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Preparation of Rice Bran Protein Solutions Using a Water-Based Extraction Process

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ABSTRACT

Rice bran (RB), which is a waste product of the rice industry, has great potential for use as a source of new protein supplements. In this study, the extraction of soluble proteins from rice bran was conducted using a water-based extraction method with the aid of sonication extraction and a hybrid sonication/thermal treatment approach, known as the soni-auto hybrid method. Both extraction methods were explored and compared to determine the most efficient extraction process using the one-factor-at-one-time (OFAT) method. The parameters studied and their experimental ranges for both extraction methods were as follows: sonication time = 5–45 min, sonication temperature = 30–80 °C, and feed-to-solvent ratio = 1:5–1:80 (g:mL). The most efficient extraction method was then used for optimization by means of response surface methodology (RSM) based on the central composite design (CCD) model. It was found that the soni-auto hybrid method exhibited a superior extraction performance compared with sonication alone, wherein the protein concentration was increased by up to 18% while maintaining a comparable quality. The use of this hybrid treatment approach also reduced the sonication time from 35 to 30 min and the sonication temperature from 50 to 45 °C. The optimal soni-auto hybrid conditions were determined by RSM to be a temperature of 50 °C, a feed-to-solvent ratio of 1:20, and an extraction time of 30 min; these conditions produced a protein concentration of 17.174 mg/mL. Finally, evaluation of the surface morphology and functional groups on the protein confirmed that the hybrid soni-auto approach provided a higher protein concentration without significantly affecting the protein structure or quality.

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Rice bran; Protein; Soni-auto hybrid method; Response surface methodology; Extraction improvement

1. Introduction

In the rice industry, large volumes of rice bran are produced through the rice milling process, and this waste material is either discarded or sold at a very low price as animal feed or fertilizer (Gul et al. 2015; Jiamyangyuen et al. 2005). Such under-utilization leads to slow growth of the rice industry, since the rice grains are the only product considered to be a profitable source, and as a result, it can be difficult to compensate for the high investment required for the rice milling process (Rajamoorthy et al. 2015). However, rice bran is known to have many health benefits, thereby leading to potential applications in the food, nutraceutical, and pharmaceutical industries, such as in the case of rice bran oil (Pandey and Shrivastava 2018). It is therefore desirable to investigate new application prospects for rice bran, such as in the production of rice bran protein, to add value to rice bran and eventually enhance the profits of the rice industry.

Rice bran contains high concentrations of oil (≤23%) and free fatty acids (Patil et al. 2016), and it is known that the free fatty acid concentration can increase dramatically over time due to the enzymatic reaction between rice bran oil and the lipase enzyme (Beatriz et al. 2019). For example, Patil et al. (2016) reported that within the first 24 h of storage, relatively large quantities of free fatty acids (i.e., ∼5–7%) can be produced in the rice bran. However, it should be noted that nutritionists consider rice bran containing ≥5% free fatty acids to be unsuitable for human consumption due to the rancid flavor and soapy taste yield (Patil et al. 2016). In addition, rice bran contains fiber and phytates, which are extensively associated or bound to proteins, thereby rendering protein separation a particular challenge. Such high fiber contents are also not suitable in terms of

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human consumption, as an excessive intake of these fibers may lead to kidney problems (Kim et al. 2014). Therefore, various extraction techniques, such as alkaline, enzymatic, and physical extraction methods, have been developed to extract proteins from rice bran fibers while also inhibiting the activity of lipase (Bhat and Riar 2017).

For the extraction of rice bran proteins, dilute alkaline solutions, such as potassium hydroxide (KOH) and sodium hydroxide (NaOH), are commonly employed due to their wide availability. In addition, these alkaline solutions can effectively solubilizing rice bran proteins through cleavage of the hydrogen, amide, and disulfide bonds of the protein structure (Hamada 1997). As reported previously, alkaline hydrolysis carried out between pH 7 and 12 can produce protein extraction yields of 30–80% (Fabian and Ju 2011). Due to the fact that alkaline solutions are also commonly used in the rice bran protein extraction process, the protein yields tend to increase with an increasing pH value. However, the exposure of proteins to strongly alkaline conditions can alter their nutritional characteristics and produce toxic products though Maillard reaction that are not suitable for human consumption (Jiamyangyuen et al. 2005). In contrast, enzymatic extraction allows for the extraction of rice bran protein at neutral and slightly basic pH values. For example, the use of enzymes that can break cell wall components can increase protein yields by releasing greater amounts of proteins from the bran polysaccharide matrix (Ansharullah et al. 1997); however, this process is time-consuming and expensive, thereby hindering its industrial application (Tang et al. 2002).

An alternative means to promote the extraction of proteins is based on the use of physical processes to induce cell disruption. To date, various physical processes have been employed for this purpose, including colloid milling, freeze-thawing, autoclaving, stirring, and subcritical water extraction (Anderson and Guraya 2001). Subcritical water extraction was proven to improve various extraction process by improving the solubility between solute and solution (Syed Jaapar et al. 2015). In general, these approaches are relatively facile and cost-effective, and as such, they are commonly applied in industry. Their application is also boosted by the fact that low-cost modifications can be easily carried out (Phongthai et al. 2017). Besides that, ultrasonic assisted extraction or sonication are also one of the alternatives for the solid liquid extraction which are proven to improve various solute extraction from botanical including bioactive component in phytopharmaceutical extraction industry (Vinatoru 2001). Therefore, the application of sonication for the protein extraction in this study are believed to promote the positive finding.

