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Preparation of Rice Bran Protein (RBP) Powder Using Spray Drying Method at the Optimal Condition and Its Protein Quality

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Abstract: Rice bran is a by-product of the rice milling process. It contains a high concentration of protein. Rice brans are frequently utilized as feed cattle, fertilizer, and fuel. However, their application as human nutrition supplements is uncommon, and the necessary process for this purpose is yet to be established, including the drying process. This study aims to evaluate the effect of the spray-drying parameters, the inlet temperature, inlet flowrate, and inlet air flowrate, on rice bran protein (RBP) powder and optimize it using response surface methodology (RSM). A thermal water-based extraction method was utilized prior to the drying process. The correlation between the spray-drying parameters, i.e., the inlet temperature (120 to 210 °C), feed flowrate (5 to 55%), and air flowrate (246 to 670 L/h), and the RBP yield were investigated. The quality of the RBP powder was evaluated based on acid amino profiling in the mixture through de novo peptide sequencing. The optimized operating conditions for the maximum yield of RBP powder (25.7 g RBP/100 g RRB) are 178 °C, feed flowrate of 25%, and air flowrate of 450 L/h. The main peptides that contribute to RBP powder protein are globulin and glutelin; meanwhile, prolamin is believed to degrade during the drying process. The process also produced protein sugar, helping to produce fine particles powder without the drying agent.

Keywords: optimization; spray-drying process; rice bran; protein quality

Citation: Mansor, M.R.; Md Sarip, M.S.; Nik Daud, N.M.A.; Saidi, S.A.; Mohd Nawi, M.A.H.; Jamlos, M.A. Preparation of Rice Bran Protein (RBP) Powder Using Spray Drying Method at the Optimal Condition and Its Protein Quality. *Processes* **2022**, *10*, 2026. https://doi.org/10.3390/pr10102026

Academic Editor: Won Byong Yoon

Received: 26 August 2022
Accepted: 3 October 2022
Published: 7 October 2022

1. Introduction

A large amount of rice bran produced through the rice milling process is regularly squandered as waste. This product is sold as animal feed or fertilizer at a low price [1]. This underutilization of a large amount of rice bran in Malaysia causes a slight decrease in the production of rice in Malaysia because most investors feel that only the rice grains are valuable in the rice milling process. Therefore, the sales revenue obtained from these rice grains is unable to compensate for the high investment capital required for the rice milling process [2]. Rice bran has many health benefits, thus leading to its application in food, nutraceutical, and pharmaceutical industries [3]. The utilization of rice bran in a beneficial way, such as producing the rice bran protein (RBP), would give added value to the rice industry.

The extraction and drying process plays an important role in preserving the protein quality in the rice bran for the development of supplementary protein for human beings. However, the soluble protein in an aqueous solution is not suitable for commercialization

when compared to the powdered form. Hence the use of the drying process is necessary to achieve this outcome. Drying, by definition, is a common technique in producing protein supplements, converting protein concentrates into powders. The drying process is a dehydration method that has been widely used in the food industry for decades. This process is crucial for longer storage life, food quality improvement, easier handling, and ease of further processing, as well as cleanliness and safety [4].

Technically, the usable properties of the RBP are mainly influenced by the drying technique since it involves the heat and mass transfer mechanisms. Several drying techniques can be used for manufacturing RBP powder, including drum, spray, freeze, vacuum, and conventional oven-drying. However, each of these techniques has its advantages and disadvantages. RBP powder yield and quality may also differ in terms of their physicochemical or nutritional properties, including their microstructures, subject to the drying methods [5]. Furthermore, most drying techniques are carried out under high temperatures, which is inappropriate for heat-sensitive substances, such as protein, which may be damaged. One of the best options for this process is spray-drying, which offers rapid evaporation.

Through rapid evaporation, the possibility of proteins denaturing is lower, despite its exposure to high temperatures [6]. However, this possibility needs to be evaluated to ensure that the high-quality powder is produced at the optimum spray-drying operating conditions. Among the main parameters affecting this process are inlet temperature, inlet feed flowrate, and inlet gas flowrate. The drying rate and duration of the drying cycle are determined by the capacity of the air (gas) stream to absorb and take away moisture. Higher inlet temperature will create better vapor holding capability, while higher the drying cycle will expose the solute or protein for possible degradation, thus degrading its quality. On the other hand, lower vapor holding capacity will increase the powder losses by sticking to the drying wall.

Therefore, the effect of inlet temperature, inlet feed flowrate, and inlet gas flowrate were evaluated. Moreover, the optimization process for this spray-drying process is also crucial to obtaining high-quality yields without comprising total yield, as stated by Emami et al. [7]. For this reason, response surface methodology (RSM) was utilized for the optimization process. Protein quality for the product were also analyzed based on amino acid profiling.

2. Methodology

2.1. Raw Material

In this study, rice bran was collected from the Kilang Beras BERNAS Sdn Bhd, Kuala Perlis, Perlis. The raw rice bran was heated at 95 °C for 3 min to prevent the hydrolytic rancidity of rice bran [8] and stored at the temperature of 4 °C prior to the experimental procedure.

2.2. Extraction Process

The rice bran was extracted using a hot water extraction process. The hot water extraction process was conducted using sterilizer (Hirayama HG-80, Saitama, Japan) at 121 °C and 0.26 MPa for 20 min as a modified method from the previous study [9]. The rice bran sample was mixed at a ratio of 1:20 (g/mL) with distilled water in a Schott bottle before the extraction process.

2.3. Spray-Drying Process

The experimental spray-drying process was conducted using a Mini Spray Dryer (Buchi B 290, Essen, Germany) at the Faculty of Chemical Engineering &Technology, UniversityMalaysia Perlis (UniMAP). For each experimental spray-drying process, 500 mL of the rice bran extract was dried, and the protein yield was measured and recorded. The protein yield was calculated using Equation (1).

$$\text{Yield} = \frac{W_{final} - W_{initial}}{W_{RRB}} \times 100 \quad (1)$$

where Yield (g RBP/100 g RRB) = yield of rice bran protein (RBP) in 100 g raw rice bran (RRB);

W_{final} (g) = Weight of the cylone after the spray-drying process;
$W_{initial}$ (g) = Weight of the cylone before the spray-drying process; and
W_{RRB} (g) = Weight of raw rice bran.

