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Optimization of Glycerol Production from Mango Seed Oil Using Response Surface Methodology and Central Composite Design

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ABSTRACT

The objective of this research was to optimize the glycerol production from mango seed oil using RSM and CCD. The glycerol was produced through the transesterification process where biodiesel and glycerol were produced as the products of the reaction. The experimental design for this study was a four factor five levels CCD. This research used oil from mango seed because it is a non-edible oil and available. The study established quadratic effects were statistically significant at 5% level of significance. The study controlled variables were oil: methanol ratio, catalyst concentration, reaction temperature and time and the response variable was glycerol yield. From the analysis using the second order model, the value of R-squared was 79.39 % (R- squared = 0.7939) and Adjusted R-squared = 60.16 % (0.6016) and this implied that 79.39 % of the variation in the response variable could be accounted for by the study variables. The F statistic value 0.004962, (at 5% level of significance) was statistically significant. So the model was fit for estimating the response from the experimental data. The findings further showed that to obtain minimum (Optimal) glycerol yield of 10.6%, 1: 6.95 oil to methanol ratio, 7.7% catalyst concentration, 65.99 ^{O}C of reaction temperature and 87.45 minutes of reaction time are required to produce glycerol from mango seeds oil. The study has established that the mango seed has a substantial amount of oil which can be extracted and used to make products which are valuable to human These adds value to the mango seeds which would otherwise would be left to litter hence causing pollution to the environment. This could lead to reduction in environmental pollution and improvement of livelihoods of mango farmers

Key words: Glycerol Production, Central Composite Design, Mango Seeds Oil, Optimization, Response Surface Methodology

1.0 INTRODUCTION

Glycerol is a naturally occurring form of fatty esters in fats and oils. It is an important intermediary in the metabolic process of living organisms. Glycerol production as a byproduct in the production of biodiesel is becoming more and more common [4]. Glycerol is being used in a wide variety of applications due to its unique chemical and physical characteristics, which are physiologically safe. Glycerol is used in many ways, with huge quantities being the production of medicine, makeups, toothpastes, among others.

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Glycerol is often regarded as a secure all-purpose food additive, and it is allowed in several types of food packaging materials. Acetyls, amines, esters, and ethers are examples of glycerol derivatives. The salts of glycerophosphoric acid are used in the medicine world.

Once the mango fruits are consumed, only few seeds are used for propagation but most of them are left to decompose. The seed inside the fruit, represent 10 - 25% of the total fruit weight. The kernel inside the seed represents 45-75% of the total seed weight [6]. Depending on the variety, mango seeds contain 9–13% oil [9] The oil has a high percentage of unsaturated fatty acids, such as oleic acid, at 46.22%, which is greater than palm oil's 15.4%, sunflower oil's 21.1%, and soybean oil's 23.4% as well as the oil from the Jatropha curcas plant, which has 44.7% oleic acid [5]. This shows that, mango was a good source of oil since it is non-edible and has no effect on food chain. While extracting oil from the mango seed, there was a need to get the optimum set of factors so that we get a maximum amount of oil. The study used ANN and RSM as tools to achieve this optimum conditions. The conversion of vegetable oil esters into biodiesel esters is called trans-esterification [2]. As a result, combustion is improved since the oil's viscosity is decreased to a level similar to that of diesel [1]. The trans-esterification is done by adding alcohol to the oil. This initiates a reaction which eliminates the glycerol backbone and substitutes it with a methyl group. This results in breaking of the large molecules to methyl esters (biodiesel) and glycerol, as shown in equation (1). R′, R″ and R‴ in the equation stands for hydrocarbon chains [2].

$$\text{OIL} + \text{METHANOL} \xrightarrow{\text{NaOH (aq)}} \text{BIODIESEL} + \text{GLYCEROL} \qquad (1)$$