Thus, we herein report the use of a water-based extraction process to extract a protein-rich rice bran solution for use in food-based applications mainly aimed at human consumption. Furthermore, the target compound in this process is a soluble protein, while the undesired oil component is non-polar and hydrophobic. We therefore expect that these factors will enhance the degree of soluble protein extraction from rice bran without the requirement for oil extraction. Moreover, due to the fact that the protein solubility is highly dependent on the amount of water used for the extraction (Islam et al. 2013), we aim to reduce this dependence by investigation of the sonication effect and a combined thermal treatment and sonication approach to optimizing the extraction process using a constant volume of water. Finally, optimization of the extraction method is also conducted using response surface methodology (RSM).

2. Methodology

2.1 Collection and storage of rice bran

The rice bran was collected from Kilang Beras BERNAS Sdn Bhd, Kuala Perlis, Perlis, Malaysia. The raw rice bran was heated at 95 °C for 3 min to prevent hydrolytic rancidity (Pandey and Shrivastava 2018) and the stored at 4 °C under required for use.

2.2 Sonication method

The rice bran specimen (5 g) was mixed with distilled water (100 mL, 1:20 rice bran/water, g:mL) in a conical flask. Then, rice bran mixture in the flask was soaked and sonicated using a WUC-D22H 230 V ultrasonication bath (Witeg, Germany) with a frequency of 40 kHz, power output of 70% and a temperature of 30 °C. The bath was equipped with a temperature controller to maintain the water temperature during the extraction process. After sonication, the rice bran mixture was subjected to centrifugation at 4000 rpm and 25 °C for 30 min. Finally, the volume of the supernatant was measured and analyzed. The extraction procedure was repeated three times for each experimental condition. Water distiller (Favorit, Thailand) were used to produce the distilled water.

2.3 Soni-auto hybrid method

The rice bran specimen (5 g) was added to a conical flask, mixed distilled water (100 mL), and heated in an autoclave at 121 °C for 20 min. After this time, the autoclaved rice bran solution was allowed to cool to room temperature. Subsequently, the mixture of rice bran was subjected to sonication and centrifugation as described in Section 2.2. Finally, the volume of the supernatant was measured and analyzed. The extraction procedure was repeated three times.

2.4 Analysis of the protein content in the rice bran solution

The protein concentration in the extracted rice bran solution was measured using a UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan) based on the Bradford method (Bradford 1976). The amount of protein in each sample was evaluated by measuring the absorbance at 595 nm and comparing the samples to bovine serum albumin (BSA) (Sigma, USA). The

Table 1. Experimental range of parameters studied using the OFAT method.

Parameter	Unit	Range
Time	Minutes (min)	5–45
Temperature	Celsius (°C)	30–80
Feed-to-solvent ratio	g:mL	1:5, 1:10, 1:20, 1:40, 1:60, and 1:80

protein concentration was measured at a constant solvent volume for all experiments.

2.5 Experimental design for the extraction of rice bran protein using the OFAT technique

The experimental design for comparison between the sonication and soni-auto hybrid methods for the extraction of protein from rice bran was carried out according to the "one-factor-at-a-time" (OFAT) method, in which only one parameter is examined in each set of experiments, with all other parameters remaining constant. The OFAT technique then identified the method that resulted in the highest protein concentration. The results obtained from the OFAT method were also used to narrow down and determine the range of values for each optimization parameter during the RSM technique, as listed in Table 1.

2.6 Response surface methodology (RSM)

The experimental design for optimization of the rice bran protein extraction method was conducted using the RSM technique in Design Expert software (StatEase, USA). A central composite design (CCD) was used as the model for the optimization process. The experimental design with three variables (parameters) was set to five levels for each numeric factor. These include plus and minus alpha (axial points), plus and minus 1 (factorial points), and the center point. CCD was used in this study to investigate the effects of the sonication temperature, the sonication time, and the feed-to-solvent ratio on the protein concentration extracted from the rice bran specimen. According to Eq. (1), the dimensionless x_i is coded instead of the independent variable X_i:

$$X_i = \frac{x_i - X_0}{\Delta X_i} \tag{1}$$

where,

x_i = Coded value of the independent variable
X_i = Real value of the independent variable
X_0 = Real value of the independent variable at the centre point
ΔX_i = Step change value

The experiments were randomized to minimize bias. All experimental conditions predicted by the CCD model were implemented in triplicate and the average rice bran protein concentration was taken as the response, Y. Additionally, the following predictive quadratic polynomial (Eq. 2) was used to fit the experimental results as the correlation between the response and independent variables:

$$Y = A_0 + \sum A_i X_i + \sum A_{ii} X_i^2 + \sum A_{ij} X_i X_j \tag{2}$$

where,

Y = Response variable
A_0 = Regression coefficients of the variables for the intercept terms
A_i = Regression coefficients of the variables for the linear terms
A_{ii} = Regression coefficients of the variables for the quadratic terms
A_{ij} = Regression coefficients of the variables for the interaction terms
X_i, X_j = Independent variables

The range of values for each parameter was determined from charts obtained using the OFAT technique. Based on the CCD model, a set of experimental conditions were implemented, and the results were collected and analyzed based on statistical analysis.

2.7 Statistical analysis

All data were analyzed using the SAS statistical package (Design Expert, Stat Ease, USA). Analysis of variance (ANOVA) was used to demonstrate the significant effect of each manipulated variable on the protein concentration. Any specific parameter was considered to result in a statistically significant difference during the process when a p-value of <0.05 was obtained based on the 95% confidence level. In addition, a higher F value indicates a highly significant effect on the process. In this study, the optimal conditions for the best extraction method were predicted using model optimization to achieve higher protein concentrations. The model was validated by comparing the experimental and predicted data based on the variance and standard deviation values.

2.8 Production of rice bran protein powder

The extracted rice bran protein solution obtained from each extraction method was dried in a laboratory spray dryer (B-290, Buchi, Switzerland) with an inlet temperature of 150 °C, and with an aspiration of 100%. The feed pump was operated at 30% full power, and the flow rate was set at ~9 mL/min. The dried protein powders were collected in Mylar bags and stored for further analysis.

