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Optimization of Biodiesel Synthesis from Avocado Seeds Using Response Surface Methodology (RSM)

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Abstract

The synthesis of biodiesel from avocado seed oil has been successfully conducted. This study aimed to determine the optimized parameters for the transesterification reaction using Response Surface Methodology (RSM). The transesterification reaction was carried out with variations in the methanol mole ratio of 1:4, 1:6, and 1:8 at temperatures of 40 °C, 50 °C, and 60 °C, utilizing 1 wt% KOH relative to the oil. The avocado seed oil, obtained from the extraction process, has a free fatty acid content of 1.95%. The RSM results indicated that the optimized parameters for biodiesel production were at a temperature of 60 °C with a methanol mole ratio of 1:6. The final yield achieved was 82%, calculated based on the initial weight of the oil. The biodiesel produced had a cetane number of 75, a density of 877.4 kg/m³, and a viscosity of 4.768 cSt. These values meet the SNI standards, which require a minimum cetane number of 51, a density of 850–890 kg/m³, and a viscosity of 2.3–6 cSt.

Keywords: Biodiesel, avocado seeds, RSM, cetane Number, KOH

1. Introduction

Avocado plants are extensively cultivated in Indonesia, ensuring a reliable supply of seeds from avocado fruit for biodiesel production. Indonesia produces over 410,000 tons of avocados annually, providing abundant seed waste for biodiesel production. Pujon, located in Malang, East Java, is one of the largest producers of avocado fruit, significantly contributing to the availability of avocado seeds. Avocado seeds are rich in fatty acid methyl esters, which render them suitable for use as raw materials in biodiesel production. The oil content of avocado seeds is higher than that of many grain crops but lower than that of palm oil (renita & Safitri, 2020). Research by (Wang et al., 2020) indicates that avocado seeds possess a comparatively high oil content relative to other vegetable seeds, with oil yields ranging from 25.15% to 34.63% which is higher than that of many grain crops but lower than palm oil. Similar findings have been reported by (Eze et al., 2025), who observed that avocado seed oil yield varies depending on factors such as avocado variety, fruit maturity, and environmental conditions. In comparison, castor beans contain approximately 20% to 30% oil based on their fruit weight (Satriana et al., 2018). The variability in avocado seed oil yield highlights the need for further research to optimize extraction methods and improve yield efficiency.

The free fatty acid (FFA) content in avocado seeds is categorized as low, making them suitable for use as a raw material in the biodiesel production process via transesterification. The FFA content in avocado seed oil typically ranges from less than 2%. This low FFA level allows for the production of biodiesel without the need for prior esterification. Biodiesel exhibits superior properties compared to petroleum, including higher biodegradability, non-toxicity, excellent lubricity, and the absence of sulfur and aromatic compounds. As a result, biodiesel fuel has the potential to reduce pollutant levels and mitigate the presence of carcinogens. This reduction occurs because the cultivation of plants used for biodiesel production tends to lower atmospheric carbon dioxide levels through their metabolic processes. The production of biodiesel via transesterification involves converting the triglycerides in avocado seeds into methyl esters and glycerol as the final byproducts.

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The quality of the resulting biodiesel can be evaluated based on several characteristics, including cetane number, density, viscosity, flash point, pour point, and acid number. If these characteristics meet the standards outlined in the Decree of the Director General of EBTKE Number 189 K/10/DJE/2019, the biodiesel produced can be considered successful and ready for use (Budiastuti et al., 2022).

The biodiesel production process is influenced by several factors, including operating temperature, alcohol-to-oil ratio, and type of catalyst. The alcohol-to-oil ratio significantly affects the conversion rate of vegetable oil into methyl esters and glycerol. If the alcohol ratio is too low, the conversion may not reach the desired level; conversely, if it is too high, it can lead to the formation of excess glycerol. An increase in methanol usage results in a greater quantity of unreacted methanol in the final product, as not all methanol reacts with the triglycerides in the oil. Higher operating temperatures can accelerate the transesterification reaction. However, increasing the reaction temperature also raises the risk of soap formation due to saponification. Additionally, the choice of catalyst plays a crucial role in the biodiesel production process. Homogeneous catalysts are generally more reactive than heterogeneous catalysts, resulting in improved process efficiency and higher yields (Dadge, 2019).

