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Methyl Linoleate Synthesis from Cottonseeds Oil: An Optimization Study

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Abbreviations:
RSM: Response Surface Methodology

Abstract: Methyl esters are derivatives of triglycerides (oils or fats) that can be produced through the esterification and transesterification process. One example of the methyl esters are widely used as an industrial raw material is methyl linoleate. Methyl linoleate is a colorless liquid with molecular formula $C_{19}H_{34}O_2$. Methyl linoleate is a fatty acid ester commonly used as a biodiesel ingredient, textiles, medical research, emulsifiers, and lubricants. The raw materials commonly used to synthesize methyl esters are palm oil, coconut oil, soybean oil, etc. However, these oils are edible materials, so that in the present study, the raw materials from cottonseed was used instead. The methyl linoleate synthesis's optimization reaction through enzymatic transesterification from cotton (*Ceiba pentandra* L.) seed oils and methanol by Response Surface Methodology (RSM) was carried out. Immobilized lipase (lipozyme TL IM) is used as a catalyst. This research aims to optimize the reaction by analyzing a variety of conditions that are influenced by several variables, such as reaction time, molar ratio, the amount of enzyme, and the reaction temperature. Design Expert v.7 software was used to view the interaction between the variables via RSM. The mathematical equations and statistical methods showed that the optimum condition of the enzymatic transesterification was obtained at 0.15 gram of the enzyme, the ratio of cottonseed oil : methanol of 1 : 2.05 (g/g), a reaction time of 14 hours, and a temperature of 49.95°C with the predicted and actual percentages yield of 37.87 and 38.24%, respectively. The model recommended for obtaining optimum results is a quadratic model with an R-squared value of 0.6957.

Keywords: cottonseed oils, Design Expert v.7, methyl linoleate, lipozyme, optimization study,

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INTRODUCTION

Cottonseed has the potential to be used as raw material for the manufacture of methyl ester. [1-4] Cottonseed is waste manufacture of mattresses. The content of cotton seeds is 40% oil by weight, readily available, and relatively abundant availability. The seed cotton yield's oil content is 25-40% with a linoleic acid content of 29-41%, [5,6], so that it has the potential to be converted into methyl ester or biopolymer. [7]

In previous studies, cottonseed oil can be converted to methyl esters by enzymatic transesterification with an optimal condition [1,3]. The optimum condition was determined using one variable at a particular time by varying one variable, while the other variables are constant. This method has not been practical to observe the interaction between one another's variables because the variation is only made of one variable while others are held constant. Interactions between variables can affect the amount of methyl ester produced in a reaction

[8]. In the case of one variable's technique at a time, this is used to determine the optimum state of the four variables that influence each other, then the number of experiments will be very high. It will be less efficient in terms of time, cost, and the number of experiments.

Therefore, we observe the relationship or interaction of four factors using RSM, a statistical and mathematical technique used to develop, improve, and optimize processes in several variables' primary response. [9,10] The objective to be achieved in this study is to optimize responses and to observe interactions between variables with a small number of experiments so that it impacts on the use of lower costs

MATERIALS AND METHODS

Equipment and Materials

Equipment used in this study was a variety of glassware, water bath shaker, rotary evaporator, and Gas Chromatography (Shimadzu QP2010 MS, Japan), software Design Expert v.7. [11] Materials were cottonseed oil, lipozyme TL IM, methanol, n-hexane, distilled water, and reference methyl linoleate.

