



Research Article

Biodiesel Preparation by Optimization of Rubber Seed *In Situ* Esterification

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PAPER INFO

Paper history:

Received: 03 February 2025

Revised: 01 January 2026

Accepted: 14 February 2026

Keywords:

In-situ Esterification,
Methyl Ester,
Biodiesel,
Rubber Seed,
Acid Number,
Yield,
Central Composite Design,
N-hexane Cosolvent

ABSTRACT

Optimization of the *in situ* esterification of rubber seed was investigated in a batch process. Rubber seed is a non-edible seed containing a high vegetable oil content ($54.64 \pm 1.80\%$), and therefore, it does not compete with the food sector. The oil in rubber seeds, however, contains a substantial level of free fatty acids, approximately 10.4%. The oil recovery processing step can be reduced by applying *in situ* esterification. The use of n-hexane as a cosolvent can facilitate oil extraction. Consequently, *in situ* methanolysis-esterification of rubber seed with n-hexane as a cosolvent was employed to produce a high-yield, low-acid-number methyl ester, which was optimized using a central composite design of the response surface method. The acid number was determined by AOCS Cd 3d-63 titration, and the methyl ester composition was verified using Gas Chromatography-Mass Spectrometry analysis. Optimization of rubber seed *in situ* esterification resulted in a maximum yield of $89.92 \pm 0.99\%$ and a low acid number of 0.45 mg KOH/g at a solution volume of methanol and n-hexane to rubber seed mass ratio of 7:1 mL/g, a methanol volume fraction of 0.44 in the blended methanol-n-hexane solution, and 12.37 wt.% H_2SO_4 for a reaction time of 5 hours. The methyl ester yield increased and the acid number decreased with increasing reaction time up to 5 hours. Beyond this duration, extending the reaction time did not significantly enhance the methyl ester yield or reduce the acid number. The obtained methyl ester properties complied with the SNI-7182 standard.

<https://doi.org/10.30501/jree.2026.499402.2232>

1. INTRODUCTION

In the Indonesian Energy Outlook 2022, energy consumption in Indonesia was still dominated by fuel oil, accounting for 26.8% (33 million TOE), with the transportation sector consuming the most at 44.2%, higher than the industrial sector at 33.5%. To address this, the Indonesian government has implemented an energy diversification program, blending 35% biodiesel with petrodiesel (Ibrahim et al., 2022).

Other fuel types include fatty acid alkyl esters (biodiesel), which are still under development in Indonesia. Biodiesel is derived from plant-based oils and fats from livestock, making it a resource that can be recycled and regenerated rapidly. It is also more environmentally friendly than diesel oil, as it emits fewer harmful substances and greenhouse gases (Othman et al., 2017; Sakthivel et al., 2018). Biodiesel is typically produced by transesterification from raw materials with low free fatty acid content, such as edible plant oils. A major barrier to biodiesel production is the higher production cost, which includes raw material and processing expenses. When biodiesel is produced primarily from edible plant oils, its cost becomes a critical concern due to their extensive use in the food industry (Ahmed et al., 2022; Al-Humairi et al., 2022; Mani et al., 2020; Oyekunle et al., 2024).

Indonesia possesses numerous plants with high vegetable oil content, including rubber seeds, which have significant potential as a biodiesel source. As the world's largest rubber

producer, Indonesia has approximately 3.4 million hectares of harvested rubber area, an estimated rubber seed production of 5.4 Mt, and a rubber seed oil potential of 0.89 Mt (Zhu et al., 2014; Oyekunle et al., 2024). In addition to producing rubber latex, rubber trees yield rubber seeds, which currently have little market value beyond their use as seeds. Rubber seeds are a promising biodiesel feedstock due to their high vegetable oil content, around 54%, and their non-edible nature, as they contain cyanide, which is toxic to humans. Therefore, rubber seeds can be utilized as a biodiesel feedstock, enabling large-scale biodiesel production without compromising food security (Khazaai et al., 2017; Oyekunle et al., 2024).

