JFAChE
Journal of Fundamentals and Applications of Chemical Engineering

Article

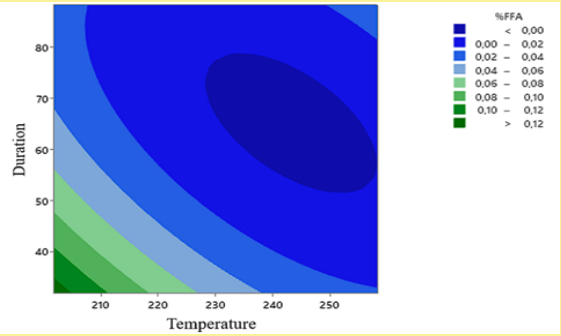
iptek.its.ac.id/index.php/ekstrak

Optimization of Esterification in the Synthesis of Surfactants Feedstock from Polar Lipid Fraction of Crude Palm Oil

Syah Sultan Ali Muzakhar[1], Gloria Islamy Assidiqie[1], Albar Sutan Bahari Siregar[1], Hakun Wirawasista Aparamarta [2], Fahmi[2], Setiyo Gunawan[2,*]

Received October 31, 2023; Revised December 08, 2023; Accepted December 10, 2023
DOI: 10.12962/j2964710X.v4i1.19220

***Abstract*—**Surfactants (monoglycerides and diglycerides) can be obtained by converting triglycerides and glycerol with a NaOH as catalyst. A low free fatty acids (FFA) content starting material is needed due to the formation of soap as side product. The aim of this research was to optimize the esterification of (Polar lipid fraction) PLF to eliminate the FFA content with $ZnCl_2$ as catalyst. Optimization was conducted on the variables of duration (ranging from 40 to 80 min) and reaction temperature (210–250°C) using the Response Surface Method with Central Composite Design with starting FFA content of 28.4%. It was found that an FFA content of 0.285% and a yield of 97.25% were achieved at a temperature of 258.3°C and a heating duration of 59.71 min. Moreover, the influence of temperature and heating duration during the esterification reaction on the FFA content was highly significant, as indicated by the P-Value of 0.00000102. Meanwhile, the influence of temperature and heating duration during the esterification reaction on the yield is not significant (P-Value = 0.130).



***Keywords*—** Esterification, Free Fatty Acids, Response Surface Methodology, Surfactant Feedstock, Triglycerides

I. INTRODUCTION

Oil palm trees are a type of tropical plant that grows well in areas with high rainfall, sufficient sunlight, and humid conditions [1]. Therefore, they are grown in many countries in Africa, South America, and Southeast Asia, such as Indonesia. Crude palm oil (CPO) is an important trade commodity and is widely traded globally. It is also used in almost all food products (such as cooking oil, and margarine) and is an important component for industry (such as soap, skin care products, candles, perfume, cosmetics, wood care products, electrical insulators, and emulsifiers). This trend contributes to a continual increase in global demand for palm oil. According to available data, a substantial 84% of the world's crude palm oil production is attributed to Indonesia and Malaysia [2].

Crude palm oil can be further processed using the liquid-liquid extraction principle. Extraction of liquids is carried out based on solubility. Liquid-liquid extraction is used to separate the desired components (cooking oil, triglycerides) that are still present in the n-hexane by contacting it with an insoluble liquid (methanol) [3]. The equipment used for liquid extraction can be divided into two types, namely batch [1] and continuous processes [3]. Both processes use two types of solvents, namely polar and nonpolar solvents. The choice of the two types of solvents is based on the polarity properties of the compounds contained in crude palm oil. Nonpolar solvents dissolve nonpolar compounds, such as triglycerides, so that a nonpolar lipid fraction (NPLF) is produced. Vice versa,

[1] Departement of Chemical Engineering, Institut Teknologi Sepuluh Nopember, Sukolilo, Surabaya, 60111, Indonesia.
[2] Departement Food Engineering Program, Departement of Chemical Engineering, Institut Teknologi Sepuluh Nopember, Sukolilo, Surabaya, 60111, Indonesia. *E-mail: gunawan@chem-eng.its.ac.id

polar solvents dissolve polar compounds, resulting in polar lipid fractions (PLF), such as monoglycerides, diglycerides, and free fatty acids [4]. Even though it uses the same solvent, a continuous process was preferred because the batch process took a long time and produced a low yield so it was only suitable for use on a laboratory scale [3].

In the production of mono-diacylglycerol, the glycerolysis reaction is a method that is often used because it is the easiest and most economical. However, PLF has a high level of free fatty acids (FFA) of 28.404%, it cannot only carry out the glycerolysis reaction. This is due to the formation of soap between free fatty acids and the base catalyst used in the glycerolysis reaction. Significant soap formation disrupted the reaction process by forming an emulsion, causing yield losses [5].

To avoid soap formation, free fatty acids must be removed from the system or converted into more valuable products. The reaction can only tolerate free fatty acid levels of a maximum of 3% without affecting the process [5]. In addition, free fatty acids less than 3% are recommended for higher conversion of the glycerolysis reaction [6]. One way to reduce free fatty acids is by esterification reaction [7]. In general, the factors that influence the esterification reaction are temperature and reaction time. The temperature used in the esterification process will affect the results of the esterification. Meanwhile, reaction time will affect the level of product purity [8]. Therefore, it is necessary to optimize these two variables.

Therefore, the objective of this work was to optimize the esterification of PLF to eliminate the FFA content with $ZnCl_2$ as catalyst. Optimization was conducted on the variables of duration (ranging from 40 to 80 min) and reaction temperature (210–250°C) using the Response Surface Method with Central Composite Design.

II. METHOD

A. Materials

Crude palm oil was obtained from PT. Buana Karya Bhakti. A thin-layer chromatography plate was obtained from Germany. Polar Lipid Fraction was produced by Batchwise Solvent Extraction. Glycerol with a purity of 99.7% obtained from PT. Wilmar Indonesia. Zinc chloride anhydrous ($ZnCl_2$) Pro Analisa from Merck was also used as a catalyst. Acetic acid, ethyl acetate, n-hexane, food-grade ethanol, and phenolphthalein 1% were purchased from local market sources.

B. Polar Lipid Fraction

Polar lipid fraction (PLF) was obtained through Batchwise Solvent Extraction. It derived from batchwise solvent extraction needs to be distilled first because it contains ethanol. This is because batchwise-solvent extraction (BSE) uses ethanol to dissolve crude palm oil (CPO). In the BSE process, two types of products are created: the non-polar lipid fraction (NPLF) and the PLF. Ethanol, as a polar solvent, will dissolve polar compounds from CPO, such as free fatty acids, monoglycerides (MG), and diglycerides (DG), leaving hydrocarbons and triacylglycerols (TG) in the NPLF.

