content of γ-oryzanol decreased in rice bran which was given defatting treatment. This could be caused by several γ-oryzanol carried by n-hexane during the defatting process. This indicates that naturally γ-oryzanol was in the lipid matrix in white, red, and black rice bran. Beside γ-oryzanol, rice bran has another antioxidant compounds such as ferulic acid, tocopherol, tocotrienol, cafeic acid in white rice bran, proanthocyanidin in red rice bran, and anthocyanin in black rice bran (Gul et al., 2015; Thitipramote et al., 2015).

Table 3. The content of γ-oryzanol in white, red, and black rice bran

Treatment combinations	γ-oryzanol (mg/g DB)		
	Rice bran types		
	White	Red	Black
No defatting, no fermentation (control)	8,60±0.59[b]	13,83±1.99[b]	19,90±5.09[b]
Defatting, no fermentation	2,17±0.06[a]	3,54±1.71[a]	3,63±1.16[a]
No defatting, fermentation	14,14±3.19[c]	18,89±0.85[c]	24,83±5.25[b]
Defatting, fermentation	2,09±0.02[a]	2,46±0.66[a]	5,37±1.68[a]

Each value is expressed as the mean ± SD. The values in each column with the same letter are not significantly different at the level of 0.05 ($p > 0.05$). Dry basis (DB).

CONCLUSIONS

There was a change in TPC, antioxidant activity, and bioactive components (γ-oryzanol) in white, red, and black rice bran which were treated with defatting and fermentation even though the defatting process did not provide a significant change in these parameters. The combination of defatting and fermentation treatment only had a significant effect on white rice bran.

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