Numerous investigations on biodiesel production from oil extracted from rubber seeds have examined transesterification using homogeneous or heterogeneous catalysts. Dhawane explored the conversion of rubber seed oil into biodiesel using lipase-immobilized carbonaceous catalysts. The transesterification conditions included a 10:1 methanol-to-oil molar ratio, a 10-hour reaction time, and 5 mass% catalyst at 30°C. The highest biodiesel yield was 84.05%, with an acid number of 0.13 mg KOH/g (Dhawane et al., 2018). The synthesized heterogeneous SO_3H -MCM-41 catalyst was used to produce biodiesel from rubber seed oil. A biodiesel yield of $83.10 \pm 0.39\%$ was achieved using 14.5 wt.% catalyst for a 48 h reaction time at 129.6°C (Samart et al., 2019). Activated limestone-based cement intermediate, or clinker, was utilized

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URL: https://www.jree.ir/article_241373.html

Please cite this article as: Jalil, F. A., Asif, K., Prabaningrum, N. & Kusnanto, K. (2026). Biodiesel Preparation by Optimization of Rubber Seed *In Situ* Esterification *Journal of Renewable Energy and Environment (JREE)*, 13(2), 01-11 <https://doi.org/10.30501/jree.2026.499402.2232>.



as a heterogeneous catalyst to transesterify rubber seed oil. At a 4:1 methanol-to-rubber-seed-oil molar ratio, a maximum biodiesel yield of 96.9% was achieved using 5% catalyst activated at 700°C during a 4-hour reaction at 65°C ([Gimbun et al., 2013](#)). Pretreated *Hevea brasiliensis* (rubber seed) oil (HBO), with an acid number of 2.02 mg KOH/g, was converted to *Hevea brasiliensis* oil methyl esters (HBOME) via alkaline heterogeneous transesterification. A HBOME yield of 96.97 wt.% was achieved using a methanol-to-HBO ratio of 6:1 and 3.5 wt.% calcined kola nut husk pod catalyst, with a reaction time of 75 minutes at 65°C. The acid number was reduced to 0.22 mg KOH/g ([Oladipoa et al., 2020](#)). A carbon-based KOH-impregnated heterogeneous catalyst derived from flamboyant pods (*Delonix regia*) was synthesized for biodiesel production from rubber seed oil. The maximum yield of 89.3% and an acid number of 0.13 mg KOH/g were achieved at an oil-to-methanol molar ratio of 1:15, using 3.5 wt.% catalyst at 60°C and 750 rpm for 1 hour ([Dhawane et al., 2015](#)).

A methyl ester yield of 93% was achieved by transesterifying rubber seed oil with 430 mg of lipase immobilized on spherical silica aerogels, using an 8:1 methanol-to-oil molar ratio and 15% (v/v) water content at 35°C for 7 hours ([Arumugam et al., 2018](#)). A heterogeneous catalyst composed of alumina (Al₂O₃) impregnated on calcined eggshells (Al₂O₃/calcined eggshells) was utilized in the transesterification of rubber seed oil. Optimal transesterification conditions were identified as a methanol-to-oil molar ratio of 12:1, a catalyst concentration of 3 wt.%, and a reaction duration of 4 hours. Under these conditions, a methyl ester conversion of 98.9% was achieved, and the acid number was reduced from 67.6 to 0.33 mg KOH/g ([Lakshmi et al., 2020](#)).

The extracted oil from rubber seeds was esterified by reacting it with a 6:1 methanol-to-oil molar ratio and 2.5% H₂SO₄ for 30 minutes at 60°C. The free fatty acid content decreased from 5.70% to 1.99%. The esterified rubber seed oil was subsequently transesterified using a 1.4:1 (v/v) mixture of tetrahydrofuran and methanol, catalyzed by 1% KOH. At 52°C for 30 minutes, a maximum biodiesel yield of 97% was achieved ([Ridwan et al., 2023](#)).

Alkaline transesterification of rubber seed oil was investigated using 1% (wt.) NaOH, with an oil-to-methanol molar ratio of 1:6 at 60°C for 1 hour. Under these conditions, the acid number decreased from 4 to 0.9 mg KOH/g, and a methyl ester yield of 84% was obtained ([Ikwaugwu et al., 2000](#)). Sulfuric acid-catalyzed esterification of *Hevea brasiliensis* was conducted to reduce the acid index using methanol at 45°C for 45 minutes. The acid index decreased by 99.5%. A biodiesel ester content of 98% was obtained after the subsequent alkaline-catalyzed transesterification step ([Lüneburger et al., 2022](#)). An oil content of 61.3 wt.% was extracted from rubber seeds and used to synthesize biodiesel via a two-step esterification-transesterification process.

