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2 Antioxidant Activity of Hydrophilic Extract Prepared from Defatted Rice Bran var. *Menthikwangi*¹⁾

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ABSTRACT

This research was aimed to measure antioxidant property of hydrophilic component extracted from defatted rice bran (DRB) of Menthikwangi local variety. The initial stage of the research was production of DRB by selected method of defatting and stabilization of rice bran. Defatting was carried out by maceration with material and solvent ratio of 1:4 (w/v), varied in washing methods of without-washing (WW) and washing with two kinds of material and solvent ratio of 1:2 w/v (WS_{1:2}) and 1:4 w/v (WS_{1:4}) to produce DRB with lowest fat content. Then, the selected methods used to produce DRB using various stabilization methods, which were without-stabilization (WS), microwave (MW) and oven (OV). The effectiveness of DRB's stabilization measured after 144 hours (35 °C) storage and extracted to get hydrophilic content extraction. Antioxidant activity of DRB hydrophilic extract was analyzed using radical DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging method. The results showed that WS_{1:4} has been selected as defatting method, while the effectiveness of stabilization both microwave (MW) and without stabilization (WS) method was not different. The highest antioxidant activity of 43.30% RSA (radical scavenging activity) was obtained from DRB hydrophilic extract without stabilization (WS).

Keywords : antioxidant, hydrophilic extract, defatted rice bran (DRB), defatting, stabilization

3 INTRODUCTION

Rice bran was a major by-product obtained from the polishing process that produces white rice. After extraction of the edible oil, defatted rice bran was used to reduce the final cost in animal feeds or was discarded as agricultural waste. However, it still contains significant amounts

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² of protein, carbohydrate, dietary fiber, and phenolic substances, which are beneficial as health promoting and functional substances in foods (Zhou *et al*, 2004; Saunders, 1985). The substances possess properties such as radical scavenging, antioxidative, and emulsifying activities (Iqbal *et al*, 2005; Hamid-Abdul and Luan, 2000). ³ Recently, these substances could be recovered by organic solvents, such as methanol and ethanol, and by supercritical carbon dioxide extraction (Iqbal *et al*, 2005; Kim *et al*, 1999). ³ Although these methods are convenient, they are time-consuming and sometimes produce toxic wastes after use.

Rice bran that easily deteriorate (rancid) due to lipase and lipoxygenase enzyme content, hindered to utilize as food material. Several measurements has been conducted to solve such problem, one of the most feasible among them was oil separation from the bran, resulting ¹⁴ rice bran oil and defatted rice bran (DRB). Many researchs related to rice bran oil published, whereas those related to DRB potential were still limited. Except its high content of fiber and free-fat bran of DRB, its was reported had high amount of bioactive component, such as phenolic acid, dominated by ferulic acid (Loukuldilok *et al*, 2011, Jung *et al*, 2007). Phenolic groups could be obtained by hydrophilic component extraction. Previous study indicated that hydrophilic extract from local variety Menthikwangirice bran had highest total phenol and antioxidant activity among other varieties. However whether the potential still remain in free-fat bran was yet to be known.

Various bran defatting method has been published with diverse results. Damayanthi and Listyorini (2006) made low fat bran powder with 2.13% fat content using hexane solvent that material and solvent ratio of 1:5, while similar research by Hadipernata *et al*. (2007) resulting 2.46% fat content. The minimal utilization of solvent in order to obtain DRB with lowest fat content was critical thus this research study should be do.

The process of bran stabilization has been commonly used for enzyme inactivation naturally present in bran to prevent deterioration-induced rancidity. However, the effect of stabilization method on effectiveness and antioxidant property of DRB hydrophilic component obtained were still unknown. Thus, the objectives of this research were to determine defatting method with minimum solvent and stabilization method to produce lowest free fatty acids indicating the effectiveness of stabilization, as well as to measure selected defatting and stabilization method on antioxidant activity of DRB hydrophilic extract.

