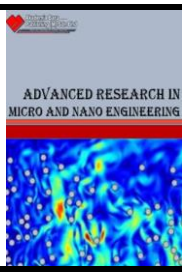
Penerbit Akademia Baru

Journal of Advanced Research in Micro and Nano Engineering

Journal homepage:
https://www.akademiabaru.com/submit/index.php/armne/index
ISSN: 2756-8210



A Three-Level Factorial Model for Maximising Protein Extraction from Rice Bran with Choline Chloride: Glycerol

Kamalea Amaleena Farhana Kamal Ramlee[1], Nik Muhammad Azhar Nik Daud[1,2,*], Mohd Sharizan Md Sarip[1], Amirul Ridzuan Abu Bakar[1,2], Mohd Asraf Mohd Zainudin[1]

[1] Faculty of Chemical Engineering & Technology, UniCITI Alam Campus, Universiti Malaysia Perlis (UniMAP), 02100 Sungai Chuhcuh, Padang Besar, Perlis, Malaysia
[2] Centre of Excellence for Biomass Utilization, Universiti Malaysia Perlis (UniMAP), Kompleks Pusat Pengajian Jejawi 3, 02600, Arau, Perlis, Malaysia

ARTICLE INFO

Article history:
Received 28 January 2024
Received in revised form 17 March 2024
Accepted 26 April 2024
Available online 30 May 2024

Keywords:
Three level factorials; protein; rice bran; deep eutectic solvents

ABSTRACT

Rice bran, a by-product of rice milling, contains four protein types – albumin, globulin, prolamin, and glutelin – known for their hypoallergenic nature and nutritional advantages. To enhance rice bran's worth, its protein was isolated using deep eutectic solvent (DES). The influence of three operational factors (temperature, extraction time, and rice bran to DES ratio) on protein yield was evaluated post-conversion using three level factorial design. Optimal conditions at 80°C, 3 hours, and 1:5 rice bran to DES ratio yielded the highest extracted rice bran protein (RBP) at 17.49%. Temperature and solvent-to-sample ratio show the lowest p-value, indicating a significant effect on the RBP yield. The validation test shows an error of less than 5% between the experimental and the predicted value, showing the model can be used to predict the RBP yield. The RBP was then characterised using Fourier Transform Infrared Spectroscopy (FTIR), Bradford Assays and SDS-PAGE electrophoresis to confirm the protein presence in rice bran powder. FTIR analysis showed the existence of amide I, amide II, aliphatic groups and amine or hydroxyl groups in the RBP. Furthermore, Bradford assays analysis indicated 23.89 ± 0.75% protein content in rice bran powder. SDS-PAGE electrophoresis shows the existence of Albumin and globulin in the RBP. In conclusion, a full three level factorial can be used to model and predict the optimal condition for protein extraction from rice bran using ChCl-Gly DES.

1. Introduction

Rice (Oryza sativa) holds a prominent position among the world's essential grains and serves as a primary dietary staple for a significant portion of the global populace, with a particular focus on Asian communities [1,2]. As of the beginning of 2019, Malaysia had produced approximately 2.9 million metric tons of paddy and 1.88 million metric tons of rice [3]. It is ranked in third place after crude palm oil and rubber in Malaysia's top 20 commodities production. The process of rice milling will

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

https://doi.org/10.37934/armne.19.1.6377

produce a by-product, namely rice husk and rice bran, constitute of 20% and 8% of total rice production. This by-product is normally discarded as a waste rather than being commercialized.

Rice bran is the cuticle between white rice and the paddy hull and serves as a contained layer for the embryo and endosperm of the rice. Rice bran possesses a remarkably nutritious chemical composition, comprising approximately 12% to 17% protein, 13% to 23% fat, 34% to 54% carbohydrates, 6% to 14% fiber, and 8% to 18% ash [4]. Several researchers have documented valuable bioactive properties within rice bran proteins, underscoring the significant worth inherent in this specific by-product [5-8]. The high protein content in rice bran can make rice bran a source of alternative protein. Several ways can be used to extract protein such as alkaline, water extraction and solvent extraction. However, the variability in protein solubility found in rice bran presents a considerable challenge during the extraction process. This is because solute solubility plays an important role in improving the mass transfer mechanism [9]. Gadalkar *et al.,* [10], for instance, utilised an alkaline extraction technique to extract protein from rice bran at pH 9, yielding approximately 50% yield from untreated rice bran [10]. This extraction method demands up to 180 minutes. In 2012, Bandypadhyay *et al.,* successfully extracted around 82.6% of protein from rice bran meal using papain and viscozyme enzymes for the process [11]. Wang and colleagues adopted a multistep extraction approach in 2014 to extract distinct protein types [12]. They achieved up to 13.7% protein yield using a combination of sodium hydroxide, sodium chloride, and alcohol as extraction solvents. Each method employed has its own merits and demerits. The alkaline approach selectively draws glutelin and albumin proteins, but higher pH conditions can lead to undesired protein modifications [13]. Enzymatic extraction, while yielding the most protein, incurs higher costs. Multistep extraction can capture various protein types but is time-intensive. A novel option, deep eutectic solvents (DES), can mimic multistep extraction, though limited to a single solvent due to its versatility.

A deep eutectic solvent (DES), constituted by a synergistic amalgamation of multiple compounds, exhibits a melting point lower than its individual constituents owing to robust hydrogen bonding interactions amongst its composite elements [14]. Typically, DES comprises a quaternary ammonium salt containing cations and halide anions, serving as a hydrogen bond acceptor (HBA) that is exemplified by choline chloride. This intricate solvent engages with a metallic salt denoted as a hydrogen bond donor (HBD), typified by glycerol and urea. The employment of DES for protein extraction is not a recent development. For instance, Grudniewska *et al.,* [15] successfully extracted protein from rapeseed cake and evening primrose cake, utilising choline chloride (ChCl): glycerol (Gly) DES, achieving yields of approximately 20% and 35%, respectively [15]. In a similar vein, Lin *et al.,* [16] conducted a study wherein Choline chloride: levulinic acid DES was employed for protein extraction from bamboo shoots and processing waste, comparing its efficacy against alkaline extraction. The findings indicated a notable enhancement in extraction yield when utilising the DES approach [16].

Response Surface Methodology (RSM) constitutes an assemblage of statistical and mathematical techniques predicated on fitting a polynomial model to a dataset [17]. This model aims to portray the behavior of the dataset, serving the purpose of facilitating statistical prognostications. This methodology proves advantageous in scenarios necessitating optimisation, design, development, and enhancement of processes where one or multiple responses are influenced by several variables [18]. Several models can be used to perform RSM. This paper will focus on the full three-level factorial design model. Three parameters shall be investigated at three factorial levels. The polynomial equation shall be computed from the model and used to determine the optimised condition for the RBP extraction. The RBP was then characterised to determine the functional group, protein content and protein size.

