



Optimization of Acid Degumming of Crude Rice Bran Oil (CRBO) on Phospholipid Removal Using Response Surface Methodology (RSM)

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Abstract

Rice bran, a byproduct of milling rice, has 60% nutritional content and potential for processing into higher-selling goods like rice bran oil (RBO). Degumming is the first stage of refining crude oil. This research aims to remove phospholipids in crude rice bran oil (CRBO) by optimizing acid degumming. Three variables were optimized in this study using Design Expert v13's response surface methodology (RSM) employing central composite design (CCD) experimental design: the amount of phosphoric acid (0.1, 0.15, and 0.2 %w/w), the degumming temperature (50, 70, and 90 °C), and the degumming time (20, 30, 40 minutes). The experimental values and predicted results did not correlate well with percentages (%) of error, with more than 50% occurring in 4 of 19 runs. The model's p-value of 0.4939 (> 0.05) is non-significant, with the coefficient determination, R^2 being 0.5026. Optimization with desirability value (> 0.5) results showed a phospholipid removal response of 40.479% under conditions of adding 0.1%w/w phosphoric acid, temperature of 83.21 °C, and degumming time of 40 minutes, which were determined by Derringer's methodology.

Keywords: Degumming, phospholipids, response surface methodology, rice bran, crude rice bran oil

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1. Introduction

According to data from the Central Statistics Agency of Indonesia (BPS, 2022), Indonesia will produce 55.7 million tons of rice annually in 2022. Paddy comprises the main, rice, and side products, namely husks, bran, and seeds (Bodie et al., 2019). Rice bran of 8-10% (Alauddin et al., 2017) is often only used as animal and poultry feed (Begum et al., 2015; Limtrakul et al., 2020). Bran contains approximately 65% of the nutrients of the total grain of rice (Garofalo et al., 2021), antioxidants (Alauddin et al., 2017), and 12-23% of the oil, depending on geography, seed type, and extraction procedures (Mingyai et al., 2017; Phan et al., 2019). Content oil consists of lipids, proteins, minerals, vitamins, and non-fatty acid saturates

(UFAs), including oleate, linoleate, and linolenate (Phan et al., 2019).

Rice Bran Oil (RBO), which can be obtained from results extraction among them using enzyme *cellulase* (Damayanti et al., 2023), contains γ -oryzanol and vitamin E (tocopherol and tocotrienol) (Mingyai et al., 2017) has been declared "oil healthy" by the organization food and health, The World Health Organization (WHO) (Garofalo et al., 2021) causes has 47% fat no saturated (Punia et al., 2021) where composition the has by standard oil foods determined by The WHO (Pal & Pratap, 2017). However, crude RBO (CRBO) is the most complex oil for purifying and fulfilling plant-based specifications (Phan et al., 2019; Engelmann et al., 2017). This is because raw vegetable oil contains various types of dirt and is also influenced by storage

and extraction processes (Sengar et al., 2014). One impurity in the CRBO is phospholipids (Phan et al., 2019), which contribute to the decline of stability color, oil taste, and reduced age oil vegetables, namely the oil that can be eaten, so it is a crucial step in purification (Sengar et al., 2014; Gaber et al., 2020; Gharby, 2022).

The purification of CRBO can be done physically (acidification with distillation) or chemically (Engelmann et al., 2017). The main thing is the purification of oil vegetables, including the degumming and bleaching processes (Damayanti et al., 2023), because No influence composition of fatty acids from glycerides, but removes phospholipids and free fatty acids, as well as lower content of several more constituents such as sterols, tocopherols, and squalene (Gaber et al., 2020).

Finally, several chemical degumming techniques, such as enzymatic, membrane, and soft degumming procedures, have been studied. Alternatively, alternatives are costly for the sector and neither worthy nor feasible (Gaber et al., 2020). Acid degumming is one of these degumming techniques that uses a number of tiny phosphoric acids (0.05 to 0.1% by weight). Because it uses both water and acid to remove phosphatides that can be hydrated and those that cannot, it is the most popular degumming method and can be thought of as an extension of the water degumming process (Cheong & Xu, 2019; Nidzam et al., 2022). In addition, phosphoric acid is a powerful chelating agent that reduces the amount of remaining phospholipids in the oil and transforms dehydrated gums into hydrated gums that are easier to remove (Nidzam et al., 2022).

