Optimization of Rice Bran Protein Extraction via Microwave-Assisted Alkaline Method for Drug Delivery Applications using Response Surface Methodology

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Rice Bran, Protein Extraction, Response Surface Methodology (RSM), Optimization

ABSTRACT
Rice bran, a by-product of rice milling, contains 10%–16% protein, 15%–22% lipids, and 7%–11.4% fiber. Rice bran protein (RBP) is hypoallergenic and has shown potential in cancer prevention, making it valuable for food and pharmaceutical applications. This study extracted RBP using microwave-assisted alkaline extraction in a shorter time for potential pharmaceutical applications, with or without modification. Response surface methodology (RSM) was used to optimize four parameters: microwave power, extraction time, pH, and solvent-to-defatted rice bran (DRB) ratio. The optimal extraction conditions were determined as 200 watts for 150 s at pH 12, with a solvent-to-DRB ratio of 5:1 (v/w). Under these conditions, the predicted RBP recovery was 47.60%, while the actual recovery was 47.76%, validating the optimization model. The recovered RBP concentrate (RBPC) had a particle size of approximately 285.13 nm, a polydispersity index of 0.33, and a zeta potential of -33.33 mV, indicating good electrostatic stability against coalescence and flocculation. Additionally, SDS-PAGE analysis revealed that glutelin was the predominant protein fraction in the RBPC, confirming the efficiency of the extraction method in isolating specific protein fractions. These findings demonstrate that microwave-assisted alkaline extraction is an effective approach for enhancing RBP recovery while maintaining its stability and making it suitable for pharmaceutical applications.

1. Introduction:

Rice bran is commonly used as an animal feed and fertilizer. It contains beneficial proteins, lipids, vitamins, and minerals. However, its high fiber content results in low digestibility, which can cause digestive issues. Additionally, the high fiber content contributes to a coarse texture and an unappealing taste, making it less suitable for direct human consumption. Furthermore, the oil content in the rice bran must be removed, as it can lead to rancidity, resulting in the production of defatted rice bran (DRB)[1]. Despite these limitations, rice bran remains a valuable resource due to its high protein content, which holds great potential for various applications, including drug delivery systems. Rice bran protein (RBP) is hypoallergenic, exhibits anticancer activity, and is highly nutritious, making it a sustainable protein source for various applications, including food and pharmaceuticals [2]. The composition of rice bran includes 10%–16% protein, 15%–22% lipids, and 7%–11.4% fiber [1]. Conventionally, protein extraction from DRB involves the use of alkaline solutions (KOH or NaOH), which effectively disrupt the hydrogen, amide, and disulfide bonds in the native protein structure. However, this method has yet to achieve an optimal rice bran protein (RBP) recovery [3]. Recent studies have integrated alkaline methods with Microwave-Assisted Extraction (MAE), offering advantages such as shorter extraction times, lower energy consumption, and higher RBP recovery [4]. The factors influencing extraction recovery in MAE include microwave power, extraction time (within limits, as excessive duration may degrade proteins), alkaline pH (which enhances protein-solvent interactions through electrostatic repulsion), and the solvent-to-sample ratio (which facilitates mass transfer from solids to the solvent) [5]. The optimization of the MAE method is expected to enhance the efficiency and quality of the RBP extraction recovery. A prevailing challenge in this field is the paucity of research that has systematically investigated the interplay among all four critical factors. This study aims to optimize the extraction process by simultaneously accounting for four factors (microwave power: 400–800 watts, extraction time: 60–120 seconds, pH: 9–11, and solvent-to-sample ratio: 10–20) using the Response Surface Methodology (RSM) with the aid of Minitab software. The extracted RBP is anticipated to be used in protein-based drug delivery systems, enhancing the

therapeutic efficacy and bioavailability.

