

Optimization of Glycerolysis

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Optimization of Glycerolysis of Free Fatty Acids from Cocoa Bean with MgO Catalyst Using Response Surface Methodology

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Abstract

Diacylglycerol (DAG) is one of the oil derivatives that have relatively high economic value. It has considerable prospects in the global market, which can be synthesized chemically by glycerolysis of oil/fat containing triacylglycerols. The process uses an alkaline catalyst such as sodium hydroxide (NaOH) at the temperature of 210-260 °C. The present study utilized a free fatty acid compound from cocoa bean processing waste as alternative raw material for producing DAG. The reaction system using a MgO catalyst and tert-butanol as solvent was known to be more favorable. The solvent can increase the solubility of oil in glycerol so that the reaction temperature can be lowered to 70-90 °C. This study aims to determine the effect of temperature and the ratio of FFA/glycerol on the glycerolysis process and to determine the optimized variables that result in a maximum conversion. The glycerolysis reaction was optimized with two factors using a central composite design, i.e., reaction temperature and FFA/glycerol ratio. The selected fixed variables were catalyst loading (3.5 wt%), the mass of FFA (10 grams), stirring speed (400 rpm), reaction time (4 hours), and volume of solvent (20 mL). The optimization process was evaluated using the response surface method, which shows that the optimum condition was achieved at a FFA/glycerol ratio of 5 g/ml and a reaction temperature of 90 °C. The experiment carried out under these optimum conditions resulted in 97.5% conversion, while the two-order polynomial model developed using RSM was able to predict the conversion of 96.7% under the same condition.

17 INTRODUCTION

Cocoa beans are seeds from the fruit of the *Theobroma cacao* L. tree, which contains cocoa butter, the main vegetable fat used by chocolate manufacturers (Simplice et al., 2008; Naik & Kumar, 2014). Crude cocoa butter is produced by pressing the cocoa bean. To comply with the standard, cocoa butter must contain a small amount of free fatty acid (FFA) and must be free of foreign, moldy, or rancid flavors to be acceptable on the market (Calliauw et al., 2008). Free fatty acids are known as carboxylic acids released from triglycerides due to the effect of oxidation or the enzymatic activity of lipase. Higher FFA content has been observed due to the effect of storage

management, in which its content in cocoa beans increases with storage time (Jonfia-Essien & Navarro, 2010). As investigated by Guehi et al. (Simplice et al., 2008), higher content of FFA in cocoa beans causes a serious quality defect. It reduces the technical and economic value of cocoa butter. For quality reasons, the maximum accepted level of FFA content is 1.75% oleic acid equivalent in the cocoa butter (EEC, 1973).

The excessive amount of FFA content in the cocoa beans can be removed by refining using injected steam at temperatures between 170 °C and 260 °C (Calliauw et al., 2008). This process results in a large amount of FFA waste, which should be utilized to produce more valuable products, such as diacylglycerols (DAG). This compound can be used

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13 for the production of emulsifiers, stabilizers, and carriers in the food, cosmetics, and pharmaceutical areas (Feltes et al., 2012; Liu et al., 2016). DAG plays a significant role in edible oil products; therefore, it is highly expected for economic, efficient, and eco-friendly production. DAG is commonly produced chemically through glycerolysis between oil/fat (triacylglycerols) and glycerol at 210-260 °C in the presence of various alkaline catalysts, such as sodium hydroxide (NaOH), potassium hydroxide (KOH), sodium methoxide (NaOCH₃), and potassium acetate (KC₂H₃O₂) (Satriana et al., 2016; Zhang et al., 2016). The production process at high temperature is unfavorable, therefore the possibility of DAG production through chemical glycerolysis at low temperatures is of great interest.