The primary factors influencing the degumming process are the amount of water and acid added, the temperature throughout the operation, and the duration of the process (Engelmann et al., 2017; Chew et al., 2017; Paisan et al., 2017; Nekouei & Rezaei, 2020). Because phosphoric acid is an efficient chelating agent and leaves the oil with few remaining phospholipids, it is crucial to know the ideal dosage to utilize during the degumming process (Zulkurnain et al., 2012). According to earlier research, the degumming process of wheat germ oil et al., 2013 used an 85% concentration of phosphoric acid solution at a dosage of 0.15% (Wang et al., 2001), canola oil at a 0.1% dosage (Ghazani & Marangoni, 2013), and safflower oil at a 0.1% dosage using a 75% concentration of

phosphoric acid solution (Ortega-García et al., 2006).

Reduced temperature led to a decrease in phosphorus concentration, especially when the temperature dropped from 85 to 40 °C (Chew et al., 2017). According to Egbuna (2015), 80 °C was the optimized temperature for the degumming stage of palm oil. According to Nekouei & Rezaei (2020), phosphorus reduction is only slightly impacted by the degumming time as a single parameter. At lower temperatures (30–40 °C), however, time has virtually no effect on the total phosphorus reduction.

Nekouei and Rezaei (2020) have successfully applied response surface methodology (RSM) in degumming wild almond oil. The RSM is an effective mathematical tool for optimizing experimental conditions from a process (Nidzam et al., 2022). RSM's potential feature is its ability to identify influencing variables with little to no practical effort (Nidzam et al., 2022; Nekouei & Rezaei, 2020). Therefore, a study must utilize RSM to optimize process variables and determine the interaction between or between process variables (Nidzam et al., 2022). This study's primary purpose is to determine the parameters of acid degumming of CRBO using RSM to remove phospholipids. The optimum condition of the degumming acid process could result in a better quality RBO.

2. Methodology

2.1. Materials

The materials used in this research include RBO obtained from supermarkets with added impurities in phospholipids because CRBO is challenging in Indonesia. Phospholipid type L- α -Phosphatidycholine 14–29% (Merck, Germany), phosphoric acid 85% (Merck, Germany), and distilled water.

2.2. Degumming of CRBO

A total of 0.5 grams of L- α -Phosphatidycholine was added to 49.5 grams of rice bran oil and heated for 5 minutes at 70°C, at a stirring speed of 300 rpm to make CRBO. CRBO is used for the degumming process. CRBO was placed in a 3-neck flask, heated at varying temperatures, and stirred at a speed of 300 rpm. After reaching the desired temperature, add a variety of phosphoric acid (85%) and continue stirring for 10 minutes. Then add 1.5 mL of distilled water and continue going for varying times. The mixture was centrifuged at 2000 rpm for 15 minutes.

Samples of rice bran oil that had been degumming were taken and collected in 50 mL glass bottles and stored at 4 °C.

Analysis of phospholipid content using the vanadomolybdate method with a spectrophotometer (Szydłowska-Czerniak & Łaszewska, 2017). Analysis was carried out at the Chemmix laboratory (Bantul, Yogyakarta). The analysis results in phosphorus levels are then converted into phospholipid levels using Equation (1).

$$\begin{aligned}\%PL &= \%P \times (MWPL/MWP) \\ &= \%P \times 26\end{aligned}\quad (1)$$

where PL (%) is Phospholipids removal, P (%) is phosphorus content, MWPL is the molecular weights of PL, and MWP is the molecular weights of P.

