



Spray Drying Optimization for Rice Bran Protein (RBP) Powder Using Response Surface Methodology (RSM)

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ABSTRACT

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Rice bran is a by-product of the rice milling process which contain a high concentration of protein. It's are often used as a feed cattle, fertilizer, and fuel. Its application as a source of human nutrition is rare due to high lipid concentration. This lipid concentration can be reduced through the extraction process. After the extraction process, the rice bran extract needs to be converted into powder form through a drying process for the quality preservation. In this study, spray drying is utilized as drying technique. The aims of this study were to optimize the spray drying parameter; inlet temperature, feed flowrate and air flowrate for rice bran protein (RBP) powders production. Box Behnken Design (BBD) model in response surface methodology (RSM) are utilized in this study to maximize the RBP powder yield and protein concentration. Raw rice bran (RRB) was extracted using thermal water-based extraction method before the drying process. The optimum condition suggested by the model are at the inlet temperature of 120°C, feed flowrate of 18.38% and air flowrate of 670 L/hr which produced RBP powder yield of 19.42 g RBP/100g RRB and protein concentration of 17.32 mg/ml. The model obtains in this study show a low error between the predicted value and experimental data at 1.68 % and 1.14 % for RBP powder yield and protein concentration respectively. The model can be used to evaluate the process characteristic and understanding.

1. Introduction

Rice (*Oryza sativa*) is known as one of the most valuable cereal crops in the world. It is a dietary core that provides daily calories for animals and humans [1]. In low- and middle-income countries, rice is by far the most important crop, and it most closely associated with the South, Southeast, and

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East Asian nations extending from Pakistan to Japan [2]. Annually, about 480 million metric tonnes of milled rice are produced [3] and from that is approximately about 50–60 million tons of rice bran are produced per year which mainly used as animal feed [4]. Its potentials are currently increasing as an alternative and cost-effective source of hypoallergenic and high-quality protein from plant-based nutrients [5]. The use of rice proteins from these sources as foods with good nutrition and functionality is extremely versatile and potential ingredient for food production and would minimize waste resources and the related pollution in the ecosystem [6].

As for the remedial benefits of rice bran, it consists of natural antioxidant compounds that promotes benefits to human health namely tocopherols, tocotrienols, vitamin C, anthocyanins, isoflavones, beta-carotene, polyphenols and oryzanol [7]. Additionally, the nutritive value of rice bran is interestingly used as food ingredient in any healthy beverages [8]. Rice bran also has a high total protein content, with the second greatest proportion of metabolites that can be easily denatured and hydrolysed under various variables of temperature, pH, and salt concentration [9]. Proteins play a main role specifically in the development and care of the human body and other functions in the body, such as enzymatic activity and nutrient transportation across cellular membranes [10]. However, the rice bran needs to be process for the lipid dan fibre reduction or convert to the other product such as yellow noodles [11] before it can be consumable for human being. This process normally involving with the extraction or other product development process depending on its application. Based on previous study, rice bran can be extracted using water extraction through autoclaving and sonication and it's demonstrating the potential for protein extraction and produce 14.9% of crude protein in 89.5% dry matter [9]. After extraction, the rice bran extract needs to be dried into powder form for quality preservation and longer shelf life. The study, such as instant soymilk powder by ultrafiltration, spray drying, and fluidized bed agglomeration in Thailand, have shown that rice bran extract can be preserved using spray drying techniques [12].

Spray drying is a method of drying into dried particles through rapid evaporation. The spray drying technique involves distributing the product's droplets inside a chamber to convert it from a liquid to a solid state in the form of powder [13]. The advantages of this technique are fast drying, high yield, large quantity, constant operation and time reduction for product exposure to the high temperature which reduced the possible thermal damage of the products [14]. Through rapid evaporation, the possibility for proteins denatured is lower, although it has been exposed to high temperature [15]. The main parameter affecting this process are inlet temperature, feed flowrate and air flowrate. Based on this data, the relationships between the solutes such as rice bran and an energy balance of vapor and air combination can be obtaining to improve the evaporation rate, radiation rate, and convection of the water vapor. The drying rate and duration of the drying cycle are determined by the capacity of the air (gas) stream to absorb and remove moisture. The higher the inlet temperature creates better vapor holding capability [16] and a higher drying cycle will expose the solute or protein for possible degradation [17]. The lower vapor holding capacity will increase the powder losses in the process by sticking on the drying wall. Therefore, the optimization process is crucial in order obtain the best spray drying condition which involve the interaction with various parameters namely inlet temperature, feed flowrate and air flowrate in rice bran protein (RBP) extract drying process.

