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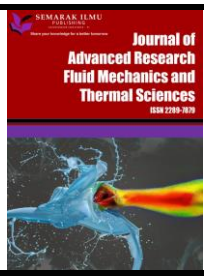

Parametric Study on The Rice Bran Protein Extraction Process Using Water as a Solvent

Malini Kanapathy[1], Mohd Sharizan Md Sarip[1,*], Syahrul Affandi Saidi[2], Mohd Aminudin Jamlos[3], Nor Ilia Anisa Aris[4]

[1] Faculty of Chemical Engineering Technology, Universiti Malaysia Perlis, Kompleks Pusat Pengajian Jejawi 3, Universiti Malaysia Perlis (UniMAP), 02600 Arau, Perlis, Malaysia
[2] Faculty of Electrical Engineering Technology, UniCITI Alam Campus, Universiti Malaysia Perlis (UniMAP), 02100, Sungai Chuchuh, Padang Besar, Perlis, Malaysia
[3] Faculty of Electronic Engineering Technology, UniCITI Alam Campus, Universiti Malaysia Perlis (UniMAP), 02100, Sungai Chuchuh, Padang Besar, Perlis, Malaysia
[4] Chemical Engineering Program, School of Engineering, Faculty of Technology and Innovation, Taylors University Lakeside Campus, Subang Jaya, Malaysia

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ABSTRACT

Rice bran is a by-product resulting from the milling process that is frequently underutilized as cattle food or disposed through open-burning despite of its high nutritional and nutraceutical properties. Thus, this research aims in recognizing and exploring rice bran and its extraction methods that could further cultivate in the industry. This study focuses on the rice bran extraction process using water assisted with ultrasonication. The relationship between the operational parameters such as the temperature, extraction time and sample-to-solvent ratio to the protein yield were studied. The rice bran protein was subjected to the surface functional group analysis using Fourier-transform infrared spectroscopy (FTIR). As a conclusion, the extraction temperature of 60°C, sample to solvent ratio of 10 % and extraction time of 25 mins were chosen as the best conditions for the protein extraction. The extraction of the protein from rice bran is highly profitable due to its nutritional and nutraceutical properties as well as it is readily available at low cost.

1. Introduction

Rice bran is a by-product from rice milling industry. It can be utilized as a source of hypoallergenic proteins which contribute to the healthy growth of infants formulated in baby food [1]. Generally, during the milling process, up to 10 % of the paddy yielded the bran, which is mostly consumed as animal feed or burnt directly to the environment via open burning [2,3]. The uses and benefits of the rice bran could be explored and developed to benefit Malaysian. Rice bran protein is branded to be nutritionally enhanced product and proven to pose an antidiabetic, lipid-lowering, hypotensive,

* *Corresponding author.*
E-mail address: sharizan@unimap.edu.my

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antioxidant, and anti-inflammatory effects [4]. Normally, the protein intake was mainly focused on animal protein which able pose threats to dietary intake assessment as well as health of the citizen.

Therefore, the focus was narrowed towards plant-based proteins to enhance the health status of the community and reduce the dependent on animal - derived protein to ameliorate protein malnutrition [5]. Rice bran proteins are characterized with high nutritional value and functional properties compared to other plant proteins due to its hypoallergenic and anti-cancer properties [1,6]. Furthermore, rice bran protein also comprises of some important amino acids that considered better than other animal and vegetable protein [7]. Hence, the extraction of protein sourcing from rice bran could be a convincing manner to satisfy the high industrial demand as well as an excellent utilization of by-products and waste management. As a matter of fact, rice bran protein extraction was researched and studied since the early 1970s [8]. However, after four decades, a commercially benign protein extraction and production, as well as the utilization tactic were not found. One of possible reason are due to expensive and inefficient extraction process. Water had a potential for this process caused its known as a cheap and abundant but its utilization as a solvent were inefficient due to its high polarity with low solubility with organic compound. Some process modifications are needed such as the uses of water at its subcritical region as reported by Izzati *et al.,* [9] in *Zingiber Zerumbet* extraction. Other process modification are high pressure process, ultrasonic extraction, and addition of co-solvent to enhance the mass transfer mechanism in the extraction process. Thus, utilizing water as solvent was possible to achieve the efficient and cheaper protein extraction method with the aid ultrasonication effect.