2.3. Experiment Design

The Central Composite Design (CCD) experimental design was chosen for this investigation, and the phospholipid removal response—the dependent variable—is examined (Czyrski & Jarzebski, 2020). Examine the combined impact of the three distinct independent variables to optimize the process variables for degumming crbo (Table 1); Table 2 detailed the experimental runs, which included 19 runs (6 factorial points, 8 axial points, and 5 central points) based on CCD, the amount of phosphoric acid (0.1, 0.15, and 0.2 %w/w), the degumming temperature (50, 70, and 90 °C), and the degumming time (20, 30, 40 minutes).

2.4. Model Fitting and Statistical Evaluation

The objective was to correlate the response variable with the independent variables by fitting each of the experimental planning's response variables (Table 3) to a second-order polynomial equation produced by Stat-Ease Inc.'s Design-expert software version 13, which is shown in Eq. (2). The initial regression fits were made using the quadratic response surface model shown in Eq. (2), which took into account the linear, quadratic, and interaction effects of the variables.

$$Y = \beta_0 + \sum_{i=1}^n \beta_i X_i + \sum_{i=1}^n \beta_{ii} X_i^2 + \sum_{i=1}^n \sum_{j=i+1}^n \beta_{ij} X_i X_j + \varepsilon \quad (2)$$

where Y represents the response factor (phospholipid removal), n is the number of factors examined and optimized during the experiment, and X_i and X_j are the coded

values of the variable parameters. The coefficients of the polynomial were β_0 (constant term), β_1 and β_3 (linear effect), β_2 and β_4 (quadratic effect), β_5 (interaction effects), and ε (error term).

To determine the mean deviation from the prediction model, utilize Eq. (3)'s Mean Absolute Percentage Error (MAPE) (Moreno et al., 2013).

$$MAPE = \frac{1}{n} \sum_{t=1}^n \left| \frac{A_t - F_t}{A_t} \right| \times 100\% \quad (3)$$

For each data point, t, A_t , and F_t represent the actual and forecasted values, respectively, and n is the number of data points.

A figure has considerable accuracy if the MAPE is less than 10%, acceptable accuracy between 10% and 20%, reasonable accuracy between 20% and 50%, and greater accuracy if it is more than 50% (Moreno et al., 2013).

Analysis of variance (ANOVA), regression analysis, and response surface plots of the components' interaction effects are among the analytical techniques used to assess the ideal circumstances for phospholipid removal. The ANOVA test was used to assess the significance of the process variables' linear, quadratic, and linear interactive effects on the removal of phospholipids. The regression coefficients were evaluated for significance using the probability value (p-value) at a 95% confidence interval; a p-value of less than 0.05 indicated whether the coefficient was significant. A mathematical model, shown in Eq. (2), was developed using the response of the degumming CRBO process to correlate the phospholipid removal to the degumming CRBO variables under investigation. The model's adequacy was evaluated by comparing the corrected R^2 value to the coefficient of determination (R^2) value.

3. Results and Discussion

3.1. Experimental Design and CCD Analysis

The model was validated by determining the percentage errors between the model's predictions and the actual experimental data (Table 2). Because percentages (%) of error are more than 50% off (Moreno et al., 2013) for several experiments (e.g., runs 10–12, 18), the residuals between the predicted and experimental (actual) values (Table 2) demonstrate poor agreement with experimental data. As a result, Fig. 1 displays

Table 1. Variable independent and their levels for the CCD

Factor	Unit	Minimal	Maximum	Medium(0)	Lower Limit (-1)	Upper Limit (+1)
(A) Amount of phosphoric acid	% (W/W)	0.07	0.23	0.15	0.1	0.2
(B) Temperature	°C	36	104	70	50	90
(C) Time	Minutes	13,18	46.82	30	20	40

