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Optimization of Rice Bran Oil Bleaching via Carotenoid Adsorption onto Activated Carbon using Response Surface Methodology (RSM)

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Abstract

Rice bran oil (RBO) extracted from rice bran contains a good fatty acid profile with high oxidative stability due to the presence of oleic acid, linoleic acid, palmitic acid, stearic acid, and linolenic acid. However, it also contains carotenoids in the form of dark pigments that can be quickly denaturated by natural oxidation process. This study aims to optimize the adsorption-based bleaching of RBO refining process based on the minimum residual carotenoids content. In this study, three variables, namely bleaching temperature (50, 80, 110^{o}C), bleaching time (20, 40, 60 minutes), and activated carbon loading (0.4, 0.8, 1.2% w/v), were optimized using Design Expert v13's response surface methodology (RSM) employing central composite design (CCD) experimental design. The experimental data were subjected to multiple regression analysis in order to fit second-order polynomial model with coefficient of determination (R^2 = 0.7843). In addition, this study employed the Derringer method to determine the most influential variable that results in the lowest carotenoid contents. The lowest carotenoid content (4.87 mg/kg) was achieved under optimum bleaching conditions employing activated carbon of 1.18% w/v at 50^{o}C for 53.155 min. The regression analysis model fit the experimental data fairly well and was further used to validate the optimum parameters and findings using a standard p-value (<0.05) and desirability value (>0.05).

Keywords: *RBO, β-carotene, Bleaching, Activated Carbon, CCD*

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INTRODUCTION

Rice is the most common staple food in Indonesia and most Asian countries, which its by-products of the milling process are often disposed or processed into other products (Octania, 2021). As the world's main cereal crop, paddy (*Oryza sativa)* is cultivated to feed more than half of world's population (Sohail et al., 2017). In Indonesia, rice production in 2022 has reached around 54.07 million tons (BPS, 2022). Before consumption, paddy rice has to undergo drying and milling process to produce as much as 70% rice, 20% husk, 8% bran, and 2% germs/other

impurities (Gul et al., 2015). Proteins (12–13%, w/w), carbohydrate (48–60%, w/w), and lipids (18–25%) are the major nutrients present in rice bran oil (Silva et al., 2006).

Typically, rural people use rice bran as a raw material for animal feed (Mila and Sudarma, 2021). However, Law et al. (2017) claimed that rice bran contains various bioactive compounds with antioxidant and anticancer potential. Additionally, rice bran can be processed through an extraction process to obtain rice bran oil (RBO). One way to obtain RBO is through enzymatic extraction of rice bran using the cellulase. Damayanti et al. (2023a) reported that enzymatic extraction of rice bran can achieve RBO yield of 1.7% and a minimum target for FFA levels of 8.4% at a temperature of 51.5 °C with an incubation time of 4 hours. Although derived from rice milling by-products, RBO has a good fatty acid profile due to the presence of oleic acid (38.4%), linoleic acid (34.4%), palmitic acid (21.5%), stearic acid (2.9%), and linolenic acid (2.2%). This composition contributes to rice bran oil (RBO's) high oxidative stability (Sohail *et al.*, 2017). In addition, RBO has strong antioxidant activity because it contains phytochemicals in the form of γ oryzanol, tocopherols, and tocotrienols (Perez-Ternero et al., 2017). Hence, RBO has high economic value and is safe for human consumption (Lai et al., 2019).

However, RBO also has several ingredients, such as free fatty acids, waxes, color pigments (carotenoids), phospholipids, and peroxide numbers that need to be reduced through several purification processes to be safe for consumption (Mingyai et al., 2017). Carotenoids have an orange color, so the oil looks pale and spoils more quickly when the RBO is stored improperly due to oxidation and hydrolysis processes, which are hazardous to health (Ifa et al., 2021). β-carotene is one of the most abundant types of carotenoids found in vegetable oils, including RBO (Silva et al., 2014).

RBO should be purified to preserve its chemical composition, sensory characteristics, and stability (Ali et al., 2019). The refining process for RBO generally includes degumming, neutralization, bleaching, dewaxing, and deodorization (Strieder et al., 2017). The bleaching stage is crucial for removing carotenoids, metal ions, and oxidation products (Pohndorf et al., 2016). These carotenoids are usually purified through a bleaching process with the assistance of adsorbents (Pandolsook and Kupongsak, 2017).