2.9 Characterisation and morphology analysis of the rice bran protein powder

The types of functional groups present in the rice bran protein powder were determined using Fourier-transform infrared spectroscopy (FTIR, Perkin Elmer, Germany). For this purpose, the rice bran protein powder (~3 mg) of rice bran protein powder was mixed and finely ground with KBr (1 g)

using a mortar and pestle to reduce the scattering loss and adsorption band distortion. The mixture was then pelletized into a transparent disk and placed into the sample holder of the FTIR spectrometer. Subsequently, the KBr pellet samples were scanned in the range of 4000–400 cm^{-1} with a scan resolution of 4 cm^{-1}.

The surface morphology of the rice bran protein powder was analyzed using scanning electron microscopy (SEM, JEOL, Japan). Prior to carrying out the SEM observations, the sample was mounted on aluminum stubs using double-sided adhesive carbon tape and was then sputter-coated with a 5 nm layer of gold using a coating system. Finally, the powders were imaged using an SEM operated at an accelerating voltage of 5 kV, and images were captured at magnifications of 500×, 1000×, 1500×, and 2000×.

3. Results and Discussion

Extraction of the protein-rich solution from the rice bran specimen was carried out using two main extraction techniques, namely sonication alone and a soni-auto hybrid method. The best method for this process was further optimized using RSM, as mentioned above, and the effects of several parameters on the rice bran protein extraction yield were evaluated. Characterization of the rice bran protein was performed based on functional group analysis using FTIR spectroscopy and surface morphology observations by SEM.

3.1 Effect of the sonication time on the concentration of protein extracted using the sonication and soni-auto hybrid methods

The effect of the sonication time on the concentration of protein extracted using both the sonication and soni-auto hybrid methods at a constant feed-to-solvent ratio of 1:20 and a constant sonication temperature of 30 °C was initially evaluated, as shown in Figure 1.

As shown in Figure 1, in the initial stages of extraction for both the sonication and soni-auto hybrid methods, the protein concentration significantly increased with the sonication time. More specifically, the highest protein concentration (11.39 mg/mL) was observed after 35 min of sonication

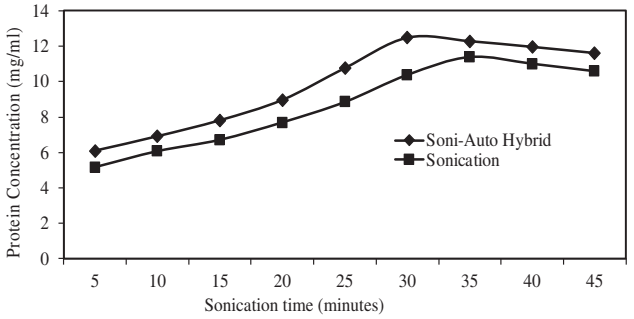

Figure 1. Effect of the sonication time on the concentration of protein extracted from rice bran using the sonication and soni-auto hybrid approaches at a constant feed-to-solvent ratio of 1:20 and a sonication temperature of 30 °C.

alone, while for the soni-auto hybrid method, the highest protein concentration (12.49 mg/mL) was observed after 30 min. However, as the extraction time was prolonged further, the protein yields from both methods decreased steadily thereafter. According to Chittapalo and Noomhorm (2009), morphological changes took place in the rice bran structure, which were caused by long-term exposure to ultrasonic waves, thereby rendering this material susceptible to the harsh extraction conditions. Ultimately, this resulted in degradation of the proteins over long sonication times (Suresh et al. 2014).

Based on the results presented in Figure 1, the incorporation of autoclaving appeared to lead to higher protein concentrations compared with the case where sonication alone was employed. This was attributed to the fact that autoclaving broke the rice bran cell walls, which allowed the solvent and the ultrasonic waves to penetrate the cells and promote protein extraction. In contrast, using sonication alone, the majority of proteins remained trapped within the rice bran cell wall. More specifically, during autoclaving, the cell wall, which mainly consists of hemicellulose and cellulose, degrades and breaks down easily, undergoes thermal hydrolysis, and produces smaller constituents, such as glucose and sucrose (Md Sarip et al. 2016). This phenomenon can be observed in the micrographs of the raw rice bran specimen before and after the autoclaving process, as shown in Figure 2.

As shown in Figure 2(a), prior to autoclaving, the cell walls remain intact, thereby reducing the possibility of protein bodies being extracted during the sonication process. In contrast, after autoclaving (Figure 2(b)), the majority of cell walls were destroyed and the protein bodies were exposed to the extraction solution, thereby increasing the mass transfer mechanism during the sonication process. These results indicate that the soni-auto hybrid method was the best of these two methods in terms of enhancing the amount of protein extracted from rice bran, in which the optimum time range for extraction is between 20 and 40 min.

