

OPTIMIZATION OF CHEMICAL NEUTRALIZATION PARAMETERS FOR ENHANCED RICE BRAN OIL RECOVERY AND QUALITY

Van Man Phan, Tiraporn Junyusen*, Pansa Liplap, and Payungsak Junyusen

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Abstract

This research investigates the effect of variable chemical neutralization parameters on the oil recovery, free fatty acid (FFA) level, and gamma-oryzanol content of neutralized rice bran oil, using response surface methodology. The neutralization parameters under study included NaOH concentrations (10-30°Be), temperatures (60-90°C), and process time (5-15 min). Quadratic response models were generated and statistical analysis performed to validate the models. The optimal neutralization condition was 19.24°Be, 74.79°C, and 11.18 min. Under the optimal condition, the predicted oil recovery, FFA level, and gamma-oryzanol were 80.00%, 0.31%, and 1.00%, respectively, while the corresponding experimental results were 80.12%, 0.29%, and 1.02%, respectively, suggesting good agreement between the predicted and experimental data and high predictive ability of the models. The response models could thus be applied to optimizing the neutralization parameters for enhanced oil yield and quality.

Keywords: Response surface methodology, rice bran oil, chemical neutralization, free fatty acids, gamma-oryzanol

School of Agricultural Engineering, Institute of Engineering, Suranaree University of Technology, 111 University Avenue, Muang District, Nakhon Ratchasima 30000, Thailand. Tel. +6644224225; E-mail: tirapo@sut.ac.th

** Corresponding author*

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Introduction

Rice bran is the cuticle between the paddy husk and rice grain obtained as a byproduct during rice processing (Amarasinghe and Gangodavilage, 2004). Rice bran contains lipids, proteins, minerals, and vitamins (Oluremi *et al.*, 2013) and is a rich source of unsaturated fatty acids (UFAs), including oleic, linoleic, and linolenic (Oluremi *et al.*, 2013). UFAs help lower cardiovascular risks, such as hyperlipidemia, hypercholesterolemia, and hypertriglyceridemia (Grundty, 1975).

According to Jung *et al.* (2017) and Ghasemzadeh *et al.* (2015), polyphenols, gamma-oryzanol, tocopherols, and tocotrienols are found in abundance in rice bran oil (RBO). These bioactive compounds are beneficial for decreasing plasma cholesterol (Wilson *et al.*, 2007), platelet aggregation, and cholesterol absorption (Seetharamaiah and Prabhakar, 1986; Sharma and Rukmini, 1986). RBO is thus commonly used in food, cosmetic, and pharmaceutical products (Lerma-García *et al.*, 2009).

In RBO production, the main challenge lies in the refining process of crude rice bran oil (CRBO) to remove impurities, such as free fatty acids (FFAs), phospholipids, pigments, and waxes, to improve the flavor, odor, appearance, and shelf life of the oil (Gunstone, 2011). According to De and Patel (2011), CRBO poses the greatest refining challenge in order to meet the standards of edible oil.

There are 2 CRBO refining processes: physical and chemical. The physical refining technique involves degumming and distillation (steam refining and deodorization) (Čmolik *et al.*, 2000). The advantages of the physical refining included low oil loss and preserved gamma-oryzanol (De and Bhattacharyya, 1998; Rajam *et al.*, 2005). However, the disadvantages are high implementation costs at the industrial level (Engelmann *et al.*, 2017) and sensitivity to the quality of input crude oil (Kövari *et al.*, 2000; Engelmann *et al.*, 2017).

The chemical refining involves degumming, neutralization, bleaching, and deodorization (Gunstone, 2011). Neutralization is crucial to remove FFAs in CRBO by saponification using a sodium hydroxide (NaOH) solution (De and Patel, 2010). Chemical neutralization

is more effective in removing FFAs and impurities (waxes and mucilage), contributing to improved RBO quality (Van Hoed *et al.*, 2006). However, chemical neutralization suffers from excessive loss of gamma-oryzanol, in which as high as 90% was lost through soapstock (Das *et al.*, 1998; Gopala Krishna *et al.*, 2001).

Previous research on improved neutralization of RBO focused on low oil recovery (Mishra *et al.*, 1988; Chumsantea *et al.*, 2012), darkening of color (De and Bhattacharyya, 1998; Van Hoed *et al.*, 2010), or low gamma-oryzanol content (Das *et al.*, 1998; Gopala Krishna *et al.*, 2001; Chumsantea *et al.*, 2012). There is currently no study on chemical neutralization optimization that is specific to RBO to improve FFA removal and oil and gamma-oryzanol recovery. This study investigates the effect of variable neutralization parameters on the recovery of RBO, FFA removal, and preservation of gamma-oryzanol, using the response surface methodology. The neutralization parameters under study included NaOH concentrations, temperature, and process time, which were varied between 10, 20, and 30°C, 60, 75, and 90°C, and 5, 10, and 15 min, respectively.