Table 2. Design a CCD experiment with experimental and predicted data

Run	Amount of phosphoric acid (% W/W, A)	Temperature (°C, B)	Time (Minute, C)	Phospholipid Removal (%)		(% Error Phospholipids Removal (%))
				Experiment	Prediction	
1	0.1	90	20	5.34	7.52	28.9893
2	0.1	50	20	22.55	26.72	15.6062
3	0.15	70	30	24.93	34.73	28.2176
4	0.15	104	30	31.45	21.08	49.1935
5	0.15	70	13,18	34.42	24.11	42.7623
6	0.15	70	30	32.34	34.73	6.8816
7	0.15	70	30	37.09	34.73	6.7952
8	0.15	70	30	41.84	34.73	20.4722
9	0.2	90	40	38.58	32.21	19.7764
10	0.15	36	30	2.97	16.44	81.9343
11	0.2	90	20	7.72	23.35	66.9379
12	0.2	50	40	11.87	7.5	58.2667
13	0.15	70	46.82	16.62	30.03	44.6553
14	0.07	70	30	26.41	29.66	10.9575
15	0.23	70	30	28.49	28.33	0.5647
16	0.1	90	40	35.61	39.83	10.595
17	0.15	70	30	37.98	34.73	9.3579
18	0.1	50	40	42.73	24.91	71.5375
19	0.2	50	20	39.17	32.76	19.5665

Table 3. CCD experiment design with experimental and predicted data

Model	Sequential p-value	Lack of Fit p-value	Adjusted R ²	Predicted R ²	Remarks
Linier	0.9449	0.0519	-0.1771	-0.7059	
2FI	0.1916	0.0623	-0.0007	-1.6334	
Quadratic	0.4269	0.0518	-0.0052	-2.5866	Suggested
Cubic	0,0201	0.4916	0.7635	-0.9021	Aliased

a poor connection between the experimental data and the model predictions.

Experimental data were obtained to find the most suitable model for a predictive response. Polynomial models that can predict response consist of 4 types: linear, interactive (2FI), quadratic, and cubic. Some parameters, like sequential p-value, lack of fit p-value, adjusted R², and predicted R² were used to conclude the most suitable model for optimizing phospholipids from degumming CRBO, as presented in Table 3.

Based on Table 3, the best model for optimizing phospholipid removal is the quadratic model. Cubic models are not recommended (aliased) because they can produce inaccurate and misleading results (Khelifa et al., 2021). Equation (4) shows the relationship between the quadratic model and the interaction of variables.

$$\text{Phospholipid removal (\%)} = 34.73 - 0.396 A + 1.38 B + 1.76 C + 2.45 AB - 5.86 AC + 8.53 BC - 2.03 A^2 - 5.65 B^2 - 2.71 C^2 \quad (4)$$

Table 4. Analysis of variance (ANOVA) for response surface quadratic model for phospholipid removal

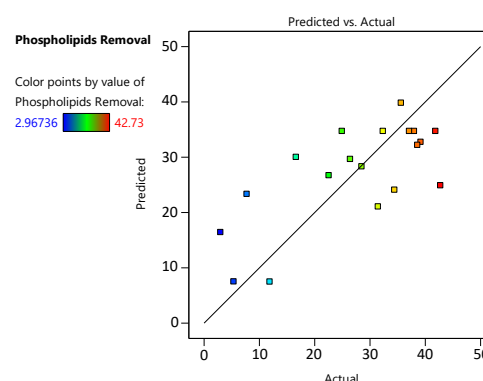
Source	Actual Coefficient	Sum of Squares	df	Mean Square	F-value	p-value	
Model	34.73	1472.87	9	163.65	1.01	0.4939	Not significant
A- Amount of Phosphoric Acid	- 0.396	2.14	1	2.14	0.0132	0.911	
B- Temperature	1.38	25.96	1	25.96	0.1603	0.6982	
C-Time	1.76	42.4	1	42.4	0.2618	0.6212	
AB	2.45	47.94	1	47.94	0.2961	0.5996	
AC	-5.86	274.77	1	274.77	1.7	0.2251	
BC	8.53	582.25	1	582.25	3.6	0.0904	
A ²	-2.03	56.07	1	56.07	0.3462	0.5707	
B ²	-5.65	435.15	1	435.15	2.69	0.1356	
C ²	-2.71	100.14	1	100.14	0.6184	0.4519	
Residual		1457.49	9	161.94			
Lack of Fit		1289.03	5	257.81	6.12	0.0518	Not significant
Pure Error		168.46	4	42.12			
Total Cast		2930.36	18				
R ²	0.5026						
CV%	46.67						