9 The enzymatic process through lipase-catalyzed glycerolysis is a potential alternative to the high-temperature chemical process usually applied at an industrial scale for DAG production (Kristensen et al., 2005; Feltes et al., 2012; Phuah et al., 2015). The enzymatic process offered attractive features, such as mild reaction conditions, selectivity, and green reaction systems. However, enzymes are expensive for industrial-scale production and a multi-step process is required to achieve a high conversion (Satriana et al., 2016). A study has been conducted for developing a heterogeneous catalytic reaction for diacylglycerols (DAG) production at low temperatures using the MgO-supported KOH (KOH-MgO) (Zhong et al., 2014). This catalyst was effective for low-temperature chemical glycerolysis. The catalytic system was applied for the glycerolysis of soybean oil in acetone at a reaction temperature of 80 °C. Another study was conducted to produce DAG through chemical glycerolysis using NaOH catalyst in acetone as the solvent medium at low temperature of 50 °C. The reaction system resulted in a total of 52.0 ± 2.2% DAG formation for 4 h (Zhong et al., 2010).

The main concern in the glycerolysis process carried out at low temperatures is the poor miscibility between the substrates, i.e., triacylglycerol (TAG) and glycerol, which leads to low mass transfer and overall reaction rate. To increase the miscibility between substrates, a proper solvent can be used. An indicator of the nature of the solvent can be expressed by log *P*, which describes the ratio of the equilibrium concentration of the compound in the 1-octanol-rich

(hydrophobic) phase to the concentration in the water-rich (hydrophilic) phase where those compounds are in equilibrium. The polarity of the solvent can be indicated by the values of log *P*. Solvents with the non-polar structure are shown by the highest value of log *P*, while the compounds with the lowest or negative log *P* value are highly polar. Solvents with small log *P* values are effective for the formation of a homogeneous glycerolysis reaction system, resulting in a higher product yield (Damstrup et al., 2006; Zhong et al., 2010).

The present study aims to produce DAG through chemical glycerolysis of free fatty acid (FFA) from cocoa beans by using MgO as the catalyst. The heterogeneous catalytic system may ease the separation process of the catalyst from the reaction mixture, thus improving process efficiency and reducing production costs. Furthermore, to overcome the poor miscibility between glycerol and fat/oil at low temperature, tert-butanol (with log *P* of 0.584) were introduced to the bulk reaction substrates. The effect of reaction temperature and FFA/glycerol ratio was also investigated.

MATERIALS AND METHODS

Materials

Magnesium Oxide (MgO) purchased from Indrasari (Semarang, Central Java, Indonesia) was used for DAG synthesis. Free fatty acid (FFA) of cocoa beans was a solid waste generated by BT Cocoa (Tangerang, Banten, Indonesia). Tert-Butanol was purchased from Happy Lab (Semarang, Central Java, Indonesia).

Experimental Design

Central Composite Design (CCD) with a total of 9 experiments (Table 1) was applied in this study. The variables and their levels were reaction temperature (R_{temp} , 70-90 °C) and substrate ratio (*S_r*, 3-5 wt/vol FFA/glycerol). The response was DAG conversion (X_{DAG} , %). This design was generated by the use of the software Design-Expert version 10.

Preparation of MgO catalyst

MgO was mixed with distilled water for 3 hours until a homogeneous mixture was formed. Then, the solid catalyst was filtered out and dried in an oven overnight. The calcination procedure at 900 °C was applied to the dried catalyst for 5 hours.

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Table 1. Experimental Runs for Central Composite Design (CCD) and the comparison between Observed and Predicted Responses for DAG conversion.

No.	R_{temp} , °C	S_r , wt/vol FFA/Glycerols
1	70	3
2	70	4
3	70	5
4	80	3
5	80	4
6	80	5
7	90	3
8	90	4
9	90	5

R_{temp} = reaction temperature (°C);

S_r = substrate ratio (wt/vol FFA/Glycerols)

Glycerolysis Reaction.