In this study, the spray-drying operating parameters, namely inlet temperature, feed flowrate, and air flowrate were evaluated in the range of 130–200 °C, 10–40% of pump rate (3.5 to 12 mL/min), and 225–525 L/h, respectively. The other parameter in the spray-drying system remained constant throughout the study, namely the aspirator percentage of 95% and the nozzle pulse of 2.

2.4. Other Drying Methods for Rice Bran Protein (RBP) Powder

Oven-drying and freeze-drying were conducted to compare the efficiency of the spray-drying method. For oven-drying, after water extraction, samples were collected and dried in the Universal Oven (Memmert, Schwabach, Germany) located at the Faculty of Chemical Engineering & Technology, UniversityMalaysia Perlis (UniMAP). Of the supernatant sample, 500 mL were taken and dried for 48 h at 100 °C within ±5 °C to remove all the water.

Another method of drying is freeze-drying and also known as lyophilization. Of the solution after water extraction, 500 mL were taken and pre-frozen at −83 °C and then freeze-dried in accordance with the operating procedure at −53 °C for 48 h with Freeze Dryer (Labogene cool safe, Allerød, Denmark).

2.5. Optimization of Spray-Drying Process Using Response Surface Methodology (RSM)

Response Surface Methodology (RSM) in Design-Expert software was used to create an experimental design for optimizing the spray-drying process for RBP powder yield. The Central Composite Design (CCD) was employed as the design model for the optimization process. Experiments were randomized to reduce bias. Furthermore, as indicated in Equation (2), the following predictive quadratic polynomial equation resulted from the relationship between the response and the independent variables and was utilized to fit the experimental results.

$$Y = A_o + \sum A_i X_i + \sum A_{ii} X_i^2 + \sum A_{ij} X_i X_j \quad (2)$$

where Y = Response variable;

A_0 = Regression coefficients of variables for intercept terms;
A_i = Regression coefficients of variables for linear terms;
A_{ii} = Regression coefficients of variables for quadratic terms;
A_{ij} = Regression coefficients of variables for interaction terms; and
X_i, X_j = Independent variables.

2.6. Statistical Analysis

Statistical analysis software was used to examine all the data, including the analysis of variance (ANOVA) based on 95% confidence level ($p < 0.005$).

2.7. Model Validation

The validation of the best model was conducted by comparing the experimental data with the predicted value. The absolute average deviation (*AAD*) for the experiment was calculated using Equation (3).

$$AAD = \frac{1}{n}\sum_{m=1}^{n}\left|Y_m - Y_{average}\right| \quad (3)$$

where Y_m = Experimental measured value; $Y_{average}$ = Experimental average value; and n = Number of experiments run (3 in this study).

The deviation between the predicted value and experimental data was calculated and evaluated based on absolute average relative deviation (*AARD*), as shown in Equation (4).

$$AARD = \left(\frac{Y_{experiment} - Y_{predicted}}{Y_{experiment}}\right) \times 100 \quad (4)$$

where $Y_{experiment}$ = Average experimental value and $Y_{predicted}$ = Predicted value.

2.8. High-Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS) for Protein Quality Analysis

Protein quality analysis was conducted through the acid amino profiling in the mixture based on de novo peptide sequencing using high-performance liquid chromatography–mass spectrometry (HPLC-MS) coupled with a tandem mass spectrometer. About 250 g of the raw sample and 250 g of the sample after the dry spray process were kept to determine the sample changes [10]. The fragmentation mode was set to collision-induced dissociation (CID) and collision activated dissociation (CAD), and the ion source was set to ESI (nano-spray) (y and b ions). For both samples, the MS scan mode was set to FT-ICR/Orbitrap and MS/MS Scan mode linear Ion Trap [11]. The system consisted of liquid chromatography (Dionex Ultimate 3000, Thermo Scientific, Waltham, MA, USA) in combination with an electrospray ionization (ESI)/quadrupole ion trap mass spectrometer (Model Amazon SL, Bruker, Germany). The separation was carried out using a reverse-phase column (Hypersil GOLD 50 mm 3 0.5 mm, 5 mm C18), protected by a guard column (Hypersil GOLD 30 mm 3 0.5 mm, 5 mm C18).

The result of acid amino profile and proteins ID from HPLC-MS was cross-checked with the UniProt database to determine the type of protein from the peptide. The mass tolerance for precursor ions was set to 10 ppm, while the fragment ion tolerance was set to 0.8 Da. Carbamidomethyl (+57.0214 Da) in cysteine and oxidation (+15.9994 Da) in methionine were two of the dynamic changes [12].

2.9. Scanning Electron Microscope (SEM)

The powder sample's morphology was observed using a Scanning Electron Microscope (SEM). SEM is done to differentiate each process physically and changes during each process. The RBP powders are first placed on aluminium stubs with double-sided sticky carbon tape and sputter-coated with a 5 nm layer of a gold coating system for this characterisation testing. The powders were then scanned using a SEM operating at a 5 kV accelerating voltage. Finally, the images of the surface morphology of the RBP were viewed at the magnification of 500×,1000×, 1500×, and 2000×. Prior to SEM analysis, each sample was stored with silica gel inside an airtight container to ensure the lower moisture content and further enhance the availability of the sample, since SEMs are very sensitive to moisture.

3. Result and Discussion

3.1. Effect of Inlet Temperature on Spray-Drying Process on RBP Powder Yield

The influence of the inlet temperature on the yield of RBP powder from the spray-drying process at a constant feed flowrate of 20% and air flowrate of 388 L/h is depicted in Figure 1.

