The Effects Of Sonication And Shaking On Yields And Characteristics Of Protein Concentrate Extract Of Deffated Rice Bran

Imam Bestari, Ratri Ariatmi Nugrahani', Ismiyati, Tri Yuni Hendrawati

Abstract: Defatted rice bran is rice bran in which oil content has been extracted. Proteins in rice bran have high content of lysine, which is one the essential amino acids. The objectives of this study were to extract and characterized the protein concentrated from defatted rice bran. The variables consisted of times of sonication at 2, 4, 6, 8, and 10 minutes, and times of shaking at 0, 10, 20, 30, and 40 minutes. Maceration was employed for three hours using n-hexane at 1:7 ratio (w/v), which was followed by pneumatic press process. Oven heating was used to remove excess n-hexane. Protein extraction was performed using ultrasonication using distilled water as solvent at 1:10 (w/v) ratio. Centrifugation and shaking were used to purify the protein concentrate. The protein content was determined with Kjeldahl Analyzer, while the characterization was performed with Differential Scanning Calorimetry (DSC) and dan Fourier Transformed Infrared (FTIR). The best yields were obtained from sonication for 8 minutes (14.06%) and shaking for 20 minutes (12.8%). There appeared to be amino acids and proteins underwent denaturation at 157.35 °C.

Index Terms: rice bran, defatted, protein, extraction, characterization

1. INTRODUCTION

Rice is widely cultivated globally because it has become a main staple for more than half of the world's population. Global production of rice reached 678 million metric tons in 2014 [1]. Meanwhile, rice production in Indonesia experienced fluctuations from 2011 to 2015. There was a steady increase from 2011 to 2013, from 65.75 million to 69.05 million, and 71.27 million metric tons. However, production dipped to 70.84 million metric tons in 2014, even though it rose significantly to 76.39 million metric tons in 2015 [2]. Rice grain contains three main parts, which are the germ and starchy center (~70%), husk (~20%), and bran (~10%) 1. Rice bran contains high nutrients, including oils, proteins, and carbohydrates [3]. Proteins in rice bran boast alluring characteristics, especially the high content of lysine, which is one of the essential amino acids. There is also a significant amount of hypoallergenic proteins, which makes it an ideal ingredient for baby foods [4]. Uses of bran proteins have steadily increased in recent years, such as in nutraceutical or cosmetics1. Defatted rice bran is a byproduct of oil extraction and known to have a significant amount of proteins, and therefore can be utilized as an additional ingredient in food products, such as biscuits [5]. The objectives of this study are to measure the protein contents in, determine the effects of sonication and shaking on yields of protein extraction of, and characterize the extracted protein concentrate in defatted rice bran.

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2 RESEARCH METHOD

2.1. Equipment and Materials

The equipment consisted of filter press, oven, digital scale, ultrasonic type, orbital shaker, centrifuge, stirring hotplate, filter cloth, Erlenmeyer, graduated cylinder, glass beaker, glass funnel, evaporating dish, and spatula. The materials included rice bran, n-hexane, and distilled water.

2.2. Procedures

The method employed in this study was the extraction process involving maceration using n-hexane as solvent at room temperature for three hours followed by sonication to obtain defatted rice bran. The supernatant and pellet phases were separated with centrifugation and dried up on evaporating dish assisted with stirring hotplate to obtain the protein extract. The extract of defatted rice bran was analyzed for protein content using Kjeldhal Analyzer test and protein characteristics using FTIR and DSC test. The steps in oil extraction are illustrated in Figure 1.

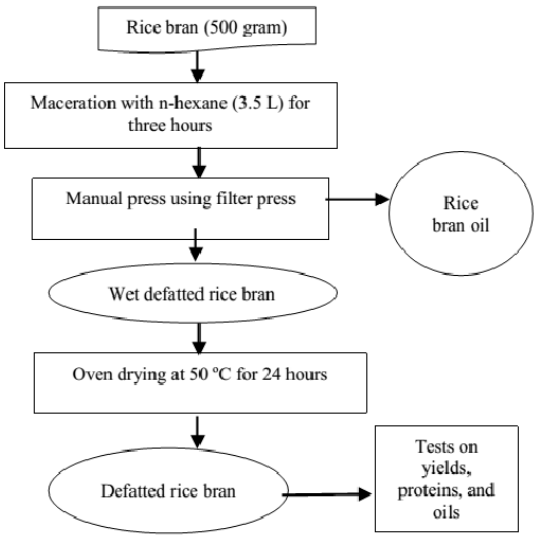

***Fig 1**. The flowchart to produce defatted Rice Bran*

The steps taken in protein extraction from defatted rice bran with varied times of sonication are illustrated in Figure 2.