In this report, rice bran protein was extracted using water as solvent assisted with ultrasonication process in evaluating the efficiency and suitability of adopting the greener methods in the industries. Besides, the research was done to determine the effect the operational conditions to carry out the water-based methods, resulting in high yield protein from rice bran.

2. Methodology

2.1 Raw Material

The rice bran was collected from Kilang Beras BERNAS (KBB) Kuala Perlis Sdn Bhd, Kuala Perlis, Perlis, Malaysia. The rice bran was heated in a microwave oven at 850 W for 3 mins to prevent the hydrolytic rancidity of rice bran during storage [10]. Then, the microwaved rice bran was kept in zipper-top-bags in refrigerator at temperature around 4-5^{o}C prior to experimental procedure.

2.2 Extraction Method

50 g of rice bran was weighed, and consequently placed in oven at 120°C on a petri dish for 5 mins. The extraction process was initiated by the mixing of the weighted rice bran with distilled water at specific sample to solvent (w/v) ratio. The mixture was then subjected to ultrasonication using the sonicator (Elmasonic, S 80 (H)) that runs at the frequency of 22 kHz. Three parameters were namely the temperature, extraction time and the sample to solvent (w/v) ratio aiming for the highest protein extraction. Three sets of experiments varying three parameters were carried out. First set of the experiment carried out was on the temperature that varied from 50 to 80^{o}C with a leap of 10^{o}C at the constant sample-to-solvent ratio of 30 (w/v %) and the extraction time of 13 minutes. Next set was carried out with the time that varied from 5 to 25 minutes with increment of 5 minutes at the constant sample-to-solvent of 30 (w/v %) and temperature of 45°C. The final sets were carried out by manipulating the weight to volume ratio that varied from 10 to 50 (w/v %) with an increase of 10% at the constant temperature of 45°C and the extraction time of 13 minutes. After extraction, the

mixture was cooled down to room temperature and subsequently subjected to centrifugation process at 4200 rpm for 30 minutes to separate the solid phase from the aqueous phase. The supernatant was collected prior to the protein analysis. Rice bran extracted solution were dried to rice bran sample powder using spray drying process based on previous study condition (11).

2.3 Protein Analysis

The protein concentration in the aqueous phase analyzed using Bradford reagent and UV-Vis Spectrophotometer (Shimadzu, UV-1800, Japan) [12]. The amount of protein in a sample was evaluated by measuring the absorbance at 595 nm comparing samples to a bovine serum albumin (BSA). The extract was filled in a cuvette after diluted 100 times with distilled water prior to the measurement. The cuvette was placed in the preheated UV spectrometer for 15 mins and the absorbance was recorded and calculated. The mass of the protein was determined using Eq. (1).

$$\text{Mass of protein (g)} = \frac{\text{Concentration } \left(\frac{\text{mg}}{\text{ml}}\right) \times \text{Volume of solvent (ml)}}{100} \quad (1)$$

From Eq. (1), protein yield was calculated using Eq. (2).

$$\text{Protein yield (\%)} = \frac{\text{Mass of protein (g)}}{\text{Mass of sample (g)}} \times 100 \quad (2)$$

2.3 Surface Functional Group Analysis

Analysis was conducted utilizing the FTIR spectrometer (Perkin Elmer FTIR/F-IR spectrometer 100) in Instrument Lab, Faculty of Chemical Engineering Technology, Universiti Malaysia Perlis (UniMAP). Rice bran sample powder and KBr were mixed, and oven dried overnight prior to FTIR test. Approximately 3mg of samples was mixed and finely grinded with 1 g of KBr by using mortar to reduce the scattering loses and adsorption band distortion. After that, the mixture was pelletised into a transparent disk, and it was placed carefully into the sample holder of the FTIR spectrometry. Lastly, the KBr pellet samples were scanned in the range of 4000 cm^{-1} to 400 cm^{-1} with resolution of 4 cm^{-1}. The FTIR spectrum of rice bran sample was recorded and analysed.

