

THE CONVERSION ENHANCEMENT OF LAURIC ACID TO N-ACYL LYSINE CATALYZED BY CALCIUM OXIDE USING BOX BEHNKEN DESIGN

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ABSTRACT

N-acyl lysine is a surface-active substance that can reduce interface tension between oil and water. N-acyl lysine was synthesized by calcium oxide amidification of lauric acid and lysine. The study aims to increase lauric acid conversion by observing the effect of substrate ratio, catalyst amount, and solvent ratio, through the preparation and observation of mathematical models using Box Behnken Design and Response Surface Methodology. Optimal conditions are observed in the ratio of lauric acid to lysine 1:2; 1:3; 1:4, catalyst amount 3%; 5%; 7%, and a solvent ratio to lauric acid 1:1; 1:2; 1:3. In this optimal condition, the conversion of lauric acid will increase by more than 80%. The variable amount of catalyst showed a more significant effect in increasing lauric acid conversion. The results showed that all three variables affect the percent conversion with an R² of 90.32%.

Keywords: lauric acid, lysine, calcium oxide, response surface methodology.

INTRODUCTION

Pharmaceutical products that are difficult to dissolve in water can be obtained by adding surfactant products, which aim to reduce the interface tension between the media and drugs and increase the solubility of drugs. In medicinal drinks, surfactants are used as solubilizers to dissolve herbal medicinal ingredients, vitamin E, and other oil ingredients [1, 2, 3].

Surfactants are generally synthesized from petroleum, but, due to their increasing needs, research on surfactants made from vegetable oils has been developed. Surfactants from vegetable oils are biodegradable; in addition, their availability can be sustainable because they are renewable natural resources [4, 5, 6].

One of the surfactants obtained from vegetable oils and their derivatives is N-acyl lysine surfactant, which is obtained from the amidification reaction between lauric acid and lysine. Lauric acid is one of the potential fatty acids, because it is an inexpensive raw material, easy to regulate its reaction conditions, and can produce high purity products [7, 8].

Amino acids have at least two functional groups, namely the carboxylic group and the amino group. These amino acids can be converted into single-chain surfactants with reactive molecules containing hydrophobic chains, such as fatty acids, fat esters, fat amines, and fatty alcohols. Amino acids with reactive side-chains such as lysine or arginine have additional opportunities for the molecular design of surfactants. They are very interesting compounds because they can be synthesized easily [5, 9]. Amino acids are much more flexible concerning the type of surfactants that are capable of being produced. Many of the 20 amino acids used in protein biosynthesis have been explored as surfactant feedstocks. However, the production of several amino acid-based surfactants is still relatively small [4].

Amino acid-based surfactants such as N-acyl lysine are an attractive alternative to conventional

synthetic surfactants. They contain several fundamental requirements for industrial development, namely multifunctionality, low toxicity, renewable raw material sources, biodegradability, and simple synthesis [9].

Metal oxides can be utilized as heterogeneous base catalysts such as CaO, MgO, ZnO, ZrO, and CuO. The nature of metal oxides is beneficial as a catalyst, but its availability is quite expensive. Metal oxides such as CaO, MgO have been widely used as catalysts for biodiesel production. The base catalyst of this metal oxide group has a heterogeneous system [10]. One of the most widely used catalysts is CaO because it has many advantages such as low prices, long catalyst life, high activity, and only requires moderate reaction conditions [11].

Calcium oxide, because it is heterogeneous, easily separated from the reaction mixture, can be recycled, and no neutralization process is needed to remove the remaining catalyst. Besides, this catalyst is also cheap, non-toxic, and widely available [12].

Previous studies reported that the yield was resulting from the sulfonation process, between palm oil methyl ester and sodium. The yield of MES surfactant products produced tends to increase with the use of CaO catalysts. It was also reported that the surfactant formation of sodium n-fatty acyl amino acid derived from dried cocoons of silkworms was degraded to fatty acids, amino acids, and other materials. The study was carried out on the ratio between amino acids and fatty acid 3:1, temperature 25°C, and 30 minutes [13].

The response surface methodology (RSM) lately is often used to model and analyze the influence between variables, so that interactions between variables can be observed to optimize the response [14, 15]. Through RSM, the value of independent variables that cause the value of the response variable to be optimal can be known [16, 17]. Designs that are often used in research include Central Composite Design (CCD) and Box-Behnken Design



(BBD). One of the advantages of the Box-Behnken design is that it is more efficient with fewer trial runs, especially for experiments with 3 or 4 factors [14, 18].