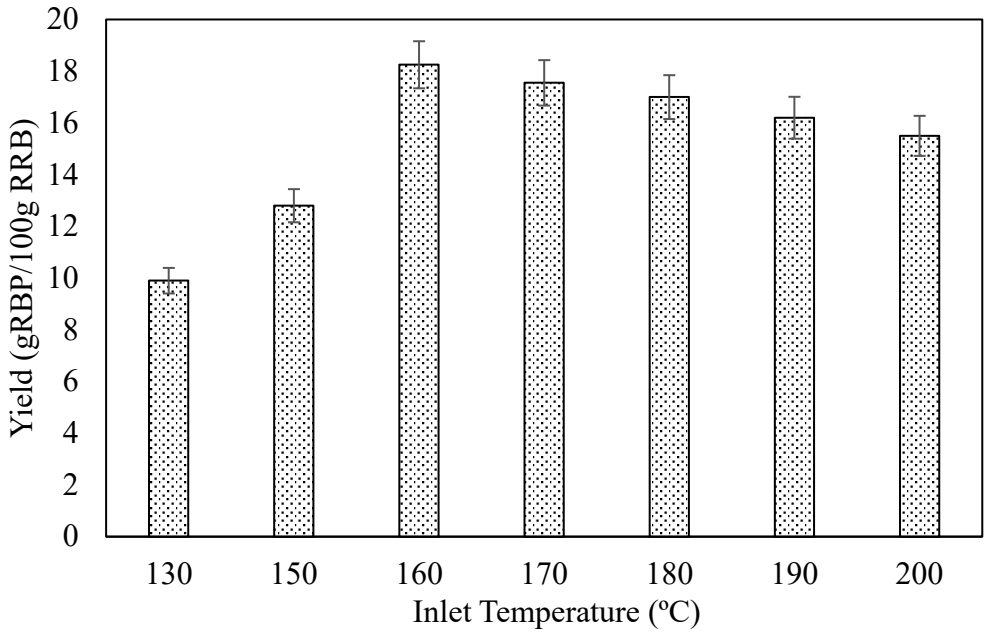


Figure 1. Effect of inlet temperature in spray-drying process on RBP powder yield at a constant feed flowrate of 20% and air flowrate of 388 L/h.

RBP powder yield increases from 9.9 g RBP/100 g RRB to 18.25 g RBP/100 g RRB when the inlet temperature increases from 130 °C to 160 °C, as shown in Figure 1. Then, it started to decrease to 15.5 g RBP/100 g RRB at inlet temperatures of 200 °C. This is because as the temperature increases, higher water evaporation rates occur and reduce its drying times due to the efficient heat transfer process. Low evaporation rates are produced at the lower inlet air temperature, which leads to the formation of microcapsules with high-density membranes, high water content, poor fluidity, and agglomeration. This will increase the possibility of the RBP powder yield losses due to the stickiness of the drying chamber wall. This can be seen by the micrograph view of the RBP powder at the different temperatures from 120 to 200 °C, as shown in Figure 2.

As seen in (Figure 2a), a bigger spherical shape protein body was observed for the sample at the temperature of 120 °C. This is possibly due to the incomplete drying of the powder at lower temperatures, thus resulting in the coagulation particles represented by the bigger droplets. According to de Oliveira et al. [13], bigger droplets also indicate less protein. The powder spherical shape becomes smaller as the temperature increases to 160 °C (Figure 2b) and 200 °C (Figure 2c), where more small spherical shape protein bodies are produced.

However, the increase in inlet temperature has also exposed the protein to the degradation process observed from 160 °C to 200 °C due to thermal degradation and oxidation. This phenomenon was also observed in tomato powders' drying process, which showed a larger loss of lycopene content with the increase of inlet drying temperature [14]. Similarly, Quek et al. [15] observed that the concentration of lycopene and β-carotene decreased in the spray-dried watermelon powder as the increase in temperature of the inlet. Tonon et al. [16] also stated that increasing the temperature causes the denaturation of protein and causes a cohesive force between the spray-dried particle and the wall of the drying chamber. This phenomenon also occurs in the previous study, such as the effect of spray-drying on black

mulberry juice [17]. Therefore, an appropriate selection of inlet air temperature is important to enhance the dryer evaporative capacity and thermal efficiency of the sample [18]. In this study, the temperature between 150 to 180 °C was considered a good temperature range for drying RBP powder.

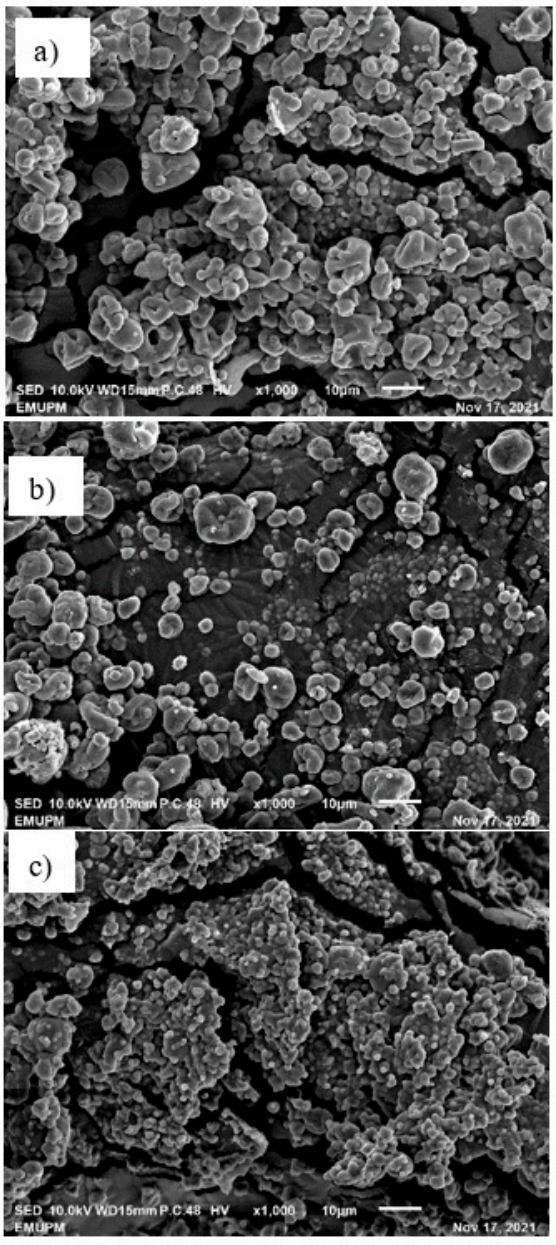


Figure 2. Change of the physical powder properties as the temperature changes from (**a**) 130 °C, (**b**) 160 °C, and (**c**) 200 °C.