The first esterification step employed a 6:1 methanol-to-oil molar ratio and 5% (v/v) H₂SO₄ at 60°C for 90 minutes. The subsequent transesterification step employed a 6:1 methanol-to-oil molar ratio and 1% NaOH at 60°C for 90 minutes. Under these conditions, a biodiesel yield of 81.55 wt.% was achieved, and the acid number decreased from 6.68 to 0.22 mg KOH/g ([Sugebo et al., 2021](#)).

Another method for biodiesel production is *in situ* transesterification, in which vegetable oil reacts directly with an alcohol in the presence of a catalyst. In this process, extraction and transesterification occur simultaneously.

Conventional (trans)esterification requires vegetable oil as the raw material for biodiesel production. Vegetable oil is obtained through extraction, or leaching, from oil-bearing seeds. The extracted oil requires pretreatment, such as neutralization, degumming, and bleaching, followed by further refining. If the vegetable oil contains high levels of free fatty acids, esterification is performed to reduce them and prevent saponification. Subsequently, transesterification converts the vegetable oil into methyl esters ([Ahmed et al., 2022](#); [Lüneburger et al., 2022](#); [Milano et al., 2025](#)). In contrast, the *in situ* (trans)esterification approach integrates extraction and reaction into a single unit operation. This method eliminates the need for intermediate solvent distillation and oil heating cycles, which are major energy-consuming steps in industrial processing. Additionally, the use of alcohol as both extraction solvent and reactant optimizes the total solvent volume and significantly reduces wastewater generation associated with intermediate purification steps.

This process offers a more cost-effective and environmentally sustainable approach for processing low-grade feedstocks compared to conventional multi-stage methods ([Ahmed et al., 2022](#); [Jain, 2025](#); [Mani et al., 2020](#)).

In situ (trans)esterification of rubber seed (*Hevea brasiliensis*) using H₂SO₄ as a catalyst was applied by [Widayat et al. \(2013\)](#) to produce biodiesel. A fatty acid methyl ester (FAME) yield of 53.61% was obtained within 120 minutes using 0.25% (v/v) H₂SO₄. A 1:3 raw material-to-methanol ratio yielded the highest FAME content of 91.05%. The density, kinematic viscosity, and iodine number of the produced FAME complied with the biodiesel standard SNI 04-7182-2006. However, the acid number of the FAME remained high, ranging from 48.42 to 66.33 mg KOH/g ([Widayat et al., 2013](#)). A methyl ester yield of 74% was achieved through *in situ* transesterification of rubber seed using a rubber-seed-to-methanol mass ratio of 1:6 (10 g/60 g) with 2.5 g KOH catalyst at 60°C for 120 minutes ([Abdulkadir et al., 2014](#)). Further process optimization increased the maximum yield from 74% to 96% by employing a seed particle size of 150 microns and 3 g (30 wt.%) KOH catalyst, while maintaining the rubber-seed-to-methanol mass ratio at 1:6, the temperature at 60°C, and the reaction time at 120 minutes ([Abdulkadir et al., 2015](#)).

Several *in situ* transesterification processes for oilseeds have been investigated using various feedstocks. A biodiesel yield of 96.62% was obtained from student mess food waste via KOH-catalyzed *in situ* transesterification. The optimal parameters, determined using a Box-Behnken design, were 1.35% (w/w) KOH, a methanol-to-food-waste ratio of 98.33%, and a reaction time of 107.06 minutes at 50°C ([Pereira et al., 2023](#)).

Acid-catalyzed *in situ* transesterification of *Madhuca longifolia* seeds was conducted to produce biodiesel. Ultrasonic pretreatment was applied to seeds with a moisture content of 1% and a particle size of 0.61 mm.

Reaction conditions consisting of 15 wt.% sulfuric acid, an oilseed-to-methanol molar ratio of 1:35, a temperature of 55°C, and a stirring speed of 400 rpm for 180 minutes produced the highest biodiesel yield of 96% ([Mani et al., 2020](#)).