MATERIAL AND METHOD

A. Materials

Material used in this research were bran from local rice variety of Menthikwangi obtained from farmer in Sawit sub district, Boyolali, and chemicals consist of hexane, methanol, 2,2-

diphenyl-1-picrylhydrazyl (DPPH) provided by Laboratory of Chemical and Biochemical, Faculty of Agricultural Technology, Gadjah Mada University at Yogyakarta.

B. Instruments

Research was carried out using microwave (Panasonic NNST 342 M), oven (Sanyo Drying oven Mov-112), waterbath, centrifuge, spectrophotometer (Spectronic 200 Thermo Scientific), test tubess, Erlenmeyer, beaker glass, and chemical analysis equipments.

C. Research Pathway

There were two stages of the research; DRB preparation and DRB hydrophilic extract evaluation. DRB preparation was started with defatting method selection (Figure 1) followed by bran stabilization method selection. Selection of defatting method was conducted to obtain defatting process with minimum solvent resulting DRB with fat content below 5%. The process was carried out through maceration with sample and solvent (hexane) ratio of 1:4 and washing variation of without washing (WW) and washing with sample ratio of 1:2 ($W_{S1:2}$) and 1:4 ($W_{S1:4}$). Fat content of DRB produced from each variation then analyzed using soxhlet method (Sudarmadji *et al.*, 2010). The selected of defatting method then applied in further stages of bran stabilization selection, which were stabilization using Microwave (MV), Oven (Ov) and without stabilization (WS). Stabilization using oven was conducted according to Nasir *et al.* (2009) where 50 g bran was heated in oven at 110°C for 15 minutes. Stabilization using microwave was following method by Mariod *et al.* (2010), where same amount of bran were heated using microwave for 2 minutes at 2450 MHz. The effectiveness of stabilization was analyzed using method by Damayanthi *et al.* (2002).

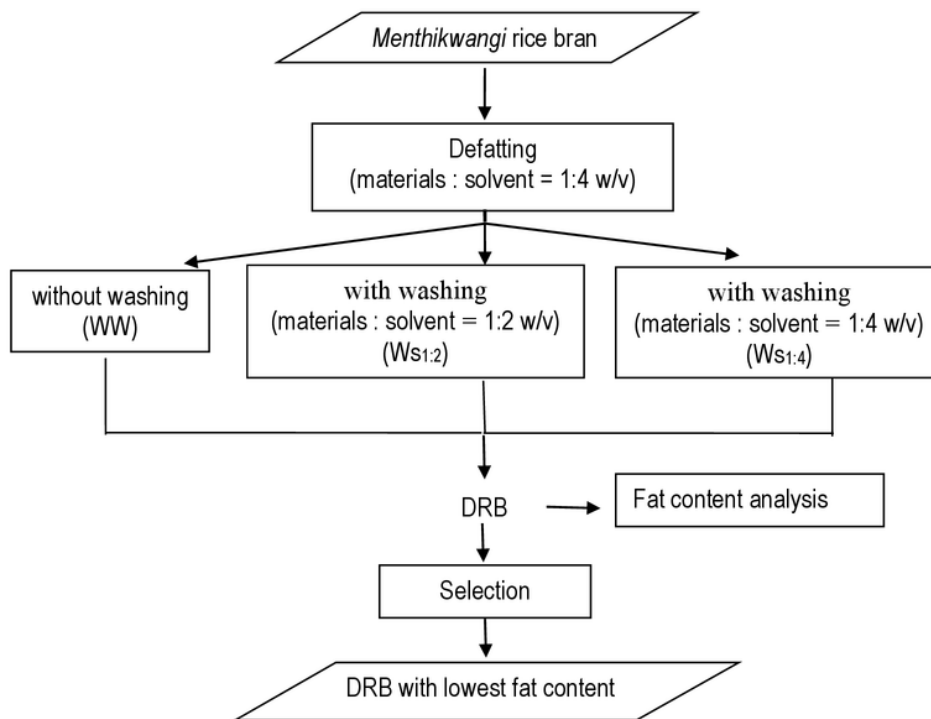


Figure 1. Defatting method selection to produce defatted rice bran (DRB)

The second stage of the research evaluated DRB hydrophilic extract obtained from previous stage to measure stabilization effect on antioxidant activity (Figure 2). The hydrophilic component of extraction was carried out according to Jang and Xu (2009), while antioxidant activity measured by DPPH radical scavenging method (Sompong *et al*, 2011).