1.1 Response Surface Methodology (RSM)

The optimization process for the spray drying process is crucial to obtain high quality protein without comprising its total yield since it was involved with three main independent variables. Response surface methodology (RSM) is one of the well-known mathematical and statistical method used for the optimization process. It had been introduced by Box and Wilson in 1951 [18]. RSM is also

effective numerical method for optimization that evaluate the effect of parameters and their interactions by using a smaller number of experiments. RSM is an approach used to obtain the surface response by fitting the data collected into a polynomial mathematical model to assess the effects of each element as well as the interactions between them [19]. RSM carries out simultaneous optimization of process variables (independent variables) to obtain the desired response variables (dependent variables) [20]. Despite the various advantages of RSM, its optimum condition depends on the mathematical design and the validity of this mathematical model are depend on the statistical analysis [17].

1.2 Box Behnken Design (BBD)

Two most common designs in response surface methodology (RSM) are central composite designs (CCD) and Box Behnken designs (BBD)[18]. The CCD can fit a full quadratic model and are often used when the design plan calls for sequential experimentation because these designs can include information from a correctly planned factorial experiment [18]. Whereas for the BBD, it usually has fewer design points than central composite designs, thus, it is less expensive to run with the same number of factors and it efficiently estimates the first- and second-order coefficients. BBD always have 3 levels per factor, unlike CCD which can have up to 5. In addition to that, BBD also never includes runs where all factors are at their extreme minimum or maximum settings [21]. As a result, Eq. (1) was introduced as the generalised polynomial model for predicting the response variables as a function of the independent factors [21].

$$Y_i = \beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_{11}x_1^2 + \beta_{22}x_2^2 + \beta_{12}x_1x_2 \quad (1)$$

Box–Behnken design is a rotating second-order design based on incomplete factor designs of three levels. The Box–Behnken design level's special arrangement allows the number of design points to rise at the same rate as the number of polynomial coefficients. For example, for three factors, the design can be designed as three blocks of four experiments consisting of a complete two-factor factor design with the level of the third factor set at zero [22]. Box Behnken design (BBD) is an independent, rotatable quadratic design with no embedded factorial or factorial fractional points where the variable combinations are in the midpoints of the vector space edges and, in the middle, [22]. Therefore, in this study the BBD model are utilized as the multivariable model for the optimization purpose due its fewer design point and proven to provide a good optimization process.

2. Methodology

2.1 Sample Collection and Pretreatment

Raw rice bran was collected from the local rice milling BERNAS factory in Perlis and undergo pre-treatment at once for rancidity prevention using a microwave oven and then keep in refrigerated at 0-4°C prior to the extraction process. Next, the rice bran was extracted using a thermal water extraction process. The thermal water extraction process was conducted using Hirayama HG-80 sterilizer at 120°C and 0.26 MPa for 20 minutes as a modified method from the previous study [10]. The rice bran sample was mixed with distilled water with the ratio of 1:20 (g:ml) in a Schott bottle before the extraction process. A cooling period of 30 minutes in room temperature were done after extraction before the slurry was filtered to obtain the filtrate as the rice bran extract.