3.2 Effect of the sonication temperature on the concentration of protein extracted using the sonication and soni-auto hybrid methods

Figure 3 shows the effect of the sonication temperature on the concentration of protein extracted from rice bran using the sonication and soni-auto hybrid methods at a constant sonication time of 30 min and with a feed-to-solvent ratio of 1:20. As shown, the extracted protein concentration increased upon increasing the temperature for both methods. However, in the case of the sonication method, a sharp increase in the protein concentration was observed when the temperature was increased from 30 to 50 °C, with the highest protein yield reaching 11.40 mg/mL. Meanwhile, for the soni-auto hybrid method, the extracted protein concentration increased significantly from 7.41 mg/mL at 30 °C to 12.62 mg/mL at 45 °C. These trends were attributed to the increased protein solubility at higher temperatures (Amarasinghe et al. 2009). Moreover, under such conditions,

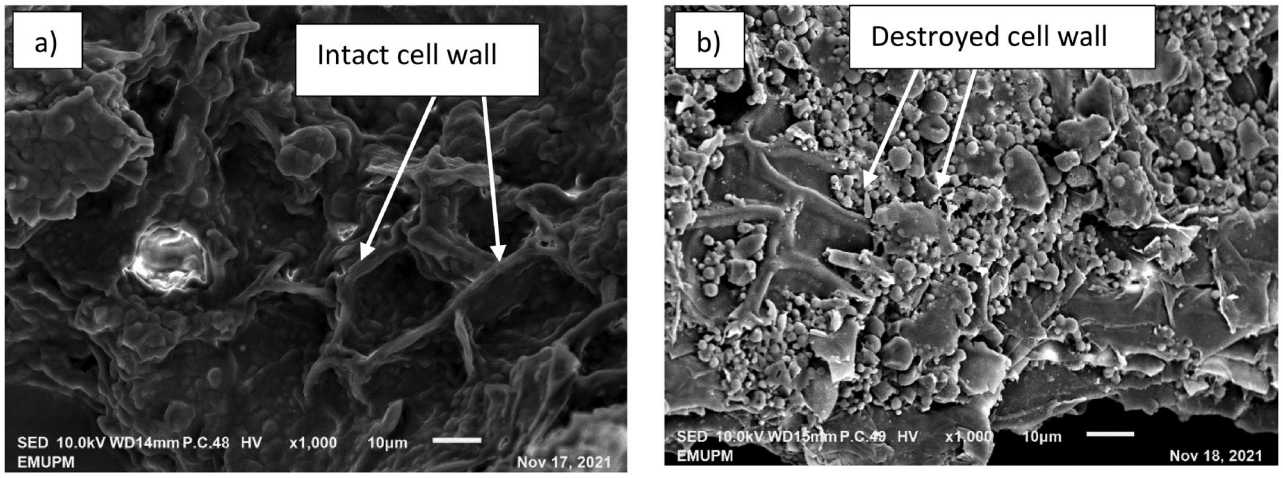

Figure 2. Micrographs showing the raw rice bran specimen (a) before and (b) after autoclaving.

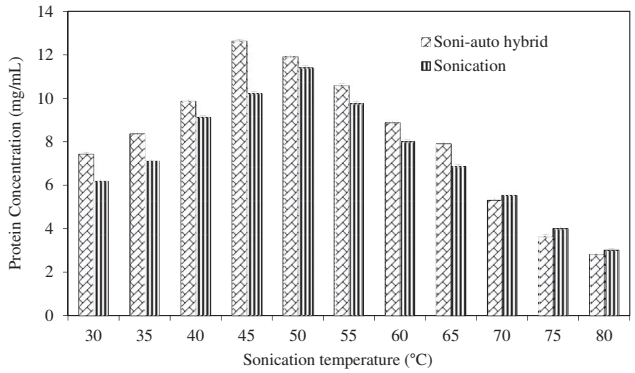

Figure 3. Effect of the sonication temperature on the extraction of rice bran protein using the sonication and soni-auto hybrid methods at a constant sonication time of 30 min and a feed-to-solvent ratio of 1:20.

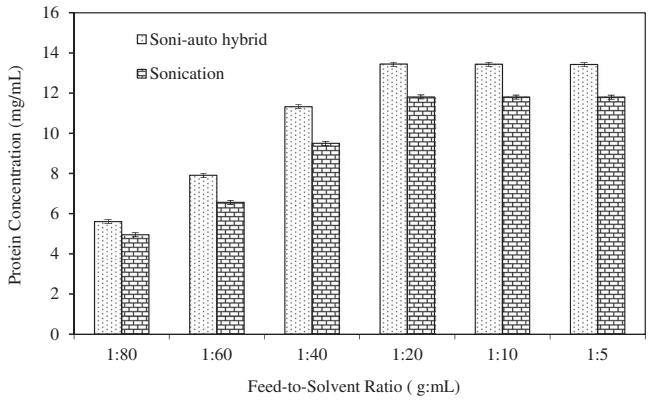

Figure 4. Effect of the feed-to-solvent ratio on the concentration of extracted protein obtained using the sonication and soni-auto hybrid methods at a constant temperature of 30 °C and a sonication time of 30 min.

the rice bran expands, and protein diffusion is promoted as a greater amount of energy accumulates in the rice bran (Suresh et al. 2014).

However, upon increasing the temperature further to 80 °C, the extracted protein concentrations decreased considerably for both methods. This was due to the proteins becoming denatured at high temperatures. More specifically, in the sonication method, the protein began to denature at temperatures >50 °C, whereas in the soni-auto hybrid method, the protein began to denature at temperatures >45 °C. However, it was found that the protein concentration obtained using the soni-auto hybrid method was higher than that obtained using sonication alone at temperatures between 30 and 65 °C. In contrast, at temperatures >75 °C, the soni-auto hybrid method exhibited a reduced extraction efficiency, and the resulting protein concentration was lower than that obtained using the sonication method. This phenomenon was attributed to the fact that during the autoclaving process, breakage of the cell wall exposes the proteins to higher temperatures (Khoei and Chekin 2016). This situation is differs from the non-autoclaving sample where the breakage of the cell during the sonication process. The exposes protein to higher temperature above 40 °C lead to the protein degradation and fractions (Čurlej et al. 2022). Čurlej et al. reported the increased α-cas content and decreased β-lg content in cow's milk protein are observed from the temperature of 40 °C due to the protein degradation process (Čurlej et al. 2022). Based on these results, the optimal temperature range for rice bran protein extraction using the soni-auto hybrid method was determined to be 40–60 °C.