Table 1. The Combination of 30 variables

No	Amount of Enzyme (g)	Time (h)	Molar ratio	Temperature (°C)	Actual Yield (%)	Predicted Yield (%)
1.	0.05	6	4	50	32.58	32.11
2.	0.05	6	2	50	33.41	32.72
3.	0.15	14	2	50	34.55	31.82
4.	0.15	6	2	50	28.03	32.42
5.	0.1	18	3	40	37.11	32.11
6.	0.05	14	2	50	27.92	32.72
7.	0.1	10	3	20	33.47	31.82
8.	0.1	10	3	40	29.20	32.42
9.	0.1	10	5	40	23.44	21.04
10.	0.05	6	2	30	37.43	37.60
11.	0	10	3	40	0	0
12.	0.1	10	3	40	31.09	37.30
13.	0.1	2	3	40	24.72	21.04
14.	0.1	10	1	40	41.88	37.60
15.	0.15	14	4	30	13.73	20.74
16.	0.1	10	3	40	32.46	37.30
17.	0.15	14	4	50	36.32	20.74
18.	0.15	6	4	30	38.73	28.68
19.	0.1	10	3	40	18.95	11.52
20.	0.15	14	2	30	26.99	23.41
21.	0.1	10	3	40	31.59	32.30
22.	0.2	10	3	40	23.19	32.30
23.	0.05	14	4	50	31.53	37.87
24.	0.15	6	4	50	30.55	33.68
25.	0.1	10	3	60	30.50	32.30
26.	0.05	6	4	30	28.25	32.30
27.	0.05	14	4	30	31.34	32.30
28.	0.15	6	2	30	32.73	32.30
29.	0.1	10	3	40	37.84	32.30
30.	0.05	14	2	30	31.49	32.30

Methods

Input data center into the Design Expert v.7. software

Data optimum one variable at a time which has been obtained previously serve as a reference.[3]. All five of data input in the software of each factor as the optimum data center. Once the required data is inputted, the obtained 30 experimental variety of data such as shown in Table 1.

Transesterification reaction

Amount of volumes (10 ml) of n-hexane is inserted into the Erlenmeyer and added amount of cottonseed oil, methanol, and the enzyme lipase in accordance with the predictions of 30 as data obtained previously by Design Expert software v.7 Each sample was incubated in a water bath shaker at a speed of 150 rpm during the period and a specific temperature. The reaction product was analyzed by using GC-MS using the parameters/ optimum condition as it has been developed by Suhendra *et al*. [8]

Statistical and data analysis

The results were analyzed with ANOVA to observe the fit model, significance, regression analysis, and lack of fit. The response surface from full factorial experiment and interaction variables were analyzed through CCRD using Design Expert v.7. The mathematical equation used to observe interactions between variables in the quadratic model is [12]:

$$Y = b_0 + \sum_{i=1}^{4} b_i x_i + \sum_{i=1}^{4} b_{ii} x_i^2 + \sum_{i=j}^{3} \sum_{j=i+1}^{4} b_{ij} x_{ij}$$

Scheme 1. The Mathematical equation for the quadratic model

RESULTS AND DISCUSSION

Data processing

The transesterification reaction results are analyzed using the GC-MS instrument to observe peak that have an equal retention time with the standard and the area produced. Data chromatogram of each trial (actual percentage yield) inputted into the Design Expert v.7 software, to obtain the predicted yield as in the following Table 1.

Based on the data in Table 2., we able to find for the suitability of the model with the assistance of ANOVA (analysis of variance). Criteria testing using an α value of 5% or 0.05. If the p-value > 0.05, the results will be significant, and if the p-value ≤ 0.05, the results were not significant. Table 3 presents statistical data based on actual results and the predicted yield. The Models were obtained by the quadratic method. The coefficient of determination or R^2 and p-value of Lack of fit is also shown in Table 3.

It can be seen that a very significant factor that was influencing the result is the amount of enzyme reactions. This finding was in agreement with Mat Radzi *et al*. [13], who states that alkyl ester production with an enzyme catalyst increases the percentage yield and reduces production costs. Furthermore, it can be observed that the model suggested that quadratic models. The model's suitability can be seen from the coefficient of determination R^2 whose value spans from zero to one. A reliable value of R^2 requires the value to be above 0.5. In the quadratic models, the R^2 value is 0.6957 (Table 3). The suitability of the model can also be seen from the Lack of fit. Lack of fit is irregularities or inaccuracies in the regression model. If the lack of fit is not significant, the model suggested is appropriate. Meanwhile, when the lack of fit is not significant, the model is not right that needs to be developed into a higher-order model. In this study p-value of lack of fit is 0.1444. This value is greater than α. So we get a significant lack of fit and show the appropriate regression model is correct. Some researchers stated that the selection of an appropriate regression model would improve the efficiency of an enzymatic reaction [14].