2.2 Spray Drying

The spray drying process was conducted using Buchi B 290 Mini spray dryer (Germany) at the Faculty of Chemical Engineering Technology, Universiti Malaysia Perlis (UniMAP). For each experimental spray drying process, 500mL of the rice bran extract are dried to obtain the rice bran protein (RBP) powder and its yield was measured and recorded. The powder yield was calculated using eq. 2

$$Yield = \frac{W_{final} - W_{initial}}{W_{RRB}} \times 100 \quad (2)$$

where, Yield = Yield of rice bran protein (RBP) in 100 g raw rice bran
 W_{final} = Weight of the cyclone after spray drying process
 $W_{initial}$ = Weight of the cyclone before the spray drying process
 W_{RRB} = Weight of raw rice bran

After that, the rice bran protein (RBP) powder obtained was then mixed with distilled water in a ratio of 1:200 (g:ml) for the protein concentration analysis using the UV-Vis spectrophotometer (Shimadzu, Model: UV-1800, Japan). By using the Bradford reagent, the protein concentration was obtained by using the standard calibration curve. The average result was taken as the protein concentration. Three main operating condition in spray drying process was evaluated in this study namely inlet temperature, feed flowrate and air flow rate. The criteria for each parameter is 120 °C - 210 °C for inlet temperature, 5% - 55% for feed flowrate and 225L/hr-525L/hr for air flowrate. The other parameter in the spray drying system are remained constant though the study namely the aspirator percentage of 95 % and the nozzle pulse of 2.

2.3 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 protein powder production using Box Behnken Design (BBD) design model. Three variables (parameters) were set to 3 to 5 distinct levels for each numeric factor and two responses are evaluated. In this study, both designs were employed to look at the effects of inlet temperature, feed flowrate, and air flowrate on yield and protein concentration. Furthermore, experiments were randomized to reduce bias. All of the software-predicted experiment conditions were carried out in triplicate. As indicated in Eq. (3), the following predictive quadratic polynomial equation will be utilized to fit the experimental results as the correlation between the response and the independent variables.

$$Y = A_0 + \sum A_i X_i + \sum X_{ii} X_i^2 + \sum A_{ij} X_i X_j \quad (3)$$

Three parameter factors are located in the middle of the edges of the experimental domain [29]. In this study, three variables were considered as three numeric factors. Thus, each numeric factor was set to 4 levels, with zero categoric factor. The BBD was also used to study the correlation between the feed flowrate, inlet temperature and the air flowrate. The response are RBP powder yield and protein concentration.

2.4 Statistical Analysis

SAS statistical tool was used to examine all the data. Sequential model sums of squares, model summary statistics, and analysis of variance were also used to analyse the response's results (ANOVA). The significance of differences between independent variables was determined using ANOVA. This analysis is often associated with the probability p-value, Fisher's F-test, sum of squares and determination coefficient R². Furthermore, the obtained data can also be subjected to Tukey's HSD test at a significance level of p<0.05 for comparison of treatment effects for each of the parameters. Therefore, the final empirical model was created after determining the relevance of each element in relation to the answer.

2.5 Model Validation

The validation of the best model was conducted by comparing the experiment data with the predicted value. The absolute average deviation (AAD) for the experimental was calculated using Eq (4)

$$AAD = \frac{1}{n} \sum_{m=1}^n |Y_m - Y_{average}| \quad (4)$$

The deviation between the predicted value and experimental data was calculated and evaluated based on absolute average relative deviation (AARD) as shown in Eq. (5).

$$AARD = \left(\frac{Y_{experiment} - Y_{predicted}}{Y_{experiment}} \right) \times 100 \quad (5)$$

3. Result and Discussion

In this optimization process, three spray drying operating parameter that are inlet temperature, feed flow rate and air flow rate were investigated to obtain the high RBP powder yield and protein concentration. There are 17 run was designated with different operating conditions of spray dryer. Table 1 shows the experimental condition design and the response in spray drying process of rice bran protein powder.

Table 1

Experimental condition design coded factors from response surface methodology (RSM) and the response

Run	A Temperature (°C)	B Feed Flowrate (%)	C Air Flowrate (L/hr)	Response 1 Yield of Rice Bran Protein Powder (g RBP/100g RRB)	Response 2 Protein Concentration (mg/ml)
1	165	5	670	10.31	12.81
2	165	30	357	10.81	13.39
3	210	55	357	9.43	13.63
4	210	30	670	10.33	12.76
5	120	5	357	14.16	12.55
6	210	5	357	9.72	10.05
7	120	55	357	8.27	11.16
8	165	30	357	9.84	12.38
9	165	55	246	5.2	10.97
10	165	30	357	10.4	13.84
11	120	30	670	20.25	17.29
12	165	30	357	10.81	13.84
13	165	30	357	10.35	12.53
14	165	55	670	10.65	12.82
15	165	5	246	8.07	9.83
16	120	30	246	6.62	10.55
17	210	30	246	7.46	12.81