3.3 Effect of the feed-to-solvent ratio concentration of protein extracted using the sonication and soni-auto hybrid methods

Figure 4 shows the effect of the feed-to-solvent ratio on the concentration of protein extracted using the sonication and soni-auto hybrid methods at a constant temperature of 30 °C and a sonication time of 30 min.

As shown in Figure 4, with a constant extraction temperature and time, the protein concentration increased with a reduction in the feed-to-solvent ratio until it reached the maximum protein concentration for each extraction method, i.e., the protein solubility was inversely proportional to the feed-to-solvent ratio. For the method employing sonication, the lowest protein concentration was 4.95 mg/mL at a 1:80 ratio, while the highest concentration of 11.81 mg/mL obtained at a ratio of 1:20 ratio. In the case of the soni-auto hybrid method, the lowest protein concentration of 5.61 mg/mL was obtained at a feed-to-solvent ratio of 1:80,

while the highest protein concentration of 13.45 mg/mL resulted from the use of a 1:20 ratio. As shown in the figure, for both methods the protein concentration reached a maximum at a ratio of 1:20 and then leveled off, thereby indicating that a further increase in the amount of distilled water used for the extraction did not significantly alter the protein concentration. This phenomenon occurs when the maximum concentration of the solute is completely dissolved in the solvent and reaches saturation, as observed at a 1:20 ratio. It was therefore considered that a feed-to-solvent ratio of between 1:10 and 1:40 was optimal for the extraction of rice bran protein.

Thus, based on a parameter study using the OFAT method, it was apparent that the extraction of rice bran protein using the soni-auto hybrid method led to a significantly improved protein concentration, with the introduction of autoclaving enhancing the overall protein concentration by up to 10% in comparison with the sonication-only approach. As described above, this was due to breakage of the cell walls under ultrasonication conditions to allow the ultrasonic waves to easily penetrate the cell bodies and release the protein. The most favorable conditions for this process were therefore determined to be a sonication time of 20–40 min, a sonication temperature of 40–60 °C, and a feed-to-solvent ratio of 1:10–1:40. Thus, these parameters and their corresponding ranges were evaluated using RSM to obtain their optimal values.

3.4 Modelling of protein extraction from rice bran using the soni-auto hybrid extraction process

The relationship between three parameters, i.e., a sonication time of 20–40 min, a sonication temperature of 40–60 °C, and a feed-to-solvent ratio of 1:10–1:40, was investigated using the RSM CCD model. For this purpose, 20 experimental conditions were considered based on the experimental design generated from the CCD model; a detailed description of this model was previously reported by Sarip et al. (2022). A quadratic polynomial model was established to represent the process, as shown in Eq. (3), wherein the adjusted R-squared value for the model was 0.8933, and the predicted R-squared value was 0.7049.

$$R1 = 17.17 - 0.26A + 0.034B - 0.67C - 0.019AB - 0.047\,AC + 0.00325BC - 0.71A2 - 1.19C2 \quad (3)$$

where,

$R1$ = Protein concentration (mg/mL)
A = Temperature (°C)
B = Sonication time (min)
C = Feed: Solvent ratio (g:mL)

Thus, Figure 5 shows three-dimensional (3D) response surface model plots for the protein concentration (R1) at a constant feed-to-solvent ratio of 1:20 (Figure 5(a)), a temperature of 50 °C (Figure 5(b)), and a time of 30 min (Figure 5(c)).

The 3D surface plot shown in Figure 5(c) exhibits a parabolic curve that is not observed in Figures 5(a,b). This type of curve was obtained when the feed-to-solvent ratio and the temperature were varied at a constant sonication time, indicating that the feed-to-solvent ratio and temperature, but not the sonication time, have a significant effect on the protein concentration in this parameter range. This was supported by ANOVA analysis of the various parameters, wherein the solvent-to-sample ratio, temperature, and time gave F values (1,11) of 32.53, 4.990, and 0.083, respectively, along with p-values of <0.0001, <0.0489, <0.7786, respectively.

In addition, as shown in Figure 5(a), at a constant feed-to-solvent ratio of 1:20 and extraction time of 20 min, the protein concentration increased from 15 to 16 mg/mL upon increasing the temperature from 40 to 52 °C. However, upon further increasing the temperature to 60 °C, the protein concentration dropped to 14 mg/mL. Theoretically, under high temperature conditions, plant tissues are softened as the cell walls break down, thereby weakening the interactions between the protein bodies and matrix surface (Tabaraki and Nateghi 2011). As a result, protein compounds can be easily extracted from the rice bran matrix into the solvent. However, if the temperature is too high, protein denaturation can occur, which therefore accounts for the lower protein concentration obtained at an extraction temperature of 60 °C (Bandyopadhyay et al. 2012).

Furthermore, as shown in Figure 5(c), at a constant time of 30 min, as the feed-to-solvent ratio was increased from 1:10 to 1:25, the protein concentration increased from 12 to 14.0 mg/mL, and this was attributed to increased swelling of the rice bran at a higher water content. Such swelling occurs when the solvent fills the empty space inside the rice bran matrix, eventually weakening the interactions between the protein bodies and the cell walls to enhance protein extraction from the matrix (Che Sulaiman et al. 2017). However, a further increase in the feed-to-solvent ratio above 1:25 had a negative impact on protein concentration. This was likely due to the inefficient sonication effect as the solvent volume increased, which resulted in a reduced mass transfer, as observed in the microwave-assisted extraction of Meghalayan cherry (Kashyap et al. 2022). Overall, these results indicate that temperatures in the range of 40–60 °C and feed-to-solvent ratios in the range of 1:10 to 1:40 have a significant effect on the extracted protein concentration, while the sonication time (20–40 min) had no clear effect.