3.2. Statistical Analysis of Optimal Conditions for Phospholipid Removal

The factors (amount of phosphoric acid, temperature, and time) were not significant in the phospholipid removal by ANOVA (Table 4). The model *F* values of 1.01, implied the model was not significant. The model's *p*-value of 0.4939 (> 0.05) is likewise considered non-significant. Here, the response is not affected by variables like temperature (B), time (C), or the amount of phosphoric acid (A). From Table 4, no variable concerning phospholipid removal was significant at the studied levels. Note in Table 2 that the values for phospholipid removal presented wide variation (2-42 %) and an average of 27,27 %. It indicates a considerable variation, non-significant at the studied levels.

Lack of fit determines the inability of the model to show experimental data points by comparing pure error with residual error, where the expected lack of fit value is *p*-value > 0.05 or not significant (Breig & Luti, 2021). The Lack of Fit is not significant in relation to the pure error, according to the "Lack of Fit *F*-value" of 6.12. A non-significant lack of fit is advantageous since we want the model to fit. The model cannot be replicated reasonably, as indicated by the CV Value of 46.67%, higher than 10% (Topkafa, 2020).

According to Chin (1998), the phospholipid removal R^2 value is 0.5026, which is considered moderate if it is more significant than 0.33 but less than 0.67. This number indicates that temperature, time, and phosphoric acid content account for 50.26% of the variance, with parameters not included in this study model influencing the remaining 49.74%. The R^2 value is explained by the regression model, which can be seen in Fig. 1.

**Figure 1.** Chart regression predictions with the experiment on phospholipid removal

The influence of process variables such as the amount of phosphoric acid, temperature, and degumming time on response variables such as phospholipids was analyzed using RSM 3D.

3.3. Effect of Phosphoric Acid Concentration and Temperature

The temperature and phosphoric acid concentration relationship strongly influence the phospholipid removal generated by Fig. 2. Figure 2 shows that the lower the temperature and the amount of phosphoric acid increases, the lower the phospholipid removal is. Phospholipid removal was at the best value when phosphoric acid was 0.12–0.16 %w/w, and the temperature was 65–75 °C. Increasing the dose of phosphoric acid in the degumming process can result in excess phosphoric acid and improve the phosphorus content in the oil. A temperature that is not too high is also enough to remove phospholipids in the oil (Chew et al., 2017).

3.4. Effect of Time and Phosphoric Acid Concentration

The relationship between the amount of phosphoric acid and time also significantly influences the resulting phospholipid removal in Fig. 3. Figure 3 shows that the longer the time and the lower the phosphoric acid amount, the higher the consequent phospholipid removal. Phospholipid removal will be at the best value when the amount of phosphoric acid is 0.1–0.2 %w/w and the time is 35–40 minutes. The degumming process takes quite a long time if a small reagent concentration is used because the reaction between phospholipids and a small amount of reagent takes quite a long time (Wang et al., 2021).