3. Results

3.1 Effect of Extraction Parameter on Rice Bran Protein Yield

In this study, three extraction parameters namely temperature, sample-to-solvent ratio and extraction time are evaluated. Figure 1 shows the effect of temperature on rice bran protein yield at a constant sample to solvent ratio of w/v 30 % and extraction time of 13 minutes.

Based on the Figure 1, the protein concentration increased from 3.98 to 4.97 % when the temperature increased from 50 to 60°C. However, the protein yield decline to 2.91 % at 80°C. As the temperature increases, the polarity of water increases thus improving the mass transfer between the bran and water and ultimately increased the extraction rate [13]. However, as the temperature increases, the proteins are exposed to deterioration as observed from 70 to 80°C [14]. Therefore, based on this study, 60°C are identified as best temperature to extract the protein from rice bran due to high protein yield.

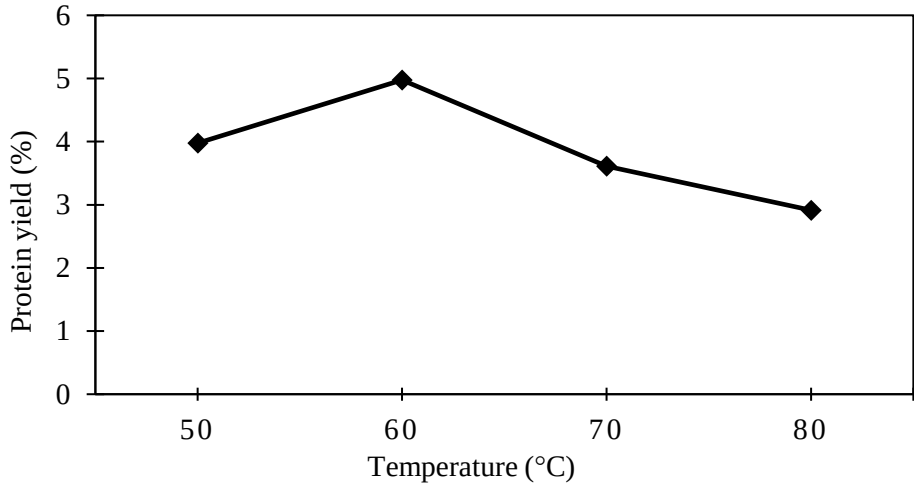

Fig. 1. Effect of temperature on the extraction of the rice bran protein yield at constant sample to solvent ratio of 30 % and extraction time of 13 mins

Figure 2 depicts the effect of sample-to-solvent ratio on the protein yield at constant temperature of 45°C and extraction time of 13 minutes.

Based on Figure 2, a trend of gradual decrease in the protein yield as the sample-to-solvent ratio increases. As the volume of the solvent decreases, the extraction rate drops leading to the reduction of the protein extraction. Lower ratio leads to enhanced mass transfer, thus increases the extraction rate. The enhance amount of solvent was affiliated to the lower protein solubility towards the amount of sample added into the solvent. Therefore, the extraction solution was saturated with the non-dissolved rice bran sample and lead to poor extraction rate. Conclusively, higher sample-to-solvent ratio leads to decrease in volume of solvent that causes reduction in protein yield. Therefore, sample to solvent ratio of 10 (w/v %) are chosen as the best condition in this study due to highest protein yield.