3.1 Sequential Model Sum of Squares

To evaluate the accurate of both models, the various statistical analysis was conducted. Table 2 shows the sequential model sum of squares (type 1) for RBP powder yield model. A general rule to follow when choosing the model would be the suggested model should be of the highest order polynomial where the additional terms are significant, and the model is not aliased. In this study, the suggested model is Quadratic vs 2FI with a sum of squares of 26.90. Furthermore, the model is significant as determined by f value (3,17) = 5.65, $p < 0.0276$.

Table 2

Sequential model sum of squares (Type 1) for RBP powder yield

Source	Sum of Squares	df	Mean Square	F value	P- value (Prob> F)
Mean vs Total	174.02	1	1754.02		
Linear vs Mean	89.72	3	29.92	4.72	0.0194
2FI vs Linear	44.48	3	14.83	3.90	0.0440
Quadratic vs 2FI	26.90	3	8.97	5.65	0.0276
Cubic vs Quadratic	10.46	3	3.49	21.67	0.0062
Residual	0.6435	4	0.1609		
Total	1926.25	17	113.31		

The analysis of sequential model sum of squares (type 1) for protein concentration is shown in Table 3. As for protein concentration, the model suggested source is Quadratic vs 2FI with sum of squares of 12.21. The model also significant as determined by f value (3,17) = 11.42, $p < 0.0044$.

Table 3

Sequential Model Sum of Squares (Type 1) for protein concentration for BBD

Source	Sum of Squares	df	Mean Square	F value	P value (Prob> F)
Mean vs Total	2674.03	1	2674.03		
Linear vs Mean	16.62	3	5.54	2.16	0.1423
2FI vs Linear	18.70	3	6.23	4.24	0.0356
Quadratic vs 2FI	12.21	3	4.07	11.42	0.0044
Cubic vs Quadratic	0.5192	3	0.1731	0.3502	0.7924
Residual	1.98	4	0.4941		
Total	2724.05	17	160.24		

3.2 Model Summary

In model summary statistic, predicted residual sum of squares (PRESS) and R-squared (R^2) value was taken into consideration for the selection of model. PRESS is a measure of how a particular model fits each design point. The coefficients for the model were calculated without the first design point. This model was used to predict the first point and then the new residual was calculated for this point. This was done for each data point and then the squared residuals were summed. The following Table 4 shows the model summary statistics for RBP powder yield.

Table 4

Model summary statistics for RBP powder yield

Source	Standard Deviation	R-Squared	Adjusted R-Squared	Predicted R-Squared	Lack of Fit P-value	PRESS
Linear	2.52	0.5212	0.4106	-0.0484	0.0007	180.57
2FI	1.95	0.7794	0.6470	-0.7216	0.0017	296.50
Quadratic	1.26	0.9356	0.8527	-0.3595	0.0062	234.15
Cubic	0.4011	0.9963	0.9851			

As shown in Table 4, linear model has the lowest PRESS value of 180.57 in comparison with quadratic polynomial and 2FI with PRESS of 234.15 and 234.15 respectively. Meanwhile, the cubic model had produced an aliased model which undesirable in the optimization process. However, the quadratic model had the highest R^2 value of 0.9356 when compared with the other non-aliased model of linear and 2FI with R^2 value of 0.5212 and 0.7794. Besides that, in term of lack of fit, the quadratic equation also show the highest P-value compare with the others. As a result, a quadratic polynomial model with a high fitted R^2 , a high lack-of-fit P-value, and a non-smoothed model was chosen to represent the experimental data in this study.

Table 5 show the model summary statistics for protein concentration. As shown in Table 5, the quadratic polynomial model has the lowest predicted residual sum of squares (PRESS) with a value of 14.29 and the highest R^2 with value 0.9501 compared to all other models that are not aliased. The fitted R^2 values were considered in determining the model best suited to represent the experimental data.