A combined ultrasound-microwave reactor was employed to facilitate KOH-catalyzed *in situ* transesterification of castor seeds. Optimization using a central composite design resulted in a maximum biodiesel yield of 93.5 ± 0.76%. The optimum conditions were a methanol-to-oil molar ratio of 350:1, a catalyst loading of 1.74 wt.%, and a reaction time of 30 minutes at 43°C ([Thakkar et al., 2019](#)).

Reactive extraction, or *in situ* transesterification, of *Calophyllum inophyllum* oilseeds was investigated using a methanol and n-hexane solvent system with a sulfuric acid catalyst. Under optimal conditions, specifically 4% (wt./v) catalyst concentration, a 1:10 flakes-to-methanol ratio, and 15 hours of reaction at 700 rpm, a biodiesel yield of 96.88% with an acid value of 0.56 mg KOH/g was achieved ([Adenuga et al., 2020](#)). Comparative studies evaluated sulfuric acid-catalyzed *in situ* transesterification and conventional transesterification for *Annona squamosa* seeds.

The *in situ* process achieved an optimal methyl ester yield of 84% using 5 wt.% catalyst and a 15:1 methanol-to-oil ratio at 60°C. However, the conventional method demonstrated slightly higher efficiency, achieving an 88% conversion with lower chemical inputs, namely 3.5 wt.% catalyst and a 9:1 molar ratio at the same temperature ([Parthiban et al., 2021](#)).

In situ esterification of freshwater *Chlorella vulgaris* microalgae, harvested via foam fractionation, was investigated using a sulfuric acid catalyst. A maximum biodiesel yield of $96 \pm 0.2\%$ was achieved under optimal conditions consisting of a 1000:1 methanol-to-oil molar ratio at 70°C for 90 minutes ([Al-Humairi et al., 2022](#)). *In situ* transesterification of *Spirogyra elongata* algae was performed using a KOH-activated wheat straw biochar catalyst. Optimization via Box-Behnken design identified the optimal parameters as a catalyst loading of 1.42%, a methanol-to-biomass ratio of 97.56% (v/v), and a reaction time of 106.45 minutes. Under these conditions, an experimental biodiesel yield of 96.96% was obtained, closely matching the predicted value of 98.82% ([Jain, 2025](#)).

In the *in situ* esterification process, methanol catalyzed by a homogeneous acid catalyst is used simultaneously for extraction, esterification, and transesterification. Methanol is the preferred alcohol for methyl ester production due to its low cost and its ability to facilitate glycerol separation. Sulfuric acid (H_2SO_4) is employed as a homogeneous acid catalyst because of its high catalytic activity and its effectiveness in converting feedstocks with high free fatty acid content into methyl esters compared to other acid catalysts ([Gomaa et al., 2021](#); [Budiastuti et al., 2022](#); [Al-Humairi et al., 2022](#); [Jain, 2025](#)). Sulfuric acid also has the ability to attract water molecules formed as byproducts during the esterification process ([Budiastuti et al., 2022](#)).

However, methanol exhibits low solubility and limited extractability in vegetable oil. Therefore, cosolvents are sometimes required to enhance oil extraction from oilseeds, such as n-hexane, acetone, petroleum ether, and chloroform ([Ahmed, et al., 2022](#); [Thanh, et al., 2022](#)).

Accordingly, in this study, n-hexane was added to facilitate the leaching of rubber seed oil, while methanol catalyzed by sulfuric acid (H_2SO_4) was used to transesterify triglycerides and esterify free fatty acids. The *in situ* esterification of rubber seeds was optimized using a central composite design within the response surface methodology framework to achieve the lowest possible methyl ester acid number and the highest methyl ester yield.

2. MATERIALS AND METHODS

2.1 Research Materials

Rubber seeds were purchased from the Banyumas local market in Central Java, Indonesia. The chemicals used included anhydrous methanol (Merck), n-hexane (Merck), 2-propanol (Merck), toluene, sulfuric acid (96%, pro analyst, Merck), potassium hydroxide (85%, pro analyst), and phenolphthalein

(99%, pro analyst). Salt was purchased from a local market in Yogyakarta, Indonesia.