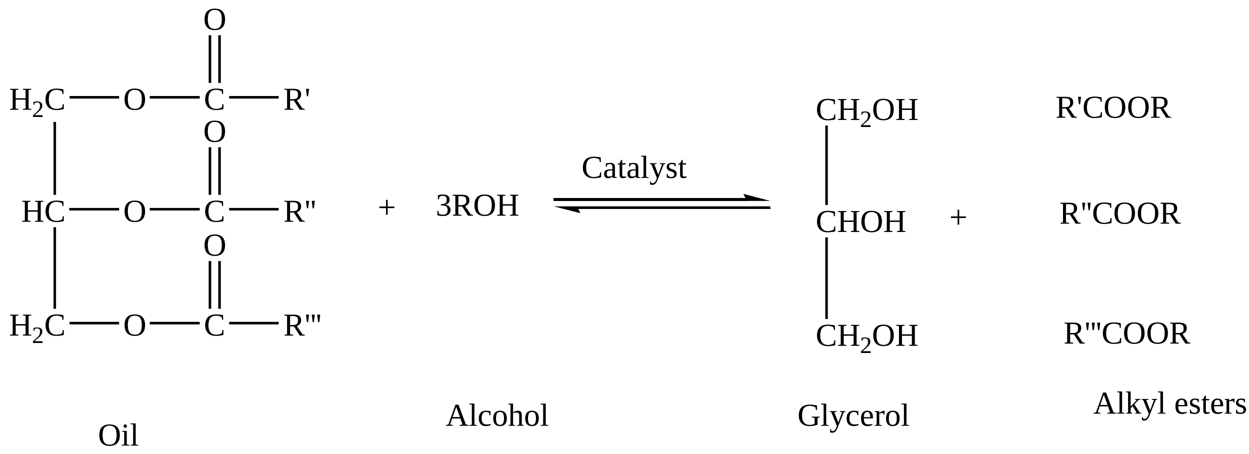


Figure 1: Overall Equation for Trans-esterification [7]

During the trans-esterification reaction each mole of the oil, reacts with three moles of alcohol for complete reaction. Excess alcohol is normally required to shift the equilibrium of the reversible process forward (towards the products side) to increase the yield [3].

The general objective of study was to optimize the glycerol production from mango seed oil using Response Surface Methodology and Central Composite Design

The study was guided by the specific objectives given below

1. To investigate the appropriate mathematical model of second order polynomial that best fits the experimental data
2. To determine the optimum conditions for glycerol production from mango seed oil using Response Surface Methodology.

2.0 MATERIALS AND METHODS

2.1 Materials and Equipments

Mango seed oil, methanol, potassium hydroxide, separating funnel, conical flasks, water bath, magnetic stirrer.

2.2 Method: Trans-esterification

Trans-esterification is reaction between an oil and an alcohol to give glycerol and biodiesel. During this study, trans-esterification began by mixing potassium hydroxide with methanol. The solution was stirred by use of a magnetic stirrer for 5–10 minutes at a constant temperature of 30°C. At this point the reaction was complete because all KOH had dissolved. This reaction gave a product known as potassium methoxide, that reacted successfully with the oil molecules. Then, the potassium methoxide and mango seed oil were mixed while being continuously and vigorously stirred at various temperatures for the predetermined durations of time. After the predetermined time lapses, the mixture was allowed to settle. Two layers were formed, glycerol layer and the biodiesel layer which were separated by use of a separating funnel. The whole procedure is illustrated in Figure 1

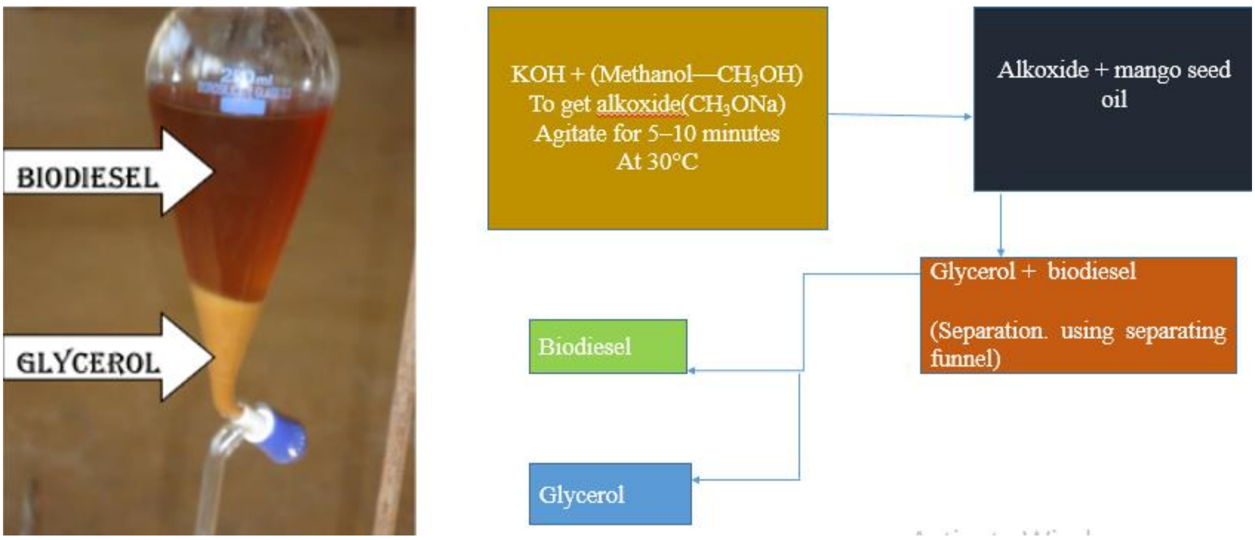


Figure 2: General Flow Diagram of Trans-esterification

2.3 Central Composite Design (CCD)

The most popular design for fitting a second-order model is CCD [11]. The design is made up of Factorial design composed of central points, and axial points that are equally spaced from the center point. In this research a set of thirty, 30, experiments which included, 2^4 factorial designs, eight-star points and six center points was used. The distance of the star points from the center point was given by $\alpha = 2^{n/4}$, where n was the number of variables. In this study the value of $\alpha = 2^{4/4}=2$