3.5 Optimization process based on the protein concentration

RSM was then employed to determine the optimal conditions for protein extraction. All constraints were set to obtain the highest possible protein concentrations, and the goals were set in the range of the lower and upper limits for all factors (A, B, and C). Sixteen solutions were suggested as optimized conditions. However, the solution with the highest desirability (i.e., 1.0) and the lowest standard error of the protein concentration (i.e., 0.150) was determined to be a

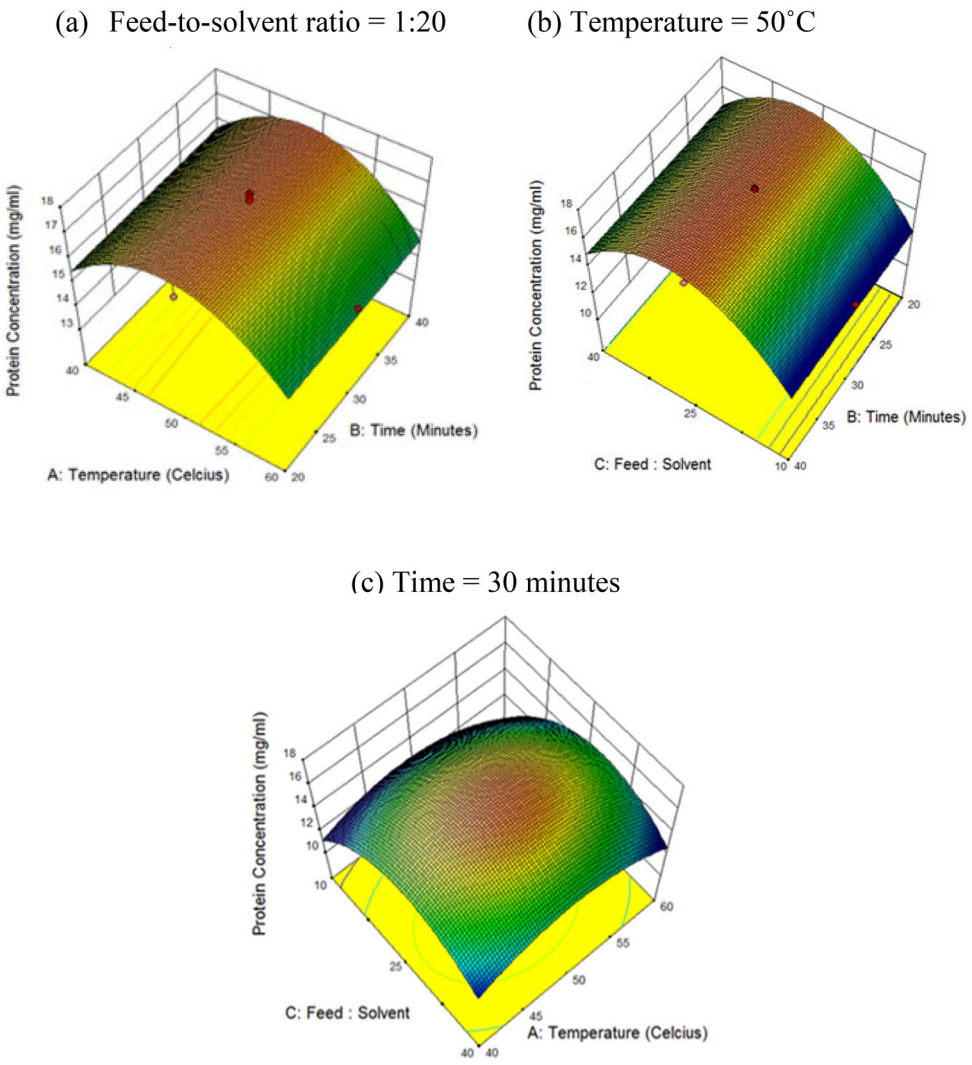

Figure 5. Three-dimensional response surface model plots for the protein concentration (R1) at (a) a constant feed-to-solvent ratio of 1:20, (b) a constant temperature of 50 °C, and (c) a constant time of 30 min.

Table 2. Statistical analysis for the confirmatory runs of the optimized conditions.

Statistical analysis	Experiment 1	Experiment 2	Experiment 3
Protein concentration (mg/mL)	16.991	17.200	16.794
Difference from predicted value	−0.183	+0.026	−0.380
Percentage difference (%)	−1.066	+0.151	−2.213
Mean (mg/mL)	16.995		
Variance (mg/mL^2)	0.187		
Standard deviation (mg/mL)	0.433		

temperature of 50 °C, a feed-to-solvent ratio of 1:20, and an extraction time of 30 min. validate the model, the experimental procedure was carried out in triplicate under the optimized conditions suggested by the model, and compared with the predicted extraction concentration of 17.174 mg/mL. The results are presented in Table 2.

As shown in Table 2, an acceptable error of 1% was observed between the predicted and experimental values, and the differences between the predicted response and the experimental responses were within a confidence level range of 95%. In addition, the three experimental results showed a small standard deviation of 0.433 mg/mL, indicating that the experimental process exhibited a high repeatability and a low degree of error.

3.6 Functional group analysis of the extracted protein

Functional group analysis of the raw rice bran specimen and the protein samples extracted using the sonication and soni-auto hybrid methods was then carried out to observe any changes in the protein structure during the extraction process. For protein extracted sample, both samples are for the temperature of 50 °C, a feed-to-solvent ratio of 1:20, and an extraction time of 30 min. Thus, the FTIR spectra of the three samples were recorded, and are shown in Figure 6.

The characteristics of the various samples were subsequently identified based on their FTIR absorbance bands, as outlined in Table 3, which lists the observed peaks and their respective functional groups.