3.2. Effect of Feed Flowrate on Spray-Drying Process on the RBP Powder Yield

Figure 3 shows the effect of feed flowrate from 10% to 40% on the spray-drying process for RBP powder at a constant inlet temperature of 160 °C and a 388 L/h air flowrate.

The effect of feed flowrate indicates an upward trend with insignificant differences in yield up to 20% of feed flowrate between 15.75 to 17.55 g RBP/100 g RRB. However, as the feed flowrate increased to 25% and higher, the yield began to decline to 7.75 g RRB/100 g RRB at a feed flowrate of 40%. Generally, the increase in feed flowrate causes a decreasing RBP powder yield. This is due to decreasing contact times between solution and hot air in the drying chamber (vaporisation chamber) as the increased feed flowrate.

The shorter contact time increases the chance of incomplete vaporisation of water, resulting in a lower yield of RBP powder [16]. This inverse proportional effect of feed flowrate to the spray dryer yield is also reported by Karaca et al. [19] on sour cherry juice concentrate formulation and Fazaeli et al. [17] on black mulberry juice powder production. Although rapid vaporization is spray-drying positive features compared with other drying processes, such as oven and sun dying, enough vaporisation time is required to obtain an optimum RBP powder yield. Moreover, larger droplets are produced at a higher feed flow, which contains more moisture and results in more stickiness on the glass drying chamber [20]. The insignificant difference in the RBP powder yield at a lower flowrate of 10, 15, and 20% is due to the saturation of the RBP powder yield available in the feed solution, and maximum powder is already being produced.

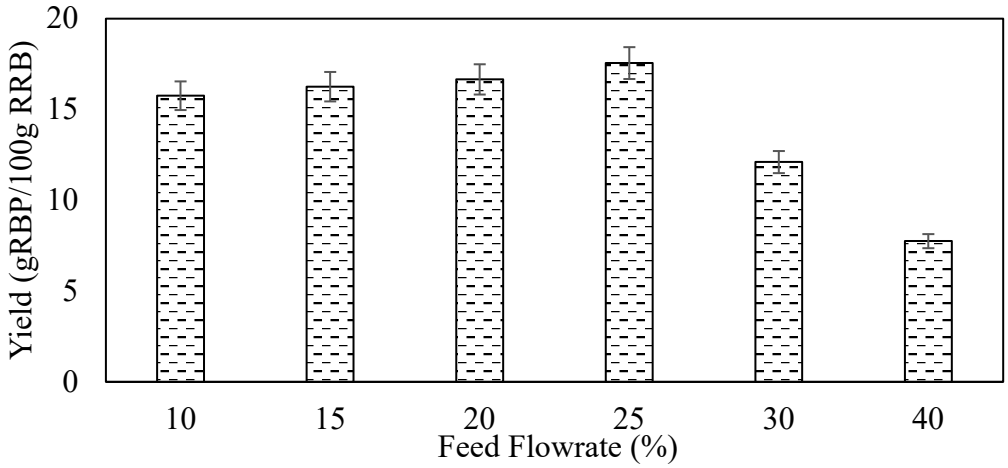


Figure 3. Effect of feed flowrate on the spray-drying process of the Rice Bran Protein (RBP) powder yield at a constant inlet temperature of 160 °C and a 388 L/h air flowrate.

3.3. Effect of Air Flowrate on Spray-Drying Process on the RBP Powder Yield

Another important parameter in the spray-drying process is air flowrate. This is because the drying air supply to the drying chamber indicated the energy supply for evaporation [21]. The effect of air flowrate on the spray-drying process to RBP powder yield at the constant inlet temperature of 160 °C and feed flowrate of 20% is shown in Figure 4.

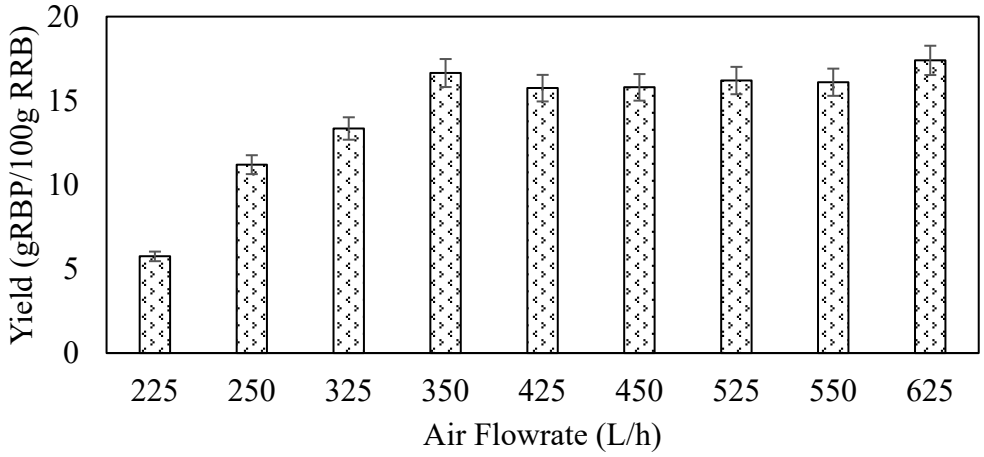


Figure 4. Effect of air flowrate on the Rice Bran Protein (RBP) powder yield at the constant inlet temperature of 160 °C and feed flowrate of 20%.