Activated carbon can be used as an adsorbent in the bleaching process because it is highly porous and easy to enlarge its surface area to facilitate extensive adsorption of carotenoids present in the RBO (Guliyev et al., 2018). Ikumapayi et al. (2020) reported that coconut shells produce the highest activated carbon among agricultural waste materials that can be used as adsorbents. This finding is in good agreement with Ghahjaverestani et al. (2022), who reported that the bleaching of soybean oil using activated carbon derived from coconut shells successfully reduced its carotenoids content. However, there were no studies on carotenoids removal from RBO using activated carbon.

Several parameters in the bleaching process, such as bleaching time, bleaching temperature, and the adsorbent percentage (w/v) must be optimized to obtain efficient removal of carotenoid contents (Ifa et al., 2021). These parameters can be optimized using the Response Surface Methodology (RSM) and the Central Composite Design (CCD) (Strieder et al., 2017).

MATERIALS AND METHODS

Materials

A refined RBO was obtained from a local market in Semarang. The β-Carotene used was obtained from Merck, Germany (≥93% (UV), powder). Prior to adsorption-bleaching experiment, the β-carotene was mixed with RBO and the resulting mixture was used a model of crude RBO (CRBO). The activated carbon (Borneo, local market). Prior to use used as the adsorbent in the bleaching experiment, it underwent size reduction to a uniform size of 200 mesh. This activated carbon has specifications in the form of surface area (m^2/g), pore size (A), and pore volume (cc/g) which were 50.374, 10.8, and 0.027, respectively (Damayanti et al., 2023b). n-hexane from Merck, Germany (>99%) was used as a diluent for carotenoids quantification via spectrophotometry.

Methods

Bleaching Experiment

A thoroughly weighed 0.445 g of β-carotene powder was put into a 50 mL glass beaker, and 50 mL RBO was also introduced accordingly. Then, the mixture was stirred to form a homogeneous mixture. Next, the mixture was transferred into a 50 mL measuring flask and RBO was further added to at an exact volume of 50 mL to allow the formation of crude rice brand oil (CRBO). The bleaching experiment was carried out by introducing CRBO and activated carbon into a three-neck with the help of a glass funnel and a spatula with activated carbon weight to CRBO volume ratio or activated carbon loading (% w/v) was varied. The mixture was stirred using a magnetic stirrer at a speed of 200 rpm (time was varied). Upon adsorption experiment, the mixture was filtered, put in a glass bottle, and stored at 4°C for carotenoid quantification.

Determination of Carotenoid Content

As much as 2.5 g of RBO was put into a 50-mL beaker glass, and 25 mL of 10% hexane was added using a volumetric pipette. The mixture is homogenized in a 25 mL measuring flask and stored in a glass bottle. Then, the cuvette was filled with the homogeneous solution to its maximum capacity. Absorbance of the mixtures were observed using a UV-Vis spectrophotometer. The blank solution (hexane) is placed in the cuvette and placed in the blank position (B) in the spectrophotometer. This is followed by other samples at positions 1 to 5. The

blank solution and the sample were measured for adsorption at a wavelength of 446 nm. The adsorption value of each sample was recorded for further the carotenoid content calculation. The equation used for carotenoids content quantification was suggested by the Malaysian Palm Oil Board (MPOB, 2008) in equation (1):

$$\text{Carotenoid Content } \left(\frac{\text{mg}}{\text{kg}}\right) = \frac{383 \text{ x A446}}{\text{l x c}} \quad (1)$$

where A 446 is the value of adsorption at a wavelength of 446 nm, l is the length of the cuvette (cm), c is the sample concentration, and 383 is the carotene calibration factor.

Experimental Design

The response surface methodology (RSM) is an effective method for predicting optimal experimental parameters and exploring the relationship between certain variables and response results (Barad, 2014; Chew et al., 2017).This RSM technique was applied to optimize operating parameters, such as bleaching temperature, pressure, activated carbon loading (w/v), and bleaching time to increase the yield of bleaching products through the design expert software (Sedghamiz et al., 2019).