2.2 Design of Experiment

The ranges for the central composite design were determined based on preliminary single-factor experiments, which indicated that the optimal reaction conditions were located near the selected center point. Four independent variables were considered: the ratio of mixed solvent volume, methanol and n-hexane, to rubber seed mass (R_{V-m} , 7:1 to 15:1 mL/g), the volume fraction of methanol in the mixed solvent (f_{vol} , 0.15 to 0.75 v/v), the H_2SO_4 catalyst mass percentage ($C_{H_2SO_4}$, 5 wt.% to 25 wt.%), and the reaction time (t , 1 to 5 hours). Two responses were evaluated, biodiesel yield (Y_B) and acid number (AN).

The mixing speed was maintained at 1000 rpm, the rubber seed mass (m_s) was fixed at 20 g, and the reaction temperature was set at 50°C. The experimental design for rubber seed *in situ* esterification was based on the Central Composite Design (CCD) of Response Surface Methodology (RSM) ([Myers & Montgomery, 2002](#)).

2.3 Feedstock Preparation and Degumming Process

Feedstock preparation involved separating the rubber seed kernel from the shell by cracking the shell with a hammer. Only seeds with white flesh and without signs of spoilage were selected. The degumming process was conducted to reduce gum content, as gums can interfere with the oil separation during *in situ* esterification and consequently decrease methyl ester yield.

Degumming was performed by soaking the rubber seeds in a 5 wt.% salt solution. After 24 hours, the salt solution was discarded, and the seeds were steamed. After approximately 50 minutes of steaming, the seeds were removed and oven-dried at 80°C to eliminate moisture. The dried rubber seeds were then crushed to obtain particles smaller than 0.1 cm.

2.4 Extracting oil from rubber seeds

Oil from rubber seeds was extracted using a Soxhlet extractor. Crushed rubber seeds with a particle size of less than 0.1 cm were weighed to 50 g and placed into a thimble. A total of 250 mL of n-hexane was poured into a round-bottom flask. The extraction process was conducted for six hours, with five continuous cycles.

After extraction, n-hexane was removed from the oil by batch distillation. The oil content (OC) of the rubber seeds was calculated as the ratio of the extracted oil mass to the total seed mass. The acid number of the rubber seed oil was determined using the AOCS Cd 3d-63 titration method.

2.5 *In situ* esterification of rubber oilseeds

In situ esterification of rubber oilseeds was carried out in a magnetically stirred batch reactor equipped with a condenser and a thermometer. A total of 20 g of crushed rubber seeds was placed into a round-bottom flask.

The H_2SO_4 catalyst was first dissolved in methanol, after which a specified volume of n-hexane was added. The methanol and n-hexane mixture was transferred into the flask once the solution reached 50°C. The reaction was maintained at 50°C for the designated time and agitated at 1000 rpm.

After completion of the reaction, the mixture was filtered to separate the solid residue from the liquid phase. The filtrate was transferred into a separating funnel and washed with water. After allowing sufficient settling time, two phases were formed. The lower phase contained impurities, including glycerol,

catalyst, alcohol, and water, while the upper phase consisted of methyl ester, and n-hexane. The lower phase was removed, and the upper phase was washed with water and subsequently distilled to separate n-hexane and residual water. Remaining traces of water and n-hexane were removed by heating the methyl ester at 70°C for 12 hours.

The purified methyl ester was stored in a clean, dry bottle at room temperature for further analysis. The methyl ester yield from rubber seed *in situ* esterification was calculated as the ratio of the methyl ester mass to the theoretical oil mass determined by Soxhlet extraction.

3. RESULTS AND DISCUSSION

The oil content of rubber seeds was determined because it was used to calculate the mass percentage of the H₂SO₄ catalyst. Table 1 presents the oil content and acid number of rubber seed oil obtained from five extraction experiments. The average oil content was 54.64 ± 1.80%.

The acid number of the rubber seed oil was 20.87 ± 0.61 mg KOH/g, corresponding to approximately 10.44% free fatty acid content, which exceeds 1%. Therefore, *in situ* esterification of rubber seeds using an H₂SO₄ acid catalyst was necessary to reduce the acid number or free fatty acid content.