MATERIALS AND METHODS

Materials

Lauric acid, lysine, calcium oxide, isopropanol, and n-hexane were purchased from E. Merck, Darmstadt Germany. All other chemicals and solvents used in this study were of analytical grade.

Methods

The study design was carried out using Box Behnken Design, which is shown in Table-1. The selection of this run was carried out using the Minitab 17 trial version software with Response Surface Methodology. The design in Table-1 is used as a combination of independent variables, and data collection is done in the laboratory by conducting experiments following the design. Observation starts by mixing lauric acid and lysine in various substrate ratios according to the experimental design in 250 mL vials. Iso-propanol and hexane as the mixed solvent are added at a ratio of 1:1; 1:2; and 1:3 (w: v lauric acid:mix solvent). Different amounts of calcium oxide were subsequently added, with amounts of 3%, 5%, 7% w/w lauric acid. The reaction was performed in a controlled temperature horizontal water bath shaker at 250 rpm at different temperatures and for different periods generated by RSM.

The mixture is then separated from the catalyst by filtering, and the solvent is evaporated. The reaction was terminated by dilution with 5 mL of 10% citric acid, and the catalyst was removed by filtration. Products that are mixed with the solvent are separated by evaporating the solvent at 90°C. Products containing excess lysine are then washed with acetone, twice the volume of the product mixture. Excessive lysine will dissolve with acetone as the top product, while the final product will be obtained as the bottom layer [19].

The percentage of actual lauric acid conversion obtained is given in Table-2. From this data, analysis of variance, regression model predictions, and model verification tests are performed to obtain the maximum percent of lauric acid conversion and determine the effect of interactions between variables [20].

Table-1. Variables and levels developed using Box-Behnken design.

Variable	Code Levels of Variables			
variable	-1	0	1	
Substrate Ratio (Lauric Acid:Lysine)	1:2	1:3	1:4	
Catalyst Amount (w:wLauric Acid)	3	5	7	
Solvent Ratio (wLauric Acid:vSolvent)	1:1	1:2	1:3	

Table-2. The optimization results of the conversion of lauric acid.

Experiment no.	Substrate Ratio (X1)	Catalyst Amount (X2)	Solvent Ratio (X ₃)	Lauric Acid Conversion (Y)
1	-1	-1	0	79.25
2	1	-1	0	79.72
3	-1	1	0	78.85
4	1	1	0	80.04
5	-1	0	-1	77.03
6	1	0	-1	78.30
7	-1	0	1	84.66
8	1	0	1	87.91
9	0	-1	-1	78.00
10	0	1	-1	77.45
11	0	-1	1	85.07
12	0	1	1	79.80
13	0	0	0	81.15
14	0	0	0	81.12
15	0	0	0	81.19



RESULTS AND DISCUSSIONS

There are three variations of variables chosen to increase the acquisition of N-acyl lysine surfactants in this study, namely the ratio of the substrate, the amount of catalyst, and the solvent ratio. The substrate, in this case, as a reactant, based on lauric acid (LA) and lysine amino acid, with a calcium dioxide catalyst and as a solvent mixture, is isopropanol-hexane. The substrate ratio is the ratio between lauric acid and lysine notated with X₁, X₂ denotes the weight of the CaO catalyst, and X₃ denotes the solvent ratio. The study results in conversion of each combination of levels from Table-2 shows that the best optimization results are found in the fourth run with the condition of substrate ratio of 1:4 (w:w LA:lysine), the solvent ratio of 3:1 (v mix solvent: w lauric acid) and 5% of catalyst amount (w:w LA), produced a conversion percentage of 87.91%.

A total of 15 research data treatments were analyzed using Response Surface Methodology. The response variable in this synthesis is the percent conversion of lauric acid, and the predictor variables are the substrate ratio, the amount of catalyst, and the solvent ratio. The form of the relationship between these variables can be determined by regression analysis [16]. The equations obtained are then tested with an analysis of variance (ANOVA). ANOVA tests the acceptability of the regression model from a statistical perspective in the form of diversity analysis [18]. The initial stage in the Response Surface Methodology is to predict the regression model and proceed with the variance analysis and then proceed with the model verification test [15].