The RSM has several experimental designs, including the Central Composite Design (CCD) and the Box Behnken Design (BBD), which are often used to optimize processes (Pereira et al., 2021). In its application, the CCD method can include more than 3 points on each variable. It contains an embedded factorial design or a fractional factorial design (two levels), supplemented by a group of axial points (star points), which allows for the estimation of the surface curvature of the resulting model (Latha et al., 2017; Gujral et al., 2018). In its application, the CCD uses a second-order mathematical model, as shown in equation (2):

$$Y = b_0 + \sum_{i=1}^{n} b_i X_i + \sum_{i=1}^{n} b_{ii} X_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} b_{ij} X_i X_j \quad (2)$$

Tabel 1. Independent Variables with Coding Level Using CCD

Factor	Unit	Minimal	Maximum	Lower Limit (-1)	Upper Limit (+1)
Activated Carbon (A)	%w/v	0.1273	1.47	0.4	1.2
Temperature (B)	°C	29.55	130.45	50	110
Time (C)	min.	6.36	73.64	20	60

where Y is the expected response, b_o is the constant coefficient, b_i is the linear coefficient, b_{ij} is the interaction coefficient, b_{ii} is the quadratic coefficient, and X_i and X_j are coded values. Activated carbon (A), time (B), and temperature (C) are the independent variables studied in this work using the CCD method (Table 1).

RESULTS AND DISCUSSION

Analysis Central Composite Design (CCD)

This research employed the CCD method and a set of experiment was obtained as presented in Table 1. The MAPE (Mean Absolute Percentage Error) that indicates was used to measure the percentage average error of the deviation between the predicted and the actual data or the connection between the variables to the response of the prediction model (Surono et al., 2022). The MAPE can be calculated using equation (3):

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

where A_t is the values that was observed at data point t, F_t is the values that was predicted to occur at data point t, and n is the total number of data points. A MAPE value of less than 10% signifies the accuracy of the model (Moreno et al., 2013). Table 2 shows that the MAPE value obtained from the response rate of carotenoids was 5.1056%. Thus, it confirms the good accuracy of the model (5.1056% < 10%).

The experimental results from Table 2 were then analyzed to obtain an appropriate model to predict the response. The resulting model as shown in Table 3, which contains linear, 2FI, quadratic, and cubic models. Based on Table 3, the quadratic model is the most recommended model for optimizing the carotenoids content of rice bran oil. Meanwhile, both linear and 2FI models failed to fit the experimental data as their accuracy are very low. Moreover, the cubic model resulted in the *aliased* remark, which means that this model cannot be used for carotenoids content estimation due to their poor accuracy (Khelifa et al., 2021).

Equation Model for Carotenoid Content Estimation

The carotenoid content equation model was expressed by a quadratic model with interactions converted into second-order polynomial equations and referred to as coded variables. The second-order polynomial equation is the interaction between the response variable and the input variable in this study, as presented in equation (4).

$$\text{Carotenoid Content} = 5.88 - 0.2513A + 0.2594B - 0.2408C + 0.0439AB - 0.2049AC - 0.1299BC + 0.2224A^2 - 0.5644B^2 + 0.2345C^2 \quad (4)$$

Equations with coded variables can predict responses to different levels of each factor. Code equations are useful for identifying the impact of factors by comparing the factor coefficients. Meanwhile, the regression equation for responses to the variables is in code obtained from the empirical interactions between factors.

Table 2. Experimental Design of CCD Method with Experimental and Predictive Data

Run	Activated Carbon, %w/v (A)	Temperature ºC (B)	Time Min (C)	Carotenoid Content mg/kg		% Error
				Predicted	Experiment	
1	0.4	50	60	5.903	6.089	3,15094
2	1.48	80	40	6.087	6.779	9,86849
3	0.4	110	20	6.406	6.97	8,804246
4	0.8	80	40	5.881	6.051	2,890665
5	0.13	80	40	6.932	6.204	9,50202
6	0.8	80	6.37	6.949	6.511	6,303065
7	1.2	50	60	4.903	4.366	9,85248
8	0.4	50	20	5.715	6.128	7,226597
9	0.8	80	40	5.881	6.089	3,536813
10	0.8	131	40	4.721	4.672	1,037916
11	1.2	110	20	6.401	6.242	2,483987
12	1.2	110	60	5.250	4.864	7,352381
13	0.8	80	73.64	6.139	6.54	6,532008
14	0.8	80	40	5.881	6.051	2,890665
15	0.8	80	40	5.881	5.821	1,020235
16	0.4	110	60	6.074	6.16	1,415871
17	0.8	80	40	5.881	5.4	8,178881
18	0.8	30	40	3.8485	3.86	0,31185
19	1.2	50	20	5.534	5.476	1,048066
					MAPE %	5,105641