Materials and Methods

Materials

Rice bran (RB) of the jasmine variety was acquired from Korat Rice Mill factory in Thailand's northeastern province of Nakhon Ratchasima. The RB was passed through a 60-mesh sieve (0.25 mm) and pretreated according to Sun *et al.* (2017), with minor modifications. The rice bran was dried at 100°C for 15 min to inactivate endogenous lipase. Dried samples were thermally cooked at 100°C for 5 min (pretreated), followed by cold press extraction (Phan *et al.*, 2019). The crude rice bran oil was centrifuged at 10,000 rpm for 10 min and the supernatant (CRBO) stored in an amber plastic container at -20°C for further analysis.

Methanol was from Mallinckrodt Pharmaceuticals (Staines, UK), and *n*-hexane,

ethanol, chloroform absolute value (<98.5%), Hanus reagent, ammonium molybdate, and ammonium metavanadate were from Sigma-Aldrich (St. Louis, MO, USA). Citric acid (99%) was acquired from Acros Organics (Morris Plains, NJ, USA). Standards of fatty acid methyl esters (37-component FAME Mix of Supercool, Lake Worth, FL, USA) and gamma-oryzanol (Oryza Oil & Fat Chemical Co., Ltd, Ichinomiya City, Japan) were used for quantification.

Experimental Methods

Degumming of Crude Rice Bran Oil (CRBO)

The degumming was carried out according to Ringers and Segers (2000) with some modifications. In degumming, 50 g of the CRBO was heated to 70°C in a water bath, and then hydrated by adding 0.3% wt of 50% citric acid solution. The degumming was conducted at 70°C for 30 min with continuous slow agitation. Degummed rice bran oil (DRBO) was then centrifuged at 10,000 rpm for 10 min to remove the gum and decanted for further refining.

Neutralization of Degummed Rice Bran Oil (DRBO)

Free fatty acids in the DRBO were neutralized following the procedures in Engelmann *et al.* (2017) and Andersen (1962) with some modifications. NaOH concentrations were varied between 10, 20, and 30°Be, and the mixtures heated at different temperatures (60, 75, and 90°C) and durations (5, 10, and 15 min). Ultra-pure water was introduced into the mixture with a ratio of 0.15 water: 1 DRBO (v/v) and centrifuged at 10,000 rpm for 10 min to precipitate the sludge before the neutralized rice bran oil (NRBO) was collected (Andersen, 1962). Residual water in the NRBO was vacuum-evaporated prior to physicochemical analysis. The NRBO recovery was calculated by:

$$\text{NRBO recovery (\%)} = \frac{\text{Weight of neutralized oil}}{\text{Weight of initial degummed rice bran oil}} \times 100 \quad (1)$$

Experimental Design for Oil Neutralization

In this study, response surface methodology (RSM) with a central composite

face-centered (CCF) design was used to optimize the neutralization parameters for RBO refining. Statistical software (Modde version 5.0, Umetrics, Umea, Sweden) was used to analyze the experimental data. The 3 independent variables were the NaOH concentration (°Be, X_1), temperature (°C, X_2), and time (min, X_3). The responses (Y) were the NRBO recovery, FFA level, and gamma-oryzanol content. Each independent variable was coded -1, 0, and +1 (Table 1), and the design of the experiment consisted of 17 experimental runs (Table 2). The experiment was carried out in triplicate and averaged.

Multiple regression analysis was utilized to derive the second-order polynomial equation to predict the oil recovery (Y_1), FFA (Y_2), and gamma-oryzanol content (Y_3):

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

where Y is the predicted response, β_0 is a constant, X_1 , X_2 , and X_3 are the independent variables, β_i is the linear coefficient, β_{ij} is the interactive coefficient, and β_{ii} is the quadratic coefficient.

The model adequacy was evaluated by the lack of fit, F-test, and regression coefficient of determination (R^2) from analysis of variance (ANOVA), and the 3D response surface plots were generated. Model validation was subsequently carried out. The significance level was based on the 5% significance level ($p < 0.05$).

Analytical Methods

Fatty acids Analysis

The fatty acids of the CRBO, DBRO, and optimized NRBO were analyzed by gas chromatography with flame-ionization detection (GC-FID, 7890C axis detector, Agilent Technologies, Abingdon, UK), following Stanisavljević *et al.* (2009) with some modifications. The fatty acids were transformed into methyl esters in BF_3 -methanol at 90°C for 15 min in a water bath. The methylated oil was then extracted with *n*-hexane, and the solvent removed by nitrogen gas.

The temperature scheme used was as follows: the injector temperature was 180°C,

the oven temperature started from 50 to 250°C at a rate of 40°C/min, and the ion source temperature was 230°C. The injection volume was 1 µL in splitless injection mode with a Luer Tip syringe (LT) (Merck KGaA, Darmstadt, Germany). The FA methyl esters were identified by comparing the retention time of the oil components against the standards of the National Institute of Standards and Technology.

Thermal Behavior Analysis

Differential scanning calorimetry (DSC 7, Perkin Elmer Corp., Norwalk, CT, USA) was utilized to characterize the thermal behavior. In the analysis, the baseline was first established using 2 empty sealed aluminum

crucibles under a thermal program in which the initial temperature was 30°C and cooled down to -60°C before heating to 60°C at a rate of 10°C/min. Then, 3-5 mg of CRBO, DRBO, and optimized NRBO were individually transferred into 1 of the crucibles (i.e., the sample crucible) and the analysis carried out and the melting curve plotted.