2. Methodology

2.1 Material Preparation

Rice bran was procured from Kilang Beras BERNAS Sdn Bhd in Kuala Perlis, Perlis. To inhibit hydrolytic rancidity, the unprocessed rice bran underwent a 3-minute heating process, following the method described by Mansor *et al*., [19]. Subsequently, it was kept at a temperature of 4˚C until the commencement of the experimental process. Using a grinder, the rice bran was then finely ground into smaller particles measuring 0.7µm. Before extraction, the sample underwent a defatting procedure using n-hexane.

A deep eutectic solvent (DES) was created by mixing choline chloride (ChCl) and glycerol (Gly) in a 1:2 ratio. These components were blended and stirred at a temperature of 60 ºC for one hour until a uniform and transparent liquid was obtained. Afterward, the mixture was allowed to cool to room temperature. Characterization of the ChCl-Gly DES involved employing Fourier-Transform Infra-Red (FTIR) analysis and a viscosimeter.

2.2 Extraction of Protein Powder from Rice Bran

This method was adapted from reference [15] with slight modifications. A 1-gram sample of ground rice bran was combined with varying amounts (5g, 10g, 15g) of deep eutectic solvent (DES) in a 50 ml screw-top glass bottle, resulting in rice bran to DES ratios of 1:5, 1:10, and 1:15 (weight/weight). The mixture was stirred at 500 revolutions per minute (rpm) for different durations (60, 120, and 180 minutes) within an oil bath maintained at distinct temperatures: 40°C, 60°C, and 80°C.

After stirring, the mixture was allowed to cool to room temperature and then subjected to centrifugation at 3500 rpm for ten minutes. The resulting supernatant, which was the liquid fraction, was vacuum-dried in an Erlenmeyer flask. The remaining residue underwent re-centrifugation at 3500 rpm after being treated with fresh 1-gram portions of DES, totaling three times. The supernatant obtained from each re-centrifugation was combined with the liquid fraction in the Erlenmeyer flask. The rice bran residue was washed with 5 ml of deionized water on three separate occasions, vacuum-filtered, and subsequently dried at 105°C for 16 hours. The dried residue was then cooled in a desiccator.

To the liquid fraction obtained after DES treatment, an additional 50 ml of deionized water was added. The resulting mixture was incubated at approximately 4°C for 12 hours. Subsequently, centrifugation was carried out at 6500 rpm for ten minutes to separate the precipitates. These precipitates were washed three times with 5 ml of deionized water and underwent an additional centrifugation step at 6500 rpm for ten minutes. Finally, the formed precipitates were subjected to freeze-drying.

2.3 Full Three Level Factorial Design Modelling

A comprehensive three-level general factorial design was employed to explore the primary and interactive influences of key parameters on the extraction of protein powder from rice bran. This design enables us to incorporate factors with varying levels, thus encompassing all possible combinations of these levels in the experiment. To account for potential nonlinearities in the response function, particularly the yield of Rice Bran Protein (RBP), three-level designs were utilized. To mitigate unexpected errors, the experiments were randomly allocated to a single block, ensuring that any variations were distributed across the entire range of experimental conditions.

The study assessed the effects of temperature (A), solvent-to-sample ratio (B), and time (C), as well as their interactions and curvature effects, on the yield of protein extraction, using Analysis of Variance (ANOVA) with Design-Expert software version 12.00. A confidence level of 95% (or a 5% significance level) was employed as the critical P-value. The comparison of critical and obtained P-values was used to evaluate the impact of these parameters on processed thermal conductivity.

Each parameter was investigated at three levels: high (+1), center (0), and low (-1), as outlined in Table 1. The experimental design was structured to analyze the primary factors and their interactions with respect to RBP extraction yield. Table 2 provides the layout of the design and the experimental results for the 32 General Factorial designs. The RBP yield was calculated using Eq. (1).

$$\text{Percent yield}, \% = \frac{\text{Mass of rice bran protein after extracted}}{\text{Mass of defatted rice bran}} \times 100\% \quad (1)$$

Table 1
General parameters used

Factor	Code	Level		
		-1	0	+1
Temperature (◦C)	A	40	60	80
Solvent: sample	B	5	10	15
Time (minutes)	C	60	120	180

Table 2
Design layout for three factorial model

Run	Parameter			Yield (%)
	A	B	C	
1	40	5	60	6.54
2	60	5	60	11.42
3	80	5	60	13.17
4	40	10	60	4.94
5	60	10	60	14.88
6	80	10	60	10.50
7	40	15	60	6.41
8	60	15	60	7.47
9	80	15	60	14.53
10	40	5	120	7.25
11	60	5	120	10.94
12	80	5	120	15.14
13	40	10	120	3.94
14	60	10	120	11.94
15	80	10	120	17.63
16	40	15	120	3.45
17	60	15	120	12.76
18	80	15	120	10.72
19	40	5	180	6.35
20	60	5	180	14.01
21	80	5	180	18.39
22	40	10	180	1.37
23	60	10	180	11.13
24	80	10	180	6.61
25	40	15	180	4.59
26	60	15	180	5.39
27	80	15	180	16.07
28	60	10	120	7.76
29	60	10	120	7.12

30	60	10	120	7.31
31	60	10	120	3.19
32	60	10	120	7.44

2.4 RBP Characterisation

RBP sample was characterised using FTIR Spectroscopy, Bradford assay analysis and SDS-PAGE electrophoresis to determine the functional group, total protein content and molecular weight of the protein.

2.4.1 Fourier-Transform Infra-Red (FTIR) spectroscopy

The characterisation of protein derived from rice bran and DES was accomplished through FTIR analysis. The FTIR spectrometer was equipped with the DES and RBP samples were subjected to spectral analysis, employing a resolution of 8 cm^{-1} within the wavenumber range of 650 to 4000 cm^{-1}. To elucidate specific functional group information regarding the protein's molecular structure, the precipitated rice bran protein was introduced into the FTIR spectroscopy setup for comprehensive analysis. The formation of pellets for analysis was achieved by integrating rice bran precipitate with potassium bromide (KBr).

2.4.2 Bradford assay analysis

Protein content was determined according to Borremans *et al.,* [20] with some modifications. Briefly, 0.02 g of freeze-dried protein sample was dissolved in 1 mL solution containing 5% SDS, 0.01 M Tris. The solutions were heated on a thermal block at 70°C for 60 minutes and centrifuged (8000rpm, 10 minutes, 4°C). The Bradford method was employed to determine the protein concentration in the supernatant, with bovine serum albumin serving as the reference standard. This assessment involved combining 800 µL of protein extracts with 200 µL of the Bradford reagent and allowing them to react for 20 minutes at room temperature. The absorption at 595 nm was measured against a blank, which was created by mixing 800 µL of a solution identical to the sample with 200 µL of Bradford reagent, using a UV/Vis spectrophotometer.