2. Materials And Methods:

i. Materials

Rice bran (Oriza sativa L.) was obtained from a local rice milling facility in Sidoarjo, Indonesia. The other chemicals, including ethanol, sodium hydroxide, and hydrochloric acid, were obtained from commercial suppliers.

ii. Preparation of defatted rice bran (DRB)

The preliminary procedure in the extraction of protein from defatted rice bran entails the elimination of the oil content present in the rice bran through the process of oil extraction from the rice bran. This is due to the action of lipase, which hydrolyzes the neutral lipids in rice bran, leading to oxidative rancidity and a consequent reduction in quality. Typically, the Soxhlet process extracts 30 g of rice bran passing through a 32-mesh sieve size using n-hexane as a solvent for 25 cycles. This process produces two products: defatted rice bran (DRB) and crude rice bran oil (CRBO). The DRB is then stored in a freezer for subsequent use as the raw material for RBP extraction.

iii. Preparation of rice bran protein extraction (RBPC)

The procedure for extracting RBP in an alkaline solution was adapted from the method described by Bernardi et al. [5] with modifications. In general, 30 g of DRB was dispersed in distilled water, and 1 M NaOH was added to adjust the pH according to the experimental design shown in table 1. The mixture was then heated using a microwave machine (R-728(S), SHARP, Indonesia). The mixture was then separated into an upper layer (the "supernatant") and a lower layer (the "precipitate") using refrigerated centrifugation (RHSC-1215A-16K, B-ONE, Indonesia) (12,000 g, 10 min.). The pH of the upper layer was then lowered to 4.5 by adding 1 M HCl, thus forming the precipitate, which was subsequently incubated at 10°C for 30 min. Thereafter, the pH of the precipitate was adjusted to 7 and then dried using a freeze-dryer (B-ONE, Indonesia). The RBP recovery was calculated using the following equation (1):

$$RBP\ Recovery\ (\%) = \frac{RBPC\ (g) x\ Protein\ content\ in\ RBPC(\%)}{DRB\ (g) x\ Protein\ content\ in\ DRB(\%)} \quad (1)$$

iv. Kjeldahl for protein content determination

The Kjeldahl method is an analytical procedure that is used to determine the nitrogen content in organic compounds. This is an essential step in the assessment of protein levels. The procedure starts with the digestion of the known weight of the sample (0.125 g) in a Kjeldahl flask. In this flask, concentrated sulfuric acid (98% H2SO4) and a catalyst (e.g., selenium or copper) are added and heated for 2 to 4 h until the solution is clear, converting nitrogen into ammonium sulfate. After cooling, the solution was neutralized with sodium hydroxide (32% NaOH) in a distillation apparatus, resulting in the release and absorption of ammonia gas in a known volume of boric acid solution. The quantity of ammonia absorbed in the boric acid was then quantified through titration with a 0.1 N hydrochloric acid (HCl) solution. The nitrogen content was subsequently calculated and converted to the protein content using a conversion factor of 6.25, which reflects the typical nitrogen composition of RBP. The protein content was then calculated using equation (2):

$$\%Protein = \frac{(A-B)\ x\ N_{HCl}\ x\ 1.4\ x\ 6.25}{Weigh\ sample\ (gram)} \quad (2)$$

Where A is the volume of HCl used for titration, B is the volume of HCl used in the blank, and NHCl is the normality of the HCl solution. This calculation reflects the nitrogen content converted to protein content based on the typical nitrogen composition of the proteins.

v. Dynamic light scattering (DLS) for particle size and zeta potential analysis

The zeta potential, particle size, and size distribution of the samples were analyzed using a Zetasizer Nano ZS (Malvern Instruments Inc., Malvern, U.K.) equipped with dynamic light scattering (DLS) technology. The measurements were conducted at a scattering angle of 173° and a controlled temperature of 25 °C, using a material refractive index of 1.59 and a dispersant refractive index of 1.330. To ensure consistency, each sample was diluted 100 times with water, achieving a uniform dispersant refractive index despite variations in desolvating agents. The zeta potential of the synthesized nanoparticles was assessed under a pH of 7.4 ± 0.2 using the same DLS instrument. The polydispersity index (PDI), which reflects the uniformity of the particle size distribution, was calculated by the software based on the equation PDI= σ2/(2RH2), where σ represents the standard deviation of a theoretical Gaussian distribution centered on the Z-average size, and RH denotes the hydrodynamic radius.