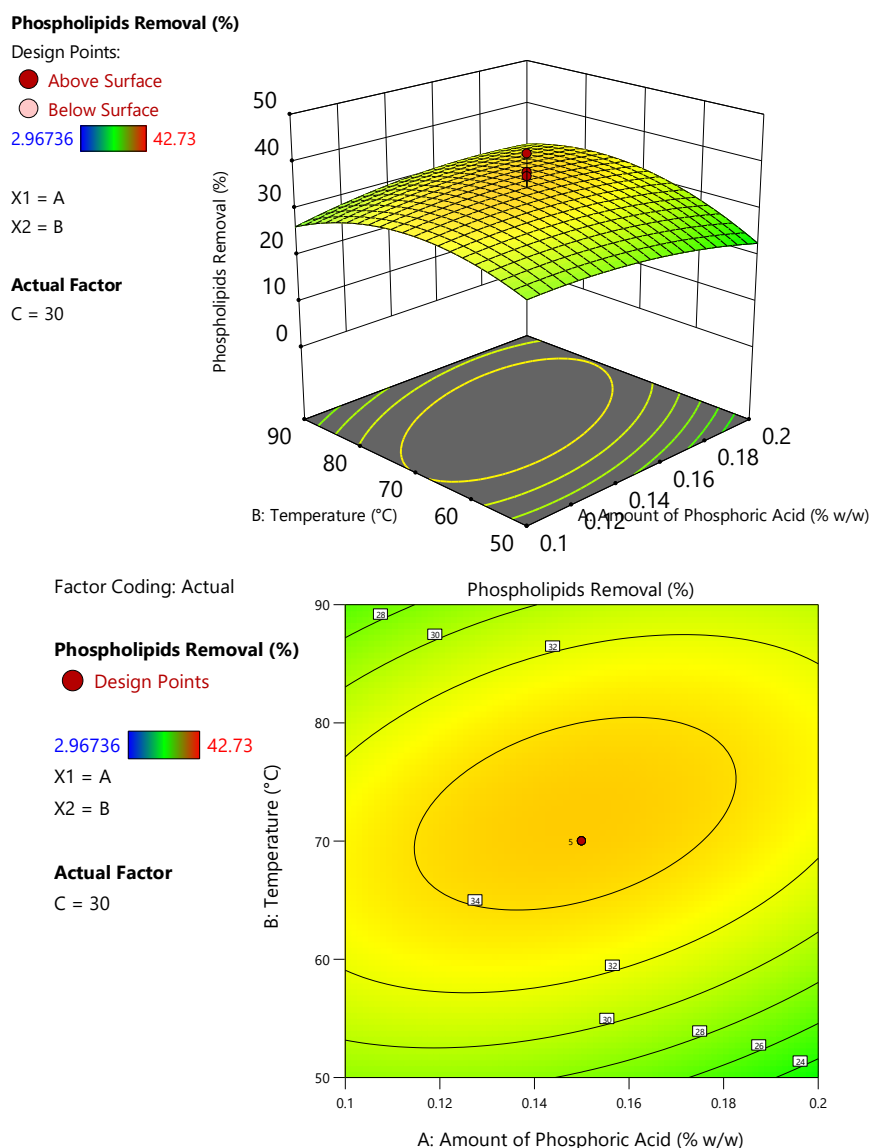


Figure 2. 3D and 2D RSM surface visualization of the amount of phosphoric acid and temperature used for phospholipid removal