Model Prediction

The regression model aims to determine the relationship between percent conversion of lauric acid (Y) with substrate ratio (X₁), amount of catalyst (X₂) and solvent ratio (X₃), and to optimize the response. In this research, the α value is determined as the level of significance indicating the permissible error. The permissible error is one minus confidence level. The confidence level used is 95%, so a value of $\alpha = 0.05$ is

obtained, meaning that the study results have a chance to correct 95% and a maximum error tolerance of 5%. P test values are used to test whether or not the relationship between variables is significant. The significance factor used is $\alpha = 0.05$. The variable is significant if the value of $P \leq \alpha$ [17].

It can be seen that the p-value for the substrate ratio, the amount of catalyst, and solvent ratio are 0.828, 0.106, and 0.693, respectively. Based on Table-3, no variable has a P-value of less than α (5%), so it can be seen that there is no statistically significant factor, but the amount of catalyst (X₂) has the smallest P-value.

After determining the P-value, then an equation model is developed that is useful to show the relationship between the reaction variable and its interaction with the percent conversion of lauric acid. The following two order equation models are obtained at X_1 , X_2 , X_3 , respectively, the substrate ratio, the amount of catalyst, and the solvent ratio. Regression equation in un-coded units:

- Conversion = 54.2 3.1 Substrate Ratio
- + 4.08 Catalyst Amount 0.96 Solvent Ratio
- + 0.103 Substrate Ratio*Substrate Ratio
- 0.1120 Catalyst Amount*Catalyst Amount
- + 0.718 Solvent Ratio*Solvent Ratio
- + 0.045 Substrate Ratio*Catalyst Amount
- + 0.495 Substrate Ratio*Solvent Ratio
- 0.295 Catalyst Amount*Solvent Ratio

There are negative and positive signs in the above equation, where the positive sign shows a significant influence on the conversion compared to other experimental variables. In contrast, the negative sign in the equation shows a relationship that is inversely proportional to the positive sign towards the conversion. The coefficient value of X_2 in the equation is the most significant coefficient value, which is 4.08, which means the coefficient value of X_2 gives the most significant influence on the conversion.

Term	Effect	Coef	SE Coef	T-Value	P-Value	VIF
Constant (Y)		65.0	13.1	4.95	0.004	
Substrate Ratio (X ₁)	-2.57	-1.29	5.64	-0.23	0.828	99.50
Catalyst Amount (X2)	10.04	5.02	2.55	1.97	0.106	81.17
Solvent Ratio (X ₃)	3.85	1.92	4.60	0.42	0.693	66.17
$(X_1)^*(X_1)$	0.207	0.103	0.832	0.12	0.906	79.01
$(X_2)^*(X_2)$	-0.896	-0.448	0.208	-2.15	0.084	55.18
$(X_3)^*(X_3)$	1.437	0.718	0.832	0.86	0.428	35.68
$(X_1)^*(X_2)$	0.180	0.090	0.400	0.23	0.831	31,50
$(X_1)^*(X_3)$	0.990	0.495	0.800	0.62	0.563	27.00
$(X_2)^*(X_3)$	-1.180	-0.590	0.400	-1.48	0.200	21.50

Table-3. The prediction of regression coefficient on coded coefficients.

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Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	119.345	13.2606	5.19	0.042
Linear	3	11.212	3.7374	1.46	0.331
Substrate Ratio (X ₁)	1	0.133	0.1333	0.05	0.828
Catalyst Amount (X ₂)	1	9.937	9.9369	3.89	0.106
Solvent Ratio (X ₃)	1	0.448	0.4476	0.18	0.693
Square	3	14.696	4.8987	1.92	0.245
$X_1 * X_1$	1	0.039	0.0394	0.02	0.906
$X_{2}^{*}X_{2}$	1	11.853	11.8526	4.63	0.084
X ₃ *X ₃	1	1.905	1.9052	0.74	0.428
2-Way Interaction	3	6.679	2.2264	0.87	0.515
$X_1 * X_2$	1	0.130	0.1296	0.05	0.831
X1*X3	1	0.980	0.9801	0.38	0.563
$X_{2}*X_{3}$	1	5.570	5.5696	2.18	0.200
Error	5	12.787	2.5575		
Lack-of-Fit	3	12.785	4.2617	3455.39	0.000
Pure Error	2	0.002	0.0012		
Total	14	132.133			

Table-4. Analysis of variance for the synthesis of N-acyl lysine.