The biodiesel production process is governed by several key parameters, including reaction temperature, alcohol-to-oil molar ratio, and the type of catalyst used. Among these, the alcohol-to-oil ratio plays a particularly critical role in determining the conversion efficiency of vegetable oil into fatty acid methyl esters (FAME) and glycerol. An insufficient alcohol ratio can hinder complete transesterification, leading to reduced biodiesel yield. Conversely, an excessive alcohol concentration may result in a high proportion of unreacted methanol in the final product, thereby complicating separation and purification steps (Yang, 2025). Reaction temperature also significantly affects the transesterification kinetics; moderate increases in temperature enhance the reaction rate by improving molecular collisions and mass transfer. However, temperatures exceeding the boiling point of methanol (~64.7 °C) can promote methanol evaporation and soap formation via saponification, thus decreasing product quality (Md. Ismail et al., 2024). In addition, the choice of catalyst is vital in influencing both the reaction mechanism and overall yield. Homogeneous catalysts such as sodium hydroxide (NaOH) and potassium hydroxide (KOH) are widely used due to their high reactivity, although they require rigorous post-reaction purification. In contrast, heterogeneous catalysts, while generally less reactive, offer advantages in terms of catalyst recovery, reusability, and environmental impact (Yang, 2025; Md. Ismail et al., 2024).

Despite extensive research on avocado seed oil as a biodiesel feedstock, previous studies have primarily focused on the oil extraction process, general biodiesel characterization, or basic transesterification parameters. Studies by (Budiastuti et al., 2023), analyzed the fatty acid composition and combustion properties of biodiesel derived from avocado seed oil. However, there remains a gap in optimizing reaction conditions to achieve maximum yield and quality of biodiesel. Many existing studies have not comprehensively examined the interaction between methanol-to-oil ratio and reaction temperature in biodiesel production from avocado seeds. Furthermore, the optimization methods used in previous research often rely on one-factor-at-a-time (OFAT) approaches, which do not consider interactions between variables. This limitation results in suboptimal process conditions and inefficient biodiesel production.

To address these gaps, this study employs Response Surface Methodology (RSM) to optimize the production of biodiesel from avocado seeds. RSM is utilized to analyze yield responses and identify optimal operational conditions that can enhance biodiesel yield. Response Surface Methodology is a combination of mathematical and statistical techniques that are useful for modeling and analyzing problems in which the desired response is influenced by multiple variables. Unlike conventional OFAT methods, RSM allows for the evaluation of multiple variables simultaneously, improving the accuracy of optimization and reducing the number of experiments required. The primary function of RSM is to optimize the response by systematically varying these factors. The general function of RSM is:

$$y = \beta 0 + \beta 1x1 + \beta 2x2 + \varepsilon \quad (1)$$

Remark:
x1, x2 = independent variable
y = respons
ε = error

In this method, the experimental matrix is designed based on the number of variables and the maximum and minimum limits

established for each variable. This approach facilitates the determination of the number of experiments required and the levels to be set for each variable in each experiment (Veza et al., 2023). This study aims to investigate the effects of methanol volume ratio and reaction temperature on the production of biodiesel from avocado seed oil via the transesterification process, with the goal of optimizing the yield of methyl esters. By employing RSM, this research provides a more precise and systematic approach to determining the optimal conditions for biodiesel production, ultimately improving process efficiency and fuel quality.

2. Methodology

2.1. Materials

The materials utilized in this research include avocado seeds, which serve as the source of avocado seed oil. The avocado seeds were peeled, sliced, and dried at 60 °C for 24 hours, reducing their moisture content by approximately 50–70%. From an initial weight of 1 kg, the dried seeds weighed between 300–500 g, depending on their initial moisture content, demonstrating the efficiency of the drying process.; n-hexane, employed as a solvent for extracting the oil; and 85% H_3PO_4, used as a degumming agent for the avocado seed oil. Additionally, 99% methanol is included as the reactant that interacts with avocado seed oil to produce methyl esters, while potassium hydroxide (KOH) acts as the catalyst in the formation of these methyl esters.