C. Esterification

10 g of glycerol was weighed using an analytical balance and put into an Erlenmeyer flask as described in Figure 1. 10 g of PLF was weighed and then added to the flask. Zinc chloride ($ZnCl_2$) catalyst of 0.3% of the PLF-glycerol mixture was weighed and added to the flask. $ZnCl_2$ must be weighed quickly because $ZnCl_2$ is an anhydrous material that can absorb water or air humidity. Esterification performed by heating the flask contained materials on a hot plate stirrer according to the temperature and time variables being carried out with a stirring speed of 500 rpm. After the desired time has been reached, the flask was removed from the hot plate and allowed to let the temperature drop. The results of this esterification were divided into 2 phases, namely the upper phase and the lower phase. Before the esterification results solidify, they must be separated first. Separation was done manually with a dropper pipette.

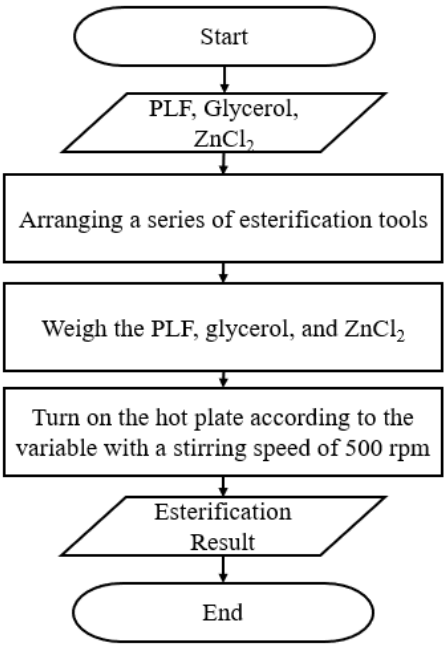


Figure 1. Esterification Procedure Flow Chart

D. Free Fatty Acid Analysis [9]

The sample was weighed then dissolved in 50 mL of 95% ethanol (alcohol). This solution was then titrated with 0.1 M NaOH with 1% PP indicator until a pink color appeared for 10 seconds. Free fatty acid levels were calculated using the calculation formula:

$$FFA, \% = \frac{V\ NaOH\ x\ M\ NaOH\ x\ 25.6}{m} \quad (3)$$

Information:

V NaOH = Volume of NaOH solution required (mL)

M NaOH = Molarity of the NaOH solution used (M)
m = The mass of the oil sample being titrated (gram)

To convert free fatty acids to acid value, multiply the percentage by 2.19.

E. TLC Analysis

Quantitative analysis of TG, DG, MG, and FFA content in PLF after esterification was pipetted using a micropipette and placed on Thin Layer Chromatography (TLC), and immersed in a mobile phase with n-hexane, ethyl acetate, and acetic acid in proportion of 80:20:1 composition (v/v/v). The immersion was carried out in a transparent vessel. During immersion, the mobile phase was controlled so that it did not exceed the finish line, which is the line created on the TLC plate. The immersed TLC plates were then dried at room temperature and visualized under 254 and 365 nm UV light [10].

F. Statistical Analysis

An experimental design using Response Surface Methodology-Central Composite Design (RSM-CCD) was simulated using Minitab version 21.2. This design considers two factors: temperature and heating time. The response variable analyzed in this study was the purity of the FFA.

III. Results and Discussion

The objective of this research was to produce a surfactant feedstock with a minimum level of free fatty acids from the polar lipid fraction obtained through batchwise solvent extraction, by optimizing the esterification reaction using Response Surface Methodology - Central Composite Design (RSM-CCD). The raw material for the polar lipid fraction (PLF) was obtained from batchwise solvent extraction (BSE). Moreover, Batchwise Solvent Extraction is a multistage solvent-based purification process that simplifies steps in the refining process, such as degumming, neutralization, and bleaching. The polar solvent used, ethanol, dissolves polar compounds in crude palm oil (CPO) like free fatty acids, monoglycerides (MG), and diglycerides (DG). This fraction was referred to as the polar lipid fraction (PLF). Polar compounds in CPO have transitioned to PLF, leaving behind nonpolar compounds like hydrocarbons and triacylglycerides (TG). This fraction was known as the nonpolar lipid fraction (NPLF) [1].

Ethanol in the PLF was separated from the polar compounds through distillation. The ethanol recovered from distillation, also known as ethanol recovery, can be reused in subsequent batchwise solvent extraction processes, making it more economical. Distillation involves heating to vaporize volatile components, which are then condensed back into a liquid phase and collected separately. The principle of distillation is based on the differences in boiling points of components in the mixture, which determine their ability to vaporize and condense at different temperatures. The polar lipid fraction (PLF) was heated using a heater to a temperature of 80°C. The choice of 80°C as the temperature is based on the fact that the ethanol boiling point of 78.5°C. Therefore, with a heater temperature of 80°C, it was expected that the ethanol in the polar lipid fraction was vaporized. This distillation process works by heating the PLF located at the bottom of the distillation apparatus until ethanol vaporizes. Free fatty acids, MG, DG, and TG were not evaporated because these compounds have higher boiling points than that of ethanol. Free fatty acids have a boiling point ranging from 173 to 227°C, depending on their source and purity [11]. MAG, DAG, and TAG have respective boiling points of approx 287.8±30°C, 240.3±7.0°C, and 397.44°C. The ethanol vapor came into contact with a coil located inside (around the tube). Inside the coil, cooling water was flow with a temperature of 10-15°C. As a result, the ethanol vapor that comes into contact with the coil condenses into a liquid and falls into the ethanol collector on the inner edge of the tube. Distillation was carried out until the temperature reached more than 80°C, where it was expected that the majority of ethanol had been distilled. From the distillation data, the yield of the distilled polar lipid fraction produced was 2.66%.

The PLF obtained from each distillation has varying colors and characteristics, thus requiring homogenization. This is because the polar lipid fraction used comes from a combination of stages 1 to 8, where the content of polar compounds decreases, as indicated by the observed color changes. In the initial stages, the PLF fraction was a darker yellow color. However, as the stages progress, the color of the PLF fraction gradually became clear. Homogenization of the Distilled Polar Lipid Fraction (DPLF) was necessary to ensure consistency in the raw material used. Homogenization was carried out on a hot plate at a temperature of 120°C to ensure that any remaining ethanol from the distillation process is completely removed during this homogenization step. Additionally, stirring was performed at a speed of 600 rpm for 1 h. The homogenized DPLF was then placed into a sealed container.