As shown in Figure 4, the increasing trend with a slight fluctuation at the higher air flowrate was observed on the effect of air flowrate on the RBP powder yield. RBP powder yield increased from 5.75 g RBP/100 g RRB to 16.65 g RBP/100 g RRB when air flowrate increased from 225 L/h to 350 L/h. A gradual variation of RBP powder yield is observed after 350 L/h where the value fluctuates between 15.75 g RBP/100 g RRB to 17.4 g. According to Ghosal et al. [22], the changing in air flowrate are relatively varied in the amount of heated dry air entering the spray chamber. Therefore, air flowrate is claimed to have a linear positive effect on water evaporation in the spray-drying process. By increasing the air flowrate, the powder yield is increased, showing that the energy available for water evaporation was increased and causing a more positive impact on powder production [23]. Based on the spray-drying atomizer design, the high air flowrate was producing smaller or tiny spray droplets, which boosted the drying process. According to Sadegh and Ecevit [24], the atomization process purposely increased the specific surface area of the liquid by the droplet's formation and augments heat and mass transfer from liquid to processing gas. Moreover, the dairy concentrate's droplet surface area substantially increased due to the atomization process and the droplet surface area was directly proportional to the rate of evaporation [25]. Thus, it is necessary to succeed in fine atomization for a more efficient operation of the spray dryer. Moreover, it is also essential to determine the physical properties of the resultant powders, such as bulk density, wettability, dispersibility, and solubility effect from the atomization [26]. Theoretically, as the air flowrate increases, the energy required to produce this powder will increase due to higher energy consumption during production. Therefore, it was essential to optimise this parameter. In conclusion, the maximum air flowrate of 425 L/h is believed to be the best to obtain RBP powder with the minimum energy consumption during the process.

3.4. Optimization of the Spray-Drying Process for RBP Powder Production

The optimization of the spray-drying for RBP powder was conducted using response surface methodology (RSM) based on central composite design (CCD) with three independent parameters, inlet temperature, feed flowrate, and air flowrate, and one response, which is RBP powder yield. The experimental condition matric coded based on CCD were presented in Table S1.

Based on statistical analysis, a good agreement quadratic polynomial model is obtained in this study to represent the RBP powder with a predicted R^2 value of 0.8439 and adjusted R^2 of 0.9467. The equation is generated as shown Equation (5).

$$\text{Yield} = -1.24C^2 - 0.3777B^2 - 0.6783A^2 + 0.5375BC + 0.0625AC + 6.39C + 1.23B + 0.8897A + 18.76 \quad (5)$$

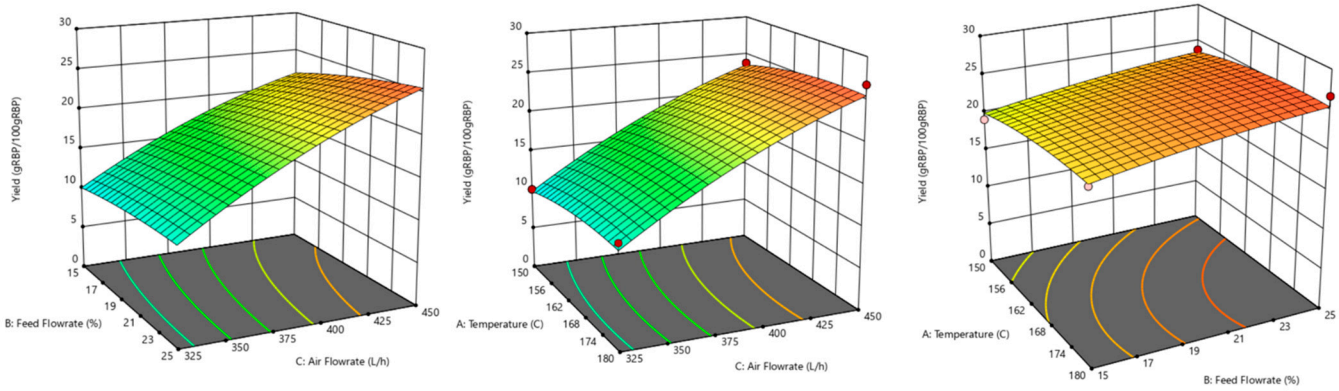
where Yield (Yield (g RBP/100 g RRB) = Yield of rice bran protein (RBP) in 100 g raw rice bran (RRB);

A = Inlet temperature (°C);
B = Feed flowrate (%); and
C = Air flowrate (L/h).

Based on Equation (5), three-dimensional response surface plots for RBP yield at different parameter relations are developed, as shown in Figure 5. Overall, the model shows a significant effect on RBP yield as determined by the f value (9,28) = 56.28, $p < 0.0001$. As shown in Figure 5a–c, it can be observed that the air flowrate and feed flowrate within this range are showing the significant effect on RBP yield as determined by f value (1,28) = 445.49, $p < 0.0001$, and f value (1,28) = 16.46, $p < 0.0007$.

Refer to Figure 5a, the maximum point was identified to be at an air flowrate of 450 L/h and feed flowrate of 25% at the RBP yield of 25 g RBP/100 g RRB. RBP powder yield's substantially increasing trend is observed as the air flowrate increased from 325 L/h to 450 L/h from 12 to 25 g RBP/100 g RRB. A similar trend with a considerable increasing pattern is also observed for feed flowrate. However, inlet temperatures from the range of 150 to 180 °C are not showing significant for this response as determined by the f value

(1,28) = 8.63, $p < 0.0085$. This can be observed in Figure 5b,c, where the increase of temperature from 150 to 180 °C caused the insignificant change of RBP yield from 24 to 25 g RBP/100 g RRB. In general, the higher inlet temperature results in higher water vaporization, swelling effect and water solubility, as shown in tomato powder study [14], tamarind powder [27], and pomegranate powder [28]. Based on this model, the optimum point of the RBP yield is determined based on the conjugate gradient method. The optimum conditions predicted are the inlet temperature is 178 °C, feed flowrate set to 25%, and air flowrate of 450 L/h. The predicted conditions were validated with the experimental as shown in Table 1.



(**a**) (**b**) (**c**)

Figure 5. Three-dimensional response surface model plot for RBP powder yield at constant (**a**) Inlet temperature of 178 °C. (**b**) Feed flowrate of 25%. (**c**) Air flowrate of 450 L/h.

Table 1. Model validation with experimental data.

Response (R1)	Experimental Data (g RBP/100 g RRB)		Predicted Value (g RBP/100 g RRB)	Error AARD (%)
	Average	AAD		
Yield	25.66	0.55	24.9	2.96

As shown in Table 1, the experimental data for the yield is 25.66 ± 0.55 g RBP/100 g RRB, and the predicted value is 24.9 g RBP/100 g RRB. The errors recorded are relatively low and acceptable based on the AARD value of 2.96%. In comparison with other drying methods, spray-drying shows relatively lower RBP yields compared with oven-drying and freeze-drying methods, which recorded yields of 61.0 and 47.8 g RBP/100 g, respectively. Spray-drying offers the industry a viable process that provides a continuous process, efficient heat utilization, and good product but will impose higher losses in comparison to oven-drying and freeze-drying.