Physicochemical Analysis

The American Oil Chemists' Society (AOCS) official methods (1989) were used: Cd 1b-87 for the iodine value, Aa 516-01 for the peroxide value, Ca 5a-40 for the free fatty acid, Ca 12-55 for the phosphorus value, and Cd 3a-94 for the saponification value. The

Table 1. Experimental levels of the three independent variables

Symbol	Variables	Levels		
		-1	0	+1
X ₁	NaOH concentration (°Be)	10	20	30
X ₂	Temperature pretreatment (°C)	60	75	90
X ₃	Time pretreatment (min)	5	10	15

Table 2. Experimental and predicted oil recovery (Y₁), FFA level (Y₂), and gamma-oryzanol of neutralized rice bran oil (NRBO) (Y₃) under variable NaOH concentrations (°Be, X₁), temperature (°C, X₂), and time (min, X₃)

Run order	Code variable						Experimental			Predicted		
	X ₁	X ₂	X ₃	X ₁	X ₂	X ₃	Y ₁ (%)	Y ₂ (%)	Y ₃ (%)	Y ₁ (%)	Y ₂ (%)	Y ₃ (%)
1	-1	-1	-1	10	60	5	80.13	0.71	1.00	80.22	0.68	1.02
2	-1	1	-1	10	90	5	80.20	0.76	0.90	80.15	0.78	0.89
3	1	-1	-1	30	60	5	79.13	0.48	0.65	79.03	0.48	0.64
4	1	1	-1	30	90	5	79.61	0.60	0.63	79.55	0.59	0.63
5	-1	-1	1	10	60	15	79.82	0.69	0.99	79.83	0.72	0.98
6	-1	1	1	10	90	15	80.12	0.79	0.88	80.14	0.81	0.88
7	1	-1	1	30	60	15	79.11	0.25	0.49	79.12	0.23	0.50
8	1	1	1	30	90	15	80.08	0.31	0.55	80.02	0.32	0.52
9	0	-1	0	20	60	10	79.69	0.33	0.96	79.72	0.35	0.93
10	0	1	0	20	90	10	80.06	0.45	0.89	80.14	0.44	0.87
11	-1	0	0	10	75	10	80.12	0.68	1.02	80.11	0.64	1.02
12	1	0	0	30	75	10	79.23	0.28	0.63	79.45	0.30	0.65
13	0	0	-1	20	75	5	80.01	0.31	0.90	80.01	0.39	0.91
14	0	0	1	20	75	15	80.05	0.30	0.81	80.05	0.28	0.84
15	0	0	0	20	75	10	80.02	0.33	0.95	79.99	0.32	0.93
16	0	0	0	20	75	10	80.09	0.30	0.90	79.99	0.32	0.93
17	0	0	0	20	75	10	80.09	0.35	0.95	79.99	0.32	0.93

relative density of the oil was measured by a density bottle. Gamma-oryzanol was analyzed by UV- spectrophotometer/ NIR (UV- 2600, Shimadzu Corp., Kyoto, Japan) (Joshi *et al.*, 2016).

Statistical Analysis

All experiments were carried out in triplicate and the results expressed as mean±standard deviation. The Modde software was used to generate experimental data and quadratic models. Multiple linear regression analysis was performed to evaluate the statistical significance of the models, and analysis of variance (ANOVA) to determine the significance of the linear, interaction, and quadratic

coefficients. One-way ANOVA was used to assess the effects of degumming and neutralization on the fatty acid profile and physicochemical properties of the DRBO, NRBO, and CRBO. A Tukey-HSD comparison was used to compare means, given $p < 0.05$.

Results and Discussion

Evaluation of Regression for Neutralization Parameters

Experimental data based on the RSM with CCF were used to optimize the neutralization parameters and evaluate the relationships between the 3 independent variables, NaOH

Table 3. ANOVA results of the response surface quadratic model on the recovery of neutralized rice bran oil

Oil yield	Degree of freedom	Sum of squares	Mean square	F-value	p-value
Total Corrected	16	2.1797	0.1362		
Regression	9	2.0744	0.2305	15.3227	0.0010
Residual	7	0.1053	0.0150		
Lack of Fit	5	0.1022	0.0205	13.4089	0.0760
Pure Error	2	0.0031	0.0016		

$R^2 = 0.9520$; Adjusted $R^2 = 0.8901$; $Q^2 = 0.5750$; $p\text{-value} < 0.05$ indicates statistical significance.

Table 4. ANOVA results of the response surface quadratic model on FFA of neutralized rice bran oil

FFA	Degree of freedom	Sum of squares	Mean square	F-value	p-value
Total Corrected	16	0.6008	0.0376		
Regression	9	0.5862	0.0652	31.059	0.0000
Residual	7	0.0147	0.0021		
Lack of Fit	5	0.0134	0.0027	4.235	0.2020
Pure Error	2	0.0013	0.0006		

$R^2 = 0.9760$; Adjusted $R^2 = 0.9440$; $Q^2 = 0.8630$; $p\text{-value} < 0.05$ indicates statistical significance.