Ethanol needs to be removed from PLF because it can react with free fatty acids, forming fatty acid ethyl ester and water. One study indicates that FAEE (fatty acid ethyl ester) has a toxic potential for myocardial ethanol metabolism and can accumulate in mitochondria, damaging cellular function, which can lead to alcohol-induced heart muscle disease [12]. The reaction between free fatty acids and ethanol is illustrated in Figure 2.

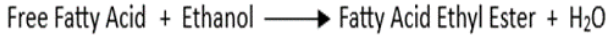


Figure 2. Reaction Between Free Fatty Acids and Ethanol

A. *Esterification*

The significant formation of soap can disrupt the reaction process by creating an emulsion, and lead to yield losses

[5]. Saponification is the formation of salts from fatty acids; such salts are called soap. The saponification reaction involved the treatment of free fatty acids and/or glycerides with a base [13]. By using a small amount of base (at a low concentration), the neutralization/ saponification that occurs primarily utilizes the free fatty acids in the reactants and does not damage triacylglycerols. Thus, the cleavage of triacylglycerol molecules can be avoided, and the formed salt (soap) primarily consists of the free fatty acids present in PLF [14]. The saponification reaction is illustrated in Figure 3.

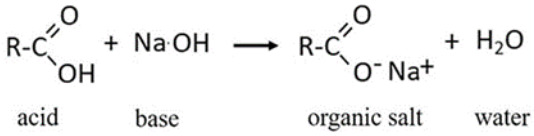


Figure 3. Saponification Reaction or Neutralization

To prevent the formation of soap, free fatty acids must be removed from the system or converted into more valuable products. The reaction can only tolerate a maximum of 3% free fatty acids without affecting the process [12]. Additionally, having less than 3% free fatty acids is recommended for a higher conversion rate in the glycerolysis reaction [6]. One of the methods to reduce free fatty acids was through the esterification reaction [7].

Esterification is a chemical reaction between an alcohol and a carboxylic acid that produces an ester and water. The reaction is typically catalyzed by an acid catalyst.

Given that this reaction is both slow and reversible, it becomes essential to eliminate the water formed in order to propel the reaction towards product formation. The main factors influencing the efficiency of the esterification process conversion were the molar ratio of alcohol to oil, the amount of catalyst, reaction temperature, type of catalyst, stirring speed, and reaction duration [15]. In this study, esterification was carried out using polar lipid fraction (PLF) containing free fatty acids as reactants, which are reacted with glycerol. Glycerol is a non-volatile alcohol, providing a significant advantage for use in the esterification reaction. The advantage was that the reaction temperature can be increased without raising the reaction pressure, as is the case when using methanol and ethanol [16]. This reaction was carried out with an excess of glycerol (compared to free fatty acids) to shift the equilibrium towards [17, 18]. There are three main reactions that occur during esterification as follows.

$$GOH + FFA \leftrightarrow MG + H_2O \quad (4)$$
$$MG + FFA \leftrightarrow DG + H_2O \quad (5)$$
$$DG + FFA \leftrightarrow TG + H_2O \quad (6)$$

With GOH representing glycerol, FFA representing free fatty acids, MG representing monoacylglycerol, DG representing diacylglycerol, and TG representing triacylglycerol, there are three additional reactions that can occur, which are linear combinations of the three reactions mentioned above. The following three reactions did not affect the composition of the final equilibrium but can influence the reaction kinetics [16].

$$TG + MG \leftrightarrow 2DG \quad (7)$$
$$GOH + TG \leftrightarrow DG + MG \quad (8)$$
$$GOH + DG \leftrightarrow 2MG \quad (9)$$

In conventional esterification processes, several factors limit conversion and reaction rates, such as poor reactant miscibility, slow kinetics, and the accumulation of by-products (like water), which can lead to dynamic system stalling. Poor interactions between reactants can result in the formation of thin layers between them, thereby restricting mass transfer. Mass transfer can be enhanced by reducing the thickness of these layers and driving the reaction in the desired direction by removing by-products, such as water. This is often achieved through methods, such as intensive stirring, conducting the reaction at high temperatures, high pressures, under supercritical conditions, and using ultrasonication. Therefore, the use of catalysts is crucial to improve productivity and expedite reactions to achieve desired outcomes in less time. Acid catalysts act as proton donors to carboxylic acids, rendering them unstable and susceptible to nucleophilic attack by alcohols [19]. In this research, zinc chloride ($ZnCl_2$) was used as an acid catalyst. Zinc chloride is considered a high-quality catalyst in organic synthesis due to its numerous advantages. The benefits of zinc chloride include being an environmentally safe catalyst, readily available, and cost-effective [20].

Mostafa et al. investigated the effects of temperature, catalyst concentration, and glycerol-to-fatty acid molar ratio on the efficiency of glycerolysis of fatty acids [15]. They concluded that the optimal esterification reaction conditions were at a temperature of 195°C, a molar ratio of 1:1, 0.3% by weight of zinc chloride ($ZnCl_2$) as the catalyst, and stirring at 500 rpm. They found that the purity of mono-, di-, and triglycerides obtained was 99%.

The esterification procedure conducted here is based on experiments conducted by Mostafa et al. with modifications [15]. In this study, the independent variables used are the reaction temperature and heating duration. The reaction was carried out at atmospheric pressure (an open system) with stirring at 500 rpm, a 0.3% by weight concentration of $ZnCl_2$ catalyst in the reactant mixture, and a mass ratio of 1:1 between PLF and glycerol. An open system was chosen over a closed system (isolated system) because the conversion in the open system (91%) is higher than the esterification conversion in the closed system (72%) [18].

B. *One Factor at a Time (OFAT)*

In this study, one-factor-at-a-time (OFAT) design experiment was conducted for the esterification reaction, one for the temperature variable and one for the time variable. The response for these OFAT experiments was the level of free fatty acids resulting from the esterification, which is analyzed using acid-base titration.

OFAT was employed to evaluate the temperature and time variables with respect to the level of free fatty acids. Additionally, the graphs generated from these OFAT experiments can be used to determine the minimum and maximum ranges for the variables to be included in the Response Surface Methodology.