Effectiveness of Stabilization Test (Damayanthi *et al*, 2002)

The effectiveness of stabilization of DRB was conducted by method used Damayanthi *et al*, (2002). Defatted rice bran (DRB) which resulted by each stabilization method kept in incubator at 35°C for 144 hours and analyzed the levels of free fatty acids (FFA) by extraction and titration method (Sudarmadji *et al*. 1994). Analysis of FFA was conducted both on immediately-stabilized bran (at 0-hour) and after DRB stored in incubator (35°C) during 144 hours. Percentage of FFA increase was measure using equation below:

$$\% \text{ FFA increase} = \% \text{ FFA at 144 hours} - \% \text{ ALB at 0 hour}$$

Extraction of DRB Hydrophilic component (Jang and Xu, 2009)

Hydrophilic component extraction was carried out according to Jang and Xu (2009), two times for each sample. Approximately 1 gram defatted rice bran was put into test tube (25×150 mm), added with 3 ml methanol and shaken using vortex for 30 second. Tubes then closed and put into 60°C water bath for 20 minutes, 2 times shaken using vortex during incubation. Methanol was separated using centrifuge at 2000 g for 15 minutes. Supernatant was taken into clean test tube previously weighed, while residue was mixed with 3 ml methanol for repeated process where supernatant then collected with those from previous step, labeled as hydrophilic extract ready for analysis.

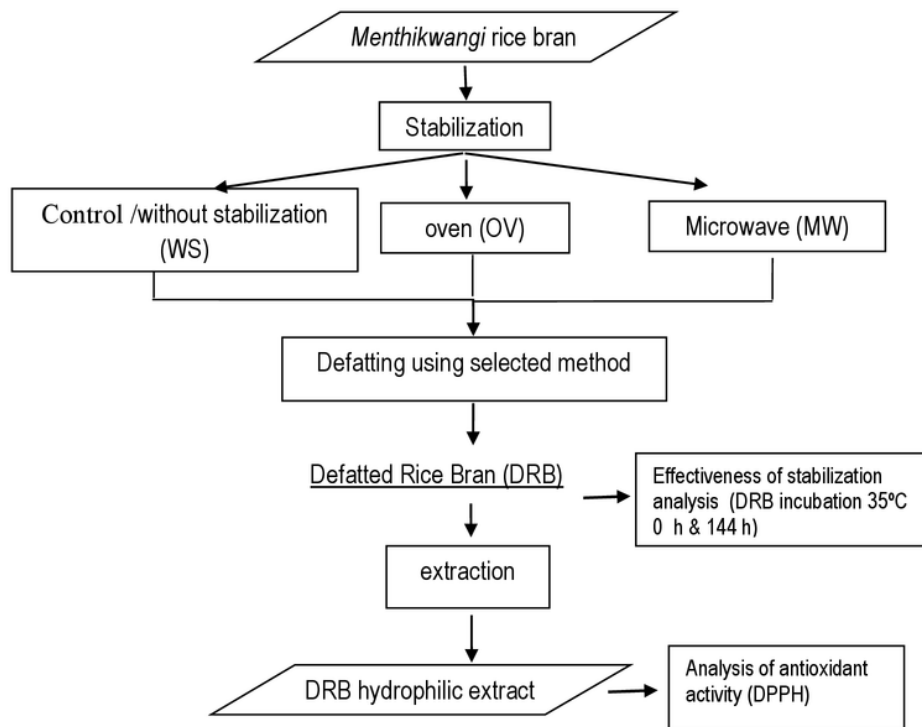


Figure 2. The evaluation of stabilization effect to antioxidant activity of DRB hydrophilic extract

DPPH radical scavenging activity analysis (Sompong *et al*, 2011)