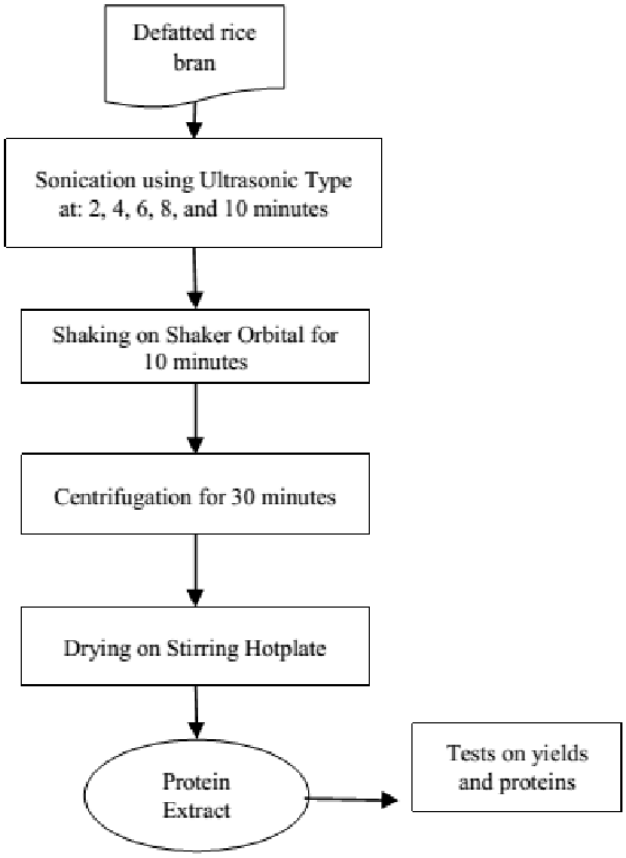

***Fig 2**. Protein extraction from Defatted Rice Bran at Different Times of Sonication*

The next variable tested was the time of shaking and the steps of this procedure is illustrated in Figure 3.

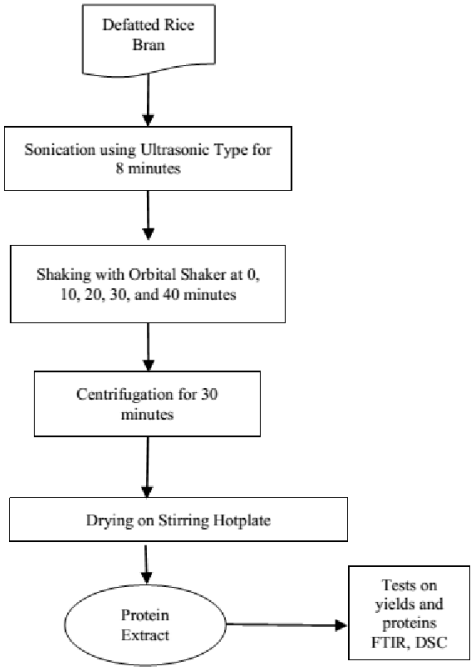

***Fig 3**. Extraction of Proteins from Rice Bran Defatted with Shaking Time Variations*

4 RESULTS AND DISCUSSION

4.1. Raw Material Analyses

This study used 500 grams of rice bran that underwent initial protein and oil content analyses using Kjeldahl Analyzer, and yielded 11.405% proteins and 16.925% oil. The sample was macerated in n-hexane for three hours and pressed manually to obtain wet defatted preparation. After drying, 395 grams of defatted rice bran was collected. The dried sample was analyzed with Kjeldahl Analyzer, and produced contents of 14.37% protein, 3.19% oil and 79% yield.

4.2. The Effects of Sonication Time on Protein Content

In this trial, the sonication time to extract proteins from defatted rice bran was investigated at 2, 4, 6, 8, and 10 minutes, while shaking time was constant at 10 minutes. Kjeldahl Analyzer was used to analyze the protein contents and the results are presented in Table 1.

TABLE 1
The Effects of Sonication Time on Protein Content

Sonication Time	*Protein Content*
2 minutes	12.46 %
4 minutes	11.85 %
6 minutes	12.06 %
8 minutes	14.06 %
10 minutes	11.63 %

The data above were plotted to determine the correlation between sonication time and protein content extraction on defatted rice bran. The graph is shown in Figure 4.