Table 5. ANOVA results of the response surface quadratic model on gamma-oryzanol of neutralized rice bran oil

Gamma-oryzanol level	Degree of freedom	Sum of squares	Mean square	F-value	p-value
Total Corrected	16	0.4635	0.0289		
Regression	9	0.4578	0.0509	63.25	0.000
Residual	7	0.0056	0.0008		
Lack of Fit	5	0.0041	0.0008	1.0848	0.5700
Pure Error	2	0.0015	0.0008		

$R^2 = 0.9870$; Adjusted $R^2 = 0.9710$; $Q^2 = 0.8260$; $p\text{-value} < 0.05$ indicates statistical significance.

concentration ($^{\circ}\text{Be}$, X_1), temperature ($^{\circ}\text{C}$, X_2), and time (min, X_3) and the responses, oil recovery (Y_1), FFA (Y_2), and gamma-oryzanol content (Y_3). Table 2 tabulates the experimental and predicted values. Specifically, the oil recovery, FFA level, and gamma-oryzanol from the experiments were 79.11-80.20%, 0.25-0.76% oleic acid, and 0.49-1.02%, respectively.

The statistical significance of the quadratic model terms was determined by ANOVA, and the significance of regression coefficients by F-value, p-value, and lack of fit. The insignificant terms ($p > 0.05$) were excluded from the quadratic models. Tables 3-5 respectively present the ANOVA results for the fitted quadratic models for the oil recovery, FFA, and gamma-oryzanol of NRBO. Specifically, there existed statistically significant relationships between the independent and response variables, with high F-values and very low p-values ($p < 0.05$), indicating that the model terms were significant. According to Quanhong and Caili (2005) and Yolmeh *et al.* (2014), larger F-values and smaller p-values indicated a strong relationship between the independent variables and the response. In this research, the p-values of the lack of fit were greater than 0.05, indicating that the models satisfactorily fitted the experimental data.

The robustness and predictive ability of the models were evaluated by the coefficient of determination (R^2), adjusted R^2 , and model predictive ability (Q^2). The high R^2 (0.952, 0.976, 0.987) and adjusted R^2 (0.891, 0.944, 0.971) of the oil recovery (Y_1), FFA (Y_2), and

gamma-oryzanol (Y_3) models suggested good agreement between the experimental and predicted data. Meanwhile, the Q^2 of the Y_1 , Y_2 , and Y_3 models were 0.575, 0.863, and 0.826, respectively, indicating that the variability of the responses was well explained by the models with a small prediction error. According to Veerasamy *et al.* (2011), a high Q^2 (> 0.5) indicates good predictive ability of a model. In this research, the oil recovery (Y_1) model possessed a high R^2 (0.952) but low Q^2 (0.575), suggesting that the predictive ability of the Y_1 model was not as high as the FFA (Y_2) and gamma-oryzanol (Y_3) models. This was probably attributable to overfitting (Makanjuola *et al.*, 2015).

The fitted quadratic relationships between the independent variables (X_1 = NaOH concentration, X_2 = temperature, X_3 = time) and responses (Y_1 = the oil recovery, Y_2 = FFA, and Y_3 = gamma-oryzanol content) were as follows:

$$Y_1 = 9908 - 0.2577X_1 + 0.1652X_2 + 0.0911X_1 \cdot X_2 + 0.0775X_1 \cdot X_3 - 0.1310X_1^2 \quad (3)$$

$$Y_2 = 0.3157 - 0.1343X_1 + 0.0386X_2 - 0.0437X_3 - 0.0462X_1 \cdot X_3 - 0.0967X_1^2 + 0.0493X_2^2 \quad (4)$$

$$Y_3 = 0.9280 - 0.1462X_1 - 0.0207X_2 - 0.0283X_3 + 0.0195X_1 \cdot X_2 - 0.0156X_1 \cdot X_3 - 0.0556X_1^2 - 0.0331X_3^2 \quad (5)$$

Table 6. Regression coefficients and p-values for the recovery of neutralized rice bran oil after backward elimination

Source ¹	Regression coefficients	p-value ²
Constant	79.988	0.0000
X_1	-0.2577	0.0010
X_2	0.1652	0.0010
$X_1 \cdot X_2$	0.0911	0.0120
$X_1 \cdot X_3$	0.0775	0.0261
X_1^2	-0.1310	0.0266

¹ X_1 , X_2 , and X_3 denote NaOH concentration ($^{\circ}\text{Be}$), temperature ($^{\circ}\text{C}$), and time (min), respectively.

² $p < 0.05$ indicates statistical significance.

Effect of Neutralization Parameters on Oil Recovery

The removal of FFAs causes the loss of NRBO. In the neutralization process, FFAs are removed as sodium soaps but some neutralized oil is entrapped in the emulsion and removed during centrifugation. Table 6 presents the effects of neutralization parameters on the oil recovery. The oil recovery was negatively affected by X_1 (NaOH concentration) and X_2 (temperature). The linear terms X_1 and X_2 , the quadratic terms X_1^2 , and the interaction terms X_1X_2 and X_1X_3 had significant effects on the oil recovery ($p < 0.05$). On the other hand, X_3 ,

X_2^2 , X_3^2 , and X_2X_3 had no significant effect on the oil recovery ($p > 0.05$).

