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Defatted Rice Bran a Byproduct of Oil Extraction with Ultrasonic Method for Protein Supplement in Cassava-Flour Biscuits

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Abstract. One of the components in rice bran is oil, which is mainly used for cooking. Because of its high uses, there is also an increase in the supply of the defatted rice bran. Currently, it is used generally as a supplement for livestock feed. Thus, there is a need to diversify their applications to increase protein content in other food sources. The objective of this study is to extract rice bran oil with ultrasonic method, characterize defatted rice bran, and as a supplement in cassava flour biscuits. The method of this research is the stabilization of rice bran by roasting for 10 minutes, extractions of rice bran with n-hexane solvent: 1:4; 1:5; 1:6; 1:7; 1:8, 10-minute ultrasonic technique to remove the oil content, and protein analyses of defatted rice bran. Defatted rice bran is used as supplement for cassava flour biscuits. Simple organoleptic tests were conducted based on respondents' preference. Results showed that there was a significant correlation between rice bran: solvent ratios and protein contents in defatted rice bran. The highest protein content was found at 18.31% in rice bran: solvent ratio 1: 5. The most preferred by respondents in its application for cassava flour biscuits was 13.6%.

1. Introduction

Currently Indonesia still imports wheat flour as one of raw materials for food products. Asosiasi Produsen Tepung Terigu (APTINDO – Wheat Flour Producer Association) reported that there was a six percent increase of wheat flour import in 2016 compared to that in 2015 at around 8.3 million tonnes. National demand for wheat flour in 2017 was 8.79 million tonnes [1]. To reduce dependency on wheat flour import, one of the alternative flours that can be used is the flour of cassava (*Manihot esculenta* Crantz), which is already frequently used in flour-based food products. Proximate analyses by [2] revealed that 100 g of cassava contains 0.3-3.5 grams of proteins, 25.3-35.7 of carbohydrates, and 0.1-3.7 grams of fiber. While, according to [3], functional nutrition containing proteins and fibers can decrease blood cholesterol level and protect from colon cancer, cardiovascular diseases, constipation, and obesity.

The biggest disadvantage of cassava flour is its low protein contents, and thus the need to enrich it with several sources of protein such as cereal, legumes and bran [3]. The advantages of cassava flour are the



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absence of cereal flavor and several characteristics needed in some applications, such as lower gelatinization and higher viscosity [4].

Rice bran is a byproduct of rice dehulling process. Its availability in Indonesia, as an agrarian country, is significantly abundant. Currently, the use of rice bran is mainly limited to livestock feed. Rice bran contains less than 90% triglycerides, comprising saturated and unsaturated fatty acids, and also antioxidants, that can be used as cooking oil and flavoring agent.

Before being extracted, rice bran has to be stabilized to prevent an increased free fatty acid (FFA) content, and extraction results can be purified by degumming [5]. In addition to cooking oil, rice bran oil can be used as oleochemical, such as base oil for uses as inhibitor and surfactant [6], [7].

Several extraction methods of rice bran oil, that are frequently used following stabilization stage by steaming, include pressing with expeller machine [8]. Another researcher used hydraulic press machine to separate the oil [9]. Rice bran that had been stabilized in a microwave for three to five minutes at 120°C was extracted using n-hexane at 50°C for eight to nine hours [10]. Ultrasonic method using low frequency sound wave around 20-50kWz for 20-40 minutes reduced extraction time by 70% compared to that of conventional hydrodistillation [11]. Rice bran oil extraction using a 24-hour maceration method in methanol and hexane solvents yields a byproduct called defatted rice bran. Previous research have been done by [12] about the extraction of cherry seed oil. The supercritical carbon dioxide showed an efficient process and a green method. Other related research have been done by [13] is about Larix Decidua Bark extraction. The highest yield is obtained by microwave with solid/liquid ratio of 1:8 (w/v).

Byproduct of rice bran extraction is defatted rice bran that can be used to increase the contents of fiber and protein, antioxidant characteristics, and storage stability in bread by adding 15% of defatted rice bran to wheat flour [14].

The objectives of this study is to extract oil from rice bran using the ultrasonic method, characterize the resulting byproduct (defatted rice bran), and test its application as a protein supplement in cassava flour biscuits.

2. Methods

2.1. Materials and Equipment

The materials used in this study are rice bran, n-Hexane, cassava flour, margarine, palm sugar, egg yolk, vanilla, and baking soda. The equipment used is a WisdTM ultrasonicator with frequency at 20 kHz, a Rotary evaporator Buchi, and a SharpTM oven.