The coefficient of determination (R^2) from the analysis of 90.32% shows that the independent variables in the experiment affect the dependent variable (% conversion) by 90.32%.

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Analysis of Variance

Analysis of variance is used to check the significance of the regression models obtained [14]. The results of the analysis of variance showed that the substrate ratio, the amount of catalyst, and the solvent ratio, affect the increase in lauric acid conversion. The influence of the three variables is shown in Table-4, in the form of the value F. F test is a simultaneous test or test model, where the F test can be seen the influence of independent variables together on the dependent variable.

Comparison of MS (mean square) regression with MS residual is a way to calculate the value of the test F. Where compared the value of F count with F table. The relationship of variables will be significant if F arithmetic is higher than the F table (F arithmetic > F table). The results of the F test in Table 4 on the F count are 3455.39, and the F value of the table with degrees of freedom $1 (N_1)$ = k - 1 = 3 and degrees of freedom 2 (N₂) = n - k = 16, which is 3.24. $F_{count} = 3455.39 > F_{table} = 3.24$ and concluded that there is a significant relationship between variables with the response so that the regression model is accepted.

The variation of squared conversions, in the form of SS value (sum of squares), is 132.133. Variation of percent conversion came from predicted variables of 119.345 (regression), and the remaining 12.787 came from

other variables, which also affected the conversion but were not included in the model (residual). If we compare the SS regression with the total SS, we will get a proportion of the total conversion variations caused by variations of the predicted variables. This comparison value is called the coefficient of determination (R^2) .

The results of the analysis of variance also indicate that the P-value of the linear model is 0.331, quadratic model (square) of 0.245, and non-linear models that include interactions between factors (2-way interaction) is 0.515. The p test value is used to test whether or not the relationship between two variables is significant.

The quadratic equation model compiled has a significance level of $\alpha = 0.05$ (or 5%). It means that linearly, quadratic (square), and non-linear (2-way interaction), which includes interactions between factors; independent variables do not contribute significantly to the model formed. Besides, the normal probability test can also be seen through the value of a lack of fit. The lack of fit test is shown through the ANAVA table where the Pvalue for the lack of fit test is 0.000. With a hypothesis:

 $H_0 =$ no lack of fit if P> α (model matches) H_1 = there is a lack of fit, if P < α (models do not match)

It can be concluded that the p-value is smaller than α (0.05), so H₀ is rejected, and H₁ is accepted, which means there is a discrepancy in the model.

Model Verification Test

The assumptions commonly taken in the analysis of variance are the assumption of normality, the assumption of homoscedasticity, and the assumption of independence.



Figure-1. Normal residual probability graph.



Figure-2. Residual plots with fitted values in the N-acyl Lysine Synthesis.



Figure-3. Residual plots with order model in N-acyl lysine synthesis.



Figure-4. Histogram of normal distribution with the conversion.

The suitability of the residuals with the assumptions required is checked to utilize a model verification test. One way to determine the normality assumption, as Figure-1, is to use the Kolmogorov-Smirnov (KS) test. This KS test was performed using a significance value (α) = 0.05. Based on Kolmogorov Smirnov statistical data, the value of 0.294 (two-way test) was obtained for α = 0.05, and the number of observations was 15. At the same time, the observations made obtained the statistical value of Kolmogorov's statistical value, so it was concluded that the residual linear regression model that was made had followed the normal distribution because of KS <KS_{1- α}.

The homoscedasticity test knows the homogeneity of the variance of N-acyl lysine conversion data in Figure-2. Residual plots with fitted values (estimated models) that show indicate that the data distribution does not form a specific pattern and tends to be random. From this homogeneity of variance plots, it can be concluded that the assumption of homogeneity of variance (homoscedasticity) can be fulfilled.

To determine whether the independent variables are interconnected or correlated, independence assumptions are used. Moreover, to check the residuals with the order model in the synthesis of N-acyl lysine used Figure-3. If the distribution of residual versus sequence data tends to be random and not patterned, as shown in Figure-3, it can be concluded that the assumption of independence is fulfilled.

In addition to the three assumptions required, a standard distribution test is also carried out to ascertain whether the study data are typically distributed. Figure-4 shows the referred histogram, which shows that the experimental data are typically distributed, marked by the shape of the histogram that resembles a bell.