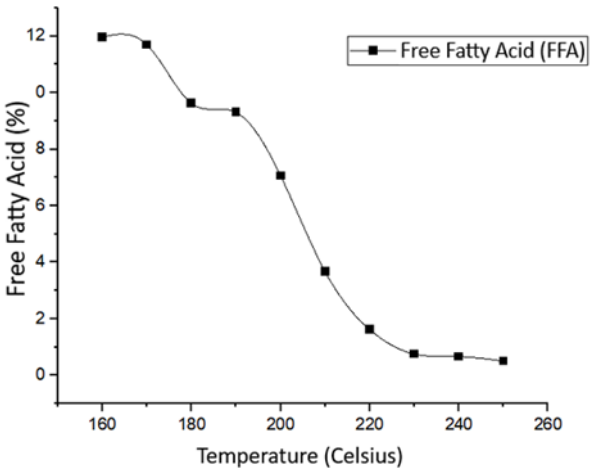


Figure 4. Graph of the Effect of Temperature on the Level of Free Fatty Acids

In the OFAT experiment with the temperature variable, the fixed variables included a 1:1 ratio of PLF to glycerol, 0.3% $ZnCl_2$ catalyst by mass of the reactant mixture, stirring speed at 500 rpm, and a heating duration of 60 min. The temperature range used started from 160°C with increments of 10°C up to 250°C. The desired outcome was to achieve the minimum level of free fatty acids. The results obtained are shown in Figure 4. From the graph, it can be concluded that the higher the temperature, the lower the level of free fatty acids in the product. The best result was obtained at a temperature of 250°C with a level of free fatty acids at 0.5059%. This reaction occurs at a high temperature, resulting in undesirable decomposition and oxidation reactions, which in turn lead to dark-colored products, a strong odor, and low monoglyceride yields. For this reason, the temperature limit is set at 250°C [21]. From this OFAT experiment, the range of operability was selected for the Response Surface Methodology-Central Composite Design (RSM-CCD). For the temperature variable, the chosen range was from a lower limit of 210°C to an upper limit of 250°C. The temperature range of 210°C - 250°C was chosen because this reaction takes place at high temperatures and results in undesirable decomposition and oxidation reactions which result in the product being dark in color, having a strong odor and low monoglyceride yields. For this reason, the temperature limit is set at 250°C.

The next step in the OFAT (One Factor at a Time) experiment was conducted on the duration variable. The tested durations were 40 min, 60 min, and 80 min of heating. Fixed variables included a PLF to glycerol ratio of 1:1, 0.3% $ZnCl_2$ catalyst by weight of the mixture, agitation speed of 500 rpm, and a temperature of 210°C. From Figure 5, the lowest level of free fatty acids was observed at the 60 min duration, which was 1.212%. Therefore, the lower range of time was chosen at 40 min, and the upper range of time was set at 80 min.

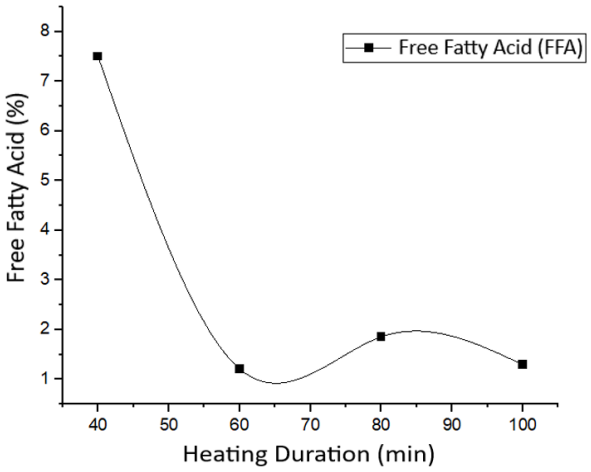


Figure 5. Graph of the Influence of Heating Duration on Free Fatty Acid Levels

C. *Response Surface Methodology - Central Composite Design*

Response Surface Methodology (RSM) was used to investigate the free fatty acid content resulting from esterification with operational parameters, namely temperature and heating duration. The composition of these two variables was designed using the central composite design (CCD) approach. With two continuous factors/variables (k) and five replications (n), the number of experiments conducted can be calculated using equation (10). Therefore, the total number of experiments (N) was 13.

For CCD, observations are divided into 2 blocks: the factorial point block consisting of nf factorial points and n0f center points, and the axial point block consisting of na axial points and n0a center points. Thus, the total number of experiments in CCD is nf + na + (n0f + n0a). In factorial design, each variable has minimum and maximum levels denoted as -1 and +1. The formula for nf factorial points is nf = 2k, where k is the number of variables. So, for this study with two variables, the total nf = 22 = 4 factorial points. The formula for na axial points is na = 2k, so in this study, it has 4 axial points. The distance between the axial points and the center points was denoted as alpha (α) [22]. The value of alpha, which was 1.41421, has been determined. For center points, typically, there are 3 to 5 points that must be included in factorial design. In this case, n0f (center points for factorial design) is set to 5, and n0a (center points for axial design) is set to 0 as chosen by default from the software.

In this research, the CCD consists of 13 experiments, comprising 4 factorial points, 5 center points for factorial design, 4 axial points, and 0 center points for axial design. The experimental design for free fatty acid (FFA) content

with temperature and duration variables can be found in Table 1. The experiments were conducted randomly (following the run order, not the standard order) to eliminate the effects of uncontrolled factors [5].

TABLE 1.
EXPERIMENTAL DESIGN FOR CENTRAL COMPOSITE DESIGN

Std Order	Run Order	Temp. (°C)	Duration (min)	FFA (%)
8	1	230	88.28	0.674
12	2	230	60	0.411
5	3	201.72	60	5.121
4	4	250	80	0.628
2	5	250	40	0.686
1	6	210	40	7.687
10	7	230	60	0.339
7	8	230	31.72	5.839
13	9	230	60	0.455
3	10	210	80	1.920
6	11	258	60	0.484
11	12	230	60	0.396
9	13	230	60	0.420

The free fatty acid content present in PLF (raw material) was 28.40%. Table 1 indicates that the product after esterification contains free fatty acids ranging from 0.339% to 7.687%. The lowest free fatty acid content (0.339%) was obtained at a temperature of 230°C and a heating duration of 60 min. Meanwhile, the highest free fatty acid content (7.687%) was obtained at a temperature of 210°C and a heating duration of 40 min.

D. *Analysis of Variance (ANOVA)*

The data were analyzed using Analysis of Variance (ANOVA) to evaluate Fischer's Test (F-value), probability value (p-value), lack of fit, coefficient of determination (R^2), adjusted R^2 (R^2adj), and predicted R^2 (R^2pred) from the experimental model, RSM-CCD. Data analysis was carried out using the Minitab application (version 21.2).