3.5. Comparison between Other Drying Methods

Although oven-drying and freeze-drying produce the higher yields of RBP powder, the process does not produce good powder products as, ideally, powders tend to have smooth and spherical morphology with little or no surface distortion. The morphology during drying can have a direct effect on active ingredients and volatile substances like flavour. This can be observed through a micrograph of the RBP sample produced with spray-drying, oven-drying, and freeze-drying, as shown in Figure 6.

As shown in Figure 6a, the freeze-dried sample showed a plate of layered particles where the granulated protein had adhered to it. Meanwhile, the oven-dried sample shows the coagulated particle and forms a large powder structure, as shown in Figure 6b. As discussed earlier in the previous section, spray-drying produced a good sphere-shaped

powder where all the protein bodies can be seen in the fine granulated form; smooth and spherical with little to no surface distortion. To obtain the same powder characteristic as a spray-dried sample, an extra method is required to convert freeze-dried and oven-dried products into a granulated form, such as grinding or milling [29].

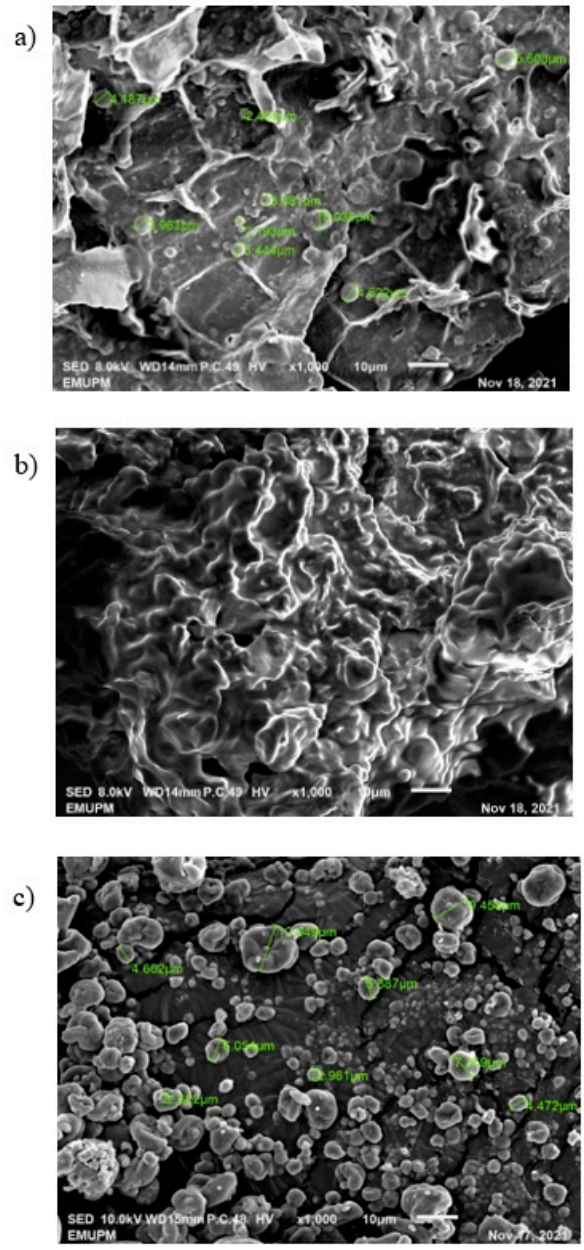


Figure 6. SEM micrograph for RBP powder produced with the different drying methods at 1000× magnification, (**a**) freeze drying, (**b**) oven drying, and (**c**) spray-drying.

3.6. Protein Identification Analysis Based on Amino Acid Profiling

Amino acid profiling for rice bran protein (RBP) and raw rice bran (RRB) was conducted for protein quality identification. Based on this amino acid profiling, the type of peptide responsible for protein structure was identified. Overall, there are an increasing number of protein types from the RRB sample to RBP powder. This is due to the peptides produced after the spray-drying process being much purer compared to the RRB, thus creating an increased signal protein identification [30]. Table 2 shows the summary of peptides of interest detected in RRB and RBP.

Table 2. Raw rice bran (RRB) and rice bran protein (RBP) in protein identification summary results.

Protein	Raw Rice Bran (RRB)	Rice Bran Protein (RBP)
Globulin	D	D
Glutelin	D	D
Prolamin	D	ND
Glucose	ND	D
Fructose	ND	D

D = Detected, ND = Non-detected.

The analysis identified Globulin, Glutelin, and Prolamin with 1, 6, and 1 groups identified, respectively, in RRB. For RBP, the analysis identified Globulin, Glutelin, Glucose, and Fructose with 1, 6, 1, and 12 groups identified, respectively, and no prolamin was detected in RBP. The sequence data for Globulin, Glutelin and Prolamin detected in RRB are shown in Figures S1, S3 and S5 respectively. Meanwhile, the sequence data for Globulin, Glutelin, Glucose, and Fructose detected in RBP are shown in Figures S2, S4, S6 and S7, respectively.

Based on the analysis, globulin and glutelin were identified as the main monomers in RRB and RBP. Globulin comprises about 15–25% of the protein stored in rice bran. Globulin is soluble in salt solution and can be identified with a molecular weight of 16 kDa and 25 kDa, respectively [31,32]. From the de novo peptide sequencing of globulin structure in RRB and RBP, carbamidomethylation and oxidation are compared with RBP powder. Carbamidomethylation is usually cystine blocked from oxidation [33]. The supporting peptide for carbamidomethylation is peptide KVAYVLDGEGEAEIVCPHLSRG with 9 ppm and peptide DGEGEAEIVCPHLSRG with 8.8 ppm, while the oxidation peptide are RMYLAGMNSVLKK with 9.4 ppm and RMYLAGMNSVLKK with 9.0 ppm. For illustration purposes, the structure of the peptide obtained from the analysis was modelled based on the structure modelling in the UniProt database. Figure 7 shows the structure of globulin protein in the rice bran product. The model shows the confidence level per residue confidence score. The bodies of the protein have a high confidence level, while the tail is in low confidence levels, as shown in the UniProt database.