2.4.3 SDS-PAGE analysis

The molecular weights of rice bran proteins were determined utilising a modified version of the SDS-PAGE method as outlined by Uraipong and Zhao [21]. Freeze-dried rice bran protein samples were first diluted to a concentration of 20 mg/mL using 5% SDS in 0.01 M Tris buffer. This mixture was then combined with a Laemmli sample buffer in a 1:1 volume ratio. Subsequent to this, the samples were preheated to 85°C for a duration of 10 minutes. The prepared samples, alongside a prestained protein standard with a broad molecular weight range (10-250 kDa) (Biolabs), were loaded onto a 4-15% Mini-Protein TGX precast gel. The electrophoresis process was conducted employing a Mini Protean Tetra Cell system (Bio-Rad, Australia) with a constant voltage set at 200 V and a current of 75 mA for a duration of 40 minutes, using a tris/glycine/SDS buffer at pH 8.3. Upon the completion of electrophoresis, the gel was subjected to staining for 24 hours, followed by a destaining process. The staining solution was prepared by dissolving 0.125 g of Coomassie brilliant blue R-250 in a mixture of 50 mL of 12.5% acetic acid, 37.5% methanol, and 50% MilliQ water. Staining was carried out with a mixture of 2 mL of staining buffer in 100 mL of MilliQ water. For destaining, a buffer composed of 10% methanol, 10% acetic acid, and 80% MilliQ water was utilised. This process

was conducted for a duration of 4 to 24 hours, enabling the removal of the Coomassie brilliant blue stain until the background became clear. After electrophoresis and staining, the gel was subjected to imaging and subsequent analysis of the spectra.

3. Results

3.1 The Model Equation and Statistical Evaluation

By employing a multiple regression analysis on the design matrix along with the responses presented in Table 2, we have successfully formulated a second-order polynomial equation in coded representation (Eq. (2)) using Design Expert version 12 software. This equation elucidates and expounds upon the relationship pertaining to the extraction yield of RBP.

$$\mathrm{Y} = -0.7879A^2 + 0.9093B^2 + 0.6147C^2 - 1.49AB + 0.3951AC - 0.6541BC + 3.07A - 2.74B + 0.1645C + 8.24 \quad (2)$$

In the context of predicting RBP yield (%), the variables are represented as follows: Y for the predicted yield, and A, B, and C as the coded terms for temperature, solvent-to-sample ratio, and time, respectively. To determine the optimal values for these variables, Eq. (2) was solved, and response surface contour plots were analyzed. The models used to fit the data performed well, with minimal and unbiased discrepancies between observed and predicted values. The coefficient of determination (R2) for RBP yield was calculated as 0.9531, indicating the statistical significance of the regression, as depicted in Figure 1.

Figure 2 visually presents the residuals for the predicted RBP yield values. These residuals appear to be uniformly distributed, suggesting a good model fit. The significance of the model fit for RBP yield was assessed using ANOVA in Design Expert software version 12, and the results are summarized in Table 3. The analysis revealed that the models are indeed significant, as evidenced by the high F-value (40.60) and a p-value (Prob > F) less than 0.05. The standard deviation of the predicted model was determined to be 0.9649. The Lack of Fit F-value was found to be 1.25, indicating that the Lack of Fit is insignificant compared to the pure error in the model. The predicted R^2 value (0.8426) is in reasonable agreement with the adjusted R^2 value (0.9296), with the difference between these two values being less than 0.2. Notably, the parameters A, B, AB, and B2 (corresponding to temperature and solvent-to-sample ratio) are significant, as their "Prob > F" (p-value) values are all less than 0.05 in the model.