The suitability of the developed model was examined using ANOVA. ANOVA for the free fatty acid content response is shown in Table 2. A large F-value and a P-value less than 0.05 indicate that a parameter is significant [5], [23]. Significant means that there is strong evidence to reject the null hypothesis and it can be concluded that there is a significant difference between the groups being compared. Not significant means that there is not enough evidence to reject the null hypothesis and it can be concluded that there are no significant differences between the groups being compared. The null hypothesis is a proposition that states there is no difference, no relationship, or no change in general. In the context of an experiment, the null hypothesis states that there are no significant differences between the groups being compared [24].

It was found that the F-value for the model with the free fatty acid response is 127.75, indicating that the model is significant at $P < 0.0001$, and the probability that the F-value occurred due to noise was only 0.01% [3]. The interaction between temperature and duration for the free fatty acid content response is significant. The quadratic effects of temperature and duration are also significant for the free fatty acid content response.

TABLE 2.
ANALYSIS OF VARIANCE (ANOVA) FOR FREE FATTY ACID

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	5	0.008	0.002	127.750	0.0000010
Linear	2	0.005	0.002	205.500	0.0000006
Temperature	1	0.003	0.003	230.660	0.0000012
Duration	1	0.002	0.002	180.350	0.0000029
Square	2	0.002	0.001	79.770	0.0000152
Temp.*Temp.	1	0.001	0.001	73.600	0.0000581
Duration* Duration	1	0.001	0.001	106.290	0.0000174
2-Way Interaction	1	0.001	0.001	68.190	0.0000744
Temp* Duration	1	0.001	0.001	68.190	0.0000744
Error	7	0.000	0.000		
Lack-of-Fit	3	0.000	0.000	154.330	0.0001371
Pure Error	4	0.000	0.000		
Total	12	0.008			

The comparison between residual and pure error was referred to as lack of fit. The F-values for the lack of fit for the free fatty acid content was 154.33 with P-values of 0.00013718. A lack of fit was not significant so it can be accepted. Therefore, the number of experiments was sufficient to evaluate the effects of the variables on the response [3], [25]. A significant lack of fit indicates that there may be contributions to the regression response relationship that are not accounted for by the model.

TABLE 3.
MODEL SUMMARY

Respons	S	R-sq	R-sq(adj)	R-sq(pred)
Free Fatty Acid	0.003	98.92%	98.14%	92.34%

The model summary can be seen in Table 3. The ANOVA regression model shows an R-squared (R^2) value of 98.92% for the free fatty acid content response, indicating that the model can explain 98.92% of the data's variation, with only 1.08% of the total variation unexplained by the model. The R^2 value should not be less than 75% for the model to be considered adequate. Thus, the model was adequate for the free fatty acid content response. However, a high R^2 does not invariably indicate the excellence of the

regression model. An excellent model can only be determined based on an equally high adjusted R-squared (R^2adj) value [26, 27]. The adjusted coefficient of determination (R^2adj) values for the free fatty acid content was 98.14%. A high R^2adj value indicates good agreement between experimental and predicted values. Furthermore, R^2adj and R^2pred should be within 20% of each other to be considered appropriate [28]. Therefore, the model is adequate for the free fatty acid content response.

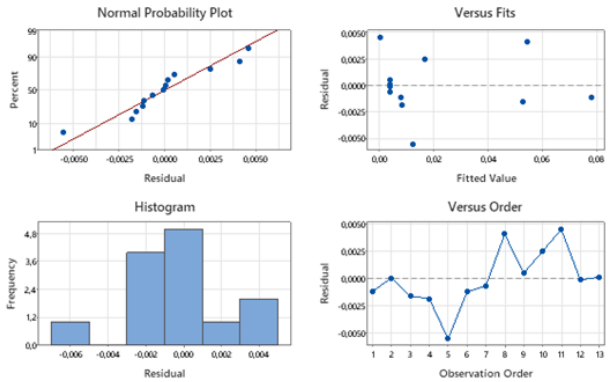


Figure 6. Residual Plot for FFA

The residual plot for free fatty acids is shown in Figure 6. In the residual plot there are four plots, namely histogram, normal probability plot, residuals versus fits plot, and residuals versus order plot. The residual histogram is used to determine whether the data is skewed or includes outliers. Histograms are most effective when there are around 20 data points or more. If the sample is too small, then each bar on the histogram does not contain enough data points to indicate skew or outliers. Because the histogram display depends on the number of intervals used to group the data, histograms are not recommended for assessing residual normality. Instead, a normal probability plot is used [29].

A normal probability plot basically straightens out the expected bell shape of a histogram so that if the sample truly comes from a normal population, then the points plotted from the sample should be fairly close to a straight line. In ANOVA, it is usually more effective (and more straightforward) to create a normal probability plot with residuals [30]. Normal probability plots of abnormal data show curves, hooks, and gaps that are usually easy to distinguish from straight lines. If the P-value for the normality test is relatively large ($P > 0.05$) then the data is normally distributed. Meanwhile, if the P-value is relatively small ($P \leq 0.01$) then the data is distributed abnormally. For intermediate P values ($0.01 < P < 0.05$), the data are inconclusive and additional data is needed [31]. The P-value for the normal probability plot for free fatty acids has a value of 0.034 (medium value) so the data is not conclusive.

In analyzing the residuals versus fits plot, it can be seen from the distribution of the points. If the model is correct and the assumptions are met, the residuals should be unstructured. The plot in the residuals versus fits plot should not show a clear pattern [30]. There are characteristics of the residuals versus fits plot, namely 1) The residuals "bounce randomly" around the 0 line. This shows that the assumption that the relationship is linear is reasonable; 2) The residuals roughly form a "horizontal band" around line 0. This indicates that the variances of the errors are the same; 3) There are no residuals that "stand out" from the basic random pattern of residuals. This shows that there are no outliers [32]. In Figure 6, it can be seen that the graph does not show a clear pattern and is not structured, but there are several points that converge for the response of free fatty acids.