The reaction was carried out in a three-neck bottle equipped with a reflux condenser as shown in Figure 1. The typical reaction condition applied were total reaction mixture of 300-gram, 400 rpm stirring speed, 4 hours reaction time, 3.5 wt% catalysts, and 20 ml of tert-butanol as solvent for every 10 grams of fat. While the ratio of FFA/glycerol and reaction temperatures varied according to the design of the experiment (Table 1). After 4 hours of reaction, the solid catalyst was filtered out from the reaction mixture. Subsequently, the DAG-containing product was separated from unreacted glycerol by decantation. The product analysis was conducted using column chromatography to identify the presence of monoglyceride, diglyceride, and triglyceride.

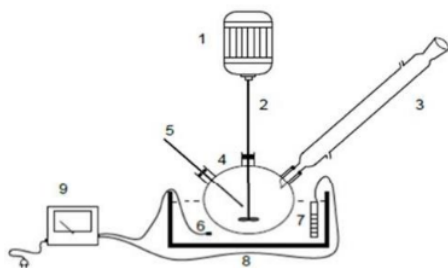


Figure 1. Scheme of reaction set-up: 1-overhead stirrer, 2-stirrer, 3-reflux condenser, 4-three neck bottle, 5-thermometer, 6-thermocouple, 7-heater, 8-heating bath, 9-temperature-controller.

DAG Composition Analysis

The obtained DAG-containing product was dissolved in 0.1 ml chloroform, then applied on the thin layer chromatography plate as a circle spot. After all the sample spots were applied, the TLC plate was eluted using a mixture of petroleum ether, diethyl ether, and glacial acetic acid in a ratio of 70:30:0.2 (v/v), which have been previously saturated in the chamber. The time required for the elution process was ± 1 hour. Then, the plate was removed from the chamber and allowed to stand for a few minutes until the remaining steam was gone. The light staining was done by spraying the plate with 2,7-dichlorofluorescein, which was subsequently observed under UV. The presence of different compounds in the sample was identified by the retention factor (Rf) value, which can be determined according to the following Eq. (1).

$$Rf = \frac{\text{distance traveled by solute}}{\text{distance traveled by solvent front}} \quad (1)$$

8 Compounds with higher Rf value have lower polarity and vice versa because the stationary phase is polar. More polar compounds will be strongly retained in the stationary phase, resulting in a lower Rf value.

28 Statistical Analysis

The experimental data were analyzed by RSM using the software Design-Expert version 10.1. Model fitting to equations of up to the two-order polynomial was performed to determine the goodness-of-fit. The responses were fitted to the variables by multiple regression. The quality of fit of the model was evaluated by the coefficients of determination (R^2 and adjusted R^2) and the analysis of variance (ANOVA).

RESULTS AND DISCUSSION

FFA raw materials

The analysis shows that the raw material composition consisted of 91.25 % free fatty acid and 0.02 % water content. Further analysis using GC-MS showed the presence of different compounds in the FFA, as can be seen in Table 2.

The fatty acid composition of the raw material is rich in palmitic acid, methyl stearate, oleic acid, and stearic acid. These results are comparable to the previous identification of the

fatty acid composition of cocoa butter as in references (Lima et al., 2011; Naik & Kumar, 2014).

Table 2. Composition of different compounds in the raw material.

No.	Relative concentration (%)	Compounds
1	49.24	Palmitic acid
2	1.05	Methyl stearate
3	25.39	Oleic acid
4	24.32	Stearic acid

Analysis of DAG using Thin Layer Chromatography (TLC)

This method was used to separate a mixture of oils and fats with different polarities in an elution. The eluent used in this study was a mixture of petroleum ether, diethyl ether, and glacial acetic acid. Each fraction of components in the mixture can be separated based on its polarity using this eluent. The more non-polar fraction will be eluted first, while the more polar fraction will be retained longer on the polar stationary phase. The result of TLC analysis of the DAG-containing product is presented in Figure 2.

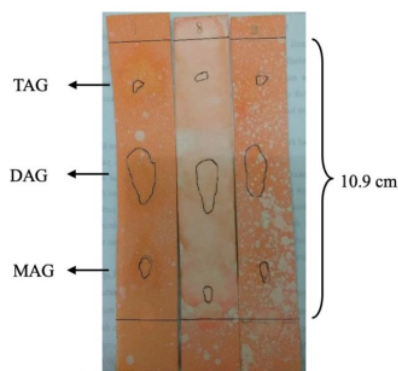


Figure 2. Separation process of Diacylglycerol using Thin Layer Chromatography method.