vi. SDS-PAGE

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to separate proteins based on their molecular weight. Protein samples were prepared by mixing them with an equal volume of SDS sample buffer containing reducing agents, followed by heating at 95 °C for 5 min to denature the proteins. A polyacrylamide gel was then cast, consisting of a stacking gel (5%) and a separating gel (10-15%), depending on the target protein sizes. After polymerization, the gel wells were loaded with the prepared samples alongside a molecular weight marker. The gel was placed in an electrophoresis chamber filled with running buffer, and a voltage was applied to facilitate the migration of negatively charged SDS-protein complexes through the gel matrix, allowing separation based on size. Following electrophoresis, the gel was stained with a protein-specific dye such as Coomassie Brilliant Blue to visualize the separated bands, which were then examined to determine their molecular weights by comparison with the marker.

vii. Statistical Analysis

This experimental design optimizes protein extraction by considering several critical factors, including microwave power, extraction time, extraction pH, and the solvent-to-sample ratio. The experimental variables are detailed in Table 1.

Table 1. Experimental variables and their coded values for central composite design (CCD)

Independent variable	Symbol	Coded Value				
		-α (-2)	-1	0	+1	+α (2)
Microwave power (watt)	A	200	400	600	800	1000
Extraction time (Second)	B	30	60	90	120	150
pH	C	8	9	10	11	12
Solvent: Sample (mL/gram)	D	5	10	15	20	25

Response Surface Methodology (RSM) using a Central Composite Design (CCD) was employed to evaluate the effects of interactions among these factors on the percentage of protein recovery. Data collection was conducted using appropriate methods to accurately measure protein recovery, and all measurements were recorded with precision. Statistical analysis was performed using Minitab software, where the Response Surface Methodology was applied to analyze the data. The significance of the factors and their interactions was assessed through analysis of variance (ANOVA), with a significance level set at $p < 0.05$.

3. Result And Discussion:

i. Impact of a Single Factor on Protein Recovery

This section discusses the impact of the individual extraction parameters on the protein recovery. Understanding the influence of each factor, including microwave power, extraction time, pH, and

solvent-to-sample ratio, is crucial for optimizing the extraction process. By analyzing these factors independently, the key contributors to protein extraction efficiency can be identified, providing valuable insights for process improvement. The effects of each parameter on protein recovery are presented in Fig. 1.

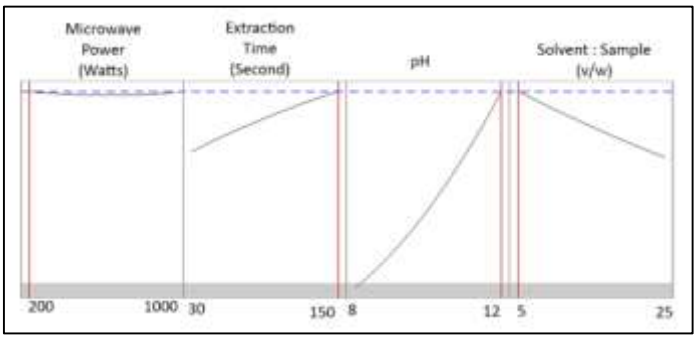


Figure 1. Impact of single factor on protein recovery

Based on the results presented in Fig. 1, the microwave power did not significantly affect the protein recovery. This is due to the absence of stirring during the extraction process. Stirring is crucial in enhancing the mass transfer kinetics during extraction by facilitating contact between the solvent and the DRB matrix. Effective stirring ensures the uniform distribution of heat and solvent within the sample, leading to improved extraction rates and recovery[6]. Studies have shown that stirring can enhance the extraction efficiency by promoting solvent penetration into the material, thereby increasing the extraction of the target compound. In addition, proper stirring can prevent localized heating and ensure consistent extraction throughout the sample, contributing to the overall effectiveness of the MAE process [7]. In contrast, the extraction time, pH, and solvent-to-sample ratio significantly influenced the protein recovery. A longer extraction time allows for a more optimal interaction between the DRB and the solvent, thereby enhancing protein release[8]. The extraction pH also plays a crucial role, as an increased pH can improve the protein solubility in the solvent, thereby enhancing extraction efficiency[9]. Interestingly, while a higher solvent-to-sample ratio is generally expected to improve protein solubility, the results indicate that the optimal ratio occurred at 5 mL/g. This may be due to the excessive solvent at higher ratios, which can lead to a dilution effect, thereby reducing the effectiveness of the interaction between the protein and the solvent. At a ratio of 5 mL/g, the system still has sufficient solvents to dissolve the protein without causing excessive dilution, thus improving extraction efficiency[10].

ii. Regression Equation of Protein Recovery

Table 2 presents the Analysis of Variance results for the studied factors, including microwave power, extraction time, pH, and solvent-to-sample ratio, and their effects on protein extraction and recovery.