Analysis of Influence of Variables

The contour response plot on the observation of the effect of the interaction between the ratio of the substrate and the amount of catalyst to the conversion of N-acyl lysine produced is shown in Figure-5. The results of the observation show that the higher the ratio of the substrate and the amount of catalyst will increase the

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conversion of N-acyl lysine, and then it will decrease. The amount of substrate is quite influential in increasing the conversion of N-acyl lysine. The higher the number of substrates, the higher the frequency of collisions between the reacting molecules [8].

The data obtained in Figure-5 shows that the increase in N-acyl lysine conversion is sharper as the substrate ratio increases compared to when the catalyst increases. The results of optimization with the number of substrates in a ratio of 1: 4 (LA: Lysine) and the amount of catalyst 5% (w:w LA) produced the best conversion of 87.91%. When viewed from a contour plot with the comparison, it is in an area with a conversion more than 82.0%.

Lauric acid dissolves in polar solvents, for example, water also dissolves in fat because of the hydrocarbon (methyl) group at one end and the carboxyl group at the other. Lauric acid also dissolves in organic solvents such as alcohol.



Figure-5. The interaction effect of substrate ratio and catalyst amount.



Figure-6. The interaction effect of substrate ratio and solvent ratio.

The use of fatty acid methyl esters of fatty acids has several advantages, including materials used cheaply for their manufacture, the final product has a high level of purity, and natural regulation of synthesis conditions [7]. The interaction between the substrate ratio and solvent ratio also influences the increase of N-acyl lysine conversion. Figure-6 shows the contour response plot in observing the effect of the interaction between the two to the conversion of N-acyl lysine produced. When viewed from a contour plot with the comparison is in an area with a conversion of more than 80%. The contour plot in Figure 6 shows that the increase in N-acyl lysine conversion is more dominant as the solvent ratio increases. From the optimization results, the ratio of solvents is at a ratio of 1:3 (S: MS), and the number of substrates is at a ratio of 1:4 (LA: Lysine), resulting in the best conversion of 87.91%.

Solvents are needed to increase the transfer of substrate and catalyst to collide with each other, thereby increasing the N-acyl lysine formed. The hexaneisopropanol mixture solvent functions to dissolve the two substrates with different properties.

Amino acids are more soluble in polar alcohol solvents, while fatty acids are more soluble in non-polar solvents such as hexane [19]. So it was concluded that the reaction using this mixed solvent would produce the best reaction with a high percent yield, as well as an increase in the ratio of solvents more dominant in increasing the conversion of lauric acid to N-acyl lysine.

The effect of the interaction of the amount of catalyst and the ratio of the solvent to the conversion of N-



acyl lysine in Figure-7 shows that the catalyst amount of 5% and the ratio of the solvent is at a ratio of 1: 3 (MS: S) resulting in the best conversion of more than 88%. It is also seen that N-acyl lysine conversion will increase as the amount of catalyst and solvent ratio increases. A large solvent ratio will cause the substrate to dissolve more efficiently, which will also increase the conversion of Nacyl lysine produced.

In general, an increase in the amount of catalyst will increase N-acyl lysine that is formed. This is because the CaO catalyst used will increase the amount of collision between lauric acid and lysine [11].



Figure-7. The interaction effect of catalyst amount and solvent ratio.

Increasing the amount of collision can be expected to accelerate the amidification reaction by decreasing the activation energy so that more lauric acid binds to lysine to form surfactant N-acyl lysine [10, 12].

CONCLUSIONS

The best of N-acyl lysine production was obtained with a ratio of the lauric acid substrate with Llysine 1: 4. The amount of catalyst (CaO) of 5% of the ratio of lauric acid to solvent (mix solvent) 1: 3.

This study uses a design according to the BBD (Box-Behnken Design) model and gives an R^2 of 90.32%.

Of the three variables used, the amount of catalyst provides a more decisive factor in the synthesis of N-acyl lysine surfactants. The P-value for the solvent ratio is the smallest compared to the P-values of the other two variables.