In Figure 1(a), the maximum oil recovery was achieved under the NaOH concentration (X_1) of 19.25°Be and temperature (X_2) of 73.68°C. In Figure 1(b), lower NaOH concentrations (5.0-19.05°Be) and shorter times (5.0-11.28 min) yielded greater oil recovery, while increased NaOH concentrations and time reduced the oil recovery. Specifically, increased NaOH concentrations accelerated the formation of rice bran soap and trapped oil, reducing oil discharge (Gunstone *et al.*, 2011; Wakelyn and Wan, 2003). Similar results were also reported

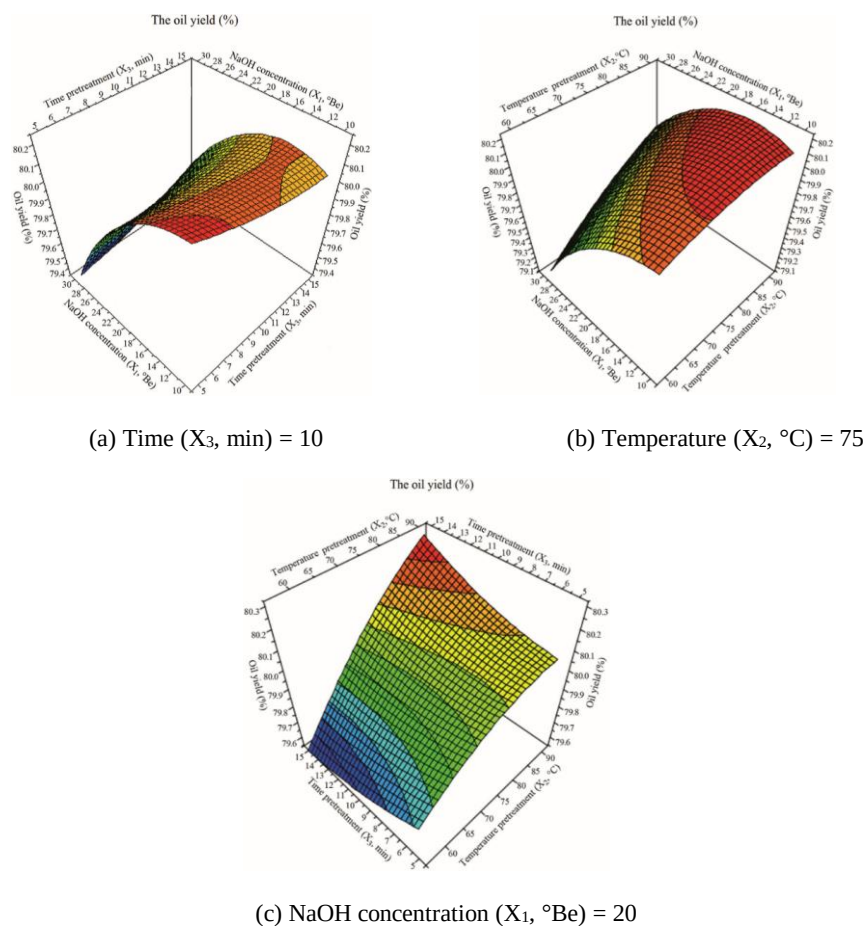


Figure 1. Response surface plots of interaction between: (A) NaOH concentration (X_1) and temperature (X_2); (B) NaOH concentration (X_1) and time (X_3); (C) temperature (X_2) and time (X_3) on the neutralized rice bran oil yield

on neutralization of sunflower and soybean oils due to higher alkali-refining loss (El Kady *et al.*, 1999).

In Figure 1(c), the maximum recovery was achieved under the 75°C and 10.63 min condition. Specifically, elevated temperatures (from 60 to 90°C) and extended time (from 5 to 15 min) had a positive effect on oil recovery. However, high temperatures (90°C) were ineffective in neutralizing the FFA, contributing to gamma-oryzanol loss (Table 2). This is consistent with Wei *et al.* (2015), who documented that the acid values slightly increased while bioactive compounds (sterols,

α -tocopherol, and Vitamin D3) were significantly reduced at high neutralization temperatures (85°C) after 30 min. According to Lee *et al.* (2014) and Chew *et al.* (2017), neutralization was normally undertaken between 60-75°C and 10-50 min to allow for the complete reaction of FFA with the NaOH solution.

Effect of Neutralization Parameters on FFA

The FFA content is used to determine the edibility and deterioration of oils and fats. The presence of FFA and other fatty materials in oil leads to offensive odor and taste caused by