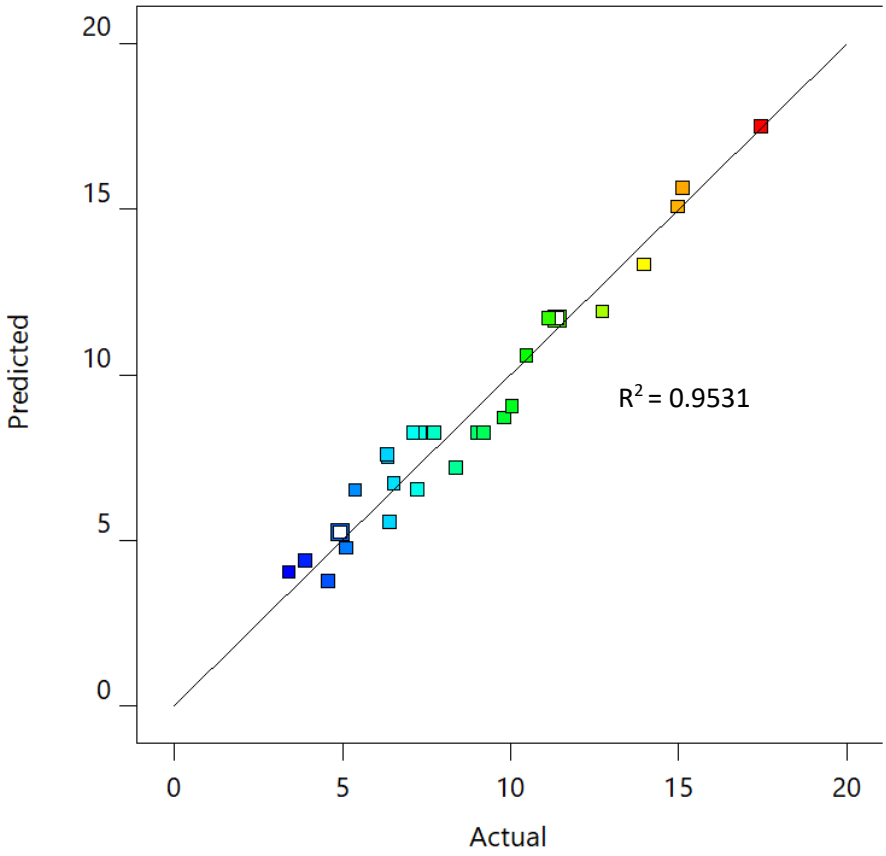


Fig. 1. Predicted versus actual data

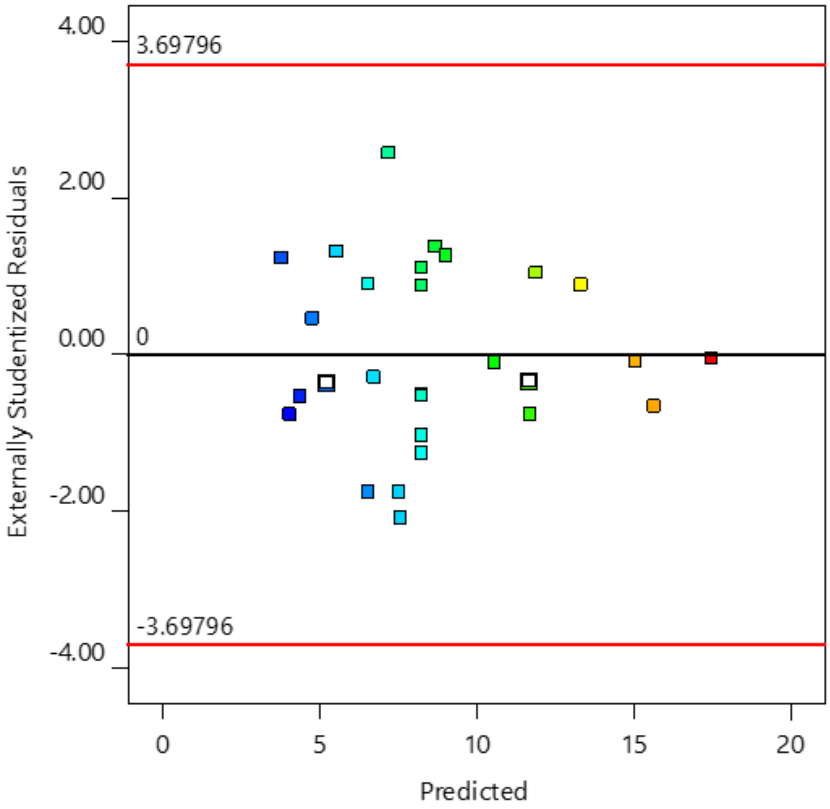


Fig. 2. Residuals for predicted values

Table 3
ANOVA analysis of RBP extraction

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	340.23	9	37.80	40.60	< 0.0001	significant
A	123.63	1	123.63	132.78	< 0.0001	
B	98.57	1	98.57	105.87	< 0.0001	
C	0.4059	1	0.4059	0.4360	0.5174	
AB	19.24	1	19.24	20.66	0.0003	
AC	1.44	1	1.44	1.55	0.2295	
BC	3.95	1	3.95	4.24	0.0542	
A^2	3.77	1	3.77	4.05	0.0595	
B^2	5.02	1	5.02	5.39	0.0322	
C^2	2.40	1	2.40	2.58	0.1254	
Residual	16.76	18	0.9311			Not significant
Lack of Fit	12.82	13	0.9863	1.25	0.4300	
Pure Error	3.94	5	0.7875			
Cor Total	356.99	27				

3.2 Effect of Parameters on RBP Yield

The coefficient values estimated for the primary effects of the process variables in Eq. (2) reveal the following trends: Temperature (A) has a positive impact on RBP yield, while the ratio (B) has a negative influence. In contrast, time (C) doesn't significantly affect the RBP yield within the tested parameter levels. Eq. (2) also suggests that interactions between variables, such as temperature (A) and solid-to-solvent ratio (B), as well as solid-to-solvent ratio (B) and time (C), have a negative impact on RBP yield. Conversely, the interaction between temperature (A) and time (C) has a positive effect. Interestingly, time (C) exhibits the most significant quadratic effect on RBP yield.

The predicted response (Y) of the process to the independent variables is visually represented in three-dimensional (3D) space to help illustrate the shape of the response surface. In Figure 3, the interaction between A and B at different experiment durations is displayed. It's evident that the yield maximizes with longer extraction times. This observation aligns with the findings by Ma *et al.,* [22], which suggest that extending the extraction time enhances peanut protein extraction. Schmidt *et al.,* [23] also arrived at a similar conclusion, emphasizing the role of residence time in influencing protein extraction yield. A longer extraction time allows for extended contact between the rice bran and the solvent, facilitating greater protein extraction and, subsequently, higher yield. Figure 4 shows the temperature–extraction time interaction at different solvent-to-sample ratio levels. The lower solvent-to-sample ratio increases the RBP yield. This agrees with [22], which observes that a higher solvent-to-sample ratio will decrease protein yield.

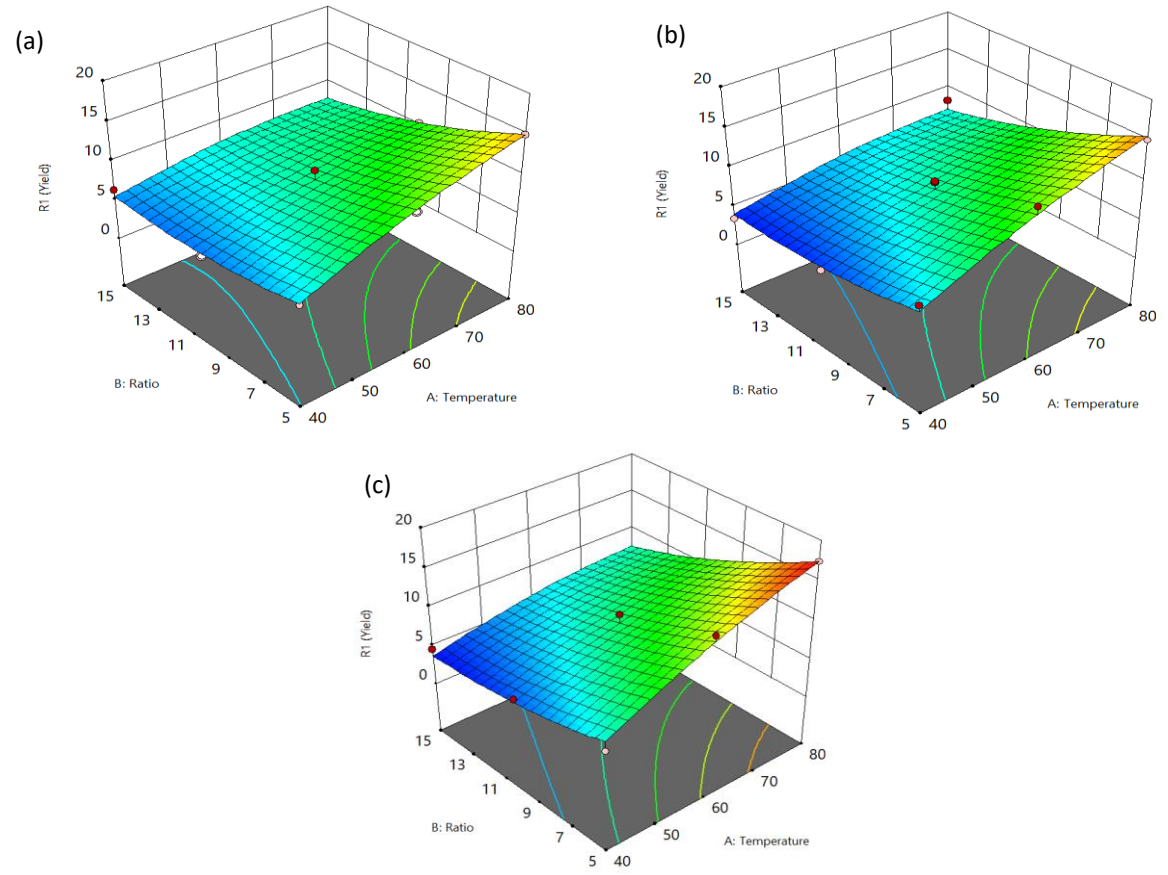


Fig. 3. A-B interaction at extraction time of : a) 60 minutes. b) 120 minutes. c) 180 minutes

