Food ScienTech Journal Vol. 2 (1) 2020 ISSN : 2685-4279 DOI:10.33512/fsj.v2i1.7563

IDENTIFICATION OF TOTAL PHENOLIC AND ANTIOXIDANT ACTIVITY OF FERMENTED RICE BRAN EXTRACTED BY ELECTROLYZED WATER

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ABSTRACT

Fermentation of rice bran is applied to facilitating the release of and may lead to higher yields of bioactive compound in its extraction product. The use of electrolyzed water as the solvent and the influence of this water on antioxidant activity of the fermented rice bran extract compounds is unclear. The bran of white (Jalawara) and red (Marahmay) rice from Banten Province are fermented using R. oligosporus and extracted using electrolyzed water at 2.5, 6.5, 7, 9.5, and 11.5 of pH value. This research presents that Marhmay contains more active antioxidant compounds with 422.11 ppm of IC50 by DPPH evaluation method and effectively extracted by electrolyzed water at pH 2 yielded of 3% extracted phenol more than water at pH 7. Furthermore, this treatment gives insights on the potency of electrolyzed water as solvent on extraction method of biological compound.

Keywords: Fermented Rice Bran, electrolyzed water, Antioxidant, Phenol

INTRODUCTION

The availability of rice bran in Indonesia is high since rice is the staple food of the citizen and paddy is the agricultural main crop. This is the favorable condition regarding to the fact that rice bran extracts may have the potential to be further exploitation as antioxidant rich products (Razak *et al*. 2017; Razak *et al*. 2014).

Rice bran is solid substrate by product of rice milling. Even though it consists of nutritive and functional component, the existences of the components are trapped on organic matrix of the bran. Fungal fermentation on enhanced the nutritional qualities and antioxidant activities on agricultural-by product solid substrates followed by both antioxidant activity and phenolic acid content of rice bran is also enhanced by solid-state fermentation using fungi (Razak *et al*, 2014). Enzymatic reaction on fermentation process also could derive the derivative components that could have the highest biological functionality (Abubakr, 2012; Korhonen and Pirlanto, 2006).

The changes of physicochemical properties of magnetized water (Mosin and Ignatov, 2014; Hasaani. *et al*, 2015) could be effective on extracting the bioactive compounds as well as done on partially remove hemicelluloses in the cell wall of Miscanthus using alkaline and acid electrolyzed water (Wang *et al*, 2009).

This research elaborates the effect of electrolyzed water in different pH value on fermented rice bran (FRB). Bioactive substance of red rice (Marahmay) and white rice (Jalawara) especially phenolic compounds are evaluated both the quantity and the quality of its antioxidative activity using DPPH assay.

MATERIALS AND METHODS

Tools and Materials

Rice bran were taken from two local varieties of paddy those are cultivated in Banten. Jalawara paddy was collected from Lebak Distric and Marahmay was collected from Pandeglang Distric, Banten Province, Indonesia. Rhizopus oligosporus obtained from Collection of Laboratory of Microbiology, PAU, Bogor Agricultural

University. The fungi were maintained on PDA (Potatoes Dextrose Agar) until sporulating stage (7 days incubation) before it inoculated on the rice bran. Electrolyzed water obtained using LeveLuk JRII which could produce the water with pH 2.5, 6.5, 7, 9.5, and 11.5.

Fermentation and Extraction

Rice bran (RB) was weighed and added by water until 30% from its dry basis mass. The RB is autoclaved and then inoculated by R. oligosporus. The mixing of RB and inoculants is fermented in incubator at 370C for three days to be the Fermented Rice Bran (FRB). The FRB than soaked by electrolyzed water using the ratio of 1:3 for dry basis FRB : electrolyzed water. The soaking is done in incubator at 600C overnight. Next, the liquor is extracted using vacuum pump. The extract was than lyophilized and both of the dry extract and precipitate were stored at -180C for further analyzes.

Proximate and total phenol

The Folin–Ciocalteu methodology was used to determine the total phenolic content in each sample. A 1 ml aliquot of the samples was allowed to react with 5 ml of Folin–Ciocalteu reagent and 4 ml of 7.5% sodium carbonate solution for 2 h at room temperature and in dark condition. Absorbance was measured at 765 nm using a spectrophotometer and the results were expressed as lg gallic acid equivalent (GAE)/gram sample.

Antioxidative test by DPPH

The antioxidant activity of the extracts was measured on the basis of the scavenging activity of the stable 1, 1-diphenyl 2-picrylhyorazyl (DPPH) free radical according to the method described by Brand-Williams with slight modifications. 1ml of 0.1mM DPPH solution in methanol was mixed with 1ml of plant extract solution of varying concentrations (50,100, 150, 200 and 250 μg/ml). Corresponding blank sample were prepared and L-Ascorbic acid (1-100 μg/ml) was used as reference standard. Mixer of 1ml methanol and 1ml DPPH solution was used as control. The decrease in absorbance was measured at 517nm after 30 minutes in dark using UV-Vis spectrophotometer. The inhibition % was calculated using the following formula.