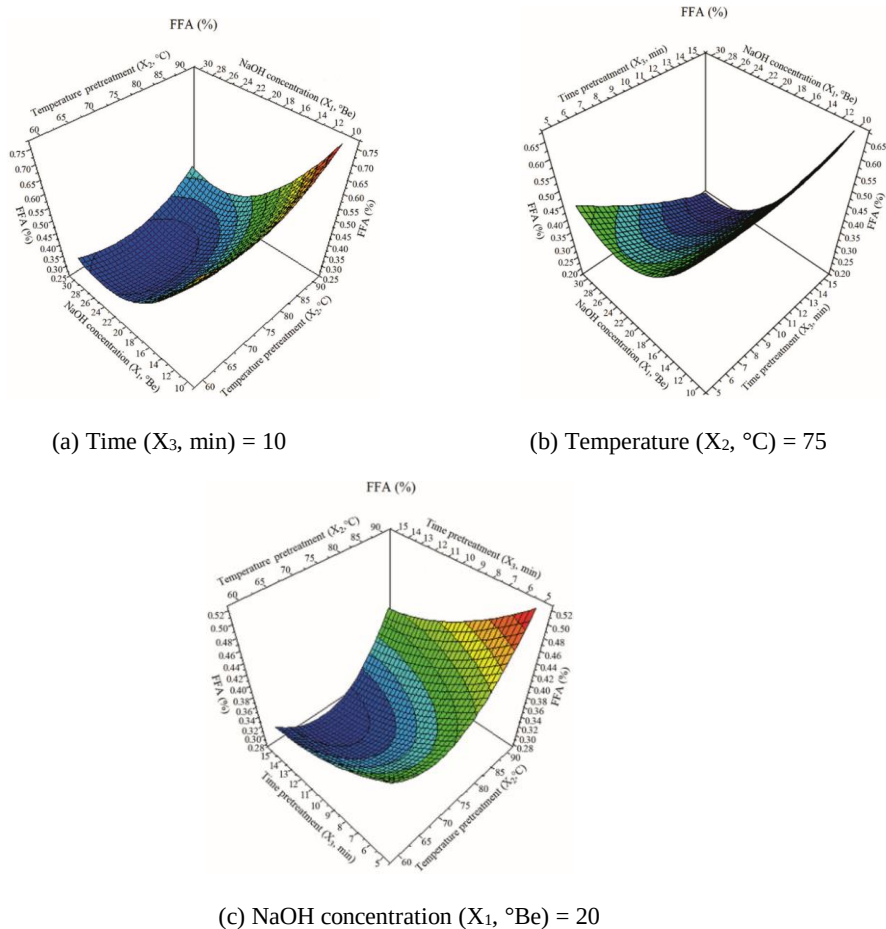


Figure 2. Response surface plots of interaction between: (A) NaOH concentration (X_1) and temperature (X_2); (B) NaOH concentration (X_1) and time (X_3); (C) temperature (X_2) and time (X_3) on the FFA of neutralized rice bran oil

enzymatic hydrolysis during processing and storage (Chemat *et al.*, 2004; Oluremi *et al.*, 2013). In this research, the variables that affected the removal of FFA were the linear terms X_1 , X_2 , X_3 , the quadratic terms X_1^2 and X_2^2 , and the interaction term X_1X_3 (Table 7).

In Figure 2(a), FFA was reduced from 0.79 to 0.28% when the NaOH concentration (X_1) and temperature (X_2) were increased to 19.26°Be and 78.36°C, respectively, given X_3 of 10 min. The neutralization efficiency nevertheless was lowered beyond the 19.26°Be (X_1) and 78.36°C (X_2) threshold. In Figure 2(b), elevated NaOH concentrations (X_1 , from 10 to 19.29°Be) and extended time (X_3 , from 5 to 11.19 min) decreased the FFA to 0.32%. Specifically, the NaOH concentrations (X_1) had more impact on the neutralization of rice bran oil. Similar results were reported for the neutralization of kenaf seed oil (Chew *et al.*, 2017) in which excessively low or high levels

of NaOH increased the FFA. In Figure 2(c), higher temperatures (X_2) and extended time (X_3) reduced the efficiency of the FFA removal.

Effect of Neutralization Parameters on Gamma-Oryzanol

In Table 8, the linear terms X_1 , X_2 , and X_3 , the interaction terms X_1X_2 and X_1X_3 , and the quadratic terms X_1^2 and X_3^2 significantly impacted the gamma-oryzanol content ($p < 0.05$). Specifically, X_1 and X_3 significantly lowered the gamma-oryzanol content. This is consistent with Engelmann *et al.* (2017) and Gopala Krishna *et al.* (2001), who reported that the decrease in gamma-oryzanol was accelerated by increased NaOH concentrations and process time.

Figure 3(a) illustrates the interaction between X_1 and X_2 on the gamma-oryzanol content in the NRBO, in which the bioactive

Table 7. Regression coefficients and p-values for FFA in neutralized rice bran oil after backward elimination

Source ¹	Regression coefficients	p-value ²
Constant	0.3157	0.0000
X_1	-0.1343	0.0000
X_2	0.0386	0.0119
X_3	-0.0437	0.0066
$X_1 \times X_3$	-0.0462	0.0026
X_1^2	0.0967	0.0009
X_2^2	0.0493	0.0255

¹ X_1 , X_2 , and X_3 denote NaOH concentration (°Be), temperature (°C), and time (min), respectively.

² $p < 0.05$ indicates statistical significance.

Table 8. Regression coefficients and p-values for gamma-oryzanol in neutralized rice bran oil after backward elimination

Source ¹	Regression coefficients	p-value ²
Constant	0.9280	0.0000
X_1	-0.1462	0.0000
X_2	-0.0207	0.0183
X_3	-0.0283	0.0046
$X_1 \times X_2$	0.0195	0.0015
$X_1 \times X_3$	-0.0156	0.0186
X_1^2	-0.0556	0.0005
X_3^2	-0.0331	0.0084

¹ X_1 , X_2 , and X_3 denote NaOH concentration (°Be), temperature (°C), and time (min), respectively.