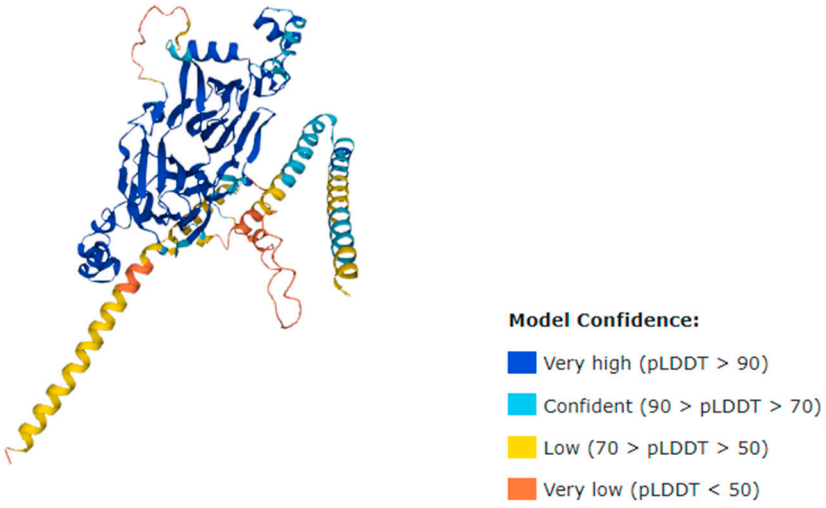


Figure 7. Globulin structure in the rice bran product developed based on structure modelling.

Another protein component found in both samples is glutelin. Glutelin is considered a major protein fraction in rice grain as it comprises 11% to 27% of the total rice bran. Glutelin is readily soluble under alkaline conditions [32]. The molecular weight of glutelin is recorded as between 45 to 150 kDa. Based on de novo peptide sequencing, the supporting peptide that has a marker for carbamidomethylation is the peptide NGLDETFCTMRV. Meanwhile, for RBP powder the peptines are NGLDETFCTMRV, but for RBP, a mutation occurs at the peptide RGLLLPHYTD (sub N) GASLVYIIQGRG. This is probably caused by

the heat shock during the spray-drying process [34]. Figure 8 shows the glutelin from rice bran products with a high confidence level of >90 per residue confidence, as mentioned in the UniProt database.

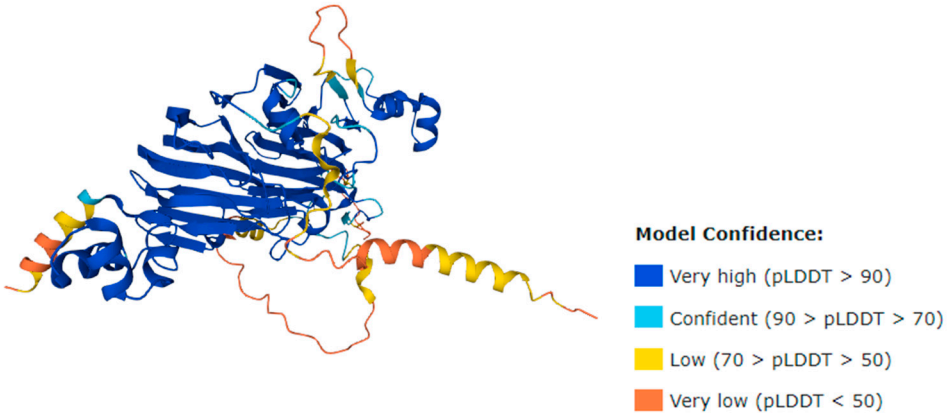


Figure 8. Glutelin in the rice bran product developed based on structure modelling.

Prolamin is the smallest fraction among the four main protein fractions in rice bran. It only comprises 4% of the rice bran. Prolamins are soluble in 60–70% aqueous ethanol and easily soluble in acid and alkali. The molecular weight of protamine is 12–17 kDa [32]. In the RRB sample, the supporting peptide that has a marker for carbamidomethylation is the peptide RNCQVMQQQCCQQLRM. Only RRB shows a prolamin, while for RBP, prolamin is non-detected (ND). Prolamin is probably denatured during the spray-drying process, since the heat for spray-drying is high in temperature [35]. Generally, rice prolamin is known as an effective agent in activating human anti-leukaemia immunity, but Kim et al. [36] suggest that the reduction of 13 kD prolamin can improve the nutritional quality of rice through the increasing of lysine level. Figure 9 shows the prolamin from rice bran product with a low confidence level of 70 > pLDDT > 50 per residue confidence, as mentioned in the UniProt database.

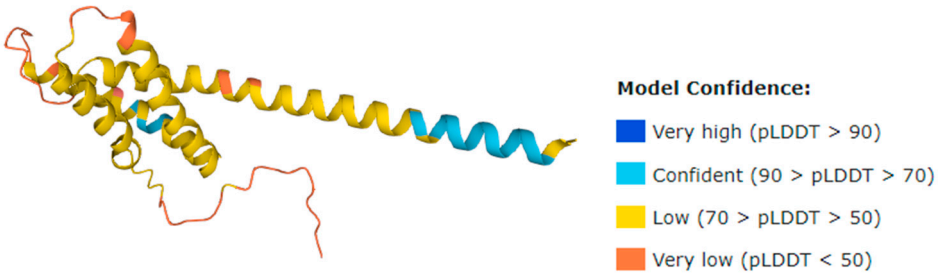


Figure 9. Prolamin in the rice bran product developed based on structure modelling.

Besides protein, the analysis also detected two types of sugar protein found in RBP but undetectable in RRB. Both are glucose and fructose, with 1 and 12 proteins identified, respectively. The formation of these components is due to the thermal hydrolysis of fibre in RRB bran during the extraction process.