As outlined in Table 3, the spectra for all three samples exhibited the same expected functional groups related to the protein structure, although their intensities varied slightly. This result demonstrates that the two protein extraction methods employed herein did not affect the protein

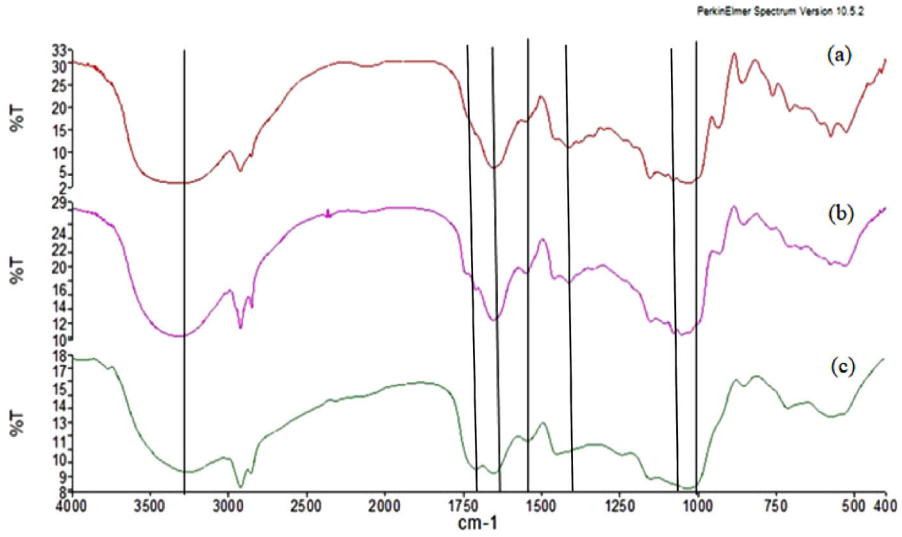

Figure 6. FTIR spectra of the rice bran products obtained using (a) the soni-auto hybrid method and (b) the sonication method at. (c) The FTIR spectrum of the raw rice bran specimen is also shown.

Table 3. Main FTIR spectral characteristics of the raw rice bran and extracted protein specimens.

Sample	Wavenumber (cm^{-1})	Bond
Raw rice bran	1035	C–O stretch
Extracted protein using sonication	1027	
Extracted protein using soni-auto hybrid	1037	
Raw rice bran	1410	Amide III band (C–N stretching)
Extracted protein using sonication	1410	
Extracted protein using soni-auto hybrid	1410	
Raw rice bran	1545	N–O asymmetric stretching
Extracted protein using sonication	1540	
Extracted protein using soni-auto hybrid	1548	
Raw rice bran	1651	α-Helix
Extracted protein using sonication	1652	β-Turn
Extracted protein using soni-auto hybrid	1652	
Raw rice bran	1745	C = O stretching
Extracted protein using sonication	1740	
Extracted protein using soni-auto hybrid	1740	
Raw rice bran	3265	O–H stretching
Extracted protein using sonication	3323	
Extracted protein using soni-auto hybrid	3303	

structure. However, as shown in Figure 6, the spectra of the extracted protein samples contain clear peaks at 1600–1700 cm^{-1}, which are not present in that of the rice bran sample. Based on previous works, it is known that these peaks correspond to the amide I band of the protein secondary structure, which in turn originates from stretching of the peptide bond C = O moiety. According to Apinunjarupong et al. (2009), the bands observed at 1651 cm^{-1} (rice bran sample), 1652 cm^{-1} (sonication method), and 1652 cm^{-1} (soni-auto hybrid method) represented a the β-sheet conformation. In addition, Meutter and Goormaghtigh (2021) reported that the absorbance band at 1656 cm^{-1} was attributed to the amide II band, corresponding to an α-helix (Meutter and Goormaghtigh 2021; Ye et al. 2017). α-Helix formation also occurred due to 180° reversion of the polypeptide chain, which comprised a segment of four amino acid residues. It should be noted that glycine, cysteine, asparagine, proline, and tyrosine are normally folded together and stabilized by hydrogen bonds. The amide II band also corresponds to the N–O asymmetric stretching, and in the three evaluated samples, the amide I bands were observed at 1545 cm^{-1} (rice bran sample), 1541 cm^{-1} (sonication method), and 1549 cm^{-1} (soni-auto hybrid method). The presence of these two amide bands thereby confirmed the presence of amino acids in all samples, especially in the samples extracted using the sonication and soni-auto hybrid methods.

Moreover, the bands observed between 3000 and 3400 cm^{-1} were attributed to the phenolic and alcoholic hydroxyl groups in the structures. More specifically, the presence of bands at 3265, 3323, and 3303 cm^{-1} for the rice bran sample, the sonication-extracted protein, and the soni-auto hybrid-extracted protein, respectively, confirmed the presence of starch, which is a long-chain polysaccharide with a large number of OH groups. This component originated from its complexation with the protein at the time of precipitation, since the degradation of hemicellulose and cellulose into smaller polysaccharides took place in the cell walls during the extraction process (Wang et al. 2021).

The FTIR results (i.e., peaks between 1740–1745 cm^{-1}, C = O stretching) also show that the lipid content was lower in the extracted rice bran protein samples compared to the

raw rice bran specimen (Shapaval et al. 2019). This result therefore indicates that the extraction temperature and sonication power can reduce the lipid concentration without affecting the protein structure.

3.7 Analysis of the surface morphology of the extracted protein

Changes in the surface morphology of the raw rice bran sample after extraction using the two methods evaluated herein were then analyzed using SEM (Figure 7) to determine the effects of sonication and heat treatment on the protein quality.