The coded variables x_i for the four variables becomes,

$x_1 = \frac{(X_1-5)}{2}$ $\quad x_2 = \frac{(X_2-8)}{2}$ $\quad x_3 = \frac{(X_3-60)}{10}$ $\quad x_4 = \frac{(X_4-80)}{20}$ The range and levels of the independent variables which were used during the experiment as given in Table 1.

Table 1: Four Factors at Five Levels Estimated Values

Independent Variable	Coded and Actual Values

	-2	-1	0	1	2
X_1 (volume to volume ratio)	1:1	1:3	1:5	1:7	1:9
X_2 (Catalyst concentration)	4	6	8	10	12
X_3 (Temperature (^{0}C)	40	50	60	70	80
X_4 (Time of reaction(min))	40	60	80	100	120

2.4 Second Order Polynomial Model

The study adopted a second order polynomial model for the yield of glycerol given by equation 2.

$$y_2 = \alpha_0 + \alpha_1 x_1 + \alpha_2 x_2 + \alpha_3 x_3 + \alpha_4 x_4 + \alpha_{11} x_1^2 + \alpha_{22} x_2^2 + \alpha_{33} x_3^2 + \alpha_{44} x_4^2 + \alpha_{12} x_1 x_2 + \alpha_{13} x_1 x_3 + \alpha_{14} x_1 x_4 + \alpha_{23} x_2 x_3 + \alpha_{24} x_2 x_4 + \alpha_{34} x_3 x_4 \quad (2)$$

2.5 Determination of the Optimal Parameter-Setting

The optimal parameter setting was achieved analytically by use of the following function;

$$\hat{Y} = \hat{\beta}_0 + \boldsymbol{X}'\boldsymbol{b} + \boldsymbol{X}'\boldsymbol{B}\boldsymbol{X} \quad (3)$$

This function can be written in matrix form as

$$\boldsymbol{X} = \begin{bmatrix} x_1 \\ x_2 \\ x_3 \\ x_4 \end{bmatrix}, \quad \boldsymbol{b} = \begin{bmatrix} \hat{\beta}_1 \\ \hat{\beta}_2 \\ \hat{\beta}_3 \\ \hat{\beta}_4 \end{bmatrix} \text{and } \boldsymbol{B} = \begin{bmatrix} \hat{\beta}_{11} & \hat{\beta}_{12}/_2 & \hat{\beta}_{13}/_2 & \hat{\beta}_{14}/_2 \\ \hat{\beta}_{21}/_2 & \hat{\beta}_{22} & \hat{\beta}_{23}/_2 & \hat{\beta}_{24}/_2 \\ \hat{\beta}_{31}/_2 & \hat{\beta}_{32}/_2 & \hat{\beta}_{33} & \hat{\beta}_{34}/_2 \\ \hat{\beta}_{41}/_2 & \hat{\beta}_{42}/_2 & \hat{\beta}_{43}/_2 & \hat{\beta}_{44} \end{bmatrix}$$

Where **b** represents a 4×1 vector of regression coefficients and B represents a 4×4 symmetric matrix with the elements in the main diagonal as quadratic coefficients β_{ii}, and the off-diagonal elements are half the mixed quadratic coefficients, β_{ij} $(i \neq j)$ and $i = 1,2,3,4$. To get the optimum point we differentiate $\hat{Y}$ with respect to X as follows.

$$\frac{\partial \hat{Y}}{\partial X} = \boldsymbol{b} + 2\boldsymbol{B}\boldsymbol{X} = 0 \quad (4)$$

The stationary point is given as

$$\boldsymbol{X_S} = -\frac{1}{2}\boldsymbol{B}^{-1}\boldsymbol{b} \quad (5)$$

And the estimated response is

$$\hat{Y} = \hat{\beta}_0 + \frac{1}{2}\boldsymbol{X}'_{\boldsymbol{S}}\boldsymbol{b} \quad (6)$$

After establishing the stationary point, we sought to determine if it was a maximum, minimum or saddle point. The study used partial derivatives to calculate the coordinates and characteristics of each stationary point, which will then be visualized using contour plots and response surface. The maximum, minimum, saddle point, or stationary ridge are typically taken into account by the second order model.