² $p < 0.05$ indicates statistical significance.

compounds decreased significantly ($p < 0.05$) with the increase in X_1 (from 15 to 30°Be) and X_2 (from 80 to 90°C). The maximum gamma-oryzanol content was achieved under the 19.86°Be (X_1) and 78.36°C (X_2) condition. In Figure 3(b), gamma-oryzanol decreased as the X_2 and X_3 increased from 75.34 to 90°C and 10.62 to 15 min, respectively, given X_1 of 20°Be. Specifically, under higher temperatures and extended time, gamma-oryzanol in the NRBO was reduced. In Figure 3(c), given X_2 of 75°C, the elliptical response surface plot indicated that the interaction between X_1 and X_3 was significant ($p < 0.05$). The maximum gamma-oryzanol content was achieved under

the 19.51°Be (X_1) and 11.18 min (X_3) condition.

Optimization of the Neutralization Parameters and Model Validation

Based on the CCF design, the optimal neutralization condition that achieved high oil recovery and quality was that of 19.24°Be for NaOH concentration (X_1), 74.79°C for temperature (X_2), and 11.18 min for process time (X_3). Under the optimal condition, the predicted oil recovery, FFA, and gamma-oryzanol were 80.00%, 0.31%, and 1.00%, respectively. Meanwhile, the experimental results for the oil recovery, FFA, and gamma-oryzanol were

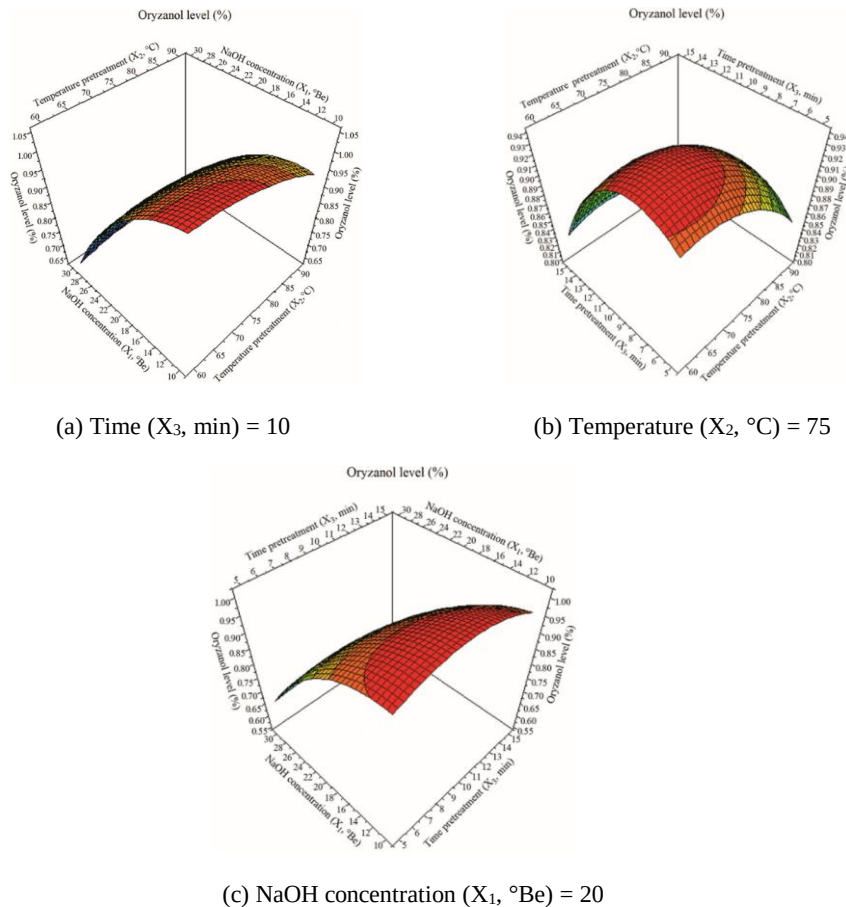


Figure 3. Response surface plots of interaction between: (A) NaOH concentration (X_1) and temperature (X_2); (B) temperature (X_2) and time (X_3); (C) NaOH concentration (X_1) and time (X_3) on the gamma-oryzanol content of neutralized rice bran oil

80.12%, 0.29%, and 1.02%, respectively. The predicted and experimental data were in good agreement, suggesting that the response models could be applied to optimizing the RBO neutralization parameters for enhanced oil yield and quality.

Physicochemical Properties and Fatty Acid Profile

Table 9 compares the fatty acid profile and physicochemical properties of the CRBO, DRBO, and NRBO under the optimal neutralization condition (19.24°Be, 74.79°C, and 11.18 min). In this study, the CRBO was obtained by thermal cooking followed by cold press extraction. Since the CRBO contains impurities, particularly phospholipids which have unfavorable effects on the flavor and shelf life of the oil, the compounds were removed by degumming (Zufarov *et al.*, 2008).