Triacylglycerol (TAG) is a fraction that is more non-polar than the other oil constituents, i.e., Monoacylglycerol (MAG) and Diacylglycerol (DAG) fractions. Therefore, during the elution process, TAG will be eluted on the top of the TLC plate, followed by DAG and MAG, respectively. The composition of the components on the reaction product was determined using the retention factor

(Rf). The Rf values of the experimental result are presented in Table 3.

Table 3. Rf value of experimental result.

No	Compound	Experimental Rf		
		1 st sample	2 nd sample	3 rd sample
1	MAG	0.22	0.16	0.21
2	DAG	0.51	0.50	0.51
3	TAG	0.80	0.82	0.80

The component of each sample point can be determined according to their location, where substances can be completely separated using thin layer plate of 10.9-cm length. Similar sample points presented by previous studies (Fuchs et al., 2011; Li et al., 2011) was used to determine of TAG, DAG, and MAG in the present study. The compound with higher polarity will adhere to the polar adsorbent (stationary phase) and the shorter the distance from the baseline, thus the lower the Rf value (Li et al., 2018). From the resulting Rf value of different components presented in Table 3, MAG is more polar than DAG and TAG. Whereas TAG has the highest Rf value, it is a non-polar compound and will be eluted first. The analysis result using TLC shows that the composition of DAG is dominant compared to other constituents, i.e., TAG and MAG. It can be seen from the larger spot area generated from DAG elution on the TLC plate.

Optimization of Diacylglycerol using Response Surface Methodology (RSM)

The experiment results from glycerolysis reaction with varying parameters, i.e., R_{temp} and S_r , using Central Composite Design (CCD) are presented in Table 4.

The fitting of the regression model and the significance of the estimated variables were determined by Analysis of Variance (ANOVA). Several values affect the fitting between the experimental results and the predicted model as tabulated in the ANOVA result. The model fits to the experiment if the p-value is less than alpha ($\alpha = 0.05$). The alpha value is the error value allowed in the experiment; the smaller the chance of error, the greater the value of confidence in the decision. Furthermore, the model fits the experiment if the lack of fit (LoF) value is greater than alpha ($\alpha = 0.05$), thus the value is significant. The lack-of-fit is

Table 4. The experiments and the predicted conversion of glycerolysis reaction following central composite design experimental method.

No.	R_{temp} , °C	S_r , wt/vol FFA/Glycerols	X_{DAG} (%)
1	70	3	62.5
2	70	4	80
3	70	5	95.8
4	80	3	57.5
5	80	4	77.5
6	80	5	90
7	90	3	72.5
8	90	4	82.5
9	90	5	97.5

Table 5. Analysis of Variance (ANOVA) of the experiment data.

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F
Model	1524.71	5	304.94	97.40	< 0.0001
A-suhu	33.61	1	33.61	10.73	0.0361
B-rasio	1374.11	1	1374.11	438.88	< 0.0001
AB	17.22	1	17.22	5.50	0.0514
A ²	98.06	1	98.06	31.30	0.0008
B ²	6.56	1	6.56	2.10	0.1910
Residual	21.92	7	3.13		
Lack of Fit	21.92	3	7.31		
Pure Error	0.000	4	0.000		
Cor Total	1546.63	12			
R-Squared	98.58%				

the value of unfitting of the model. The evaluated ANOVA result is shown in Table 5.