As shown in Figure 7(a), the raw rice bran particles were irregularly shaped with large, rough surfaces. Amagliani et al. (2016) stated that the heterogeneity observed in raw rice bran in terms of its particle size, shape, and surface characteristics may be due to the milling process employed to convert paddy rice into white rice and rice bran. In addition, it was observed that there were many polygonal hemicellulose structures on the surface of the rice bran, which were built up as cell walls in the rice bran matrix.

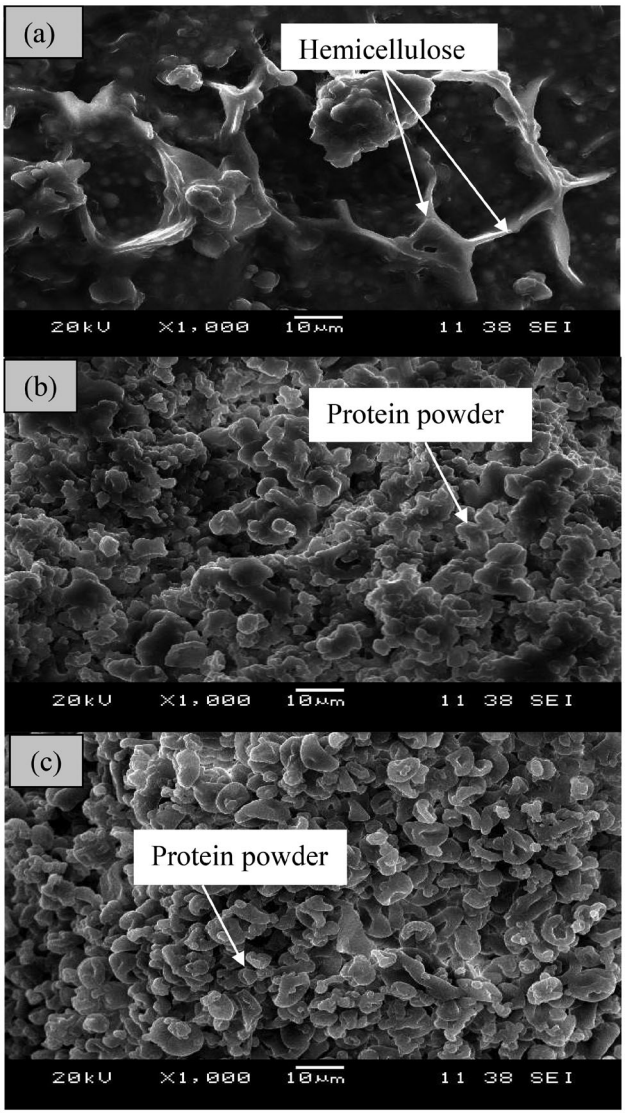

Figure 7. SEM micrographs recorded at 1000 magnification: (a) raw rice bran, (b) protein powder extracted using the sonication method, and (c) protein extracted using the soni-auto hybrid method.

In contrast, Figures 7(b,c) show the modular (rounded irregular)-shaped particles of the extracted protein powders, wherein their shriveled appearance is apparent. In both cases, the surfaces of the samples were rough, wherein the large particles exhibited mostly irregular shapes, and the smaller particles were modular. This was attributed to the fact that cohesiveness can increase the surface roughness by promoting interactions and mechanical locking between the particles. In addition, the samples extracted using both the sonication and soni-auto hybrid methods possessed similar powder characteristics, thereby demonstrating that heat treatment did not change the powder characteristics of the sample. As previously reported by Amagliani et al. (2016), spherical particles can reduce the degree of particle interlocking and resistance to flow. As a result, our extracted protein powders were expected to exhibit a higher flowability and a lower compressibility based on the obtained micrographs. Overall, these results demonstrate that the using of thermal treatment during the soni-auto process increased the protein concentration to a maximum of 17.174 mg/mL while maintaining the powder quality of the product.

4. Conclusions

In conclusion, the addition of a thermal effect via an autoclaving process during the extraction of protein from rice bran resulted in an increased degree of protein extraction (i.e., up to 18%) without altering the quality of the powder product. The implementation of this hybrid sonication/thermal (soni-auto) approach also allowed the sonication time to be reduced from 35 to 30 min (c.f., sonication alone) at a constant feed-to-solvent ratio of 1:20 (g:mL) and a sonication temperature of 30 °C. In addition, it was possible to lower the required sonication temperature from 50 to 45 °C in the soni-auto approach at a constant sonication time of 30 min and a feed-to-solvent ratio of 1:20. This was attributed to breakage of the cell wall under heating, and a subsequent enhancement of the mass transfer process. Optimization of the extraction conditions was then carried out for the soni-auto hybrid method with variation in the sonication temperature, feed-to-solvent ratio, and sonication time. It was found that an optimal temperature of 50 °C, a feed-to-solvent ratio of 1:20, and an extraction time of 30 min gave the highest protein concentration, i.e., 17 mg/mL. Furthermore, it was determined that a feed-to-solvent ratio of between 1:10 and 1:40 and a temperature of 40–60 °C had significant effects on the extracted protein concentration, as determined by F values (1,11) of 32.53 and 4.90, respectively, and p-values of <0.0001 and <0.0489, respectively. Meanwhile, variation in the extraction time between 20 and 40 min had no significant effect on the protein concentration, as determined by its F value (1,11) of 0.083 and its p-value of <0.7786. Overall, Fourier transform infrared spectroscopy and scanning electron microscopy investigations indicated that the rice bran protein produced

by means of the soni-auto hybrid method exhibited a comparable quality to that obtained using the sonication method, and higher protein yields were also obtained. Detail economic analysis are required before this technology can be implemented in this industry to ensure that the process are economicaly and industry viable.We therefore expect that our proposed protein extraction process will add value to rice bran and eventually enhance the profits of the rice industry.

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