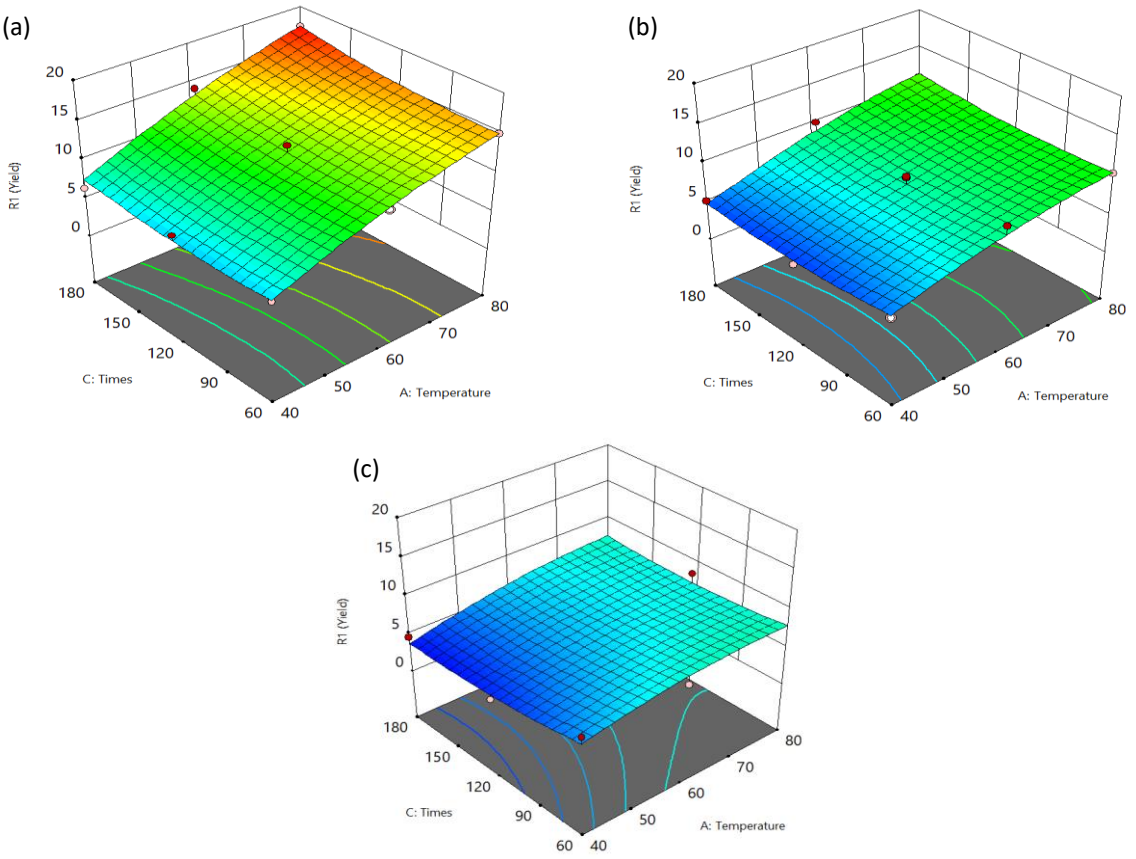


Fig. 4. A-C interaction at sample to solvent ratio of: a) 5. b) 10. c) 15

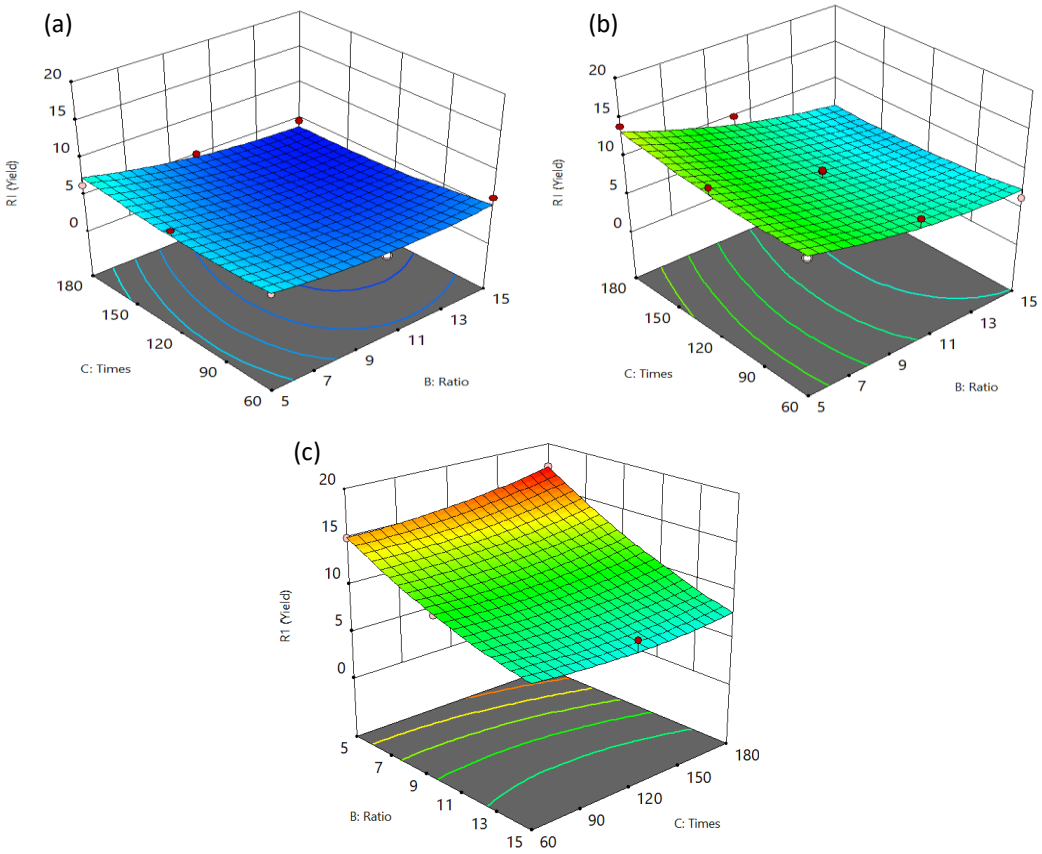


Fig. 5. B-C interaction at extraction temperature of: a) 40◦C b) 60◦C c) 80◦C

Figure 5 shows the interaction of solvent-to-sample ratio and times at three different temperatures. This is in agreement with Lin *et al.,* [24] who observed higher protein yield from bamboo shoots at higher temperatures. Higher temperature increases the protein yield. This is due to higher energy will allow more mass transfer. The viscosity of DES will also reduce, making the mass transfer more efficient [25]. The temperature was kept at 80◦C for the maximum temperature as the protein tends to denature at higher temperatures. Based on the RSM, the optimum conditions for the RBP protein extraction are at a temperature of 80◦C, an extraction time of 180 minutes and a solvent-to-sample ratio of 1:5, expected to yield 17.451 % of RBP.