2.6 Canonical Analysis

The canonical analysis is very important in systems of maximum and minima as it is used, to define the type and nature of the stationary points. Using the sign on the eigenvalues from matrix $\boldsymbol{B}$, we can determine whether the stationary point is a maximum or minimum response surface. If all the eigenvalues have a negative sign the stationary point is maximum and if the eigenvalues values have positive signs, the stationary point is a minimum. If we have mixed signs on the eigenvalues, the stationary point is a saddle point.

The canonical equation is given by

$$\hat{y} = \hat{y}_s + \lambda_1 w_1^2 + \lambda_2 w_2^2 + \cdots + \lambda_k w_k^2 \tag{7}$$

Where $\{\lambda_i\}$ are the eigenvalues of the matrix $\boldsymbol{B}$, while the $\{w_i\}$ are the transformed explanatory variables. The response surface is sloppiest in the $\{w_i\}$ direction, corresponding to the largest absolute eigenvalue.

3.0 RESULTS AND DISCUSSION

3.1 Results for the Biodiesel Yield

The experimental, predicted and deviations results for the glycerol yield are presented in Table 2. The table represents the experimental values as recorded from the average values of the three triplicate runs. The predicted values were calculated by substituting the second order regression model in the experimental data. The difference between the predicted and the experimental data gave the deviation.The difference between the actual value and predicted value ranged between -3 to 6.4 with a percentage error ranging between -15.38 to 57.14. To establish whether there was any statistical significant difference between the predicted value and experimental, a paired t-test was done. The t-test value was found to be -1.27577 with two sided critical value of $\pm$2.045 this implies that there was no statistical significance difference between the actual value and the predicted value implying that the RSM model was suitable for optimizing the conditions for glycerol production

1.0

Table 2: Results of Experimental, Predicted and Deviations in the Data

	Coded Values				Glycerol yield (%)			
Runs	X_1	X_2	X_3	X_4	Exp.	Pred	Deviation	Error (%)
1	-1	-1	-1	-1	20.0	17.6	-2.4	-12.00
2	+1	-1	-1	-1	15.0	14.7	-0.3	-2.00
3	-1	+1	-1	-1	11.2	17.6	6.4	57.14
4	+1	+1	-1	-1	17.3	14.7	-2.6	-15.03
5	-1	-1	+1	-1	19.0	17.6	-1.4	-7.37
6	+1	-1	+1	-1	14.0	14.7	0.7	5.00
7	-1	+1	+1	-1	18.0	17.6	-0.4	-2.22
8	+1	+1	+1	-1	13.5	14.7	1.2	8.89
9	-1	-1	-1	+1	18.5	17.6	-0.9	-4.86
10	+1	-1	-1	+1	15.0	14.7	-0.3	-2.00
11	-1	+1	-1	+1	18.2	17.6	-0.6	-3.30
12	+1	+1	-1	+1	16.0	14.7	-1.3	-8.13
13	-1	-1	+1	+1	19.0	17.6	-1.4	-7.37
14	+1	-1	+1	+1	15.0	14.7	-0.3	-2.00
15	-1	+1	+1	+1	16.0	17.6	1.6	10.00
16	+1	+1	+1	+1	11.8	14.7	2.9	24.58

17	-2	0	0	0	19.5	18.7	-0.8	-4.10
18	2	0	0	0	13.0	12.8	-0.2	-1.54
19	0	-2	0	0	17.0	16.1	-0.9	-5.29
20	0	2	0	0	18.0	16.1	-1.9	-10.56
21	0	0	-2	0	18.4	16.1	-2.3	-12.50
22	0	0	2	0	14.9	16.1	1.2	8.05
23	0	0	0	-2	19.5	16.5	-3.0	-15.38
24	0	0	0	2	14.5	16.5	2.0	13.79
25	0	0	0	0	11.0	11.3	0.3	2.73
26	0	0	0	0	11.3	11.3	0.0	0.00
27	0	0	0	0	12.0	11.3	-0.7	-5.83
28	0	0	0	0	11.1	11.3	0.2	1.80
29	0	0	0	0	11.0	11.3	0.3	2.73
30	0	0	0	0	11.5	11.3	-0.2	-1.74

3.2 Fitting a Second Order Polynomial Model for Glycerol Production

3.2.1 Parameter Estimates for Second Order Model for Glycerol Yield

The study sought to fit a second order polynomial model to the experimental data on the glycerol yield. The second order polynomial model was fitted to determine the optimum set of operating levels for the oil to methanol ratio, Catalyst concentration, Temperature (^{0}C) and time of reaction (min) that optimized the glycerol Yield. The regression estimates, standard error of estimate, t-statistic and probability value associated with the estimate of linear, interaction and quadratic effects were presented.