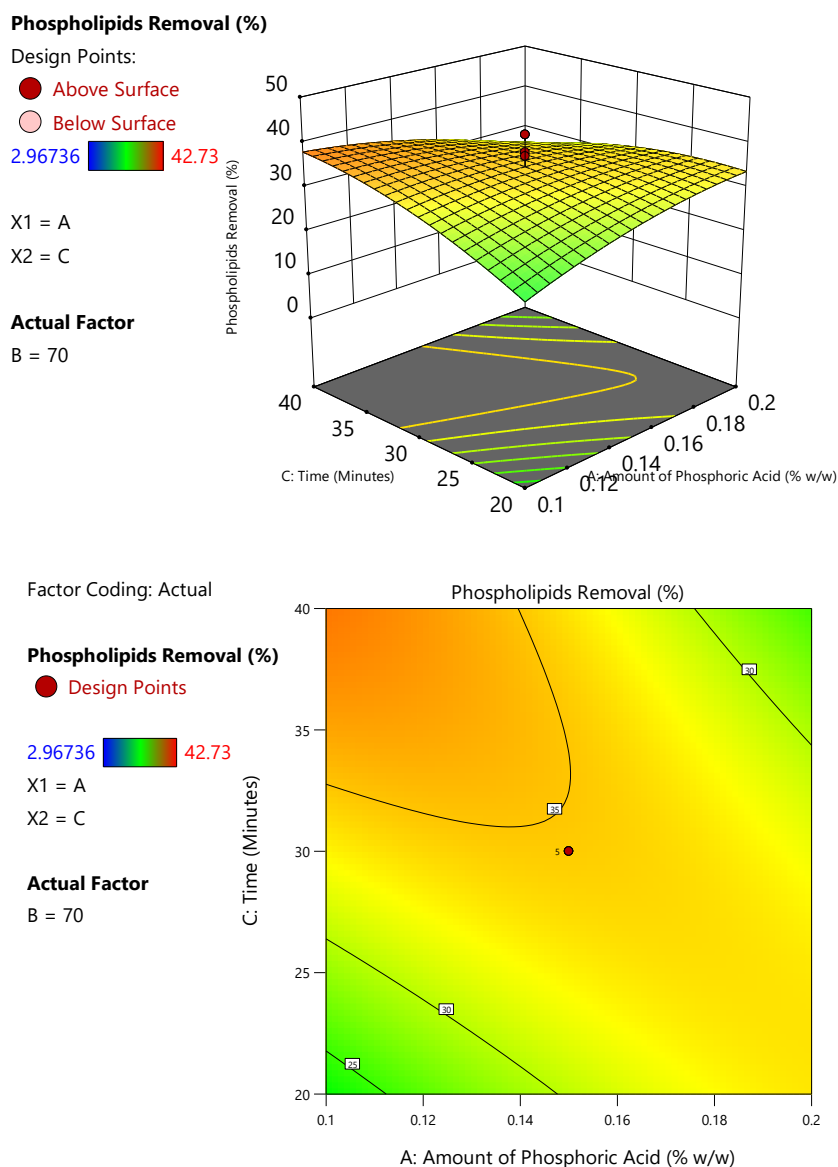


Figure 3. 3D and 2D RSM surface visualization of the amount of phosphoric acid and time to phospholipid removal

3.5. Influence of Temperature and Time

As demonstrated in Fig. 4, the relationship between temperature and time also significantly impacts the subsequent removal of phospholipids. As illustrated in Fig. 4, the elimination of phospholipids increases with temperature and duration. Phospholipid removal will be at its best value in 30–40 minutes, and the temperature is 70–90 °C. Degumming at high temperatures and for a long time produces high phospholipid reduction (Chompoo et al., 2019). High degumming temperatures can accelerate oil oxidation and form pigments that will deepen the color of the oil (Jiang et al., 2014).

3.6. Optimization Using Derringer Method

Optimization of acid degumming on CRBO to remove phospholipid content using the Derringer method is presented in Fig. 5. The Derringer method is used for response optimization, characterized by a desirability function (Damayanti et al., 2021).

Desirability shows how fulfilled or close to the optimum point. The optimization results showed that phospholipid removal was 40.479% under the conditions of adding 0.1 %w/w phosphoric acid, a temperature of 83.21 °C, and a degumming time of 40 minutes. This condition is close to the optimum point where the desirability value is 0.943.