Table 1. The oil content and acid number of extracted rubber seed oil

No.	m _{seed} (g)	m _{oil} (g)	OC (%)	AN (mg KOH/g)
1	50	28.0	56	21.32
2	50	26.5	53	20.20
3	50	28.5	57	21.32
4	50	26.5	53	20.20
5	50	27.1	54.2	21.32
		Result	54.64 ± 1.8	20.87 ± 0.6

3.1 Analysis of variance and regression analysis

The experimental central composite design results for methyl ester yield and acid number obtained from the *in situ* esterification of rubber seeds are presented in Table 2. Regression analyses were conducted on the experimental data for methyl ester yield and acid number to derive empirical quadratic models, as presented in Equation (1) and Equation (2).

$$Y = 223.1 - 18.62R_{V-m} - 90.6f_{vol} + 0.78C_{H_2SO_4} - 13.98t + 0.301R_{V-m}^2 - 1991.6f_{vol}^2 - 0.0347C_{H_2SO_4}^2 - 0.679t^2 + 9.93R_{V-m}f_{vol} + 0.1209R_{V-m}C_{H_2SO_4} + 1.708R_{V-m}t + 0.07f_{vol}C_{H_2SO_4} + 30.42f_{vol}t - 0.461C_{H_2SO_4}t \quad (1)$$

$$AN = 33.19 + 0.592R_{V-m} - 81.83f_{vol} - 1.545C_{H_2SO_4} - 1.210t - 0.0313R_{V-m}^2 + 47.43f_{vol}^2 + 0.02360C_{H_2SO_4}^2 - 0.0400t^2 + 0.721R_{V-m}f_{vol} - 0.0106R_{V-m}C_{H_2SO_4} - 0.0175R_{V-m}t + 1.243f_{vol}C_{H_2SO_4} + 2.008f_{vol}t + 0.0352C_{H_2SO_4}t \quad (2)$$

Where,

Y: the rubber seed *in situ* esterification (methyl ester) yield (%)

AN: acid number (mg KOH/g)

R_{V-m}: proportion at the mixed solution (methanol and n-hexane) in the seed mass (mL/g)

f_{vol}: methanol relative to mixed (methanol and n-hexane) volume fraction

C_{H₂SO₄}: H₂SO₄ catalyst mass percent (wt.%)

t: reaction time (hour)

These quadratic equations were then used to predict the *in situ* esterification yield and the acid number of rubber seed oil, as shown in Equations (1) and (2), respectively.

Analysis of variance (ANOVA) was used to evaluate the predicted responses for rubber seed *in situ* esterification yield and acid number, in order to determine the significance of the effects of the independent variables and their interactions. The significance of each term in the empirical quadratic models was assessed at a 95% confidence level.

An independent variable was considered to have a significant effect on the response when the F-value of a given term exceeded the theoretical F-value of 4.67 and the corresponding P-value was less than 0.05.

Table 3 and 4 present the analysis of variance for the empirical quadratic models describing rubber seed *in situ* esterification yield (Equation 1) and acid number (Equation 2). The F-values for the yield and acid number models are 44.14 and 69.87, respectively, while the corresponding model P-values are 0.000, indicating that both models are highly significant for predicting methyl ester yield and acid number.

The Lack-of-Fit test produced F-values of 0.27 for methyl ester yield and 5.79 for acid number, with corresponding P-values of 0.950 and 0.088.

Because these P-values exceed 0.05, the Lack-of-Fit is not significant relative to the pure error. This confirms that the quadratic models adequately represent the experimental data and that no significant systematic variation remains unexplained.

Table 3 indicates that the response of rubber seed *in situ* esterification yield was not significantly affected by six terms: the linear and quadratic terms of the mixed solution-to-seed mass ratio (R_{V-m} and R_{V-m}²), the quadratic term of the H₂SO₄ catalyst mass percent (C_{H₂SO₄}²), the quadratic term of reaction time (t²), the interaction between the mixed solution-to-seed mass ratio and the H₂SO₄ mass percent (R_{V-m} × C_{H₂SO₄}), and the interaction between the methanol volume fraction in the mixed solution and the H₂SO₄ mass percent (f_{vol} × C_{H₂SO₄}). For these terms, the P-values were greater than 0.05 and the F-values were lower than the theoretical F_{0.05}(1,13) value of 4.67, indicating a lack of statistical significance. In contrast, the remaining regression coefficients had a significant effect on methyl ester yield.

Table 4 presents the ANOVA results for the predicted acid number of the rubber seed *in situ* esterification products described by Equation (2).