2.2. Procedures

The study was began with the rice bran stabilization, which is to roast for 10 minutes. The method in rice bran oil extraction was using several ratios of rice bran that has been stabilized : n-hexane 1:4; 1:5, 1:6; 1:7; 1:8 (w/v) and ultrasonication for 10 minutes. Defatted Rice bran and the mixture of the oil extract and solvent are separated using a vacuum filter. The next step, n-hexane residue is separated from the oil extract using Rotary evaporator. Protein analyses in defatted rice bran was conducted with Kjeldahl methodology. The Kjeldahl method is the total nitrogen content analysis. The sample is mineralized with concentrated sulfuric acid and catalyst (copper and potassium sulfate) to convert the nitrogen into ammonium sulfate. Ammonia from ammonium sulfate is replaced with concentrated sodium hydroxide, then ammonia is distilled and collected in a solution of boric acid and titrated with hydrochloric acid. The protein content is determined by multiplying the nitrogen content with the NCF (Nitrogen conversion factor) [15]. Defatted rice bran sample with the highest protein content was use as a supplement in cassava flour biscuits, which were assessed in taste, color organoleptic tests to determine respondents' preference.

3. Results and Discussion

3.1. Oil Extractions and Protein Content Analysis in Defatted Rice Bran

Rice bran contains oil that has many kinds of bioactive compounds, including antioxidants. Rice bran oil that has been further separated from n-hexane using Rotary evaporator can be put in many uses, such as cooking oil, flavoring, and cosmetic additives. Extraction with ultrasonication in n-hexane solvents was performed in rice bran that had been stabilized by roasting for 10 minutes. The variation of weight to volume ratios between rice bran to solvent was 1:4; 1:5; 1:6; 1:7; 1:8. The residue of n-hexane in the defatted rice bran was separated by oven. Figure 1 shows the ultrasonic extraction equipment of rice bran oil and Figure 2 shows the defatted rice bran.



Figure 1. The Ultrasonic extraction equipment of rice bran oil and Figure



Figure 2. The defatted rice bran

Table 1 shows the protein contents of defatted rice bran resulted from rice bran oil extraction using ultrasonic method.

Table 1. Protein contents of defatted rice bran resulted from rice bran oil extraction using ultrasonic method at different solvent ratios.

Rice bran : Solvent (w/v)	Protein (%)
1:4	17.23
1:5	18.31
1:6	17.84
1:7	18.19
1:8	18.05

Based on Table 1, the protein contents of defatted rice bran increase with increased ratios of rice bran and n-hexane solvent (w/v) from 1:4 to 1:5 in the rice bran oil extraction processes using ultrasonic method. The highest protein content was found at rice bran and solvent ratio (w/v) of 1:5. Then the protein contents of defatted rice bran decrease with increased ratios of rice bran and n-hexane solvent (w/v) from 1:5 to 1:8.

Hexane is one of the nonpolar solvents and better suited to oil extraction, while the polar solvents are known to extract free fatty acid (FFA) and other product such as protein [16]. It was expected that the higher the ratio of rice bran and solvent, the higher oil and the lower protein is extracted, which leads to the increase in protein content of defatted rice bran. In previous research, [17] concluded that protein stable in nonpolar solvents such as cyclohexane. Based on [18], protein decomposition has occurred in

defatted soybean protein in n-hexane solvent, especially at high temperatures. The separation of n-hexane residue in the oven caused the denaturation of the protein.

3.2. Correlation Analyses Between Rice Bran to Ratio and Protein Content in Defatted Rice Bran

Regression analysis and R^2 tests were performed on the data to determine the correlation between rice bran and solvent ratios in ultrasonic extraction and protein contents in defatted rice bran. Figure 3 shows this correlation.

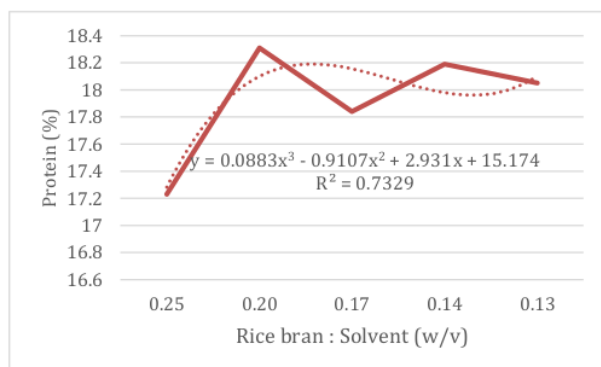


Figure 3. Protein contents in defatted rice bran (%) as a function of rice bran : solvent ratios (w/v)

The result shown in Figure 3 describe that the higher the solvent: rice bran ratio, the higher the protein content in the defatted rice bran, with the optimal ratio of solvent to rice bran at five. The more oil is extracted from the rice bran, the higher protein content is retained in the defatted rice bran. However, when the ratio value is greater than five, the protein content slightly decreases. The correlation between the solvent: rice bran ratio in x and protein content in rice bran in y can be expressed in the following formula: $y = 0.0883x^3 - 0.9107x^2 + 2.931x + 15.174$, with an R^2 value of 0.7329. This model is the equation that best suits to the real data. This result shows that there is a correlation between the ratio of solvent: rice bran and protein contents in defatted rice bran.