Table 3. Determination of the Model used for Carotenoid Content Estimation

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	significance
Model	9.81	9	1.09	3.64	0.0340	
A-Activated Carbon	0.8625	1	0.8625	2.88	0.1240	
B-Temperature	0.9190	1	0.9190	3.07	0.1138	
C-Time	0.7917	1	0.7917	2.64	0.1385	
AB	0.0154	1	0.0154	0.0514	0.8257	
AC	0.3358	1	0.3358	1.12	0.3174	
BC	0.1349	1	0.1349	0.4504	0.5190	
A^2	0.6754	1	0.6754	2.25	0.1675	
B^2	4.35	1	4.35	14.51	0.0042	
C^2	0.7504	1	0.7504	2.50	0.1480	
Residual	2.70	9	0.2996			
Lack of Fit	2.36	5	0.4721	5.62	0.0597	not significant
Pure Error	0.3360	4	0.0840			
Cor Total	12.50	18				
R square	0.7843					
Adeq Prec	7.808					

Table 4. ANOVA of Carotenoid contents

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	Remarks
Linear	0.3123	0.0185	0.0470	-0.4360	
2FI	0.8903	0.0118	-0.1330	-0.8531	
Quadratic	0.0080	0.0597	0.5686	-0.4805	Suggested
Cubic	0.0179	0.8476	0.9022	0.8958	Aliased

Statistical Analysis for Carotenoid Content

Analysis of Variance (ANOVA) has been widely used to analyze research. The statistical analysis of carotenoid content and the ANOVA regression model used for predictions are presented in Table 4.

This model has an F value of 3.64 with a p - value of 0.0340, which confirms that the model has a significant influence. Table 4 demonstrates that only B^2 was significant in this case, with a p-value of 0.0042.

Kandasamy et al. (2019) explained that although a factor may have a p-value of >1, it may still cause effects based on its carotenoid rate. However, this effect may not be significant in number. The lack of fit has a p-value of 0.0597 (5.97%). As it is higher than 5%, this suggests that the model is not inaccurate or inappropriate (Singh et al., 2020). Next, the Adeq precision value is a measure of the signal-to-noise ratio with an expected ratio of > 4 (Gul et al., 2015). In this model, the Adeq precision value is 7.808, indicating that the model can be recommended for use.

A line of unit gradient, or a line of perfect fit with points corresponding to zero error between the actual values and predicted values, is displayed in Figure 1. Accordingly, it demonstrates that the model

has reasonably accurate description of the experimental data regarding the carotenoid contents. Figure 1 shows the actual response values compared to the predicted values. The results indicate that they are in a good fit with the experimental data.

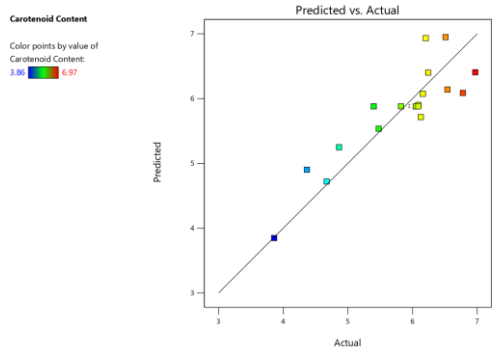


Figure 1. Plot Between Actual and Predicted Values

Effect of Activated Carbon Loading (w/v) and Temperature on Carotenoid Contents

Figure 2 displays the correlation between activated carbon loading (w/v) and bleaching temperature to the carotenoid content in RBO. An activated carbon loading between 0.4-0.6%w/v and a temperature of 20-40ºC is the best to produce the lowest carotenoid, marked with dark blue in Figure 2. Likewise, a high activated carbon loading of 1-1.2 %w/v and a temperature of 120-140ºC will result in low carotenoid content.