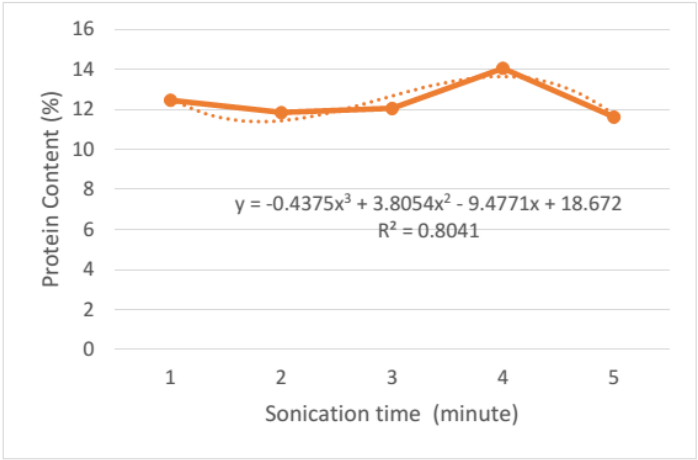

***Fig 4**. The Effects of Sonication time on Protein Content*

The graph in Figure 4 represents the interaction between sonication time and protein contents extracted from defatted rice bran. The results showed that there was a reduction of protein contents from 12.46% at two minutes to 11.85% at four minutes, followed by increases of 12.06% and 14.06% at six and eight minutes consecutively, and another reduction of 11.63% at ten minutes. The correlation between sonication time (x) and protein content (y) produce a 3rd-order polynomial formula of $y = -0.4375x^3 + 3.8054x^2 - 9.4771x + 18.672$, with $R^2 = 0.8041$. This analysis showed a strong negative correlation between those variables. Sonication delivers mechanical pressure on cells that induces cellulolytic

processes on the sample, resulting in an increased compound solubility and extraction yield [6]. Reduced protein contents might have been the result of protein denaturation at high temperature and/or prolonged drying processes.

4.3. The Effects of Shaking Time on Protein Yield

In this research, the effort to optimize the yield of protein concentrate extraction focused on the time of shaking by varying it into 0, 10, 20, 30, and 40 minutes, while the sonication time was constant at eight minutes. The yields are presented in Table 2.

TABLE 2
The Effects of Shaking Time on Protein Yield

Shaking Time	*Protein Yield*
0 minutes	11.1%
10 minutes	9.7%
20 minutes	12.8%
30 minutes	10.8%
40 minutes	12.4%

These data were plotted to determine the correlation between the shaking time and the protein yield and presented in Figure 5.

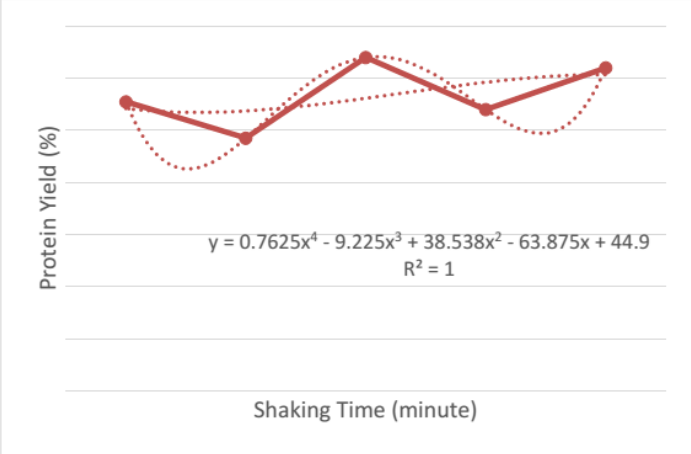

***Fig 5**. The Effects of Shaking Time on Protein Yield*

The graph in Figure 5 represents the interaction between shaking time and protein yield from defatted rice bran. There appeared to be fluctuations of protein yields along the shaking time. The yield at 0 minutes was 11.1% but decreased to 9.7% at 10 minutes, followed by an increase to 12.8% at 20 minutes and reduction to 10.8% at 30 minutes and final increase to 12.4% at 40 minutes. These data produced a 4th-order polynomial correlation of $y = 0.7625x^4 - 9.225x^3 + 38.538x^2 - 63.875x + 44.9$, $R^2 = 1$ between shaking time (x) and protein yield (y). This result showed a strong positive correlation between the variables. The shaking treatment can increase the solidification process, which maximize the desired protein yield and efficiency [7]. The reductions of protein yield might have been caused by excessive heat and/or prolonged drying time.