Table 3: Parameter Estimates for Second Order Polynomial Model

Parameters	Estimate	Std. Error	t value	Pr(>|t|)
(intercept)	11.31667	0.78872	14.3481	**3.629×10^{-10}**
X_1	-1.47083	0.39436	-3.7297	**0.002013**
X_2	-0.47917	0.39436	-1.2150	0.243129
X_3	-0.49583	0.39436	-1.2573	0.227867
X_4	-0.35417	0.39436	-0.8981	0.383334
X_{12}	0.79375	0.48299	1.6434	0.121091
X_{13}	-0.81875	0.48299	-1.6952	0.110697
X_{14}	-0.34375	0.48299	-0.7117	0.487567
X_{23}	-0.11875	0.48299	-0.2459	0.809120
X_{24}	0.15625	0.48299	0.3235	0.750780
X_{34}	-0.43125	0.48299	-0.8929	0.386026
${X_1}^2$	1.10729	0.36889	3.0017	**0.008942**
${X_2}^2$	1.41979	0.36889	3.8488	**0.001578**
${X_3}^2$	1.20729	0.36889	3.2728	**0.005138**
${X_4}^2$	1.29479	0.36889	3.5100	**0.003158**

The result in Table 3 indicates that methanol to oil ratio was statistically significant but the other three main effects (catalyst concentration, reaction temperature and time) had no statistical significant effect on glycerol yield. This means that methanol to oil ratio greatly influenced the glycerol yield. For instance, for an increase in oil: methanol ratio (X_1) by one unit, the glycerol yield would decrease by a factor of 1.47083. These results are in line with [10] findings who reported that ethanol to oil ratio influenced the reaction rate and ethyl esters yield. biodiesel production is favored, while higher glycerol formation is minimized. The two-way interaction of the controlled variables were not statistically significant at 5% level of significant. The study found that all quadratic components had significant effects on glycerol production at 5% significant level. For instance, a change in quadratic term of oil to methanol ratio, correspond to an increase in glycerol production by a factor of 1.10729. Similarly, a change in quadratic term of catalyst concentration, correspond to an increase in glycerol production by a factor of 1.41979. Further a change in quadratic term of temperature leads to an increase in glycerol production by a factor of 1.20729. lastly a change in quadratic term of time causes an increase in glycerol production by a factor of 1.29479. The predicted model glycerol production in terms of coded controlled variables is stated as shown in Equation (8)

$$\hat{y}_2 = 11.317 - 1.471\,X_1 + 1.107\,{X_1}^2 + 1.207\,{X_2}^2 + 1.207\,{X_3}^2 + 1.295\,{X_4}^2 \qquad (8)$$

Where $\hat{y}_2$ represents the % of glycerol yield
11.317=estimated glycerol yield when controlled variables are equal to zero.
X_1 =oil: methanol ratio
X_2 = Catalyst concentration
X_3 = Reaction Temperature
X_4 = Reaction Time
This coded equation is useful in identifying the relative impact of controlled variables by comparing the controlled variables coefficients. The study revealed that high oil: methanol ratio, catalyst concentration, reaction temperature and reaction time, correspond to slight increase in glycerol production hence reducing biodiesel yield.

3.2.2 Model Summary Statistic

To determine the suitability of the model the study determined the coefficient of determination, F-statistic and the p-value as shown in Table 4.

Table 4: Model Summary Statistic

Statistic	Value
R – squared	0.7939
Adjusted R – squared	0.6016
F – statistic	4.128
p- value	0.004962

Table 4 shows the value R-squared of 79.39 % (R- squared = 0.7939) and Adjusted R-squared = 60.16 % (0.6016) and this implied that 79.39 % of the variation in the response variable could be accounted for by the variables (X_1, X_2, X_3, X_4). The F statistic value 0.004962, (at 5% level of significance) was statistically significant. So the model was fit for estimating the response from the experimental data. It was important to check the adequacy of the second order model by conducting analysis of variance. By use of lack of fit test, it was concluded that the second order model was not adequate to fit the experimental data. The results from Analysis of Variance were presented in Table 5

3.2.3 Analysis of Variance

Analysis of Variance is a statistical tool used coming up with decisions that allows the detection of of goodness of fit of a fitted model by checking the variations in the overall performance of the investigated parameters [8]. The model's significance and adequacy was assessed using the F ratio at a significance probability value of 5%, using ANOVA.