2.2. Pre – Treatment Avocado Seeds

The pre-treatment process is a critical step aimed at maximizing the yield in the extraction of avocado seed oil. This process involves several stages: peeling the skins from the avocado seeds, cutting the seeds into smaller pieces, and subsequently drying and roasting them to reduce moisture content. In this study, the seeds were dried at 100 °C for 24 hours, reducing their moisture content from 70% to 15%, ensuring optimal conditions for the extraction process. The reduction in water content is essential for preventing the formation of emulsions that can occur due to the interaction between inorganic water and organic n-hexane. Moreover, decreasing the moisture content facilitates more efficient contact between the solvent and the avocado seed material, thereby enhancing extraction efficiency.

2.3. Extraction of Avocado Seed Oil

The maceration method is employed using n-hexane as the solvent. Pre-treated avocado seeds are immersed in n-hexane at a temperature of 69°C for 6 hours. Oil extraction used a solvent-to-seed ratio of 3:1 (v/w), with 3 liters of n-hexane per 1 kg of avocado seeds. During this extraction, oil is released from the avocado seeds into the solvent due to the mass transfer between the solvent and the triglycerides present in the seeds. Following this, the filtrate is separated from the avocado seed pulp using filter paper. Subsequently, the filtrate undergoes a distillation process at 69°C for an additional 6 hours to separate n-hexane from the avocado seed oil. This distillation concludes when the temperature of the condensate exceeds the boiling point of the solvent, allowing for the recovery of n-hexane, which can be reused in subsequent extraction processes. Furthermore, the avocado seed oil is degummed with the addition of 85% phosphoric acid (H_3PO_4) at a concentration of 0.1% of the oil's weight. The mixture is heated to 90°C while being stirred for 30 minutes. After heating, the resulting mixture is separated using a separating funnel, yielding a top layer of pure oil, which is then utilized in the subsequent biodiesel production process.

2.4. Avocado Seed Oil Transesterification

Following the separation of impurities, the transesterification process is initiated. A solution of potassium methoxide is prepared in an Erlenmeyer flask by mixing methanol with potassium hydroxide (KOH). This method is employed to prevent direct contact between KOH and the avocado seed oil, which could lead to unwanted saponification reactions.

Subsequently, the potassium methoxide solution is combined with the avocado seed oil in a three-necked flask equipped with a spiral cooler, thermometer, and stirrer. The mixture is continuously stirred at 300 rpm while being heated at a specified temperature for 60 minutes. Stirring plays a crucial role in enhancing mass transfer between the reactants by ensuring proper mixing of the immiscible phases of oil and alcohol, increasing the contact surface area for the reaction, and preventing phase separation during the process. After the transesterification reaction, the mixture is allowed to stand for 1 to 2 days to facilitate the separation of biodiesel from glycerol. Separation is achieved using a separating funnel, where the upper layer contains

glycerol and the lower layer consists of methyl esters (biodiesel).

Once the biodiesel is formed, a water washing process is conducted to remove residual impurities, such as catalyst, glycerol, and soap that may have formed during transesterification. This washing is performed by adding water at a ratio of 1:2 relative to the biodiesel. The mixture is then shaken for 5 minutes and allowed to settle until two distinct layers form. The two phases are separated using a separating funnel, and this washing step should be repeated 3 to 5 times to ensure thorough removal of impurities. Finally, the biodiesel is heated in an oven at 100°C for 30 minutes to evaporate any remaining water that was not separated.

3. Results and Discussion

3.1. Analysis of Biodiesel Feedstock

The oil obtained is subsequently analyzed for free fatty acid (FFA) levels to determine its FFA content. This testing is conducted using the titration method, where a potassium hydroxide (KOH) base solution is added to the heated oil sample, resulting in a color change of the indicator. The volume of KOH solution required is then used to calculate the FFA content in the sample, which was found to be 1.95%.