Table 2. Analysis of variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	14	0.1009	0.0072	9.80	0.00
Linear	4	0.0913	0.0228	31.07	0.00
Microwave Power (watt)	1	0.0003	0.0003	0.38	0.55
Extraction time (second)	1	0.0130	0.0130	17.66	0.00
pH	1	0.0690	0.0690	93.93	0.00
Solvent: sample (v/w)	1	0.0090	0.0090	12.28	0.00
Square	4	0.0029	0.0007	0.98	0.45
Microwave Power (watt)*Microwave Power (watt)	1	0.0001	0.0001	0.12	0.73
Extraction time (second)*Extraction time (second)	1	0.0001	0.0001	0.10	0.75

pH*pH	1	0.0026	0.0026	3.59	0.08
Solvent: sample (v/w)*Solvent: sample (v/w)	1	0.0000	0.0000	0.05	0.83
2-Way Interaction	6	0.0067	0.0011	1.51	0.24
Microwave Power (watt)*Extraction time (second)	1	0.0019	0.0019	2.62	0.12
Microwave Power (watt)*pH	1	0.0001	0.0001	0.18	0.67
Microwave Power (watt)*Solvent: sample (v/w)	1	0.0007	0.0007	0.97	0.34
Extraction time (second)*pH	1	0.0007	0.0007	1.01	0.33
Extraction time (second)*Solvent: sample (v/w)	1	0.0009	0.0009	1.18	0.29
pH*Solvent: sample (v/w)	1	0.0023	0.0023	3.11	0.10
Error	16	0.0118	0.0007		
Lack-of-Fit	10	0.0093	0.0009	2.22	0.17
Pure Error	6	0.0025	0.0004		
Total	30	0.1126			

The analysis of variance (ANOVA) results indicate that the model used significantly explains the variation in protein recovery, with an F-value of 9.80 and a p-value of 0.00. Among the linear factors tested, pH exhibited the most dominant influence on protein recovery, with an F-value of 93.92 and a p-value of 0.00. This suggests that increasing the pH enhances the protein solubility and facilitates its release from the DRB matrix. Additionally, the extraction time was found to have a significant effect, with an F-value of 17.66 and a p-value of 0.00, indicating that longer extraction durations allow for more efficient protein release. The solvent-to-sample ratio also showed a significant impact, with an F-value of 12.28 and a p-value of 0.00; however, excessive solvent use should be carefully considered as it may lead to dilution effects, reducing the extraction efficiency. In contrast, microwave power was not a significant factor affecting protein recovery (p-value = 0.55), possibly due to the absence of stirring during the extraction process, which may have resulted in uneven heating and reduced interaction between the protein and solvent.

The quadratic analysis results revealed that most quadratic terms did not significantly influence protein recovery, except for pH^2, which approached significance with a p-value of 0.08. These results indicate that the relationship between pH and protein recovery is nonlinear, forming a curve with an optimal point before declining. Meanwhile, other quadratic factors such as microwave $power^2$ (p-value = 0.73), extraction $time^2$ (p-value = 0.75), and solvent-to-sample $ratio^2$ (p-value = 0.83) showed no meaningful effects. Among the tested interaction terms, none were statistically significant; however, the interaction between pH and the solvent-to-sample ratio (pH*Solvent: sample) had a p-value of 0.10, approaching significance. These findings indicate a potential synergistic effect between these two variables on protein recovery.

The lack-of-fit analysis, with an F-value of 2.22 and a p-value of 0.17, indicates that the model does not exhibit a significant deviation from the experimental data. The proposed model adequately explains the relationship between the process variables and protein recovery. The experimental results showing the response values for each design point are presented in Table 3.