Table 2 Statistical Value

Statistical	Value
Standard deviation	7.25
Mean	31.03
Coefficient Varian	23.38
Press	4010.27
R-Squared	0.6957

Based on the model and ANOVA analysis were performed statistical equations second order obtained in this study are

Yield = +33.69 + 4.29 A - 0.15 B - 1.38 C - 1.55 D - 1.25 AB + 1.21 AC + 3.99 AD -1.62 BC - 0.51 BD - 0.91 CD - 3.22 A^2 - 2.32 B^2 - 1.22 C^2 + 3.44 D^2

(A = Amount of enzyme, B = Reaction Time, C = Molar ratio, D = Temperature)

Table 3. Data Significance Model

Source	Sum of Square	dF	Mean Square	F Value	p-value Prob > F	Note
Model	1805.23	14	128.25	2.45	0.0481	**Significant**
A-Enzyme amount	441.70	1	441.70	8.39	0.0111	Significant
B- Time	0.53	1	0.53	0.010	0.0215	Significant
C-Molar Ratio	45.82	1	45.82	0.87	0.3656	Not Significant
D-Temperature	57.54	1	57.54	1.09	0.3123	Not Significant
AB	25.20	1	25.20	0.48	0.4995	Not Significant
AC	23.43	1	23.43	0.45	0.5148	Not Significant
AD	254.40	1	254.40	4.83	0.0440	Significant
BC	41.93	1	41.93	0.80	0.3862	Not Significant
BD	4.10	1	4.10	0.078	0.7840	Not Significant
A^2	285.20	1	285.20	5.42	0.0343	Significant
B^2	148.06	1	148.06	2.81	0.1142	Not Significant
C^2	40.71	1	40.71	0.77	0.3930	Not Significant
D^2	325.37	1	325.37	6.18	0.0252	Significant
Residual	789.43	15	52.63			
Lack of Fit	665.16	10	66.52	2.68	0.1444	**Not Significant**
Pure Error	124.28	5	24.86			
Cor Total	2594.67	29				

The above equation shows the interaction between variables. The interaction effect of this variable is discussed later.

Table 4. Solutions with optimum condition

No.	Amount of enzyme (mg)	Time (h)	Molar Ratio (g)	Temp (°C)	Yield (%)
1.	0.15	14.00	2.05	49.99	37.87
2.	0.15	14.00	2.12	50.00	37.85
3.	0.15	14.00	2.26	50.00	37.74
4.	0.15	14.00	2.22	49.95	37.73
5.	0.15	14.00	2.09	50.00	37.86

Determination of optimum condition

Following the statistical tests performed, the software Design expert revealed a large selection condition by the equation of order 2 with a predicted yield for each condition.

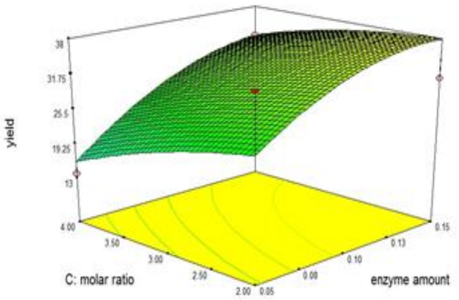
Experiments with predicting the highest percent yield on the recommended optimum condition solution have been tried to find out the actual percent yield. The actual percent yield at the optimum conditions selected was 38.24%.

In previous studies, [4] the synthesis of methyl linoleate from kapok oil under optimal conditions using the one variable at a time method produced an ester of 35.43%. An increase in methyl linoleate of 2.81% would be very significant if large-scale ester production was carried out.

The interaction between the variables was observed at the optimum conditions to produce the highest yield percent. The optimum conditions observed are the amount of enzyme of 0.15 g, the reaction time of 14 hours, the molar ratio of 1: 2.05 (oil : methanol, w/w), and 49,99°C. Following the test, it can be seen the influence of interaction among variables as will be explained below:

Interactions of enzyme amount and molar ratio

Figure 1. shown the interaction between the enzyme with a molar ratio. Percentage yield would be improved by increasing the amount of enzyme, and optimum conditions are reached on a weight of 0.15 g enzyme. Increasing the amount of lipase will lead to an increase in the transesterification reaction results if there are no limiting factors such as low concentrations of substrate, inhibitor, mass transfer effects, or the presence of an activator [15].