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REFERENCES

- [1] Colomer A., Pinazo A., Garcia M. T., Mitjans M., Vinardell M. P., Infante M. R., Martinez V. and Perez L. 2012. pH-sensitive surfactants from lysine: assessment of their cytotoxicity and environmental behavior. Langmuir. 28: 5900-5912.
- [2] Szczesniak P., Agnieszka P. H., Klimczak U. and Stecko S. 2014. Synthesis of b- and g-hydroxy aamino acids via enzymatic kinetic resolution and cyanate-to-isocyanate rearrangement. The Journal of Organic Chemistry. 81: 380-395.
- [3] Sekhon B. S. 2013. Surfactants: pharmaceutical and medicinal aspects. Journal of Pharmaceutical Technology, Research and Management. 1: 11-36.
- [4] Bordes R. and Holmberg K. 2015. Amino acid-based surfactant - do they deserve more attention? Advances in Colloid and Interface Science. 222: 79-91.
- [5] Preiss L. C., Wagner M., Mastai Y., Landfester K. and Munoz-Espi R. 2016. Amino-acid-based polymerizable surfactants for the synthesis of chiral nanoparticles. Macromol. Rapid Commun. DOI: 10.1002/marc.201600210.
- [6] Takassi M. A., Hashemi A., Rostami A. and Zadehnazari A. 2016. Lysine amino acid-based surfactants: application in enhanced oil recovery. Petroleum Science and Technology. 34: 17-18.
- [7] Dayrit F. M. 2014. The properties of lauric acid and their significance in coconut oil. J. Am. Oil Chem. Soc. 48: 134-139.
- [8] Boemeke L., Marcadenti A., Busnello F. M. and Gottschall C.B.A. 2015. Effects of coconut oil on human health. Open Journal of Endocrine and Metabolic Diseases. 5: 84-87.
- [9] Perez L., Pinazo A., Pons R. and Infante M. 2014. Gemini surfactants from natural amino acids. Advances in Colloid and Interface Science. 205: 134-155.
- [10] Zhang, J., Cai D., Wang S., Tang Y., Zhang Z., Liu Y. and Gao X. 2014. Efficient method for the synthesis of fatty acid amide from soybean oil methyl ester



catalysed by modified CaO. The Canadian Journal of Chemical Engineering. 92: 871-875.

- [11] Istadi I., Prasetyo S. A. and Nugroho T. S. 2015. Characterization of K₂O/CaO/ZnO catalyst for transesterification of soybean oil to biodiesel. International Conference on Tropical and Coastal Region Eco-Development 2014. Procedia Environmental Sciences. 23: 394-399.
- [12] Kumar D., Kuk H. and Ali A. 2016. One-pot solventfree synthesis of fatty acid alkanoamides from natural oil triglycerides using alkali metal droped CaO nanoparticles as heterogeneous catalyst. Journal of Industrial and Engineering Chemistry. 38: 43-49.
- [13] Wu M. H., Wan L. Z. and Zhang Y. Q. 2013. A novel sodium n-fatty acyl amino acid surfactant using silkworm pupae as stock material. Scientific Reports. 4: 4428-4433.
- [14] Montgomery D. C. 2013. Design and analysis of experiments. 8th edition. John Willey & Sons.
- [15] Masyithah Z., Ashari M., Annisa N. and Syukri M. 2020. Synthesis of fatty ethanolamides from lauric and palmitic acid: optimization using response surface methodology. ARPN J. Eng. App. Sci. 15(1): 1-8.
- [16] Natthapon S. and Krit S. 2014. Optimization of methyl ester production from palm fatty acid distillate using single step esterification: a response surface methodology approach. ARPN J. Eng. App. Sci. 10: 7075-7079.
- [17] Masyithah Z., Swasono A. W. P., Sianturi P. D. E., Leanon R., Wirawan W. and Riyadi R. 2020. Modeling and optimization of alkyl polyglucoside surfactants from fatty alcohol by response surface methodology. ARPN J. Eng. App. Sci. 15(12): 1313-1318.
- [18] Masyithah Z., Syukri M., Ashari M., Annisa N. and Ginting, A. 2020. Optimizing process condition for amidification of stearic acid and urea using response surface methodology. Rasayan Journal of Chemistry. 13(2): 868-875.
- [19] Wang X., Han Z., Chen Y., Jin Q. and Wang X. 2016. Scalable synthesis of oleoyl ethanolamide by chemical amidation in a mixed solvent. J. Am. Oil Chem. Soc. 93: 125-131.

[20] ASTM International. 2006. Standart Test Method for Determination of the Saponification Value of Fats and Oils. D. 5558-95.