Fatty acid profile is an important indicator of the nutritive value and quality of the oil. According to Oluremi *et al.* (2013) and Muniz *et al.* (2015), high-quality RBO contains high

percentages of unsaturated fatty acids, mainly oleic and linoleic. In this study, oleic acid (C18:1) and linoleic acid (C18:2) were the dominant fatty acids, followed by palmitic acid (C16:0). In addition, the degumming and neutralization had a minimal impact on the quality of oil in terms of fatty acid composition. This is consistent with Karabulut *et al.* (2005) and Chew *et al.* (2017).

The peroxide value, iodine value, FFA, saponification value, gamma-oryzanol value, and relative density of the DRBO were not significantly different from those of the CRBO ($p>0.05$). However, Ortega-García *et al.* (2006) documented that the peroxide value of degummed safflower oil was higher than that of its crude oil, probably due to the presence of moisture and high temperature (70°C) during degumming promoting the primary oxidation compounds. In this study, the phosphorus value of the DRBO (33.59 mg/kg) was drastically decreased, compared with the CRBO (318.52 mg/kg) ($p<0.05$). The finding suggested that the citric acid degumming process at 70°C

Table 9. Fatty acid profile and physicochemical properties of crude rice bran oil (CRBO), degummed rice bran oil (DRBO), and neutralized rice bran oil (NRBO)

Quality of oil	CRBO ¹	DRBO ²	NRBO ³
Fatty acid profile (%)			
Myristic (C14:0)	3.59	3.57	3.43
Palmitic (C16:0)	16.75	16.51	17.11
Stearic (C18:0)	3.45	3.58	3.44
Oleic acid (C18:1)	41.91	41.59	42.04
Linoleic (C18:2)	24.19	24.49	24.21
Dihomo γ -linolenic (C20:3)	4.59	4.46	4.43
Heneicosylic (C21:0)	4.52	4.62	4.34
Nevonis (C24:0)	1.00	1.10	1.00
Saturated fatty acid	29.31	29.38	29.32
Unsaturated fatty acid	70.69	70.62	70.68
Physicochemical properties			
Peroxide Value (meqO ₂ /kg)	5.27 ^a ±0.03	5.29 ^a ±0.05	5.31 ^b ±0.05
Iodine value (g I ₂ /100g)	99.98±1.22	99.72±0.50	99.96±0.25
Free fatty acid (% oleic acid)	4.44 ^b ±0.05	4.37 ^b ±0.25	0.31 ^a ±0.15
Saponification value (mgKOH/g oil)	176.98 ^b ±0.98	176.68 ^b ±0.50	173.12 ^a ±0.50
Gamma-oryzanol value (%)	1.81 ^a ±0.02	1.80 ^a ±0.03	1.00 ^b ±0.25
Phosphorus value (mg/kg)	318.52 ^c ±1.04	33.59 ^b ±0.15	6.25 ^a ±1.15
Relative density	0.92±0.01	0.92±0.02	0.91±0.25
Melting point (°C)	11.25 ^c ±0.02	10.05 ^b ±0.01	9.90 ^a ±0.50

^{1,2,3} Different letters in each row of physicochemical properties denote statistically significant differences between treatments ($p<0.05$). The values are mean of three replications $\pm$ standard deviation.

effectively removed phospholipids from the CRBO, consistent with Smiles *et al.* (1988) and Zufarov *et al.* (2008). Mei *et al.* (2013) also reported that the phosphorus value of *Silybum marianum* seed oil was reduced by 53.10% after citric acid degumming. In addition, the melting point of the DRBO was lower than the CRBO's. The presence of impurities (phospholipids, wax, and peroxide) in the CRBO contributed to a higher melting point (Taghvaei *et al.*, 2013).

The peroxide value, FFA, saponification value, gamma-oryzanol content, phosphorus value, and melting point of the NRBO were significantly lower than those of the CRBO and DRBO ($p < 0.05$). In comparison with the CRBO, the phosphorus value, FFA, and gamma-oryzanol of the NRBO were reduced by 98.03%, 91.12%, and 44.75%, respectively. The reduction in the phosphorus value, FFA, and gamma-oryzanol was attributable to the formation of soapstock (De and Patel, 2010; Van Hoed *et al.*, 2006). The lower saponification value and melting point were probably due to the absence of impurities in the NRBO (Taghvaei *et al.*, 2013). Nevertheless, the neutralization had no effect on the iodine value and relative density of RBO, consistent with Oluremi *et al.* (2013).

Conclusions

The effect of neutralization parameters on the oil recovery (Y_1), FFA level (Y_2), and gamma-oryzanol content (Y_3) of neutralized rice bran oil, using response surface methodology (RSM) with a central composite face-centered design was investigated. The ANOVA results validated the predictive power of the models, given fitness to the experimental data. The optimal neutralization condition was 19.24°Be, 74.79°C, and 11.18 min, achieving the predicted and experimental oil recovery, FFA level, and gamma-oryzanol of 80.00%, 0.31%, and 1.00% and 80.12%, 0.29%, and 1.02%, respectively. The findings verified the high predictive ability of the response models and thus could be deployed for optimization of rice bran oil neutralization parameters to enhance the oil yield and quality.

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