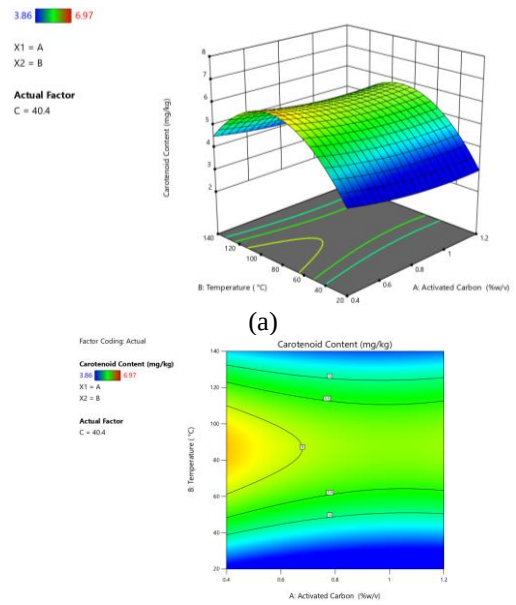


Figure 2. Response Surface Methodology (a) 3D and (b) Contour Plot for Effect of Percentage (w/v) of Activated Carbon and Temperature on Carotenoid Contents

Chetima et al. (2018) state that the adsorbent loading (w/v) is directly proportional to bleaching temperature. Thus, when the percentage (w/v) of activated carbon is low, the bleaching temperature should also be low and vice versa. Guliyev et al. (2018) also emphasize that carotene is a thermolabile color pigment. Therefore, a bleaching process using activated carbon will not be optimum at high temperatures because it will destroy the carotenes.

The more activated carbon is used in the bleaching process, the more optimal amount of carotenoid is absorbed. This is because a larger surface area would promote a greater adsorption capacity. Figure 2 demonstrates that the highest carotenoid content was obtained at temperatures between 60-110^{0}C and an activated carbon of 0.4-0.7%w/v. This is because the adsorbent has reached its highest temperature, resulting in the desorption of the previously adsorbed substances (Xu et al., 2020).

Effect of Time and Temperature on Carotenoid Contents

As seen in Figure 3, the highest carotenoid content of 6 mg/kg is obtained at temperatures between 90-140ºC, marked as the red area. At a temperature of 20-50ºC, the carotenoid obtained is relatively low as greater carotenoid content can only be obtained from prolong bleaching duration.

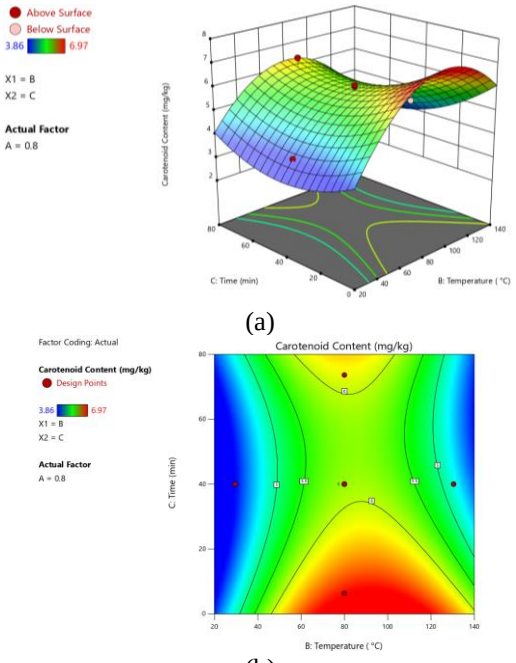


Figure 3. Response Surface Methodology (a) 3D and (b) Contour Plot for Effect of Activated Carbon Temperature and Time on Carotenoid Contents

However, a long bleaching process may also reach a saturation point where the adsorbent can no longer absorb impurities from oil. This is supported by Ifa et al. (2021), who explained that when the temperature increases, a longer adsorption time is also needed to produce optimal carotene content.

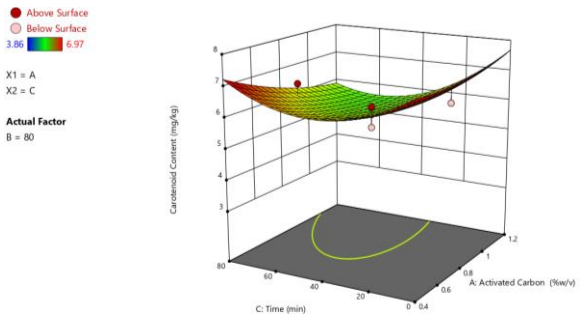
Furthermore, temperature and heating time will increase the reaction rate because, in such conditions, the particles are more actively moving, resulting in higher collisions and particle movement.

The higher the temperature, the greater the collision. Thus, the adsorbent's ability to adsorb impurities will also increase (Gao and Fatehi, 2018). Bleaching is recommended not to be conducted at high temperatures as research by Pohndorf et al. (2016) showed that using a too high temperature can cause oxidation and reduce the oil's effectiveness.