Six factors were found to have no significant effect on the acid number response: the linear and quadratic terms of the mixed solution-to-seed mass ratio (R_{V-m} and R_{V-m}²), the interaction between the mixed solution-to-seed mass ratio and the H₂SO₄ mass percent (R_{V-m} × C_{H₂SO₄}), the interaction between the mixed solution-to-seed mass ratio and reaction time, and the interaction between H₂SO₄ mass percent and reaction time. The other regression coefficients significantly influenced the methyl ester acid number.

Figure 1 illustrates the relationship between the predicted and experimental results for rubber seed *in situ* esterification (yield and acid number).

The coefficients of determination (R²) are 0.9794 for methyl ester yield and 0.9869 for acid number, indicating that 97.94% of the variability in experimental yield and 98.69% of the variability in experimental acid number are explained by the respective empirical models (Equations 1 and 2).

Table 2. Central composite design, yield, and acid number of rubber seed *in situ* esterification

No	R_{V-m} (mL/g)	f_{vol}	$C_{H_2SO_4}$ (wt.%)	t (h)	Yield (%)		AN (mg KOH/g)	
					Experimental	Predicted	Experimental	Predicted
1.	15	0.45	15	3	71.46	79.68	0.56	0.32
2.	11	0.15	15	3	78.72	78.44	7.29	7.00
3.	13	0.6	20	4	73.75	81.44	0.45	1.13
4.	9	0.6	10	2	65.42	53.17	0.90	1.47
5.	13	0.6	10	2	42.84	47.51	2.47	2.25
6.	13	0.6	10	4	85.77	83.09	2.02	2.01
7.	9	0.6	10	4	71.92	75.09	0.67	1.37
8.	11	0.45	15	3	70.88	77.23	0.90	0.76
9.	13	0.3	20	2	80.18	73.33	0.90	0.94
10.	11	0.45	15	1	48.58	59.50	0.90	1.02
11.	9	0.3	20	2	84.86	86.07	1.12	1.45
12.	13	0.3	10	2	75.49	69.23	5.95	6.26
13.	11	0.45	5	3	76.82	76.59	6.28	6.01
14.	9	0.3	20	4	88.87	80.52	0.45	0.85
15.	11	0.75	15	3	33.67	41.54	3.25	3.05
16.	9	0.6	20	4	63.60	65.34	0.90	0.91
17.	11	0.45	15	3	79.98	77.23	0.56	0.76
18.	11	0.45	15	3	79.18	77.23	0.90	0.76
19.	9	0.3	10	2	85.93	86.81	6.84	6.34
20.	9	0.3	10	4	89.08	90.48	4.71	5.04
21.	7	0.45	15	3	85.04	84.42	0.45	0.19
22.	13	0.3	10	4	86.81	86.57	4.38	4.81
23.	11	0.45	15	5	89.60	89.53	0.79	0.18
24.	11	0.45	25	3	69.12	70.94	0.45	0.23
25.	11	0.45	15	3	78.61	77.23	0.67	0.76
26.	13	0.6	20	2	61.82	51.82	0.67	0.67
27.	13	0.3	20	4	80.74	78.18	0.45	0.20
28.	9	0.6	20	2	54.08	52.64	0.56	0.31