Table 5
 Model Summary Statistics for protein concentration

Source	Standard Deviation	R-Squared	Adjusted R-Squared	Predicted R-Squared	Lack of Fit P-value	PRESS
Linear	1.60	0.3323	0.1782	-0.3259	0.0377	66.33
2FI	1.21	0.7060	0.5297	-0.4505	0.0899	72.56
Quadratic	0.5971	0.9501	0.8860	0.7144	0.7924	14.29
Cubic	0.7029	0.9605	0.8420			

Table 5 shows that, in addition to the cubic model, which was aliased, the quadratic polynomial model had the highest adjusted R² value of 0.8860, compared to the linear and 2FI models with 0.1782 and 0.5297 respectively. The P value for the lack of fit of the quadratic model is the highest compared to the aliased model. Therefore, the quadratic polynomial model with a high R² value, a high P-value for lack of fit and a non-smoothed model was selected as the model representing the experimental BBD based model data of this study.

3.3 ANOVA Analysis

Analysis of variance (ANOVA) was conducted to evaluate the significant effect of the studied parameter toward the RBP powder yield and protein concentration. Table 6 show the ANOVA analysis for quadratic model of RBP powder yield.

Table 6
 ANOVA analysis for quadratic model of RBP powder yield

Source	Sum of Squares	df	Mean Square	F Value	p-value
Model	161.13	9	17.9	11.29	0.0021
A-Temperature	13.85	1	13.85	8.73	0.0212
B-Feed flowrate	0.0049	1	0.0049	0.0031	0.9574
C-Air flowrate	34.99	1	34.99	22.07	0.0022
AB	7.84	1	7.84	4.94	0.0615
AC	32.99	1	32.99	20.8	0.0026
BC	3.65	1	3.65	2.3	0.173
A ²	6.9	1	6.9	4.35	0.0754
B ²	7.42	1	7.42	4.68	0.0673
C ²	13.18	1	13.18	8.32	0.0235
Residual	11.1	7	1.59		
Lack of Fit	10.46	3	3.49	21.67	0.0062
Pure Error	0.6435	4	0.1609		
Cor Total	172.23	16			
Standard Deviation	1.26		R ²		0.9356
Mean	10.16		Adjusted R ²		0.8527
C.V. %	12.40		Predicted R ²		-0.3595
PRESS	234.15		Adeq Precision		15.8880

As shown in Table 6, two parameters namely air flowrate and inlet temperature are showing the significant effect on RBP powder yield as determined by *f* value (1,16) =22.07, *p* < 0.0022 and *f* value (1,16) = 8.73, *p* < 0.0212 for air flowrate and temperature respectively. Meanwhile, feed flowrate had shown no significant effect on yield as determined by *f* value (1,16) = 0.0031, *p* < 0.9574. To be acceptable, the model must also exhibit a little lack of fit. As a result, the Lack of Fit *f*-value (3,16) of

21.67 in this study shows that the Lack of Fit could be attributed to noise by 0.62 percent as shown in Table 6. A final quadratic polynomial model for RBP yield was generated as equation 6.

$$\text{Yield} = - 4.63C^2 - 0.3318B^2 + 1.28A^2 + 0.6529BC - 3.93AC + 6.45C + 0.0213B - 1.66A + 10.75 \quad (6)$$

where Yield is rice bice protein (RBP) powder yield, A is the inlet temperature, B is the Feed Flowrate, and C is the air flowrate. The similar analysis was conducted for the protein concentration. Table 7 shows the ANOVA results for quadratic model of protein concentration.