Residuals versus order plots can also be analyzed. If there is a funnel pattern that opens outward, it indicates that variability is increasing over time [33]. Residuals versus order plot is used to verify the assumption that the residuals are independent of each other. Independent residuals do not show a trend or pattern when displayed in a time series. Patterns in the dots may indicate that residuals close to each other may be correlated, and thus, not independent. Ideally, the dots should fall randomly around the center line [34]. In Figure 6, it can be seen that the graph does not show any pattern so it can be said that the residuals are independent of each other. The uncoded value regression equation (actual value) for the variable's free fatty acid content (%) is shown as follows,

$$Y\%FFA = 2.368 - 0.01600X1 - 0.01308X2 + 0.000028 X12 + 0.000034X22 + 0.000036 X1*X2 \quad (10)$$

where X1 is temperature and X2 is duration. A positive sign in the model indicates a synergistic effect of the factors, whereas a negative sign indicates an antagonistic effect.

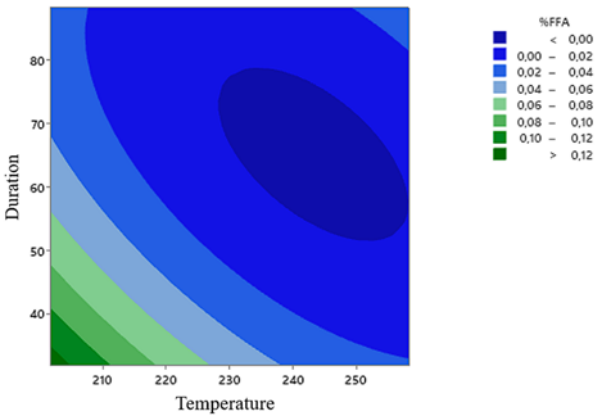


Figure 7. Contour Plot of Temperature and Duration Variables against %FFA

E. *Response Surface Analysis*

In this research, based on the regression equation, contour plots or two-dimensional (2D) contour plots and response surface plots or three-dimensional (3D) response

surface plots are drawn as a function of two independent variables with the aim of understanding the synergistic effect of temperature and duration on levels of free fatty acids. The contour plot is shown in Figure 7, while the response surface plot is shown in Figure 8. The contour plot helps to determine the level of variables that contribute to the desired response, while the response surface plot helps to determine the minimum, middle and maximum response points [35]. The desired synergistic effect between two independent variables has an elliptical shape on the contour plot [36].

Figure 7 illustrates a contour plot for the free fatty acid content response with temperature and duration as variables. It can be observed that as the temperature increases, the free fatty acid content in the product decreases. However, at temperatures above 250°C, the free fatty acid content starts to increase. Regarding the duration variable, longer durations result in lower free fatty acid content in the product, but at durations exceeding 80 minutes, the free fatty acid content increases. This can occur due to: 1) reversible reactions leading to the formation of free fatty acids, and 2) potential inaccuracies in the analysis since experiments conducted at temperatures above 250°C and durations exceeding 80 minutes were performed only once. Free fatty acid content below 0.1% can be achieved within the temperature range of 230–260°C with durations of 55–80 min.

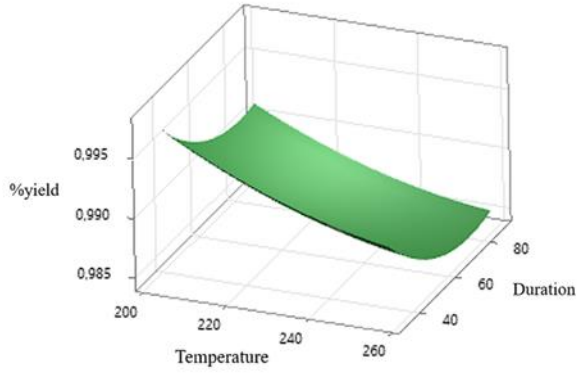


Figure 8. Surface plot of temperature and duration variables against %FFA

Furthermore, in the surface plot of Figure 8 with the %FFA response and temperature and duration variables, it can be seen that the free fatty acid content tends to decrease with increasing temperature. At lower temperatures (200-230°C) there is a relationship that the longer the duration, the lower the free fatty acid levels. However, at higher temperatures (230-260°C) there is a relationship, namely in the duration range of 60-80 min the free fatty acid levels increase.

F. *Validation of Optimization Results*

In Response Surface Methodology there is Response Optimization where the application will suggest solutions based on parameters and previous experimental results. The %FFA response is set with the aim of minimizing (minimum). A temperature of 231.4°C with a duration of 59.71 min was chosen as the main solution to produce minimum %FFA. Apart from this solution, there are several solutions which are summarized in Table 4. The first and second solutions were chosen because the first solution has the highest composite desirability, namely 0.991574, while the second solution was chosen because it has the lowest predicted free fatty acid content, namely 0.02%. Both solutions were carried out five times to validate the optimization solution.

TABLE 4.
SOLUTION FROM RESPONSE OPTIMIZATION

Solution	Temp.	Duration	%FFA Fit	Composite Desirability
1	231.4	59.710	0.003	0.992
2	258.3	59.710	0.000	0.985
3	251.4	79.800	0.009	0.906
4	218.0	87.430	0.010	0.900

The results of the optimization are summarized in Table 5 for the FFA response. There are two calculations carried out, namely error and standard deviation. The term "error" in statistics refers to the difference between the actual value and the measured or estimated value of a quantity. These differences can arise for various reasons, such as measurement error, sampling error, or modeling error [37]. Standard deviation is a statistical measure that measures the amount of variation or dispersion in a set of data points, thereby providing information about how spread out the values of the mean (average) of the data set are [38]. From the results obtained, it can be seen that solution 1 (temperature 231.4°C and duration 59.71 min) has a smaller error and standard deviation than solution 2 (temperature 258.3°C and duration 59.71 min). A small error means that the free fatty acids obtained and the predicted fatty acids have close values. A small standard deviation means that the data set is not spread out. However, solution 2 has a smaller free fatty acid content (0.285%) compared to solution 1 (0.388%). Moreover, solution 1 gave a higher yield (97.93%) compared to solution 2 (97.25%).

TABLE 5.
OPTIMIZATION RESULTS ON FFA RESPONSE

Solution	Actual FFA Average	FFA Predict.	Average Error	StDev FFA
1	0.388%	0.30%	29.45%	0.04%
2	0.285%	0.02%	1136.46%	0.05%

Apart from analyzing free fatty acid levels, the results of esterification of solutions 1 and 2 were also carried out by thin layer chromatography (TLC) to qualitatively identify the components in the sample. In this TLC, silica gel 60 F¬254 was used and the mobile phases were hexane, ethyl acetate and acetic acid in a ratio of 80:20:1.