%inhibition=((As-Ac))/Ac 100%

Where Ac is the absorbance of the control and As is the absorbance of the sample

RESULT AND DISCUSSION

Proximate analysis was done before the bran is inoculated and fermented by R. oligosporus. Table 1. present the result of proximate analysis of Marahmay and jalawara rice bran before it is fermented.

Table 1. Composition of Pre-Fermentation Rice Bran

Rice bran Type	Dry Basis Composition (%)				
	Ash	Fat	Protein	Carbohydrate	Total Phenol
Jalawara	4.65	8.14	13.95	74.42	0.35
Marahmay	13.19	12.09	9.89	63.74	0.26

The macromolecule components of these two varieties are different in all items and become the basis on the yield prediction of the extract. Rice bran could be extracted for many technical methods, but in general it is divided into two basic, polar and non-polar. Non-polar basic method is done to extract the oil component such as tocopherols, tocotrienols, and γ-oryzanol (Zhang, 2010). Polar basic method is done to extract components such as phenolic compounds, anthocyanins, and also flavonoid (Kapcum, 2016; Muntana and Prasong, 2010).

The use of electrolyzed water in this research was to evaluate its effectiveness on extracting not only polar compounds but also non-polar compounds because of its electromagnetic power changes which could interact with. Table 2. presents the composition of precipitate and extract after fermentation.

The total phenol content of precipitate or waste of extracted FRB (Fermented Rice Bran) is higher than non-fermented/pre-fermented rice bran and it indicates that fermentation is improve the quality of biological compounds composition (Razak *et al*, 2014; Oliveira, 2010). Moreover, this result also informs that more pH values of electrolyzed water shift

toward neutral the extracted phenol becomes more seditious.

Table 2. Comparison of Phenol composition between Precipitate, Extract and its antioxidative activity

No	Variety	pH	Precipitate	Extract		
			Total phenol (%)	Solid content (g/ml)	Extracted Phenol (%)	IC50 (ppm)
1	Marahmay	2,5	0.16	0.0467	0.30	422.11
2	Marahmay	6.5	0.22	0.0608	0.24	500.03
3	Marahmay	7.0	0.33	0.1097	0.13	>800.00
4	Marahmay	9.5	0.46	0.0470	0	>800.00
5	Marahmay	11,5	0.16	0.0404	0.30	689.61
6	Jalawara	2,5	0.22	0.0980	0.29	>800.00
7	Jalawara	6.5	0.51	0.1095	0	653.71
8	Jalawara	7.0	0.51	0.1593	0	>800.00
9	Jalawara	9.5	0.26	0.1095	0.25	>800.00
10	Jalawara	11,5	0.25	0.1589	0.26	>800.00

Marahmay FRB that was extracted using electrolyzed water at pH value 2.5 and 11.5 give the least mass of solid content, 0.0467 g/ml and 0.0404 g/ml. Otherwise, the extract from electrolyzed water at pH value 2.5 and 11.5 give the highest yield of extracted phenol, 0.3% of each. The same pattern also presented in yield of extracted phenol of Jalawara at the pH 2.5 and 11.5. In these pH values, the yield of phenol is also the highest on number of 0.29% and 0.26% respectively.

Mosin and Ignatov (2014) hypnotized that water magnetization influences three condition of water: purifying water because of coagulation and precipitation of metallic ion, decreasing of ionic solubility because of water-ionic polarization, and deformation of water molecule because of dissolution of hydrogen bonding between molecule and polarization of OH- and H+. Deformation of water causes the change in density, surface extension, viscosity, pH value, and other physicochemical properties (Mosin and Ignatov, 2014; Hasaani. *et al*, 2015).

This change of molecule formation and properties facilitate the water to interact with the polar component such as phenolic compounds so that in pH 2.5 and 11.5 of electrolyzed water yielded the highest amount of extracted phenol. This pH value also renders the breakage of hemicelluloses in rice bran which lead the effectiveness of the extraction (Wang *et al*, 2010). This easiness of extraction also is facilitated by the enzymatic breakage of carbohydrate chain in cell wall because of enzymes excretion by the inoculants (Jones, *et al*. 1992).

IC50 was used to describe the strength of antioxidant activity of the FRB water extract. The strength antioxidant activity of the Marahmay extract obtained by extraction with water at pH 2.5 gives the best result at 422.11 ppm. However, almost all of water extract of Jalawara give the weak antioxidant activity. Only the extract from pH 6.5 which showed the concentration of IC50 under 800 ppm. This condition is expected that the biological compounds between Marahmay and Jalawara are different. Marahmay as red rice variety is estimated contain more rich on antioxidative compounds (Zhang, *et al*, 2010).

CONCLUSION

Marahmay and Jalawara rice bran have different amount of macromolecule and total phenol composition. Fermentation by R. oligosporus improves the extracted phenol in fermented rice bran as describe by the result on yield of precipitate fermented rice bran extract. Electrolyzed water in both alkaline and acidic pH could extract more phenol at least 3% more higher than water at pH 7. Further work should be focused on analysis of the component in the extract beside phenolic compounds.

ACKNOWLEDGEMENT

This research is funded by 4 in 1 Project of Islamic Development Bank.

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