From the analysis result shown in Table 5, the p-value in the model is < 0.0001, which is lower than the degree of significance ($\alpha = 0.05$), indicating that the variable has a significant impact on the model. The correlation coefficient (R^2) was used to express the quality of the conformity of the polynomial model (Buchori et al., 2020). The results of the analysis of variance on diacylglycerol production gave an R^2 of 98.58%. The p-value and F-value determine the significance of each coefficient. The larger the F-value and the smaller the p-value, the more significant the coefficient on the model. ANOVA shows that the regression model is statistically good with a significant level of $P < 0.0001$ and the model had no significant ($P > 0.05$) lack of fit (Cheong et al., 2007). The parameter of B (the glycerol ratio) is the most influential variable because it has the largest F-value and the smallest p-value, while A (temperature), AB, A², and B² have less effect on the conversion.

The obtained mathematical model is shown in Eq. (2). The actual and predicted conversion of DAG is shown in Figure 3.

$$Y = 76.87 + 2.37A + 15.13B - 2.07AB + 5.96A^2 - 1.54B^2 \quad (2)$$

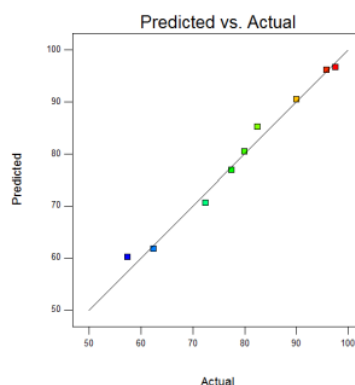


Figure 3. The actual (symbol) vs predicted (solid line) conversion of DAG.

Table 6. Calculated value of DAG conversion based on a mathematical model developed using RSM method.

Exp. run	Temperature (°C)	FFA to Glycerol ratio	$X_{DAG, predicted}$ (%)
1	70	3	61.711
2	70	4	80.461
3	70	5	96.128
4	80	3	60.194
5	80	4	83.918
6	80	5	90.461
7	90	3	70.595
8	90	4	85.194
9	90	5	96.711

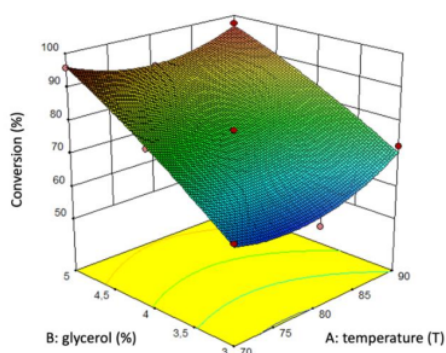


Figure 4. Surface response of the optimized parameters on the DAG production.

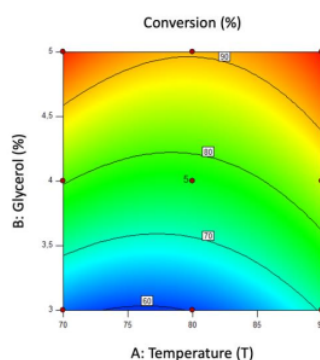


Figure 5. Contour plot of optimized parameters on the DAG production.

Figure 3 shows a linear relationship between the actual and the calculated values so that the mathematical model matches the experimental result satisfactorily. The calculation result based on the developed mathematical model is presented in Table 6.

The surface and contour responses show the optimized parameters on the DAG production (Figure 4 and Figure 5).

The red area of the graph shows the highest response of 97.5%, while the blue area shows the lowest response of 57.5%. Points that lie on the same curved line have the same response value. The optimized conditions with the highest conversion of all experimental data can be achieved by the reaction at 90°C and the ratio of FFA to glycerol 5 g/ml. While the prediction, according to the developed model, resulted in a conversion of 96.71%.

CONCLUSION

The present study was conducted to optimize the production of diacylglycerol (DAG) from free fatty acid, the waste generated from cocoa

beans processing. The MgO catalyst promoted the reaction in the tert-butanol solvent at the relatively mild reaction temperature of 70-90 °C. The experimental result shows that the highest conversion up to 97.5% can be achieved from a reaction at 90°C with a substrate ratio (FFA to glycerol ratio) of 5 g/ml. The optimization using the RSM method resulted in a good prediction for DAG production. The developed mathematical model predicted the DAG conversion of 96.7% at optimized conditions.

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