Table 5: Analysis of Variance

Source of Variation	df	SS	MSS	F-statistic	Pr(>F)
FO (X_1, X_2, X_3, X_4)	4	66.342	16.5854	4.4435	0.0144209
TWI (X_1, X_2, X_3, X_4)	6	26.289	4.3815	1.1739	0.3706660
PQ (X_1, X_2, X_3, X_4)	4	123.094	30.7735	8.2447	0.0010047
Residuals	15	55.987	3.7325		
Lack of fit	10	55.239	5.5239	36.9081	0.0004664
Pure error	5	0.748	0.1497		

The study found that the main effect terms (FO) were statistically significant in predicting glycerol production at 5% significance level (F-statistic= 4.4435 and p-value 0.0144209 < 0.05). In addition, the study revealed that the Two Way Interaction effect terms (TWI) were not statistically significant in predicting of glycerol production at 5% significance level (F-statistic=1.1739and p-value 0.3706660 > 0.05). In addition, it was observed that the pure quadratic effect terms (PQ) were statistically significant in predicting biodiesel production at 5% significance level (F-statistic = 8.2447 and p-value 0.0010047 < 0.05). The findings revealed that at least one main effect term, and pure quadratic term are included in the prediction model. The results in Table 5, further presents the lack of fit test value. The lack of fit test helps in determination of adequacy of the proposed model on the experimental data in predicting the response. If the p-value is less than the level of significance (0.005) then we conclude that the model is not adequate. To determine the adequacy of the model (No lack of fit) for the second order polynomial model, the hypothesis was set as at 5% level of significance.

H_0: There is no evidence that the model does not fit the data, i.e., there is no lack of fit.

H_1: There is evidence that the model does not fit the data, i.e., there is lack of fit

From the results in Table 5 the lack of fit F-value was 36.9081 and the p-value (0.0004664) which is less than 0.05 Therefore the null hypothesis is rejected and therefore there is lack of fit meaning that there is evidence that the fitted model does not fit the data. A higher order polynomial is needed for better prediction of the glycerol yield. This finding is in contrast with a study by [10] who reported that the second model can be considered statistically significant according to the F-test with 95% of confidence in predicting yield of glycerol.

3.2.4 Validation of the Model

The study sought to assess the validity of the second order polynomial model and for easy understanding and clarity. A plot of predicted values versus actual values from experimental data using the model was as shown in Figure 3. It shows a graph comparing actual Glycerol percentage yield to expected percentage yield. There was a positive correlation between the predicted and experimental values for the glycerol produced from mango seed oil. Additionally, the best line of fit depicted a better model fitting on the experimental results.

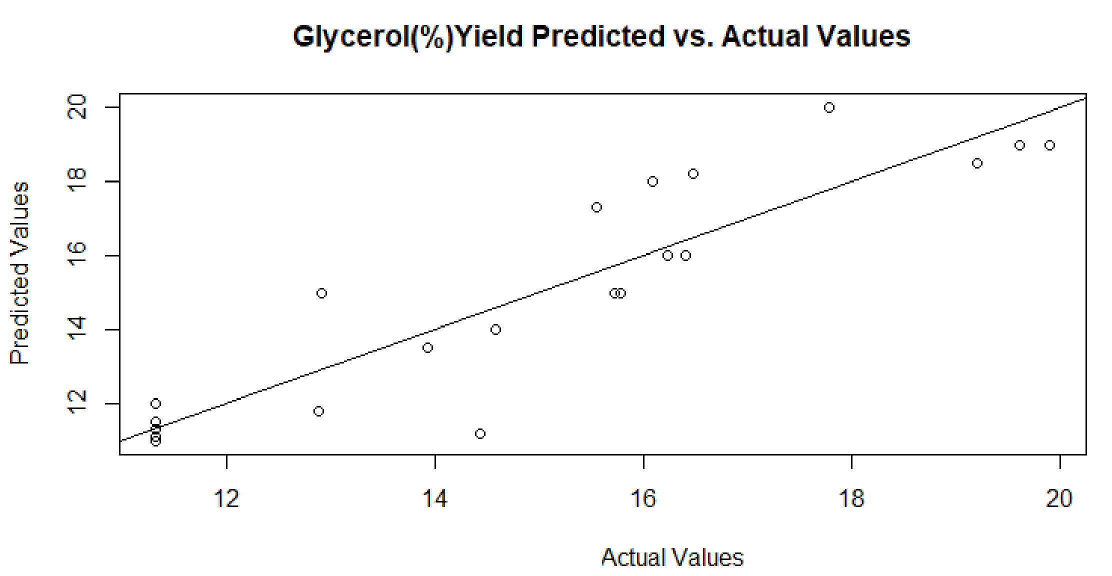
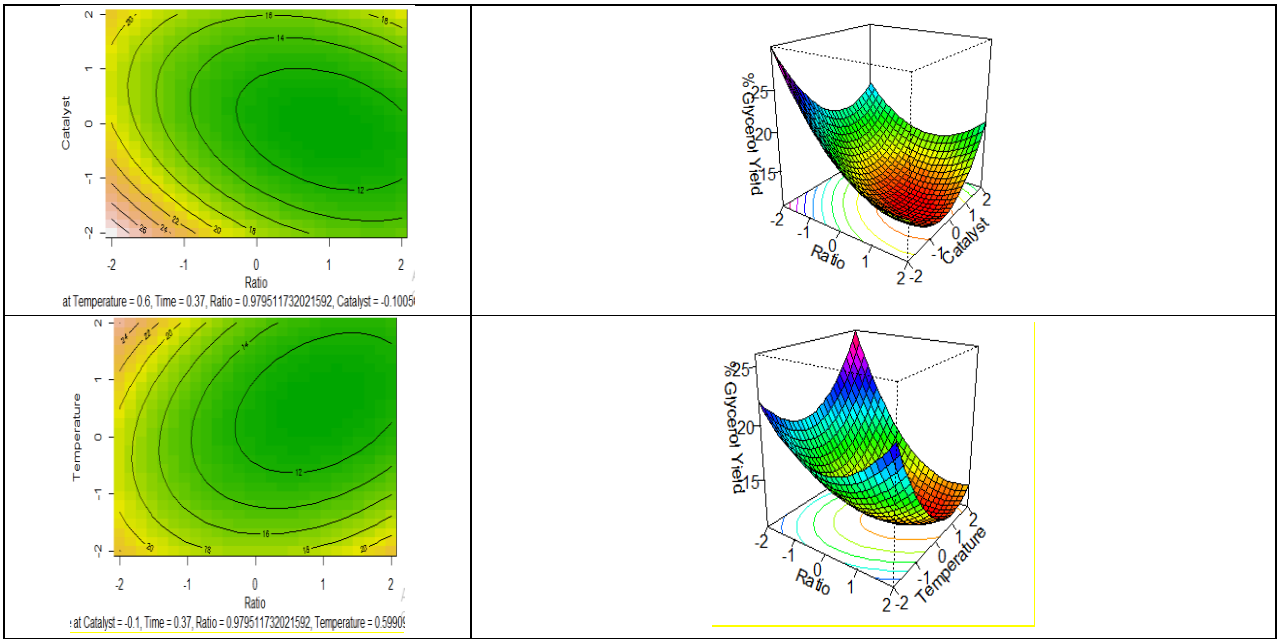