3.3 Validation of RBP Optimization

Following the completion of the optimization process, validation data were collected as per the recommendations provided by the Design of Experiments (DOE) software. The purpose of this validation was to assess whether there were any notable differences between the anticipated outcomes and the actual results obtained through experimentation. Two validation runs were carried out, as detailed in Table 4, and the results indicated minimal discrepancies between the predicted and observed protein yields. The percentage error, which was within the range of ±5%, indicates that the data utilized in this study can be considered generally reliable and acceptable.

Table 4
Total protein in rice bran protein

Protein sample	Protein yield (%)	Error (%)
Replicate 1	17.453	0.01%
Replicate 2	18.389	5.1%

3.4 Characterisation of RBP

RBP sample was characterised using FTIR, Bradford assay and gel electrophoresis to understand the functional group, protein content and type of protein inside the RBP.

3.4.1 Functional properties of RBP

FTIR spectroscopic analysis was employed to investigate the changes in extracted rice bran when treated with Choline chloride-glycerol Deep Eutectic Solvent (DES). Figure 6 displays the FTIR spectra of defatted rice bran (the raw material) and rice bran protein extracted in triplicate using Choline chloride-glycerol DES under optimal conditions. The observed peaks align with findings from Zhang *et al.,* and Ramlee *et al.,* [26,27]. Specifically, the bands within the range of 3500 to 3200 cm^{-1} are associated with O-H stretching [28] and N-H stretching, while the band at 2928 cm^{-1} corresponds to aliphatic groups within proteins. Notably, peaks at wavelengths 1649 cm^{-1} and 1542 cm^{-1} are indicative of amide I and amide II, respectively. Following the extraction process, there is a noticeable increase in the intensities of these bands associated with amide I, amide II, aliphatic groups, and amine or hydroxyl stretching. This increase suggests a rise in the protein content, demonstrating the effectiveness of the extraction process.

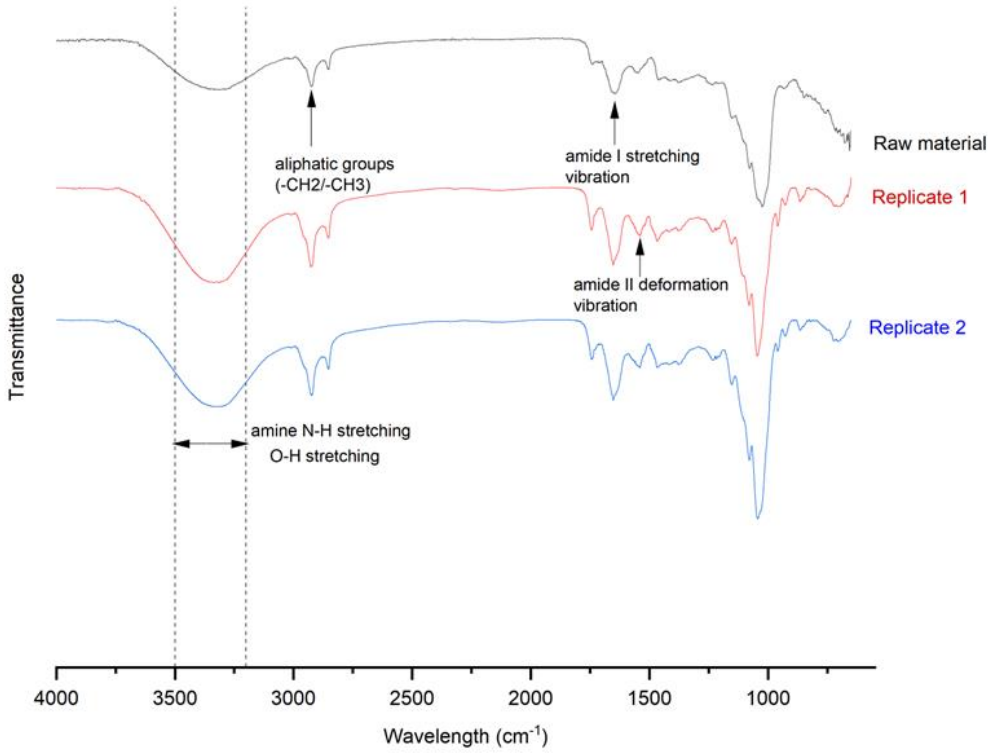


Fig. 6. FTIR Spectra of rice bran and RBP

3.4.2 Total protein content of RBP

To determine the total protein content of the rice bran protein extracts, the Folin-Ciocalteu assay was employed. This assay involved creating a standard curve using bovine serum albumin (BSA) and considering the correlation between absorbance and concentration. This allowed for the quantification of protein content in the rice bran protein extracts. The calibration curve generated from the analysis of the standard (BSA) was linear with equation y = 0.2714x + 0.1421 with R^2 =

0.8809; where x is the absorbance and y is the concentration of BSA solution (mg/mL) expressed as mg protein/g sample. The results presented in Table 5 showed that at optimum conditions, the protein yield obtained was 17.453% and 18.389%, with total protein recovery of 24.42 mg/g sample and 23.37 mg/g sample, with an average of 23.89 ± 0.75%.

Table 5
Total protein in rice bran protein

Protein sample	Protein yield (%)	Total protein (mg/g sample)
Replicate 1	17.453	24.4215
Replicate 2	18.389	23.3677

3.4.3 Protein molecular weight of RBP

Figure 7 shows SDS-PAGE examined the protein patterns of RBP protein. Raw rice bran protein had bands around 64 - 10 kDa regions. Based on lane 2 and 3, DES 1 successfully extracted protein around 64 – 15 kDa and the bands appeared higher intensity than raw rice bran protein in lane 5. The DES protein electrophoresis patterns result implied that choline chloride-glycerol can extract globulin and albumin fractions. Tang *et al.,* [29] reported that the molecular weights of rice bran albumin have bands range 14-33 kDa, while globulin showed bands around 20-50 kDa.