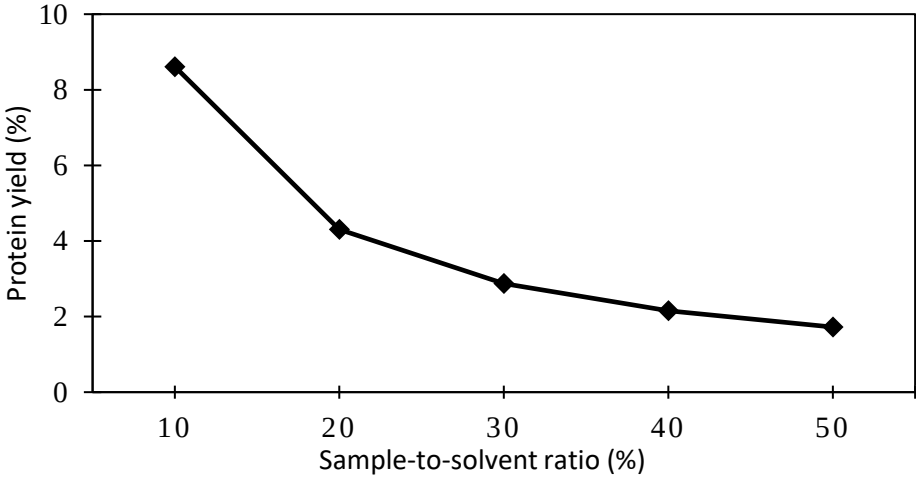

Fig. 2. Effect of sample-to-solvent ratio on rice bran protein yield at constant temperature of 45°C and extraction time of 13 minutes

Figure 3 depicts the effect of extraction time on the bran protein yield at constant temperature of 45°C and constant sample to solvent ratio of 30 (w/v %).

Based on the Figure 3, protein yield increased from 2.87 to 16.21 % when the extraction time was increased from 5 to 25 mins. Longer extraction time allowed more interaction between the rice bran sample and solvent, thus, boosting the solubility of the protein in the solvent [3]. Besides that, longer

ultrasonication dilution will also causes the complete breakdown of the rice bran matric cell wall and release the protein to be dissolved in the solvent. Therefore, the 25 minutes of extraction time are chosen as the best condition in this study due to highest protein yield.

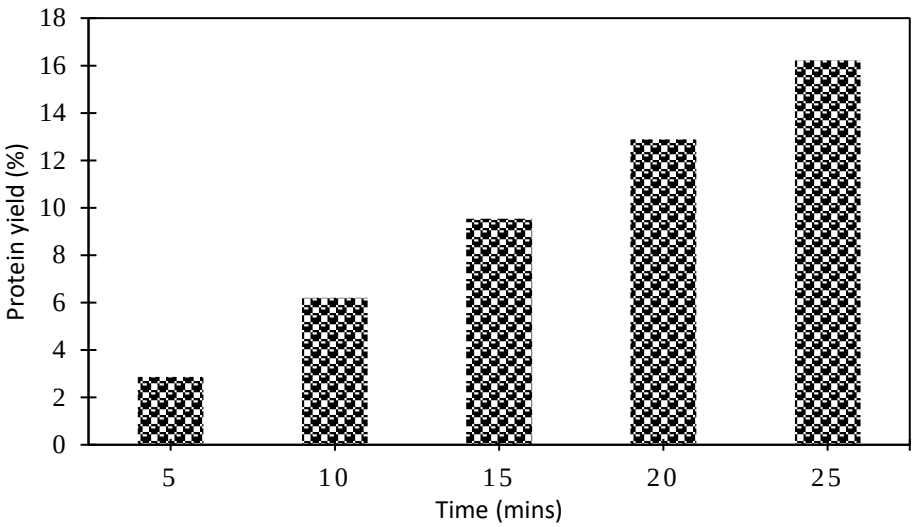

Fig. 3. Effect of time on the extraction of the rice bran protein at constant temperature of 45°C and constant sample to solvent ratio of 30 (w/v %)

3.2 Surface Functional Group Analysis of the Sample

Fourier Transform Infrared (FTIR) Spectroscopy was carried out for 4 different samples namely, the raw sample, the protein extracted at optimized conditions, protein extracted at high and low temperature. The figures on the spectrum generated that depicts on the functional group present in the samples are shown in Figure 4.