Figure 3: Predicted Value versus Experimental Value Percentage Glycerol Yield

3.3 Determining Optimal Conditions for Minimum Glycerol Production

3.3.1 Response Surface and Contour Plots for the Glycerol Yield

Figures 4, display plots of two dimension (2 D) and three dimension (3D) for different combination of control variables. They show the trend of variation of response within the selected range of control variables and also influence of each variable over the other variable.



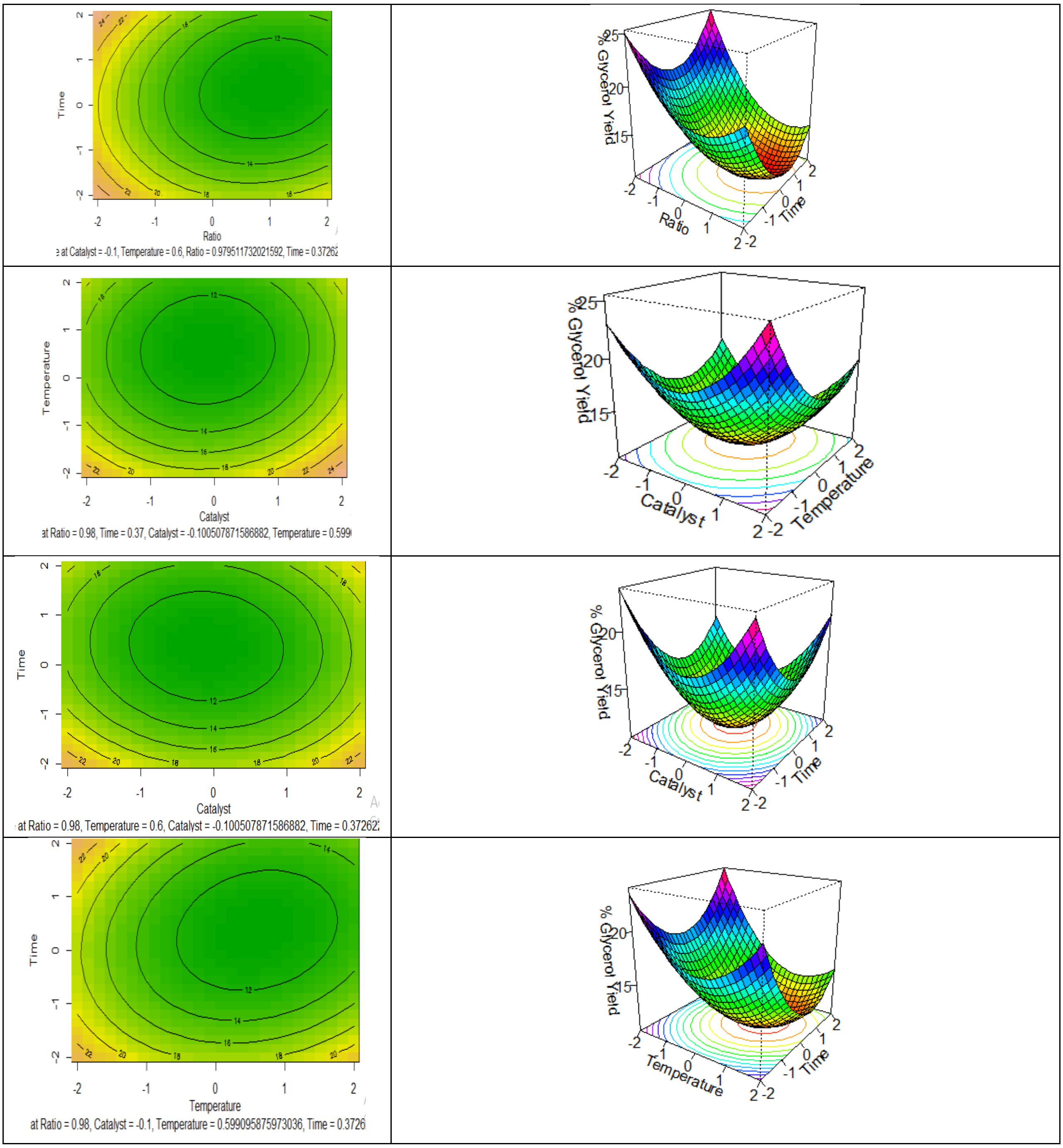


Figure 4: Contour Plot and 3D Response Surface plot for Glycerol Yield

3.3.2 Optimal Conditions for Minimum Glycerol Production

The aim of the study was to find the optimal experimental conditions for minimum glycerol production from mango seed oil. The best solution satisfying the fitted model are displayed in Table 6.

Table: 6 Optimal Conditions for Minimum Glycerol Production

Variables	description	Coded values	Actual values
X_1	Oil to methanol ratio	0.979	1: 6.95 V/V
X_2	Catalyst concentration	-0.101	7.7%

X_3	Temperature	0.599	65.99 ^oC
X_4	Time	0.373	87.45 min
Y	Optimal glycerol yield		10.0589 %.

The coded values of the stationary points in Table 6 were transformed to actual values as shown below

$$Oil: methanol\ ratio = 1:(2 \times 0.9795117) + 5 = 1:6.95V/V$$

$$Catalyst\ concentration = (2 \times -0.1005079) +) + 8 = 7.7\%$$

$$Temperature = (10 \times 0.5990959) + 60 = 65.99^oC$$

$$Time = (20 \times 0.3726230) + 80 = 87.45\ minute$$

It was found that for minimum glycerol yield, 1: 6.95 oil to methanol ratio, 7.7% catalyst concentration, 65.99 ^{O}C of reaction temperature and 87.45 minutes of reaction time are required to produce 10.6% of glycerol yield from mango seed oil. This finding were in contrast with [10] who reported an optimum concentration for molar ratio, catalysts, time and temperature were, 9:1, 1.3 wt.%, 80 min and 40.0 °C, respectively for maximum yield of glycerol.