Table 3. CCD matrix and the experimental results of protein recovery

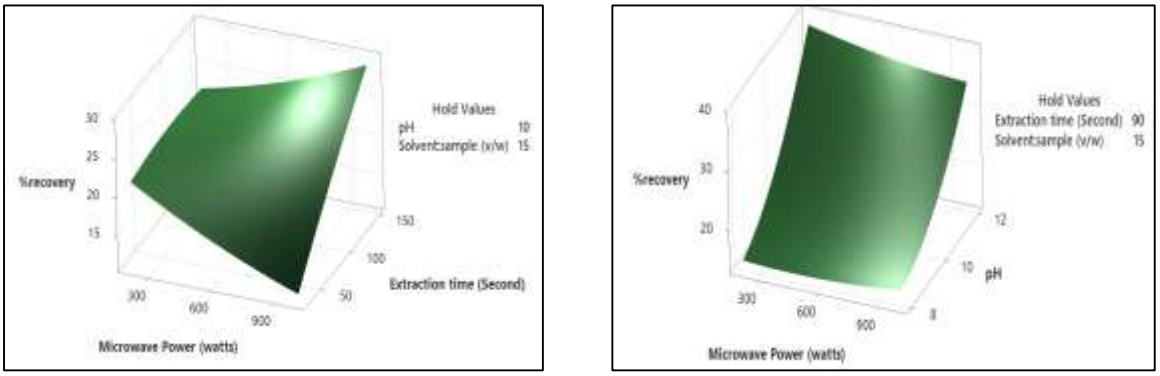
Run	A	B	C	D	%Recovery
1	800	60	9	20	27.14
2	400	120	9	10	16.60
3	600	90	10	15	23.34
4	600	90	10	15	24.41
5	600	90	10	25	23.93

6	600	150	10	15	20.81
7	800	120	11	20	33.01
8	400	60	11	20	26.41
9	400	60	9	10	14.95
10	600	90	10	15	20.72
11	600	90	12	15	35.58
12	800	120	9	20	23.05
13	1000	90	10	15	19.76
14	400	60	11	10	26.43
15	400	120	11	10	32.69
16	600	90	10	15	19.14
17	200	90	10	15	19.92
18	400	120	9	20	22.74
19	800	120	9	10	22.74
20	400	120	11	20	34.91
21	600	90	8	15	10.38
22	800	60	11	20	25.48
23	600	90	10	5	15.23
24	800	60	11	10	20.23
25	600	90	10	15	23.65
26	400	60	9	20	20.26
27	600	90	10	15	18.98
28	800	60	9	10	9.85
29	800	120	11	10	31.00
30	600	90	10	15	20.40
31	600	30	10	15	16.10

The optimization results using the Central Composite Design (CCD) method produced a mathematical model that can be used to predict protein recovery based on variations in the factors tested. This model is constructed from regression analysis, where each factor, such as microwave power, extraction time, pH, and the ratio of distilled water to the sample, contributes to the measured response as written in equation (3):

$$\begin{aligned}\text{Protein recovery (\%)} = {} & 38.0 - 0.0187\,A - 0.153\,B - 11.4\,C + 2.69\,D + 0.000004\,AA - 0.000179\,BB \\ & + 0.961\,CC + 0.0044\,DD + 0.000183\,AB - 0.00145\,AC + 0.000666\,AD \\ & + 0.0226\,BC - 0.00490\,BD - 0.239\,CD \end{aligned} \quad (3)$$

To visualize the interactions between factors and their effect on the recovery, this can be illustrated using a Response Surface, as shown in Fig. 2.



(a) (b)