As much as 1.5 ml DPPH (2,2-diphenyl-1-picrylhydrazyl) reagent 0.16 mM was added to 300µL DBR extract previously obtained from 10× dilution, shaken using vortex then incubated in dark room for 40 minutes at room temperature. Absorbance was measured at wavelength (λ) of 515 nm using spectrophotometer. Absorbance difference between sample and control (blank

sample) indicated radical scavenging activity expressed as DPPH scavenging percentage calculated using following equation.

$$\text{Radical Scavenging Activity(\%)} = \frac{[\text{Control Absorbance} - \text{Sample Absorbance}]}{\text{Control Absorbance}} \times 100$$

D. Experiment Design and Data Analysis

Direct completely randomized design was used in this study; applied in all research stages of defatting selection, measurement of stabilization effect on DRB, as well as DRB hydrophilic extract antioxidant activity analysis. Each treatment was carried out twice and sampling was taken in duplicate. Analysis of variance (ANOVA) was used to compare countable F value from experiment with those of statistic table. When F value from experiment showed significant effect on 95% level ($F_{\text{exp}} > F_{\text{table}}$ with $P < 0.05$ was classified as statistically significant), comparison among different means were analyzed using Duncan's multiple range analysis.

RESULTS AND DISCUSSION

A. Defatting Method Selection

Defatted rice bran (DRB) was rice bran whose oil was previously taken (defatting). Defatting process usually started with stabilization then followed by defatting. In this research, selection of defatting was carried out to determine the best method which furthermore applied in DRB preparation. Bran defatting method was basically oil extraction with maceration using hexane, according to method by Hadipernata (2007). Three variations applied, which were defatting without washing (WW) and washing with sample and solvent ratio of 1:2 ($W_{S1:2}$) and 1:4 ($W_{S1:4}$), to produce DRB with lowest fat content. The result presented in Figure 3.

Results showed that DRB fat content for WW, $W_{S1:2}$ and $W_{S1:4}$ were 11.6552%, 5.1355 % and 3.5515%, significant different statistically. Sample was soaked and macerated into hexane with material-solvent ratio of 1:4 (w/v). Bran was then filtered and washed using same solvent and ratio. This result was comparable to the result obtained by Damayanthi and Listyorini (2006) and Hadipernata et al., (2007). Damayanthi and Listyorini (2006) made low fat bran powder with 2.13% fat content using hexane using material and solvent ratio of 1:5, while study on various solvent by Hadipernata et al. (2007) resulting 2.46% fat content obtained by hexane treatment. Thus selected defatting method further applied in research was $W_{S1:4}$.

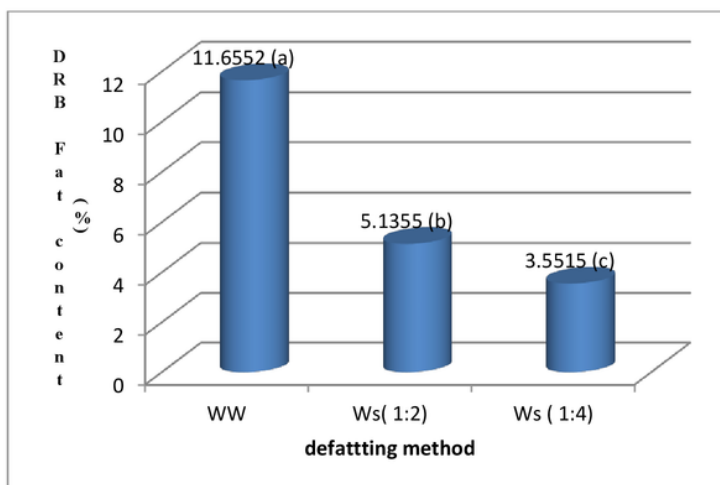


Figure 3. The effect of defatting method on DRB fat content

(WW = Defatting without washing; Ws_{1:2} = Washing with material : solvent ratio of 1:2; Ws_{1:4} = Washing with material : solvent ratio of 1:4)

B. Effect of Stabilization Method on DRB

The effect of bran stabilization on DRB to measure stabilization effectivity was analyzed, according to the method by Damayanthi et al (2006). There were 3 variations as seen in Figure 2: without stabilization (WS), stabilization using oven (OV), microwave (MW). Stabilization has been commonly used for enzyme inactivation of lipase which naturally present in bran and able to accelerate bran rancidity.