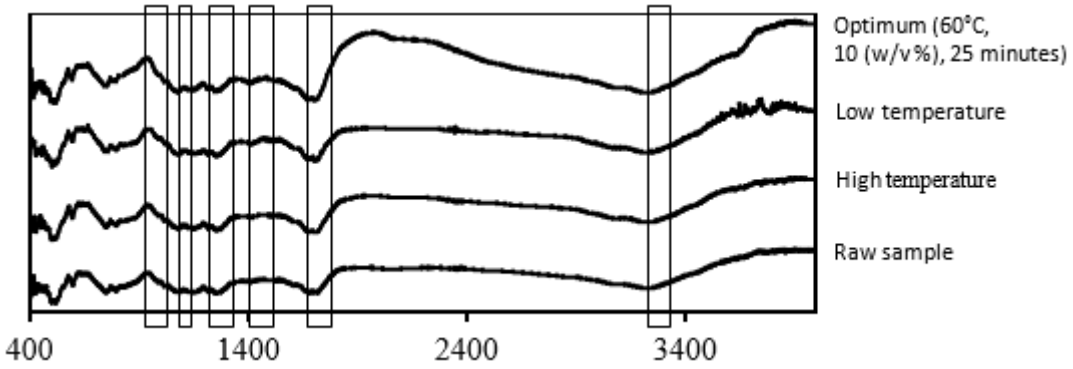

Fig. 4. FTIR absorbance band for the raw sample, the protein extracted at optimized conditions, and the protein extracted at high and low temperatures

The different parameters that lead to different protein yield have not significantly affected the functional groups in the protein yield at different conditions. Based on the Figure 4, the wavelength that is noted at a range of 1600 cm^{-1} to 1700 cm^{-1} was observed in all four spectrums of four different samples that indicates the presence of amino acid which proves on the presence of protein [15]. Existence absorbance at 1410 - 1260 cm^{-1} contributed by -OH deformation [16]. CH_3 symmetric deformation vibrations occur at observance band of 1190 - 1370 cm^{-1} [16,17]. It also can be observed the strong valance vibrations between 1150-1040 cm^{-1} and 1125-950 cm^{-1} which it indicated the

overlap aromatic impressions [16]. Wavelength of 3000 cm^{-1} to 3400 cm^{-1} indicates the presence of the functional group phenol and alcohol as observed in sample. Thus, the presence of starch due to the complexation with protein can be deduced from the observation made due to the presence of polysaccharide chains that comprises of O-H groups that is been found along the chain. The significant peak are observed for protein extracted sample in comparison with raw sample at 1410-1500 cm^{-1} which indicate characteristic of -NH absorption groups of the amino acids and amide I and II bands of proteins which indicated pure protein are observed in extracted sample in comparison with raw sample [16]. The sharp band centered at 1082 cm^{-1} for raw bran sample indicated Si-O-Si bond in the structural framework of the siloxane in the silica [18]. Various element such as fibre, silica, cellulose, and others impure component interfere the FTIR absorbance in raw rice bran sample.

4. Conclusions

As a conclusion, water-based extraction method with sonication was successfully extract the rice bran protein with maximum protein yield of 16.21 %. Three parameters in this process; namely temperature, sample to solvent ratio and time were giving the influence on the protein yield. Based on this study, the extraction temperature of 60°C, sample to solvent ratio of 10 % and extraction time of 25 mins were choosing as the best condition for the extraction protein from rice bran using water extraction process assisted with sonication technology. Furthermore, the presence of pure protein in rice bran extracted powder was further validated with the sharp -NH absorption groups of the amino acids and amide I and II bands of proteins observed in the sample FTIR spectrum.

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