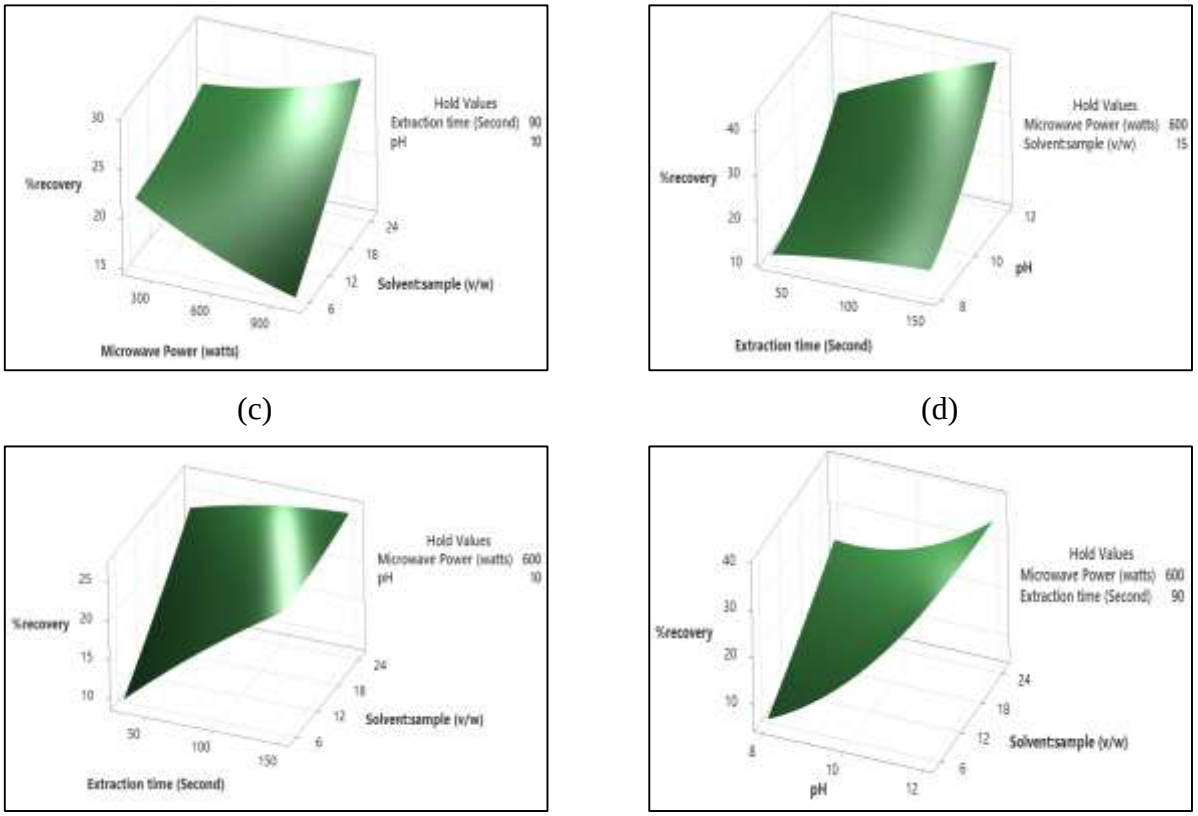


Figure 2. Response Surface results showing interactions between factors: (a) %recovery vs extraction time, microwave power; (b) %recovery vs pH, microwave power; (c) %recovery vs solvent: sample ratio, microwave power; (d) %recovery vs pH, extraction time; (e) %recovery vs solvent: sample, Extraction time; (f) %recovery vs solvent: sample, pH

Based on the optimization analysis conducted using Minitab with the CCD method, the optimized conditions for maximum protein recovery from rice bran extraction were found to be a microwave power of 200 watts, an extraction time of 150 seconds, pH 12, and a solvent-to-sample ratio of 5. The predicted protein recovery under these conditions was 47.60%. For further details, refer to Table 4.

Table 4. Optimal conditions for extracting protein from rice bran.

	Microwave Power (Watt)	Extraction time (second)	pH	Aquadest/ sample	R-sq	Prediction (%)
Optimization	200	150	12	5	89.56%	47.60
Actual						47.76 ± 3.11

iii. Particle Size and Zeta Potential

Dynamic Light Scattering (DLS) was used to evaluate the particle size distribution, offering insights into the sample's homogeneity. The analysis of particle size and zeta potential was conducted using data from the experiment with the highest % recovery, corresponding to sample 11, as presented in Table 5.

Table 5. Dynamic Light Scattering (DLS) of RBPC*

Run	Particle Size (nm)	PDI[b]	Zeta potential (mV)
Optimized	285.13 ± 57.6	0.33 ±0.027	-33.33±0.40

**RBPC obtained under operation conditions: microwave power of 200 watts, an extraction time of 150 seconds, pH 12, and a solvent-to-sample ratio of 5*

Based on the DLS results, the PDI value indicates that the particle size distribution is relatively homogeneous. Generally, a PDI value below 0.3 indicates a very homogeneous distribution, while values up to 0.33 are still considered acceptable for certain applications, such as drug delivery systems. A low PDI is crucial in drug delivery because it ensures uniformity in particle size, which can enhance the stability of the formulation and improve the bioavailability of the drug [11]

The size of the particles is also a critical factor in drug delivery. Smaller particles typically exhibit higher bioavailability as they can more easily penetrate the cell membranes. Additionally, particle size influences the distribution of the drug within the body; smaller particles can circulate more freely in the bloodstream, whereas larger particles may become trapped in specific tissues or organs. This characteristic is particularly important for targeted drug delivery systems, where the ability to reach specific sites, such as tumor cells, is essential for therapeutic efficacy [2].