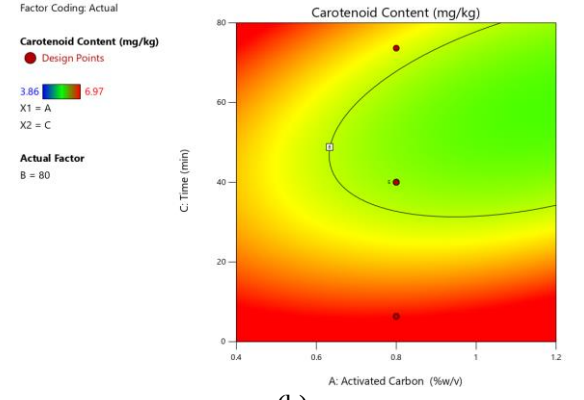
An optimal temperature must be determined to obtain optimal bleaching results because this factor will influence capacity and selectivity adsorption, at higher temperatures, the adsorption capacity of activated carbon can decrease and reduce the effectiveness of activated carbon in adsorbing the carotenoids from the CRBO. In addition, temperatures that are too high can decrease the selectivity adsorption of activated carbon, causing it be unable to differ between the impurities and the useful components in the oil (Rahman et al., 2019).

Effect of Activated Carbon Loading (w/v) and Time on Carotenoid Contents

The effective absorption of carotenoids in the bleaching process decreases along with increasing time and amount of activated carbon used. Figure 4 shows that the best carotenoid content value is reached when the bleaching time is around 30-50 minutes and activated carbon is 0.7-1.2%w/v, marked as a green area. The green area signifies the optimal variables for bleaching time and activated carbon loading (w/v) to obtain the lowest carotenoid content.



(a)



(b)

Figure 4. Response Surface Methodology (a) 3D and (b) Contour Plot for the Effect of percentage (w/v) of Activated Carbon and Time on Carotenoid Contents

The adsorbent activity of activated carbon is proportional to the duration and activated carbon loading (w/v) used (Oliver et al., 2018). A high activated carbon loading (w/v) will require a longer time to bind impurities. Likewise, if only a small amount of activated carbon is loaded, the optimum time required for absorbing impurities will also be low.

Furthermore, if properly used, the activated carbon loading has a good effect on RBO carotenoid content The high of adsorbent percentage (w/v) used allows for the maximum absorption of impurities. However, this also allows the natural antioxidants contained in the pigments to be absorbed and affect the oxidation stability of RBO (Guedidi et al., 2017). Conversely, if the activated carbon loading (w/v) is too low, less impurities will be adsorbed because the active side of the adsorbent would already be covered by impurities (Islam et al., 2018).

Optimization Results

RBO bleaching decreased carotenoid contents in RBO, which was influenced by several interaction factors, such as activated carbon loading (w/v), temperature, and time. The factors affecting the response are determined by Derringer charts. The Derringer chart is used to show the level of compatibility of the data analysis with the variables used. In this study, the Derringer graph (Figure 5) was chosen to optimize the bleaching process with the temperature, time, and activated carbon loading (w/v), which has a desirability value of 0.673.

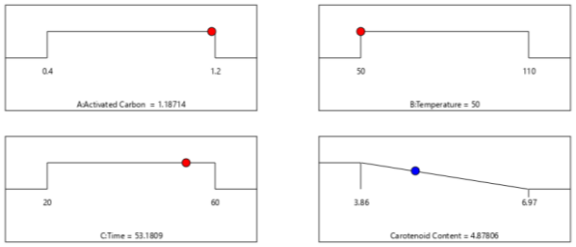


Figure 5. Derringer Optimization

The optimization results showed that the optimum carotenoid content produced is 4.878 mg/kg. This value is within the standard range of carotenoid contents commonly found in refined RBO, which is in the range of 4.5 - 5.3 mg/kg (Pohndorf *et al*., 2016).

CONCLUSION

The parameter of temperature has a significant effect on the RBO carotenoid content in the bleaching process using activated carbon as an adsorbent. Meanwhile, time and activated carbon loading (w/v have low effects. Statistically, the data error (MAPE) is 5.105641%, and the regression analysis of the fit data model uses a p value of 0.0340, an R^2 value of 0.7843, and a desirability value of 0.673. The optimum yield of carotenoid content was 4.878 mg/kg obtained at 1.18% w/v activated carbon,

a temperature of 50 °C, and a bleaching time of 53.155 min.

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