3.3.3 Eigen Values and Eigen Vectors

The Eigen values and vectors for the second order polynomial model were computed and displayed in Table 7

Table 7: Eigen values and vectors

		Eigen values		
	λ_1 = 1.8401234	λ_2 = 1.4180637	λ_3 = 1.2263139	λ_4= 0.5446657
		Eigen vectors		
X_1	-0.59228014	-0.2283590	0.3031396	0.7107481
X_2	-0.64376823	-0.2031284	-0.6658382	-0.3177433
X_3	0.47541352	-0.3885317	-0.5908721	0.5233500
X_4	-0.09353441	0.8692722	-0.3400530	0.3463830

The nature of the stationary points is determined by the signs of the Eigen values. All the Eigen values were positive meaning that the stationary point was a minimum optimal region. The canonical equivalence form for the fitted model can be stated as follows;

$$\hat{y} = 10.0589 + 1.840w_1 + 1.418w_2 + 1.226w_3 + 0.544w_4 \quad (9)$$

4.0 CONCLUSION AND RECOMMENDATIONS

The second order polynomial model indicated that 79.4% variation in glycerol yield was accounted for by controlled variables in the model. However, the main effects were not statistically significant at 5% level of significance. All quadratic effects were statistically significant. Therefore, there is evidence that the fitted model fairly fits the data. The study comes to the conclusion that due to the mango seed's potential to produce a lot of oil and its limitations as an edible oil, the industrial use of mango seed oil for the production of glycerol is desirable after the study's objectives were met. The study recommends use of oil to methanol ratio, catalyst concentration and reaction temperature on glycerol production.

CONFLICT OF INTEREST

There is not conflicting interest.

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