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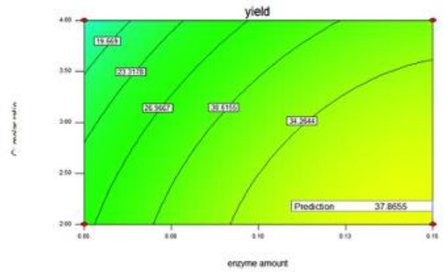

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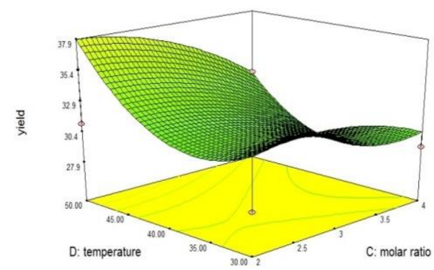
Figure 1. Response surface (a) and contour plot (b) of interactions of the amount of enzyme and molar ratio

The effect of the molar ratio of methanol to the oil of seed cotton on the transesterification reaction shows the competitive nature of methanol and fatty acids in cottonseed oil. The molar ratio of 2.05 : 1 (methanol : cottonseed oil) produced the highest percentage (37.87%). The decline in the ester yield in a higher molar ratio will damage the layer of water on the enzyme, and it can inhibit the enzyme activity [16]. The interaction between the enzyme and the molar ratio produces a positive interaction even though the molar ratio's effect on yield is negative.

Interactions of temperature and molar ratio

Figure 2 shows that the optimum conditions were achieved at a molar ratio of 1 : 2.05 and a temperature of 49.9°C. The interaction of two factors contributes to the percentage yield. The percentage yield will decrease when the temperature is lowered. Sun *et al*. [16] reported that the enzyme's optimum temperature with the type of lipozyme activity is around 40°C. The reaction temperature changes can affect the activity and stability of enzymes. It also affects the reaction rate and the percentage of reaction products. The effect of temperature can be apportioned to its effect on substrate solubility as well as its direct influences on the reaction and the enzyme [18-19].

The arrangement of temperature should be concomitant with the setting of the molar ratio. The molar ratio increase will decrease the yield percentage even though the reaction using an optimum temperature for the reasons that have been mentioned in the previous paragraph. Therefore, the interaction among temperature and the molar ratio is negative interactions.



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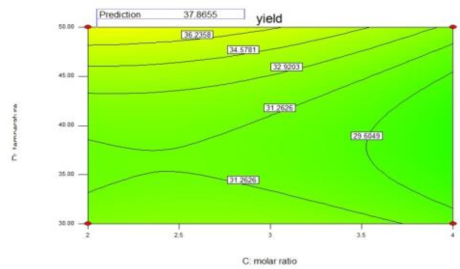
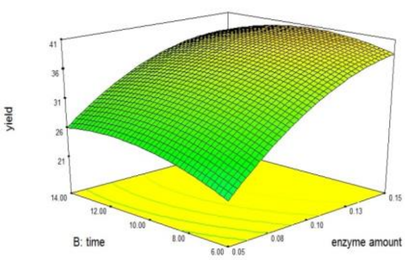


b

Figure 2. Response surface (a) and contour plot (b) of interactions of temperature and molar ratio



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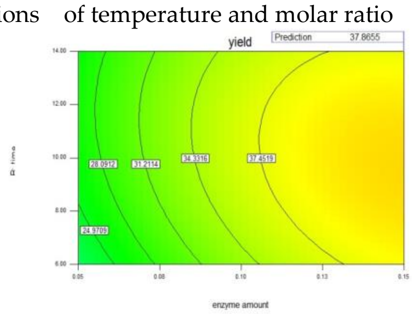

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Figure 3. Response surface and contour plot of interactions of the amount of enzyme and reaction time

Interaction of reaction time and enzyme amount

The increase in the amount of enzyme will increase the percent yield. The enzyme will catalyze the substrate more quickly and leverage, as shown in Figure 3. However, it stops at the optimum time is achieved. The reaction time is increased to make the substrate back and drive the reaction to the left and reduces the conversion results because the reaction is reversible [20].

The interaction between the reaction time and the amount of the enzyme shows that the amount of enzymes contributes more significantly. Enzymes are added to be in excess of 0.13 grams to achieve optimal results. Meanwhile, a further increase in reaction time is not significant to increase the percentage of methyl linoleic.

CONCLUSION

Quadratic models have been selected to determine the solution of the optimum condition of methyl linoleate synthesis from cottonseed oil. The interaction effects between variables have been successfully observed through the RSM method.

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