4.4. FTIR Tests on Protein Concentrate Extract from Defatted Rice Bran

FTIR tests were performed on preparations obtained from 8-minute sonication and 20-minute shaking to determine the protein characteristics based on the functional groups and polar bonds of a compound. The results are presented in Figure 6.

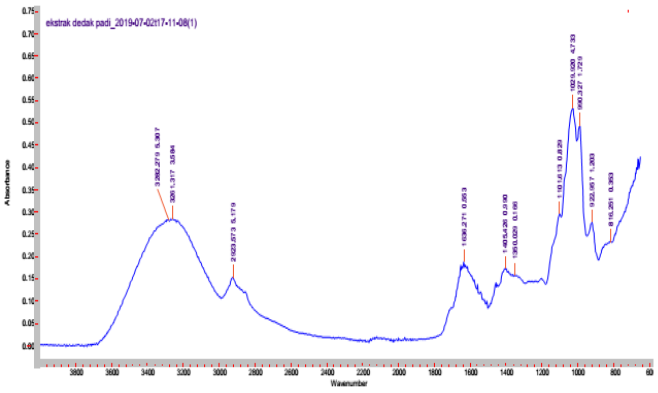

***Fig 6**. FTIR Test Results of Protein Concentrate Extract from Defatted Rice Bran*

The FTIR tests produced peaks from the frequency of 816.251/cm to 3282.279/cm. On the amino acid FTIR graph, peaks appeared on the frequency of 1000/cm to 4000/cm [8]. These results indicate that protein concentrate extract from defatted rice bran contained amino acids.

4.5. DSC Tests on Protein Concentrate Extract from Defatted Rice Bran

DSC tests were also performed on the preparations obtained from 8-minute sonication and 20-minute shaking to further characterize the protein content of defatted rice bran extraction. The samples were tested at 140°C to 165°C with 5°C increments. Reactions were observed from 156.73°C to 157.68°C, and the results are presented in Figure 7.

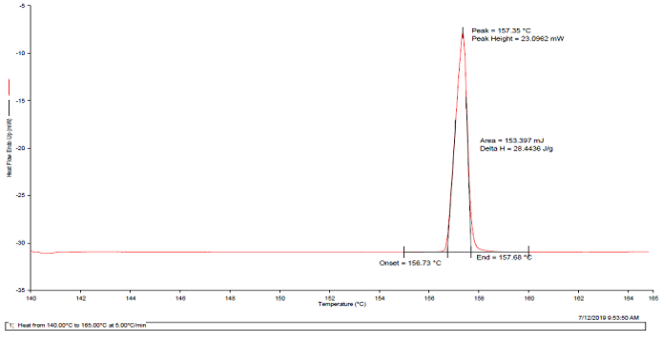

***Fig 7**. Results of DSC tests on Protein Concentrate Extract from Defatted Rice Bran*

DSC tests on the protein extract concentrate from defatted rice bran showed that there was no reaction taking place at 140 °C to 155°C indicated by the absence of peak. Reaction was observed to start at 156.73 °C, peak at 157.35 °C with h = 23.0962 mW, area = 153.397 mJ, and ΔH = 28.4436 J/g, and end at 157.68°C. The peak at 157.35°C indicated that protein denaturation in the sample had taken place 9. The test temperature only reached 165°C because the tested sample contained sugar groups, which tend to result in an increased noise.

4 CONCLUSION

The results of this study can be concluded as follow:

1. Compared to the raw material, there was an increase of protein content from 11.405% to 14.37%, and a reduction of fat content from 16.925% to 3.19% in defatted rice bran with the yield of 79%.
2. The effects of sonication time showed the highest protein content produced at 14.06% for 8 minutes.
3. Sonication time and protein content have a negative and the formula is $y = -0.4375x^3 + 3.8054x^2 - 9.4771x + 18.672$ with $R^2 = 0.8041$.
4. The effects of shaking time on protein yield produced the highest value of 12.8% for 20 minutes.
5. There is a strong positive correlation between shaking time and protein yield and the formula is $y = 0.7625x^4 - 9.225x^3 + 38.538x^2 - 63.875x + 44.9$ with $R^2 = 1$.
6. FTIR tests showed the presence of amino acids in defatted rice bran.
7. DSC tests exhibited the appearance of a peak at 157.35 ^oC indicating that proteins in defatted rice bran began to denature at that temperature.

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