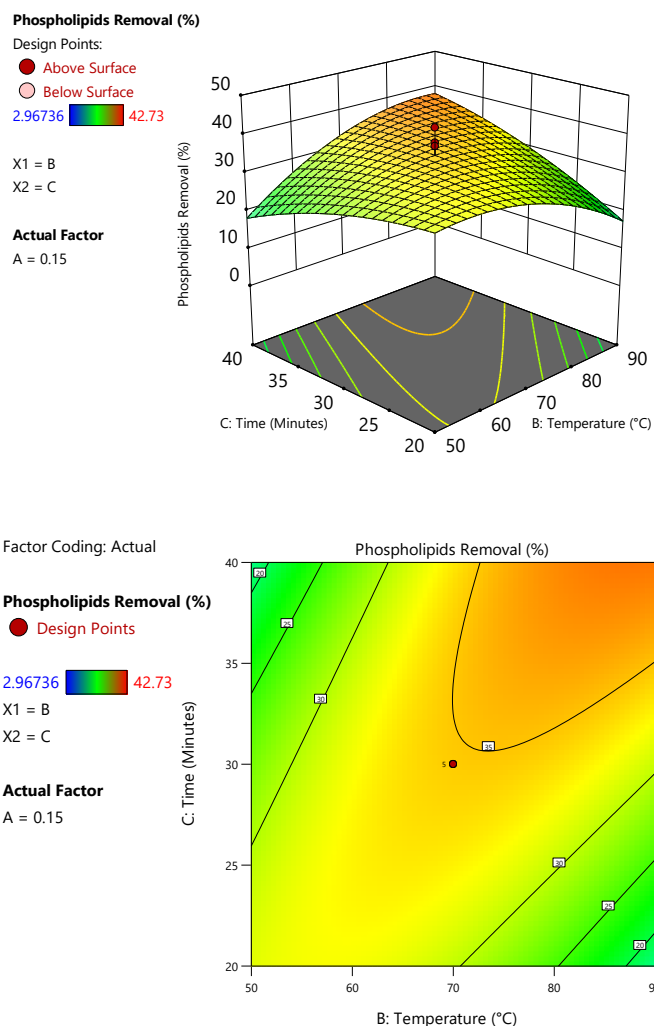
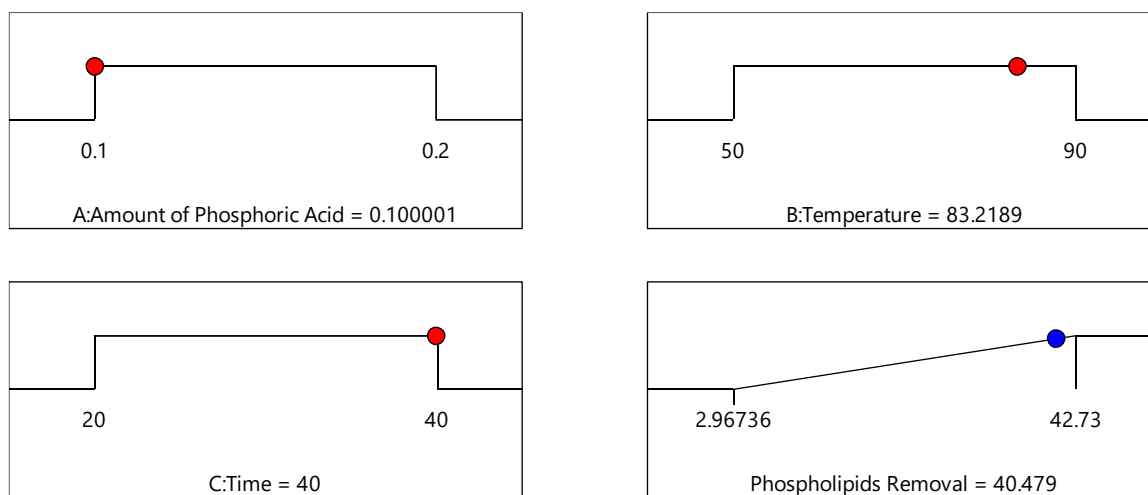


Figure 4. 3D and 2D RSM surface visualization of the amount of temperature and time to phospholipid removal



Desirability = 0.943

Figure 5. Optimization results use the Derringer method

The expected value is a desirability value close to 1 (Rao & Murthy, 2018). However, optimization aims to find a desirability equal to 1 and conditions that meet expectations (Candioti et al., 2014).

4. Conclusion

According to the study, phospholipid removal from the rice bran oil degumming process was significantly impacted by a small amount of phosphoric acid and a lengthy degumming period. Optimization results showed a phospholipid removal response of 40.479% under the conditions of adding 0.1 %w/w phosphoric acid, temperature 83.21 °C, and degumming time of 40 minutes.

Author Contribution Statement

A.D., M.M., I.W., D.R.W.: Writing – review & editing, Methodology, Conceptualization. Y.R., R.A.P.: Writing – original draft, Investigation, Data curation, investigation. M.P.R., N.N: software.

Data Availability Statement

Available on request to the corresponding author.

Declaration of Competing Interest

The author declares that there is no conflict of interest in the publication of this manuscript.

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