This process was conducted before defatting using selected method of Ws_{1:4}, thereafter DRB obtained was subjected to analysis of stabilization effectivity. DRB free fatty acid content was measured after incubation at lipase optimum temperature of 35°C for 144 hours. Free fatty acid (FFA) increase below 10% was considered as effective, whereas bran with more than 10% FFA was improper as food (Tao *et al*, 1993). The results were presented in Table 1.

Table 1. DRB free fatty acid content as affected by bran stabilization

Stabilization method	Free fatty acid (%)		
	H - 0	H-144	FFA increase (%) ^{*)}
Microwave (MW)	7.2293±0.60	8.6034±0.42	1.3742±0.20 ^a
Oven (OV)	6.5793±0.45	8.4462±0.45	1.8669±0.02 ^b
Without Stabilization (WS)	6.7308±0.12	7.9209±0.23	1.1902±0.16 ^a

^{*)} Same subscript in the same column means not significant different on significance level of 95%

Table 1 showed that all stabilization method combined with defatting resulting DRB with low FFA content, before and after storage, below 10%. FFA increase as the result WS and MW treatment also had no significant difference, indicated that bran stabilization was unnecessary during DRB preparation, and presumably caused by defatting which had its role on FFA removal from bran. The different results of studies indicated by Mariod *et al* (2010) that stabilization of rice bran has a significant effect on its methanolic extract antioxidant properties and that antioxidant potential differs among the stabilized and unstabilized samples up to a significant extent.

C. Effect of stabilization and defatting on DRB hydrophilic extract antioxidant activity

Proximate analysis was conducted to determine the chemical composition of defatted rice bran (DRB). Proximate composition of defatted rice bran produced using selected method then analyzed, resulted as follow (Table 2) :

Table 2. Proximate composition of DRB produced from Menthikwangi

No	Component	Content (%wb)
1	Moisture	9.73 ± 0.03
2	Ash	8.39 ± 0.02
3	Fat	3.32 ± 0.15
4	Protein	12.18 ± 0.09
5	Starch	32.21 ± 0.53
6	Soluble fiber	4.42 ± 0.16
7	Insoluble fiber	30.02 ± 0.47
8	Total fiber	34.44 ± 0.63

Proximate composition of DRB resulted in this research was slightly different compared to low-fat bran from the research by Damayanthi and Listyorini (2006). This was probably due to different variety of the bran as well as defatting procedure. Bran moisture content and ash content in this research was higher than previous research of 7.48% and 8.87 % (db), respectively. Fat content was also higher, 3.32% compare to previous research of 2.13 % (db), as also seen in protein (12.18% compare to 10.41%). Previous research also noted soluble fiber content, insoluble fiber, and total fiber content of 3.55 % (db), 35.51 % (db), and 39.06 % (db), respectively, while total carbohydrate was 70.57 % (db).

According the results which Daou and Zhang (2011), that the defatted rice bran dietary fiber fractions (Total Dietary Fiber, TDF), Insoluble Dietary Fiber, IDF and Soluble Dietary Fiber, SDF) have important physiological and functional properties. IDF showed the higher cation exchange and bile salt adsorption capacity, however GDRI and cholesterol lowering effect were

greater for SDF. They also showed an antioxidant activity. These properties are influenced by their Physico-chemical properties. Therefore, defatted rice bran dietary fiber can be used in food system as a natural additive possessing health, technological and antioxidant properties.

This analysis was conducted to measure the effect of stabilization and defatting on antioxidant activity of DRB hydrophilic extract previously prepared without stabilization and defatting $W_{S1.4}$ using methanol as solvent. Hydrophilic extract was analyzed using DPPH radical scavenging method, expressed as % RSA (radical scavenging activity), on 0 hour after extracted and after extract incubation at 35°C for 144 hours. Previous sample was the extract of freshly stabilized and defatted DRB, while the later was obtained after similar sample stored for 144 hours at 35°C. Results were presented in Figure 4.