The TLC of PLF and esterification optimization of solutions 1 and 2 is shown in Figure 9. It can be seen that in PLF there are large amounts of free fatty acid (FFA) compounds, indicated by the large FFA area compared to solutions 1 and 2. In solution 1 there are few lines. in the TG area which shows solution 1 has little TG. Solution 1 also still has a small amount of FFA and increased levels of MG and DG compared to PLF which has a fainter color. In solution 2, TG and FFA are not visible and the MG line becomes longer indicating high MG levels.

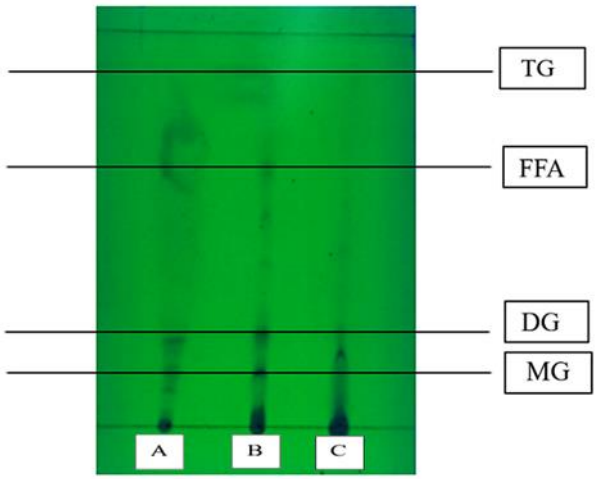


Figure 9. TLC of PLF (A), Optimization of Esterification Solutions 1 (B) and 2 (C)

IV. CONCLUSION

Optimizing the esterification reaction obtained optimal temperature and heating duration with a fatty acid content of 0.285% for esterification results at a temperature of 258.3°C and a heating duration of 59.71 min. In this condition, the resulting yield was 97.25%. The effect of temperature and duration of heating during the esterification reaction on free fatty acid levels was very significant which can be seen from the P-Value, namely 0.00000102. Meanwhile, the effect of temperature and heating duration during the esterification reaction on the yield was not significant (P-Value = 0.130).

ACKNOWLEDGMENTS

The authors would like to acknowledge the financial support from Institut Teknologi Sepuluh Nopember for this work under the Chemical Engineering Department Funding project scheme (1322/PKS/ITS/2023).

REFERENCES

[1] H. W. Aparamarta, T. Saputra, A. Claratika, Y.-H. Ju, and S. Gunawan, "Separation and Purification of Triacylglycerols from Nyamplung (Calophyllum inophyllum) Oil by Batchwise Solvent Extraction," Ind Eng Chem Res, vol. 55, no. 11, pp. 3113–3119, Mar. 2016, doi: 10.1021/acs.iecr.5b04877.

[2] R. Ostfeld, D. Howarth, D. Reiner, and P. Krasny, "Peeling back the label—exploring sustainable palm oil ecolabelling and consumption in the United Kingdom," Environmental Research Letters, vol. 14, no. 1, p. 014001, Jan. 2019, doi: 10.1088/1748-9326/aaf0e4.

[3] M. Hawashi, A. Altway, T. Widjaja, and S. Gunawan, "Optimization of process conditions for tannin content reduction in cassava leaves during solid state fermentation using Saccharomyces cerevisiae," Heliyon, vol. 5, no. 8, p. e02298, Aug. 2019, doi: 10.1016/j.heliyon.2019.e02298.

[4] P. C. Sadek, "The HPLC solvent guide," 2nd ed. New York: Wiley-Interscience, 2002.

[5] G. G. Kombe, A. K. Temu, H. M. Rajabu, G. D. Mrema, and K. T. Lee, "Low Temperature Glycerolysis as a High FFA Pre-Treatment Method for Biodiesel Production," Advances in Chemical Engineering and Science, vol. 03, no. 04, pp. 248–254, 2013, doi: 10.4236/aces.2013.34032.

[6] M. P. Dorado, E. Ballesteros, J. A. de Almeida, C. Schellert, H. P. Löhrlein, and R. Krause, "An Alkali–Catalyzed Transesterification Process for High Free Fatty Acid Waste Oils," Transactions of the ASAE, vol. 45, no. 3, 2002, doi: 10.13031/2013.8849.

[7] P. Jasen and J. M. Marchetti, "Kinetic study of the esterification of free fatty acid and ethanol in the presence of triglycerides using solid resins as catalyst," International Journal of Low-Carbon Technologies, vol. 7, no. 4, pp. 325–330, Dec. 2012, doi: 10.1093/ijlct/ctr049.

[8] D. Darnoko and M. Cheryan, "Kinetics of palm oil transesterification in a batch reactor," J Am Oil Chem Soc, vol. 77, no. 12, pp. 1263–1267, Dec. 2000, doi: 10.1007/s11746-000-0198-y.

[9] American Oil Chemists' Society, AOCS official method Ca 5a-40. Free fatty acids. AOCS, 2009.

[10] M. Mahardika, N. T. Susparini, D. Dewaldo, B. Situmeang, and F. Amin, "Sintesis dan Karakterisasi Cangkang Kapsul Non Gelatin dari Rumput Laut (Eucheumma cottonii) dan Kaktus Koboi (Cereus peruvianus) untuk Sistem Penghantaran Obat," KOVALEN: Jurnal Riset Kimia, vol. 9, no. 1, pp. 1–12, Apr. 2023, doi: 10.22487/kovalen.2023.v9.i1.16098.

[11] A. A.-W. Japir, J. Salimon, D. Derawi, M. Bahadi, and M. R. Yusop, "Separation of free fatty acids from high free fatty acid crude palm oil using short-path distillation," 2016, p. 030001. doi: 10.1063/1.4966739.

[12] M. E. Beckemeier and P. S. Bora, "Fatty Acid Ethyl Esters: Potentially Toxic Products of Myocardial Ethanol Metabolism," J Mol Cell Cardiol, vol. 30, no. 11, pp. 2487–2494, Nov. 1998, doi: 10.1006/jmcc.1998.0812.

[13] M. P. Penfield, S. Taylor, and A. M. Campbell, Experimental Food Science, 3rd ed. Academic Press, 1990.

[14] C. Medeiros Vicentini-Polette, P. Rodolfo Ramos, C. Bernardo Gonçalves, and A. Lopes De Oliveira, "Determination of free fatty acids in crude vegetable oil samples obtained by high-pressure processes," Food Chem X, vol. 12, p. 100166, Dec. 2021, doi: 10.1016/j.fochx.2021.100166.