The Zeta potential results indicate that the particles are in a system with good stability, as the value is below -30 mV. A Zeta potential in this range results in strong electrostatic repulsion between the particles, which is essential for preventing aggregation. Similar Zeta potential results were also obtained in the study conducted by Zhou et al., [12] ranging from -34 mV to -24 mV at different protein concentrations. This stability is particularly important in applications where prolonged shelf life and consistent performance are required.

In conclusion, the analysis of the particle size and Zeta potential demonstrates that the extracted protein particles exhibit favorable characteristics for further applications, indicating the potential for effective use in various fields.

iv. SDS-PAGE

SDS-PAGE is an electrophoresis technique used to separate proteins based on their molecular size. This technique involves the use of Sodium Dodecyl Sulfate (SDS), an anionic detergent that denatures proteins and imparts a uniform negative charge to them. The presence of SDS eliminates the influence of the protein's original charge, so the separation of proteins in the polyacrylamide gel depends solely on their molecular size [13]. The results of the SDS-PAGE analysis in this study can be seen in Fig. 3

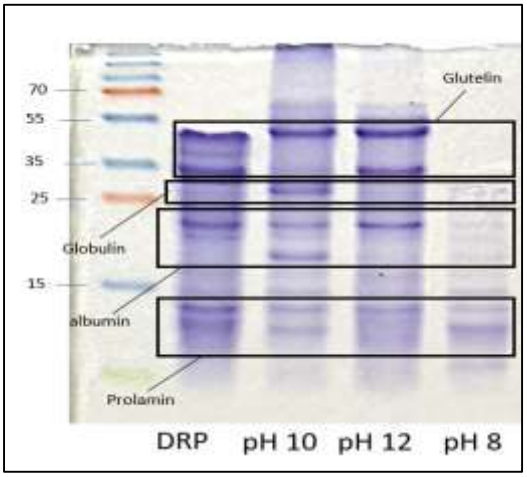


Figure 2. SDS-PAGE Profile of Protein Fractions at Different pH Levels (8, 10, and 12)

The SDS-PAGE analysis results showed that at pH 8, 10, and 12, major protein fractions such as

glutelin, globulin, albumin, and prolamin were successfully identified based on their molecular weight differences. Glutelin was dominant at pH 12, indicating that strong alkaline conditions enhanced its extraction, as glutelin is a protein fraction that is soluble in alkaline conditions. At pH 8 and 10, the bands of globulin and albumin were more prominent, indicating better solubility at these pH levels [14].

This protein profile reflects the success of the pH-based extraction method in separating specific protein fractions, while also supporting its potential use in advanced applications, such as protein modification for nanoparticle production. The identified proteins, particularly glutelin and albumin, have shown promise in drug delivery systems due to their biocompatibility and ability to encapsulate therapeutic agents. Their molecular characteristics indicate that they could enhance the stability and bioavailability of drugs, making them suitable candidates for further development in targeted drug delivery applications [15].

4. Conclusion:

This study optimized the protein extraction from rice bran using microwave-assisted alkaline extraction. The optimal conditions identified were a microwave power of 200 watts, an extraction time of 150 s, a pH of 12, and a solvent-to-rice bran ratio of 5:1, resulting in a predicted protein recovery of 47.60%. The extracted protein particles had an average size of approximately 285.13 nm, a polydispersity index (PDI) of 0.33, and a zeta potential of -33.33 mV, indicating good stability for protein-based drug delivery applications. SDS-PAGE analysis confirmed that glutelin was the main protein fraction extracted, highlighting the potential of rice bran as a valuable protein source for food and pharmaceutical applications.