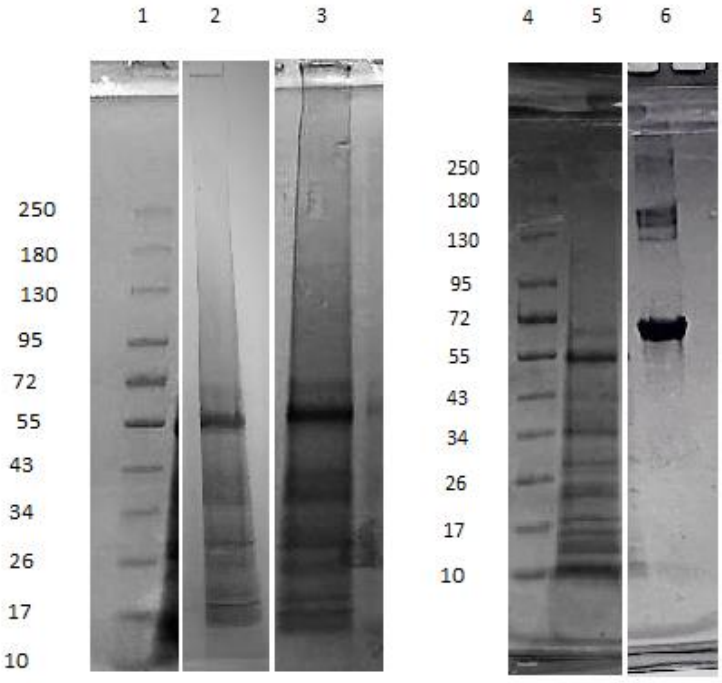


Fig. 7. SDS-PAGE profiles of proteins in rice bran extracted different solvents. Lane 1: marker; Lane 2: RBP replicate 1; Lane 3: RBP replicate 2; Lane 4: marker; Lane 5: defatted rice bran; Lane 6: bovine serum albumin

4. Conclusions

In summary, Rice Bran Protein (RBP) powder can be efficiently extracted from rice bran using Choline Chloride-Glycerol Deep Eutectic Solvent (ChCl-Gly DES). A comprehensive three-level factorial design was employed successfully to optimize the extraction process parameters, including temperature, time, and solvent-to-sample ratio. According to the analysis of variance (ANOVA),

temperature and solvent-to-solid ratio were found to have a significant impact on the yield of RBP. The optimal conditions for RBP protein extraction were determined to be a temperature of 80°C, an extraction time of 180 minutes, and a solvent-to-sample ratio of 1:5, which is expected to yield approximately 17.451% of RBP. The validation of the model indicated an error of less than ±5%, confirming the validity of the model for predicting RBP yield.

The characterisation of the RBP shows the existence of amide I, amide II, aliphatic groups and amine or hydroxyl groups in the RBP. The total protein content in the RBP at the optimised condition is 23.89 ± 0.75%. SDS-PAGE analysis shows the existence of albumin and globulin in the RBP. Finally, enhancing the utility of rice bran as a food product was achieved through protein extraction. This is particularly important because rice bran is frequently relegated to animal feed usage, being discarded as waste once the milling process is completed. Consequently, through the execution of this research, the value of rice bran was elevated by employing an extraction process to isolate its protein content. This protein, recognised for its hypoallergenic nature and possessing nutraceutical properties with excellent bioavailability, holds the potential to serve as a substantial constituent in formulations for infant food and protein supplements.

Acknowledgement
The author wishes to express gratitude for the financial support provided for this research. This research was made possible through the Fundamental Research Grant Scheme (FRGS) under grant number FRGS/1/2020/STG04/UNIMAP/02/4 from the Ministry of Higher Education Malaysia, as well as the RSC Research Fund under grant number R20-3604 from the Royal Society of Chemistry (RSC).

References

[1] Sen, Saikat, Raja Chakraborty, and Pratap Kalita. "Rice-not just a staple food: A comprehensive review on its phytochemicals and therapeutic potential." *Trends in Food Science & Technology* 97 (2020): 265-285. https://doi.org/10.1016/j.tifs.2020.01.022

[2] Fukagawa, Naomi K., and Lewis H. Ziska. "Rice: Importance for global nutrition." *Journal of nutritional science and vitaminology* 65, no. Supplement (2019): S2-S3. https://doi.org/10.3177/jnsv.65.S2

[3] Dorairaj, Deivaseeno, and Nisha T. Govender. "Rice and paddy industry in Malaysia: governance and policies, research trends, technology adoption and resilience." *Frontiers in Sustainable Food Systems* 7 (2023): 1093605. https://doi.org/10.3389/fsufs.2023.1093605

[4] Fabian, Cynthia, and Yi-Hsu Ju. "A review on rice bran protein: its properties and extraction methods." *Critical reviews in food science and nutrition* 51, no. 9 (2011): 816-827. https://doi.org/10.1080/10408398.2010.482678

[5] Gul, Khalid, Basharat Yousuf, A. K. Singh, Preeti Singh, and Ali Abas Wani. "Rice bran: Nutritional values and its emerging potential for development of functional food—A review." *Bioactive Carbohydrates and Dietary Fibre* 6, no. 1 (2015): 24-30. https://doi.org/10.1016/j.bcdf.2015.06.002

[6] Park, Ho-Young, Kwang-Won Lee, and Hee-Don Choi. "Rice bran constituents: Immunomodulatory and therapeutic activities." *Food & function* 8, no. 3 (2017): 935-943. https://doi.org/10.1039/C6FO01763K

[7] Sohail, Muhammad, Allah Rakha, Masood Sadiq Butt, Muhammad Jawad Iqbal, and Summer Rashid. "Rice bran nutraceutics: A comprehensive review." *Critical Reviews in Food Science and Nutrition* 57, no. 17 (2017): 3771-3780. https://doi.org/10.1080/10408398.2016.1164120

[8] Liu, Y. Q., Padraig Strappe, W. T. Shang, and Z. K. Zhou. "Functional peptides derived from rice bran proteins." *Critical reviews in food science and nutrition* 59, no. 2 (2019): 349-356. https://doi.org/10.1080/10408398.2017.1374923

[9] Jaapar, Syaripah Zaimah Syed, Noor Azian Morad, Yoshio Iwai, Mohd Sharizan Md Sarip, Mohd Azizi Che Yunus, and Yasmin Anum Mohd Yusuff. "Estimation of ginger bioactive compound solubilities in hot compressed water and enhancement with entrainer." *Fluid Phase Equilibria* 393 (2015): 40-47. https://doi.org/10.1016/j.fluid.2015.02.021

[10] Gadalkar, Sagar M., Parag R. Gogate, and Virendra K. Rathod. "Recovery of proteins from rice mill industry waste (rice bran) using alkaline or NaCl-assisted alkaline extraction processes." *Journal of Food Process Engineering* 40, no. 3 (2017): e12430. https://doi.org/10.1111/jfpe.12430