According to (Wang et al., 2020), oils with an FFA content of less than 2% do not require an esterification process prior to transesterification in biodiesel production. However, this study contradicts that theory, as the oil derived from avocado seeds necessitates prior esterification to prevent the occurrence of saponification reactions. The FFA content is determined using the following formula:

$$FFA\ (\%) = \frac{V\ x\ N\ x\ 28{,}2}{W} \qquad (2)$$

Remark:
V = volume of KOH solution used (mL)
N = normality of KOH solution (N)
W = weight of oil sample (g)

Even though the FFA content was below 2%, esterification was conducted to prevent potential side reactions due to prolonged storage or oxidation. Several factors influence the free fatty acid (FFA) content in oil, including oxidation and polymerization, which can compromise the integrity of essential fatty acids. Oxidation occurs when oil is exposed to oxygen, leading to the formation of peroxides and hydroperoxides, which degrade fatty acids over time. While the initial FFA content was within an acceptable range, prolonged storage or oxidative conditions could increase it, potentially causing unwanted side reactions during transesterification.

To mitigate this risk, esterification was performed as a precautionary step to convert any present or potential free fatty acids into esters, reducing the likelihood of saponification and improving the efficiency of the biodiesel production process (Wang et al., 2020).

The biodiesel produced was subsequently tested for cetane number to assess its compliance with the SNI standard. According to SNI 7182:2015, the cetane number is a measure of the combustion performance of diesel fuel, determined by comparing it to a reference fuel in a standard test engine. Diesel fuels are classified based on their cetane numbers, which indicate their ability to combust efficiently. In general, a higher cetane number correlates with quicker engine start-up and greater power output, as diesel engines require rapid combustion reactions to function effectively.

The SNI standard stipulates that the cetane number for biodiesel fuel must be at least 51. In our biodiesel production, the cetane number was tested using ASTM D4737 equipment, specifically a PAC Herzog Cetane Ignition Delay (CID 510) analyzer, manufactured by PAC (Process Analyzer Corporation). This device determines the cetane number by analyzing ignition delay time under controlled conditions. A maximum cetane number of 75 was achieved, indicating that the biodiesel meets and exceeds the standard requirements, demonstrating its suitability for use in diesel engines.

Additionally, the biodiesel exhibited a viscosity of 4.768 cSt and a density of 877.4 kg/m³. These values meet the SNI standards, which require a minimum cetane number of 51, a density of 850–890 kg/m³, and a viscosity of 2.3–6 cSt. This result indicates that the biodiesel meets and exceeds the standard requirements, demonstrating its suitability for use in diesel engines.

3.2. Optimization Process with RSM Design Expert 13

The cetane number test was conducted with ASTM-D4737 equipment and a cetane analyzer. The selection of methanol mole ratio (4:1, 5:1, 6:1, 7:1, 8:1) and reaction temperature (40°C, 45°C, 50°C 55°C, 60°C,

65°C) in this study is based on previous research findings. Studies by (Wang et al., 2020) indicate that a methanol-to-oil ratio within this range is commonly used in transesterification reactions, as it optimizes the conversion of oil into methyl esters without excessive unreacted methanol in the final product.

Additionally, research by (et al., 2021)and (Veza et al., 2023) suggests that a reaction temperature between 40°C and 60°C is optimal for biodiesel production. Lower temperatures may slow the reaction rate, while higher temperatures increase the risk of oil degradation and soap formation due to saponification.