Table 7
 ANOVA analysis for quadratic model of protein concentration

Source	Sum of Squares	df	Mean Square	F Value	p-value
Model	47.53	9	5.28	14.81	0.0009
A-Temperature	1.54	1	1.54	4.32	0.0763
B-Feed flowrate	11.05	1	11.05	31.01	0.0008
C-Air flowrate	8.99	1	8.99	25.20	0.0015
AB	6.18	1	6.18	17.32	0.0042
AC	12.10	1	12.10	33.94	0.0006
BC	0.4191	1	0.4191	1.18	0.3142
A ²	0.1655	1	0.1655	0.4642	0.5176
B ²	10.07	1	10.07	28.25	0.0011
C ²	1.67	1	1.67	4.68	0.0674
Residual	2.50	7	0.3565		
Lack of Fit	0.5192	3	0.1731	0.3502	0.7924
Pure Error	1.98	4	0.4941		
Cor Total	50.03	16			
Standard Deviation	0.5971		R ²		0.9501
Mean	12.54		Adjusted R ²		0.8860
C.V. %	4.76		Predicted R ²		0.7144
PRESS	14.29		Adeq Precision		16.5839

As shown in Table 7, two independent parameters namely feed flowrate and air flowrate had shown the significant effect on protein concentration as determined by *f* value (1,16) = 31.01, *p* < 0.0008 and *f*-value (1,16) = 25.20, *p* < 0.0015 for feed flowrate and air flowrate respectively. Meanwhile, the inlet temperature had showed the insignificant effect as determined by *f* value (1,16) = 4.32, *p* < 0.0763 which is higher than <0.05. In order, for the model to be suitable, it must have a non-significant lack of fit. Hence in this study, the Lack of Fit *f* -value of 0.3502 which implies that there is a 7.924% chance that the Lack of Fit could occur due to noise. The final equation for quadratic polynomial model for protein concentration is shown in equation 7.

Protein concentration

$$= -165C^2 - 0.3867B^2 + 0.1983A^2 - 0.2213BC - 2.38AC + 0.6213A + 3.27C + 1.02B - 0.5520A + 12.57 \quad (7)$$

where A is the inlet temperature, B is the Feed Flowrate, and C is the air flowrate.

3.4 Optimum Condition

Optimum condition for RBP powder yield was determined by using numerical optimization in RSM by the Design of Experiment software. All the parameters (A, B and C) were set within range of upper

and lower limits to obtain the maximum possible yield of RBP powder and protein concentration. There were 10 solutions that were predicted as the optimized condition. However, solution with highest desirability of yield of RBP powder was chosen. Conditions suggested where inlet temperature is 120°C, feed flowrate set to 18% and air flowrate of 357 L/hr and it has the highest desirability at 0.975 while producing RBP yield of 19.4 g RBP/100gRRB and protein concentration of 17.3 mg/ml.

3.5 Model Validation

The suggested optimum condition was validated through the experimental run and compare it with predicted value suggested by the model. Table 8 show the model validation for the RBP yield powder and protein concentration.

Table 8

Model validation with experimental data for BBD model

Response (R1)	Experimental data		Predicted value	Error AARD (%)
	Average	AAD		
Yield	19.1 (g RBP/ 100 g RRB)	1.0 (g RBP/ 100 g RRB)	19.42 (g RBP/ 100 g RRB)	1.68
Protein concentration	17.13 mg/ml	0.126 mg/ml	17.326 mg/ml	1.14

As tabulated in table 8, the experimental value for yield and protein concentration are 19.1 ± 1.0 g RBP/100 g RRB and 17.13 ± 0.126 respectively with predicted value of 19.42 g RBP/ 100 g RRB and 17.326 mg/ml. It was observed that at this optimized condition the predicted value is agreed with the experimental value with the error of 1.68 and 1.14 for yield and protein concentration, respectively. The error AARD differences between the predicted response and the experimental responses were found to be in the range of below 5%. Besides that, all model validation absolute average deviation less than 1 which indicates that the experiments have high repeatability with very minor error

4. Conclusion

As a conclusion, the BBD based model in response surface methodology (RSM) can be used to optimize the spray drying for RBP powder drying process. The optimum conditions to obtain the higher RBP powder yield and higher protein concentration are inlet temperature of 120°C, feed flowrate of 18% and air flowrate of 357 L/hr to produce RBP powder yield of 19.1 ± 1.0 g RBP/100 g RRB with the protein concentration of 17.13 ± 0.13 mg/ml. The AARD recorded for the predicted value and experimental are 1.68 % and 1.14 % and consider as the low error. The model can be utilized characteristic study and process understanding if considering the 3-D surface and contour graph.

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