[11] Bandyopadhyay, Kakali, Chaitali Chakraborty, and Amit Kumar Barman. "Effect of microwave and enzymatic treatment on the recovery of protein from Indian defatted rice bran meal." *Journal of Oleo Science* 61, no. 10 (2012): 525-529. https://doi.org/10.5650/jos.61.525
[12] Wang, Changyuan, Dan Li, Feng Xu, Tianshu Hao, and Min Zhang. "Comparison of two methods for the extraction of fractionated rice bran protein." *Journal of Chemistry* 2014 (2014). https://doi.org/10.1155/2014/546345
[13] Phongthai, S., W. Homthawornchoo, and S. J. I. F. R. J. Rawdkuen. "Preparation, properties and application of rice bran protein: A review." *International Food Research Journal* 24, no. 1 (2017).
[14] Abbott, Andrew P., David Boothby, Glen Capper, David L. Davies, and Raymond K. Rasheed. "Deep eutectic solvents formed between choline chloride and carboxylic acids: versatile alternatives to ionic liquids." *Journal of the American Chemical Society* 126, no. 29 (2004): 9142-9147. https://doi.org/10.1021/ja048266j
[15] Grudniewska, Aleksandra, Eduardo M. de Melo, Angel Chan, Radosław Gniłka, Filip Boratynski, and Avtar S. Matharu. "Enhanced protein extraction from oilseed cakes using glycerol–choline chloride deep eutectic solvents: a biorefinery approach." *ACS sustainable chemistry & engineering* 6, no. 11 (2018): 15791-15800. https://doi.org/10.1021/acssuschemeng.8b04359
[16] Lin, Zhen, Guangling Jiao, Junzeng Zhang, Giovana Bonat Celli, and Marianne Su-Ling Brooks. "Optimization of protein extraction from bamboo shoots and processing wastes using deep eutectic solvents in a biorefinery approach." *Biomass Conversion and Biorefinery* 11 (2021): 2763-2774. https://doi.org/10.1007/s13399-020-00614-3
[17] Yolmeh, Mahmoud, and Seid Mahdi Jafari. "Applications of response surface methodology in the food industry processes." *Food and bioprocess technology* 10, no. 3 (2017): 413-433. https://doi.org/10.1007/s11947-016-1855-2
[18] Ghorbannezhad, Payam, Abotaleb Bay, Mahmoud Yolmeh, Rahim Yadollahi, and Javad Yazdan Moghadam. "Optimization of coagulation–flocculation process for medium density fiberboard (MDF) wastewater through response surface methodology." *Desalination and Water Treatment* 57, no. 56 (2016): 26916-26931. https://doi.org/10.1080/19443994.2016.1170636
[19] Mansor, Mohd Rizuan, Mohd Sharizan Md Sarip, Nik Muhammad Azhar Nik Daud, Syahrul Affandi Saidi, Mohd Al Hafiz Mohd Nawi, and Mohd Aminudin Jamlos. "Preparation of Rice Bran Protein (RBP) Powder Using Spray Drying Method at the Optimal Condition and Its Protein Quality." *Processes* 10, no. 10 (2022): 2026. https://doi.org/10.3390/pr10102026
[20] Borremans, An, Sara Bußler, Sorel Tchewonpi Sagu, Harshadrai Rawel, Oliver K. Schlüter, and Van Campenhout Leen. "Effect of blanching plus fermentation on selected functional properties of mealworm (Tenebrio molitor) powders." *Foods* 9, no. 7 (2020): 917. https://doi.org/10.3390/foods9070917
[21] Uraipong, Chatchaporn, and Jian Zhao. "Identification and functional characterisation of bioactive peptides in rice bran albumin hydrolysates." *International Journal of Food Science & Technology* 51, no. 10 (2016): 2201-2208. https://doi.org/10.1111/ijfs.13204
[22] Ma, Tiezheng, Qiang Wang, and Haiwen Wu. "Optimization of extraction conditions for improving solubility of peanut protein concentrates by response surface methodology." *LWT-Food Science and Technology* 43, no. 9 (2010): 1450-1455. https://doi.org/10.1016/j.lwt.2010.03.015
[23] Schmidt, Florian, Max Blankart, Janina Wanger, Markus Scharfe, Theresa Scheuerer, and Jörg Hinrichs. "Upscaling of alkaline pea protein extraction from dry milled and pre-treated peas from laboratory to pilot scale: Optimization of process parameters for higher protein yields." *Journal of Food Measurement and Characterization* 16, no. 6 (2022): 4904-4913. https://doi.org/10.1007/s11694-022-01558-0
[24] Lin, Zhen, Guangling Jiao, Junzeng Zhang, Giovana Bonat Celli, and Marianne Su-Ling Brooks. "Optimization of protein extraction from bamboo shoots and processing wastes using deep eutectic solvents in a biorefinery approach." *Biomass Conversion and Biorefinery* 11 (2021): 2763-2774. https://doi.org/10.1007/s13399-020-00614-3
[25] Cao, Jun, Meng Yang, Fuliang Cao, Jiahong Wang, and Erzheng Su. "Tailor-made hydrophobic deep eutectic solvents for cleaner extraction of polyprenyl acetates from Ginkgo biloba leaves." *Journal of cleaner production* 152 (2017): 399-405. https://doi.org/10.1016/j.jclepro.2017.03.140
[26] Zhang, Yanpeng, Bin Wang, Weinong Zhang, Wei Xu, and Zhixiong Hu. "Effects and mechanism of dilute acid soaking with ultrasound pretreatment on rice bran protein extraction." *Journal of Cereal Science* 87 (2019): 318-324. https://doi.org/10.1016/j.jcs.2019.04.018
[27] Kamal Ramlee, Kamalea Amaleena Farhana, Intan Nurfazliyana Muhammad Nor, Mohd Sharizan Md Sarip, Mohd Asraf Mohd Zainudin, Amirul Ridzuan Abu Bakar, Mohd Azizi Nawawi, and Nik Muhammad Azhar Nik Daud. "Optimization of Rice Bran Protein Extraction Using Choline Chloride-Glycerol Deep Eutectic Solvent Using Response Surface Methodology (RSM)." In *International Conference on Biomass Utilization and Sustainable Energy*, pp. 457-474. Singapore: Springer Nature Singapore, 2022. https://doi.org/10.1007/978-981-99-1695-5_39

[28] Mat, Siti Syamimie Atirah, Sharifah Zati Hanani Syed Zuber, Siti Kartini Enche Ab Rahim, Khairunissa Syairah Ahmad Sohaimi, Noor Amirah Abdul Halim, Nor Fauziah Zainudin, Nor Aida Yusoff et al. "Malachite green adsorption by spent coffee grounds." In *IOP Conference Series: Materials Science and Engineering*, vol. 318, no. 1, p. 012015. IOP Publishing, 2018. https://doi.org/10.1088/1757-899X/318/1/012015

[29] Tang, H., T. Fu, Y. Feng, S. Zhang, C. Wang, and D. Zhang. "Effect of heat treatment on solubility, surface hydrophobicity and structure of rice bran albumin and globulin." *Quality assurance and safety of crops & foods* 11, no. 6 (2019): 499-509. https://doi.org/10.3920/QAS2018.1402