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Reference

[1] C. Fabian and Y.-H. Ju, "A review on rice bran protein: its properties and extraction methods," *Crit Rev Food Sci Nutr*, vol. 51, no. 9, pp. 816–827, 2011.
[2] F. N. A. Rahim *et al.*, "Comparison of different protein extraction methods of rice bran protein concentrates," 2021. [Online]. Available: https://www.tci-thaijo.org/index.php/APST/index
[3] S. Bedin, K. Zanella, N. Bragagnolo, and O. P. Taranto, "Implication of microwaves on the extraction process of rice bran protein," *Brazilian Journal of Chemical Engineering*, vol. 36, no. 4, pp. 1653–1665, 2019, doi: 10.1590/0104-6632.20190364s20180599.
[4] S. Bernardi, M. P. Corso, I. J. Baraldi, E. Colla, and C. Canan, "Obtaining concentrated rice bran protein by alkaline extraction and stirring-Optimization of conditions," 2018.
[5] F. N. Abd Rahim *et al.*, "The effect of alkaline extraction and drying techniques on the physicochemical, structural properties and functionality of rice bran protein concentrates," *Int J Biol Macromol*, vol. 242, Jul. 2023, doi: 10.1016/j.ijbiomac.2023.124908.
[6] R. Lucena, "Extraction and stirring integrated techniques: Examples and recent advances," Jun. 2012. doi: 10.1007/s00216-012-5826-9.
[7] A. S. Lozano Pérez, J. J. Lozada Castro, and C. A. Guerrero Fajardo, "Application of Microwave Energy to Biomass: A Comprehensive Review of Microwave-Assisted Technologies, Optimization Parameters, and the Strengths and Weaknesses," Jun. 01, 2024, *Multidisciplinary Digital Publishing Institute (MDPI)*. doi: 10.3390/jmmp8030121.
[8] M. Kanapathy, M. S. M. Sarip, S. A. Saidi, M. A. Jamlos, and N. I. A. Aris, "Parametric Study on The Rice Bran Protein Extraction Process Using Water as a Solvent," *Journal of Advanced Research in Fluid Mechanics and Thermal Sciences*, vol. 100, no. 1, pp. 165–171, 2022, doi: 10.37934/arfmts.100.1.165171.
[9] F. N. Abd Rahim *et al.*, "Effect of protein concentration, pH, and ionic strength on the adsorption properties of rice bran protein concentrates at the oil-water interface," *Food and Bioproducts Processing*, vol. 147, pp. 162–174, Sep. 2024, doi: 10.1016/j.fbp.2024.06.011.
[10] F. K. S. Lynaa, M. S. Md Sarip, S. A. Saidi, M. A. H. Mohd Nawi, and K.-I. Sotowa, "Preparation of Rice Bran Protein Solutions Using a Water-Based Extraction Process," *Journal of Chemical Engineering of Japan*, vol. 56, no. 1, Dec. 2023, doi: 10.1080/00219592.2023.2205889.
[11] M. Danaei *et al.*, "Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems," May 18, 2018, *MDPI AG*. doi: 10.3390/pharmaceutics10020057.

[12] Q. Zhou, H. Li, F. Li, B. Zhang, X. Wu, and W. Wu, "Strategy and Mechanism of Rice Bran Protein Emulsion Stability Based on Rancidity-Induced Protein Oxidation: An Ultrasonic Case Study," *Foods*, vol. 11, no. 23, Dec. 2022, doi: 10.3390/foods11233896.
[13] A. B. Nowakowski, W. J. Wobig, and D. H. Petering, "Native SDS-PAGE: High resolution electrophoretic separation of proteins with retention of native properties including bound metal ions," *Metallomics*, vol. 6, no. 5, pp. 1068–1078, 2014, doi: 10.1039/c4mt00033a.
[14] R. H. Van Wyk and E. O. Amonsou, "Physiochemical and functional properties of albumin and globulin from amadumbe (Colocasia esculenta) corms," *Food Science and Technology (Brazil)*, vol. 42, 2022, doi: 10.1590/fst.02621.
[15] S. Gammoh *et al.*, "Functional and Bioactive Properties of Wheat Protein Fractions: Impact of Digestive Enzymes on Antioxidant, α-Amylase, and Angiotensin-Converting Enzyme Inhibition Potential," *Molecules*, vol. 28, no. 16, Aug. 2023, doi: 10.3390/molecules28166012.