Figure 1. ASTM-D4737 equipment

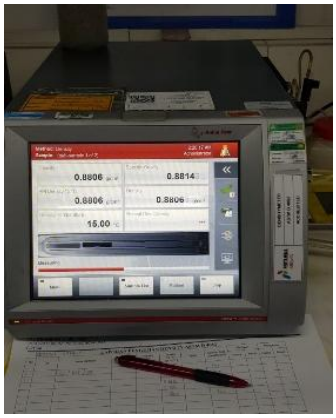

Figure 2. The result of ASTM-D4737

By referring to these previous studies, this research adopts these parameter ranges for optimization using Design Expert 13, ensuring that the selected values are relevant and have been shown to influence biodiesel yield and cetane number effectively. The yield is calculated based on the mass of biodiesel produced relative to the initial mass of oil used in the reaction, expressed as a percentage, using Equation 3.

$$Yield\ Biodiesel\ (\%) = \frac{mb}{mo}\ x\ 100 \qquad (3)$$

Remark:
mb: mass of biodiesel produced (g)
mo: mass of oil used (gr)

The Design Expert 13 application can be seen in Table 1. The data obtained indicated that the cetane number of biodiesel derived from avocado seed oil ranged from 52 to 75. Descriptive statistical analysis, including calculations of the mean, median, and standard deviation, was employed to assess the variation and reliability of the results.

ll biodiesel samples met the established RSM standards, underscoring the potential of biodiesel as an efficient alternative fuel. This confirms the viability of biodiesel from avocado seed oil for use in diesel engines, contributing to sustainable energy solutions. The positive outcomes of this study support the notion that using agricultural waste, such as avocado seeds, for biodiesel production not only enhances fuel properties but also contributes to waste reduction. Future research should focus on optimizing the transesterification process and exploring the economic feasibility of large-scale production. Additionally, life cycle assessments could be conducted to evaluate the environmental benefits associated with the use of avocado seed oil biodiesel compared to traditional fossil fuels.

Table 1. Experiment data result

Factor 1: mole ratio of methanol	Factor 2: Temperature (C)	Response 1: Yield (%)	Response 2: Cetane Number
4	40	62	52,44
4	50	66	56,47
4	60	68	62,5
6	40	80	72
6	50	82	75
6	60	84	70
8	40	70	58,32
8	50	66	56,44
8	60	60	55,74

Based on these data, the results of data processing are obtained to determine the optimization process model with the RSM method through Table 2. Model selection is based on the analysis results from the Design Expert 13 application. The quadratic model is preferred over other models due to its higher R^2 value of 0,9605. However, the cubic model exhibits the standard deviation at 3,20, while the quadratic model has a standard deviation of 2,66.

Table 2. Summary statistical model of cetane number response

Source	Std. Dev	R^2	Adjusted R^2	Predicted R^2	Press	
Linear	9,43	0,0095	-0,3206	-1,0359	1096,88	
2F	9,94	0,0837	-0,4661	-2,1520	1698,20	
Quadratic	2,66	0,9605	0,8948	0,6091	210,59	Suggested
Cubic	3,20	0,9810	-0,8483	-2,4566	1862,34	aliased

Table 3. ANOVA results on cetane number response of the quadratic model

Source	Sum of squares	Df	Mean square	F-value	P-Value	
Model	*517,52*	5	103,50	14,61	0,0257	Significant
A-Rasio Mol Metanol	0,1380	1	0,1380	0,0195	0,8978	
B-Suhu	5,01	1	5,01	0,7064	0,4624	
AB	39,94	1	39,94	5,64	0,0981	
A^2	471,14	1	471,14	66,49	0,0039	
B^2	1,29	1	1,29	0,1822	0,6983	
Residual	*21,26*	3	7,09			
Cor Total	538,78	8				
R²	0,9605					
Adj R²	0,8948					
Pred R²	0,6091					
Adec prcision	3,9771					

The quadratic model emphasizes maximizing the R² value while also considering the predicted R². In contrast, the cubic model appears to lack significance, as indicated by the Lack of Fit Test, which reveals that the adjusted R² value does not support the cubic model. Additionally, results from the cubic model are described as aliased, suggesting that not all parameters of the cubic model can be accurately estimated. A high R² value indicates a strong correlation between variables (Pinto et al., 2020). The R² value of 0,9605 signifies that the methanol mole ratio and reaction temperature account for 96,05% of the variability in the cetane number response, with the remaining 3,95% attributable to other factors outside the model. The adjusted R² value is beneficial for calibrating the R² value in the population, as it reflects an estimation of the population's variability. The quadratic model achieves the highest adjusted R² value of 0,9605. Furthermore, the PRESS (predicted error sum of squares) value for the quadratic model (Adeniyi, Ighalo and Odetoye, 2020) is the smallest at 210,59 indicating that a lower PRESS value corresponds to improved predictive accuracy. In the Predicted R² section, the model yields a value of 0,6991 which is the highest among the models evaluated.