Figure 10 shows the glucose and ribitol identified in the RBP. There are three peptides, RALALQLAEEGIR.V, RTNIFSYFFMSKH, and KGQEEKDAEETLRA, that support this protein.

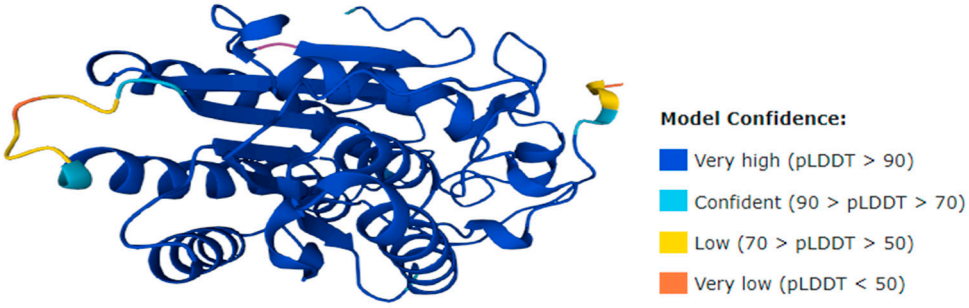


Figure 10. Glucose and ribitol in the rice bran product developed based on the structure model.

Figure 11 shows the structural model based on its peptide make-up in the UniProt database with a high confidence level of >90 per residue confidence. Fructose-bisphosphate aldolase sequence for rice bran powder (RBP) with its peptide support KGILAADEST-GTIGKRL and its structure with high confidence level > 90 in the UniProt database. The Uniprot database also stated that this protein is involved in step 4 of the subpathway synthesising D-glyceraldehyde 3-phosphate and glycerone phosphate from D-glucose. This subpathway is part of the pathway glycolysis, which is itself part of carbohydrate degradation. The existence of these sugar protein is helping the spray-drying process to produce fine powder without drying agents, such as maldodextin, arabic gum, and gelatin.

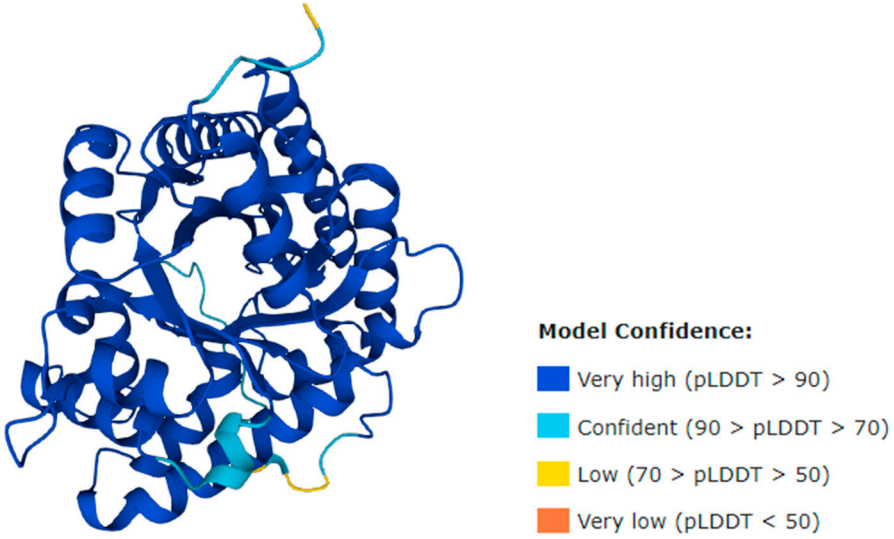


Figure 11. Fructose-bisphosphate aldolase develops based on structure modelling.

4. Conclusions

The study on the effect of spray-drying operating conditions, namely inlet temperature, feed flowrate, and air flowrate, on the RBP powder yield and protein concentration was executed. The optimum conditions obtained based on response surface methodology (RSM) are at the temperature of 178 °C, feed flowrate set to 25%, and air flowrate of 450 L/h. Predicted RBP powder yields were validated with experimental data which produced a lower and acceptable error as determined by the AARD value of 2.96%. The protein quality analysis was performed on the RBP product, and RRB demonstrated that the main peptides contributing to this protein were globulin and glutelin. Meanwhile, prolamin is believed to degrade during the drying process, as it is not detected in RBP powder. The process also produced protein sugar, helping produce a powder of fine particles without a drying agent. The spray-drying produced a good quality powder with a spherical shape, as observed in the micrograph picture.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr10102026/s1. Table S1: Experimental condition matric coded factors based on central composite design for this study. Figure S1 : Globulin sequence for raw rice bran (RRB). Figure S2: Globulin sequence for rice bran powder (RBP). Figure S3 : Glutelin sequence for raw rice bran (RRB). Figure S4: Glutelin sequence for rice bran powder (RBP). Figure S5 : Prolamin sequence for raw rice bran (RRB). Figure S6: Glucose and ribitol sequence for rice bran powder (RBP). Figure S7 : Fructose-bisphosphate aldolase sequence for rice bran powder (RBP).

Author Contributions: Conceptualization, M.S.M.S., N.M.A.N.D., S.A.S., M.A.H.M.N. and M.A.J.; Data curation, N.M.A.N.D., S.A.S., M.A.H.M.N. and M.A.J.; Formal analysis, M.R.M.; Funding acquisition, M.S.M.S.; Investigation, M.S.M.S.; Methodology, M.R.M. and M.S.M.S.; Project administration, M.S.M.S.; Supervision, M.S.M.S.; Validation, N.M.A.N.D., S.A.S., M.A.H.M.N. and M.A.J.; Writing—original draft, M.R.M.; Writing—review and editing, M.S.M.S., N.M.A.N.D., S.A.S. and M.A.J. All authors have read and agreed to the published version of the manuscript.

Funding: The financial support provided by the Ministry of Higher Education Malaysia through the Fundamental Research Grant Scheme, reference no: FRGS/1/2018/TK02/UNIMAP/02/3.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The financial support provided by the Ministry of Higher Education Malaysia through the Fundamental Research Grant Scheme, reference no: FRGS/1/2018/TK02/UNIMAP/02/3, is acknowledged.

Conflicts of Interest: The authors declare no conflict of interest.

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