3.3. Analysis of Respondents' Preferences to Different Biscuit Recipes

The test for the applications of defatted rice bran was conducted by using it as a supplement for biscuits. The biscuits also has cassava flour, margarine, palm sugar, egg yolk, vanilla, and baking soda as its main ingredients. Furthermore, the biscuits has addition of defatted rice bran for recipe 1 and both defatted rice bran and rice bran for recipe 2. Those formulas are prepared at 140°C in the oven for 25 minutes, as shown in Figure 4.



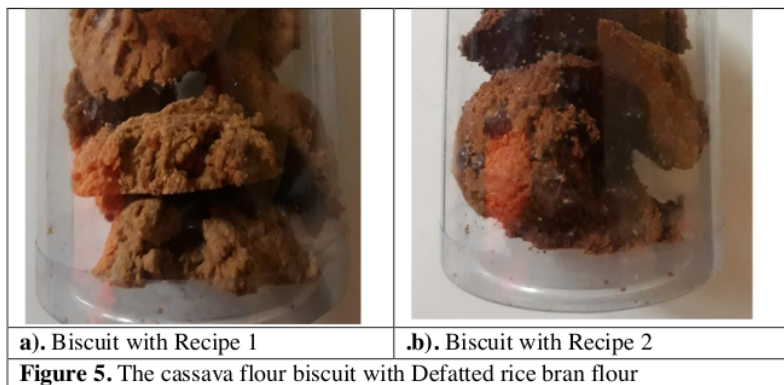
Figure 4. The cassava flour biscuit preparation with the supplementation of defatted rice bran

The results of preference tests from 10 respondents are presented in Table 2.

Table 2. Results of taste organoleptic tests based on respondents' preference

Ingredients	Composition in Biscuit		Respondents' Evaluation	
	Recipe 1	Recipe 2	Recipe 1	Recipe 2
Defatted rice bran flour	60 g	40 g		
Rice bran flour		20 g		
Cassava flour	150 g	150 g		
Palm sugar	100 g	100 g	less preferred	more preferred
Margarine	100 g	100 g		
Egg yolk	1 sp	1 sp		
Vanilla	1 tsp	1 tsp		

The results in Table 2 show that biscuits with 40% defatted rice bran in Recipe 1 were less preferred than those of 13.33% in Recipe 2. This result shows equal to the [19], in which the best-tasted biscuits is the one with addition of 10-15% w/w rice bran in wheat flour. Then, the final products of the biscuit preparations with recipe 1 and recipe 2 are shown in Figure 5a and Figure 5b.



a). Biscuit with Recipe 1

b). Biscuit with Recipe 2

Figure 5. The cassava flour biscuit with Defatted rice bran flour

While the result of the organoleptic color test in Figure 4a and 4b shows that recipe 2 biscuit with the addition of rice bran in defatted rice bran has darker color compared to recipe 1 biscuit. This is accordance to [19] that the evaluation results of biscuit sensory shows that the score of biscuit color decrease significantly with the increase of rice bran.

4. Conclusion

One of the components in rice bran is oil, which is mainly used as cooking oil and main ingredient of oleochemical products. It can also be processed further to produce active compounds for health and cosmetic products. Because of the high benefits and uses of oil and extractions of rice bran, therefore there is also an increase in the supply of defatted bran. Extraction of rice bran oil with an ultrasonic process resulted in defatted rice bran. Defatted rice bran contains proteins and can be utilized as a supplement in other food materials that have low protein contents, such as cassava flour. The highest protein content was 18.31% and was obtained from the byproduct of rice bran oil extraction with rice bran: n-hexane solvent ratio at 1:5. The more oil is extracted from the rice bran, the higher protein content is retained in the defatted rice bran. However, when the ratio value is greater than five, the protein content slightly decreases. In its application in cassava flour biscuits, the composition of 13.33% defatted rice bran was the most preferred by respondents. The cassava flour biscuits with 40% defatted rice bran in Recipe 1 were less preferred than those of 13.33% in Recipe 2. The result of organoleptic test shows that the biscuit's color turns darker when there is an increase in supplementation of defatted rice bran.

5. Acknowledgement

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