Based on the ANOVA results presented in Table 3, the relationship between the methanol mole ratio, reaction temperature, and cetane number value is analyzed. A significant relationship is indicated when the p-value is less than 0.05 (5%) (Mohmad Noor et al., 2020). In this study, the model does not demonstrate a significant influence on the response, as indicated by a p-value of 0,0257. This value suggests a 2,57% probability that noise or disturbances could be affecting the results.

Both Variable A (methanol mole ratio) and Variable B (reaction temperature) have a p-value of 1.0000, indicating a substantial influence on the cetane number response. Generally, a high R² value implies a good fit between predicted and experimental data. Figure 3 llustrates this relationship, highlighting the correlation between the predicted values and the experimental results (Bullo & Fana, 2021).

Figure 3 presents a plot of predicted versus experimental data, illustrating how well the equation model fits the process, characterized by data scatter points closely aligned with the linear regression line.

Figure 3 indicates that the distribution of data points is near the experimental values, suggesting a strong correlation between the predicted and actual results. Furthermore, the model's performance is assessed through the lack of fit analysis, which reveals the difference between the adjusted R² and predicted R² values. Additionally, the value of adequate precision meets the predetermined requirements. Collectively, these metrics demonstrate that this Response Surface Methodology (RSM) model is suitable for

application in the analysis of the cetane number based on the methanol mole ratio and reaction temperature.

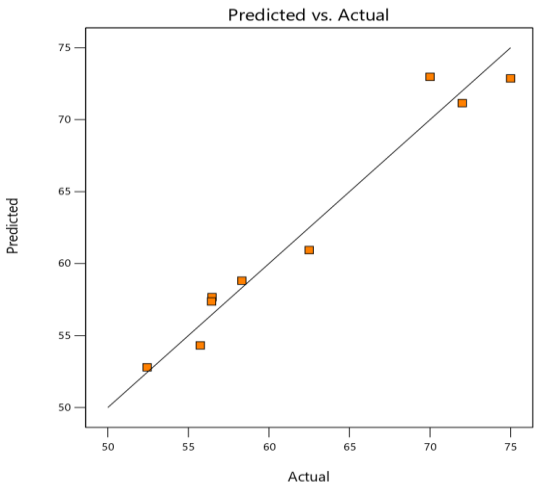

Figure 3. Relationship graph between data and prediction with experimental data

The equation obtained from the selected model of the cetane number response is as follows:

$$Y = -136{,}86111 + 53{,}86917\,A + 1{,}84267\,B - 0{,}158\,AB - 3{,}83708\,A^2 - 0{,}008033B^2 \quad (4)$$

Remark:
Y : Cetane Number
A : Mole methanol ratio
B : Temperature reaction

The equation represents the mathematical model utilized to determine the cetane number response based on varying values of the specified variables. This model enables the prediction of the cetane number under different experimental conditions, facilitating a comprehensive analysis of the factors influencing biodiesel production. By inputting diverse values for the independent variables into this equation, one can accurately calculate the corresponding cetane number, thus aiding in the optimization of the biodiesel production process.

3.3. Graph of The Effect of Methanol Mole Ratio and Reaction Temperature

The surface shape of the effect of methanol mole ratio and reaction temperature with cetane number response can be seen in Fig. 4. The surface curve model illustrating the effect of methanol mole ratio and reaction temperature on cetane number is parabolic in shape. The methanol mole ratio variable significantly influences the cetane number response, as evidenced by the color changes observed on the curve. The 3D contour plot displays these color variations, with red indicating higher response values and blue indicating lower response values, reflecting the impact of the independent variables (Abdmunaf Atta et al., 2022). The observed color changes suggest that the treatment involving the addition of entrainer volume has a substantial effect on bioethanol content. The highest bioethanol content is represented by red, while green indicates lower bioethanol content. As the volume of the entrainer and the reflux-to-distillate ratio increase, the bioethanol content obtained is correspondingly higher. This highlights the importance of optimizing these variables to enhance bioethanol production in the process (Kumar et al., 2021).