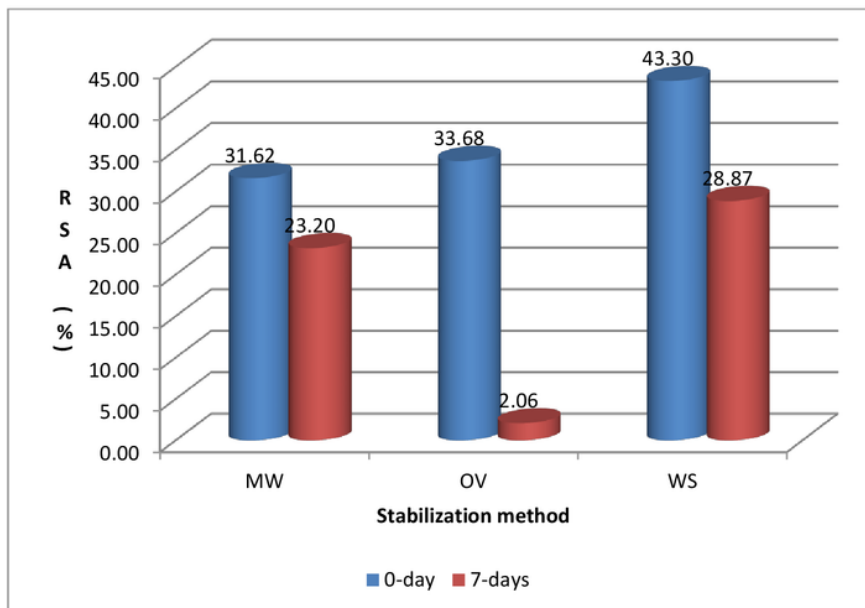


Figure 4. Antioxidant activity of DRB hydrophilic extract before (0 h) and after (144 h) storage
RSA : radical scavenging activity; MW : stabilization with Microwave, OV: stabilization with Oven
and WS : without stabilization

Figure 4 showed that highest activity was obtained by DRB hydrophilic extract prepared without stabilization compare to other stabilization methods (using microwave or oven), both before and after storage. Antioxidant activity of DRB hydrophilic extract on h-0 without stabilization was 43.30% RSA while sample prepare using microwave and oven had 33.68% RSA and 31.62% RSA, respectively. However, this result was different with the result by Mariod *et al* (2010), mentioned that antioxidant activity of previously stabilized DRB extract was higher than those without stabilization.

After 144 hours storage, hydrophilic extract DRB antioxidant activity was decreased. Significant decrease was particularly seen in hydrophilic extract stabilized using oven (OV) with only 2.06% RSA, while microwave-stabilized extract decrease into 23.20% RSA. Better result was obtained by hydrophilic extract without stabilization with 28.87% RSA.

Antioxidant activity significant decreased was the result of bran heating in oven (110 °C for 15 minutes) which affected antioxidant component, as mentioned by Pujihartati *et al.* (1999) that antioxidant property of bioactive compound with health functional might decreased by heating. Higher temperature and longer period of heating accelerate significant decrease of antioxidant activity. Mariod *et al* (2010) concluded that stabilization of rice bran has a significant effect on its methanolic extract antioxidant properties and that antioxidant potential differs among the stabilized and unstabilized samples up to a significant extent. These extracts when added in rice bran oil can reduce the changes of α -tocopherol, and γ -oryzanol contents due to lipid peroxidation during oil storage. Therefore, these extracts can provide potential sources of natural antioxidants suggesting their use in the inhibition of lipid oxidation in nutraceuticals and functional foods. The results showed that highest antioxidant activity of hydrophilic extract was obtained using DRB prepared without stabilization (WS).

CONCLUSIONS

It was concluded that:

- (1) Defatted rice bran (DRB) with lowest fat content and minimum solvent was prepared using incubation with sample and solvent ratio of 1:4 (w/v) followed by washing similar sample-solvent ratio ($W_{s1:4}$)
- (2) Stabilization was unnecessary to produce DRB with low free fatty acid.
- (3) DRB hydrophilic extract with highest antioxidant activity of 43.30% RSA (radical scavenging activity) was obtained using DRB prepared without stabilization (WS).

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