[15] N. A. Mostafa, A. Maher, and W. Abdelmoez, "Production of mono-, di-, and triglycerides from waste fatty acids through esterification with glycerol," Advances in Bioscience and Biotechnology, vol. 04, no. 09, pp. 900–907, 2013, doi: 10.4236/abb.2013.49118.

[16] M. A. Maquirriain, C. A. Querini, and M. L. Pisarello, "Glycerine esterification with free fatty acids: Homogeneous catalysis," Chemical Engineering Research and Design, vol. 171, pp. 86–99, Jul. 2021, doi: 10.1016/j.cherd.2021.04.018.

[17] Z.-Z. Cai et al., "A two-step biodiesel production process from waste cooking oil via recycling crude glycerol esterification catalyzed by alkali catalyst," Fuel Processing Technology, vol. 137, pp. 186–193, Sep. 2015, doi: 10.1016/j.fuproc.2015.04.017.

[18] S. H. Yeom and Y. W. Go, "Optimization of a Novel Two-step Process Comprising Re-esterification and Transesterification in a Single Reactor for Biodiesel Production Using Waste Cooking Oil," Biotechnology and Bioprocess Engineering, vol. 23, no. 4, pp. 432–441, Aug. 2018, doi: 10.1007/s12257-018-0209-5.

[19] Z. Khan et al., "Current developments in esterification reaction: A review on process and parameters," Journal of Industrial and Engineering Chemistry, vol. 103, pp. 80–101, Nov. 2021, doi: 10.1016/j.jiec.2021.07.018.
[20] M. A. Päsha and A. Nizam, "Zinc Chloride–Catalyzed Expeditious Route to Nitriles," Synth Commun, vol. 40, no. 9, pp. 1276–1279, Apr. 2010, doi: 10.1080/00397910903069657.
[21] P. Felizardo, J. Machado, D. Vergueiro, M. J. N. Correia, J. P. Gomes, and J. M. Bordado, "Study on the glycerolysis reaction of high free fatty acid oils for use as biodiesel feedstock," Fuel Processing Technology, vol. 92, no. 6, pp. 1225–1229, Jun. 2011, doi: 10.1016/j.fuproc.2011.01.020.
[22] S. Zhao, W. Li, and L. Gu, "Biomechanical prediction of abdominal aortic aneurysm rupture risk: Sensitivity analysis," J Biomed Sci Eng, vol. 05, no. 11, pp. 664–671, 2012, doi: 10.4236/jbise.2012.511083.
[23] R. U. Owolabi, M. A. Usman, and A. J. Kehinde, "Modelling and optimization of process variables for the solution polymerization of styrene using response surface methodology," Journal of King Saud University - Engineering Sciences, vol. 30, no. 1, pp. 22–30, Jan. 2018, doi: 10.1016/j.jksues.2015.12.005.
[24] E. Lolang, "Hipotesis Nol dan Hipotesis Alternatif," Jurnal Keguruan dan Ilmu Pendidikan, vol. 3, no. 3, pp. 685–695, Dec. 2017, doi: 10.47178/jkip.v3i3.99.
[25] R. T. Silvestrini, D. C. Montgomery, and B. Jones, "Comparing Computer Experiments for the Gaussian Process Model Using Integrated Prediction Variance," Qual Eng, vol. 25, no. 2, pp. 164–174, Apr. 2013, doi: 10.1080/08982112.2012.758284.
[26] A. Koocheki, A. R. Taherian, S. M. A. Razavi, and A. Bostan, "Response surface methodology for optimization of extraction yield, viscosity, hue and emulsion stability of mucilage extracted from Lepidium perfoliatum seeds," Food Hydrocoll, vol. 23, no. 8, pp. 2369–2379, Dec. 2009, doi: 10.1016/j.foodhyd.2009.06.014.
[27] H. Le Man, S. K. Behera, and H. S. Park, "Optimization of operational parameters for ethanol production from Korean food waste leachate," International Journal of Environmental Science & Technology, vol. 7, no. 1, pp. 157–164, Dec. 2010, doi: 10.1007/BF03326127.
[28] A. Rai, B. Mohanty, and R. Bhargava, "Supercritical extraction of sunflower oil: A central composite design for extraction variables," Food Chem, vol. 192, pp. 647–659, Feb. 2016, doi: 10.1016/j.foodchem.2015.07.070.
[29] Minitab, "Residual plots for Fit Regression Model," https://support.minitab.com/en-us/minitab/21/help-and-how-to/statistical-modeling/regression/how-to/fit-regression-model/interpret-the-results/all-statistics-and-graphs/residual-plots/#histogram-of-residuals.
[30] D. C. Montgomery, "Design and Analysis of Experiments," 8th ed. Arizona: John Wiley & Sons, Inc., 2013.
[31] P. G. Mathews, "Design of Experiments with MINITAB," 1st ed. William A. Tony, 2004.
[32] The Pennsylvania State University, "Residuals vs. Fits Plot," https://online.stat.psu.edu/stat462/node/117/.
[33] J. Lawson, "Design and Analysis of Experiments with R. Chapman and Hall/CRC," 2014. doi: 10.1201/b17883.
[34] Minitab, "Residual plots for Fit Regression Model," https://support.minitab.com/en-us/minitab/21/help-and-how-to/statistical-modeling/regression/how-to/fit-regression-model/interpret-the-results/all-statistics-and-graphs/residual-plots/#histogram-of-residuals.
[35] S. Ahirwar, H. Soni, H. K. Rawat, B. P. Prajapati, and N. Kango, "Experimental design of response surface methodology used for utilisation of palm kernel cake as solid substrate for optimised production of fungal mannanase," Mycology, vol. 7, no. 3, pp. 143–153, Jul. 2016, doi: 10.1080/21501203.2016.1229697.
[36] R. V. Muralidhar, R. R. Chirumamila, R. Marchant, and P. Nigam, "A response surface approach for the comparison of lipase production by Candida cylindracea using two different carbon sources," Biochem Eng J, vol. 9, no. 1, pp. 17–23, Nov. 2001, doi: 10.1016/S1369-703X(01)00117-6.
[37] S. Angel, F. Disslbacher, S. Humer, and M. Schnetzer, "What did you Really Earn Last Year?: Explaining Measurement Error in Survey Income Data," J R Stat Soc Ser A Stat Soc, vol. 182, no. 4, pp. 1411–1437, Oct. 2019, doi: 10.1111/rssa.12463.
[38] W. C. Eells, "A Plea for a Standard Definition of the Standard Deviation," J Educ Res, vol. 13, no. 1, pp. 45–52, Jan. 1926, doi: 10.1080/00220671.1926.10879621.