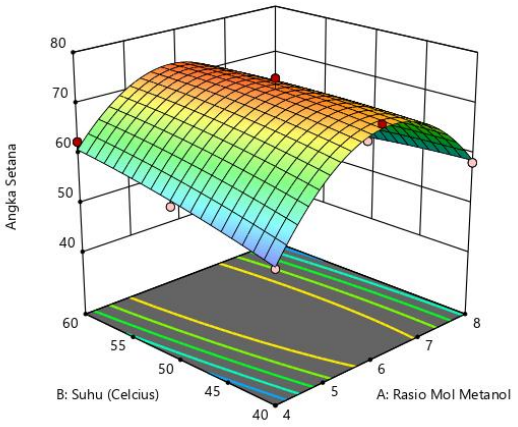

Figure 4. Effect of methanol mole ratio and reaction temperature on cetane number generated in the Design Expert 13 program

Optimization in this study aims to determine the best factor value in order to produce the optimum response value. The optimal solution can be seen in Table 4.

Table 4. Optimal solution

Methanol Ratio	Temperature	Cetane Number	Desirability
6	50	75	0.95

Based on the results presented in Table 4, the solution derived from the Design Expert 13 calculation system identifies the optimal conditions for biodiesel production. The best response is achieved with a methanol mole ratio of 6 and a reaction temperature of 50°C, yielding a cetane number of 75. Desirability is a metric used to assess how well the optimal solution aligns with the intended response, with a desirability value of 1 indicating a perfect scenario. The optimization results

indicate that both the methanol mole ratio and reaction temperature significantly influence the cetane number response, and these variables are interrelated. The parabolic graph illustrates the quadratic relationship between the independent and dependent variables. At a methanol mole ratio of 6, the response reaches its maximum, as represented by the apex of the parabolic curve, after which the yield begins to decline. Similarly, the reaction temperature shows an optimal point beyond which reaction efficiency decreases. The peak of the parabola signifies the ideal conditions for maximizing biodiesel yield. In comparison to previous research, the RSM optimization results obtained in this study demonstrate improved biodiesel yield, highlighting the effectiveness of the applied methodology.

4. Conclusion

The methanol mole ratio and reaction temperature exhibit a directly proportional relationship, as illustrated by a parabolic graph. The cetane number increases until it reaches an optimal point, after which it declines if the methanol mole ratio or temperature is excessively high. Elevated temperatures can enhance the speed of the transesterification reaction, thereby accelerating the conversion of triglycerides into biodiesel. At optimal temperatures, this leads to an increase in the cetane number, as an efficient reaction yields a more stable biodiesel product. However, excessively high temperatures may induce side reactions and degrade the feedstock, adversely affecting the biodiesel composition. While a high methanol mole ratio can enhance the conversion of triglycerides to methyl esters (biodiesel), an overly high ratio results in surplus methanol. This excess complicates the separation process necessary to obtain pure biodiesel. In this experiment, the optimal conditions for the transesterification process of biodiesel derived from avocado seeds, using the Response Surface Methodology (RSM), are identified as a methanol mole ratio of 6 and a reaction temperature of 50°C. Under these conditions, a biodiesel yield of 82% is achieved, accompanied by a cetane number of 75.

Author contribution statement

A.P.D., Formal analysis, investigation, Supervision, S.M.; Administration, writing – original draft, P.S.N.P. : Conceptualisation, writing – original draft, writing – review and editing.

Data Availability Statement

Available on request to the corresponding author.

Declaration of Competing Interest

The author declares that there is no conflict of interest regarding the publication of this manuscripts

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