Table 3. Variance analysis of rubber seed *in situ* esterification yield

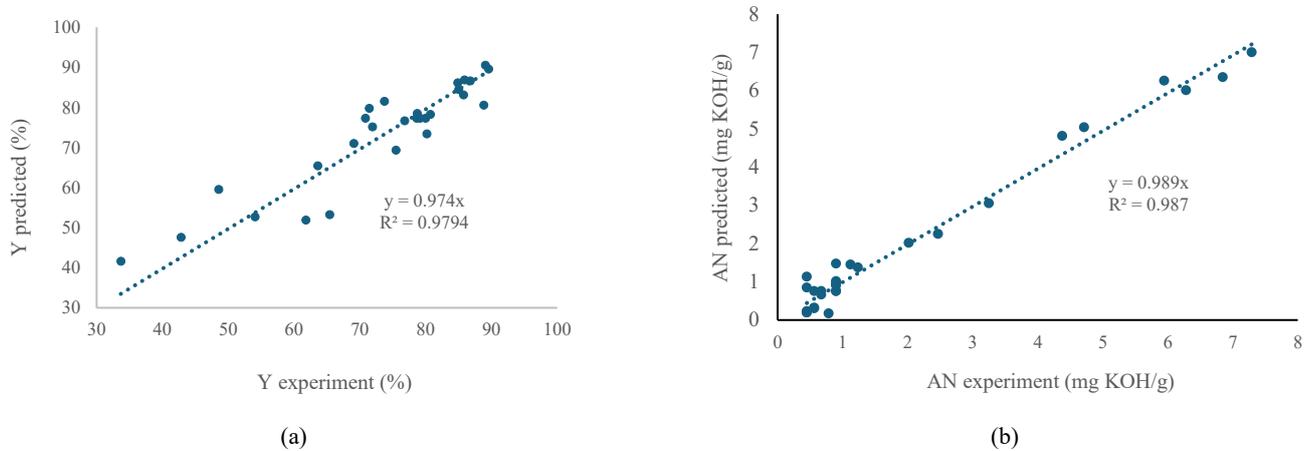
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	14	4837.11	345.51	44.14	0.000
Linear	4	3486.32	871.58	111.35	0.000
R_{V-m}	1	34.99	34.99	4.47	0.054
f_{vol}	1	2047.95	2047.95	261.64	0.000
$C_{H_2SO_4}$	1	48.56	48.56	6.20	0.027
t	1	1354.80	1354.80	173.08	0.000
Square	4	580.82	145.20	18.55	0.000
$R_{V-m} \times R_{V-m}$	1	34.70	34.70	4.43	0.055
$f_{vol} \times f_{vol}$	1	446.09	446.09	56.99	0.000
$C_{H_2SO_4} \times C_{H_2SO_4}$	1	18.06	18.06	2.31	0.153
$t \times t$	1	11.06	11.06	1.41	0.256
2-Way Interaction	6	769.98	128.33	16.39	0.000
$R_{V-m} \times f_{vol}$	1	141.85	141.85	18.12	0.001
$R_{V-m} \times C_{H_2SO_4}$	1	23.38	23.38	2.99	0.108
$R_{V-m} \times t$	1	186.73	186.73	23.86	0.000
$f_{vol} \times C_{H_2SO_4}$	1	0.04	0.04	0.01	0.941
$f_{vol} \times t$	1	333.06	333.06	42.55	0.000
$C_{H_2SO_4} \times t$	1	84.92	84.92	10.85	0.006
Error	13	101.76	7.83		
Lack-of-Fit	10	48.18	4.82	0.27	0.950
Pure Error	3	53.57	17.86		
Total	27	4938.87			

R^2	R_{adj}^2	R_{pred}^2
0.9794	0.9572	0.9245

Table 4. Variance analysis of rubber seed *in situ* esterification acid number

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	14	133.399	9.5285	69.87	0.000
Linear	4	76.519	19.1298	140.27	0.000
R_{V-m}	1	0.075	0.0748	0.55	0.472
f_{vol}	1	23.562	23.5620	172.77	0.000
$C_{H_2SO_4}$	1	51.509	51.5094	377.70	0.000
t	1	1.373	1.3728	10.07	0.007
Square	4	39.720	9.9299	72.81	0.000
$R_{V-m} \times R_{V-m}$	1	0.433	0.4334	3.18	0.098
$f_{vol} \times f_{vol}$	1	28.221	28.2209	206.93	0.000
$C_{H_2SO_4} \times C_{H_2SO_4}$	1	8.161	8.1608	59.84	0.000
$t \times t$	1	0.171	0.1709	1.25	0.283
2-Way Interaction	6	17.160	2.8600	20.97	0.000
$R_{V-m} \times f_{vol}$	1	0.980	0.9801	7.19	0.019
$R_{V-m} \times C_{H_2SO_4}$	1	0.160	0.160	1.17	0.298
$R_{V-m} \times t$	1	0.046	0.0462	0.34	0.570
$f_{vol} \times C_{H_2SO_4}$	1	13.727	13.727	100.66	0.000
$f_{vol} \times t$	1	1.638	1.6384	12.01	0.004
$C_{H_2SO_4} \times t$	1	0.608	0.6084	4.46	0.055
Error	13	1.773	0.1364		
Lack-of-Fit	10	1.686	0.1686	5.79	0.088
Pure Error	3	0.087	0.0291		
Total	27	135.172			

R^2	R_{adj}^2	R_{pred}^2
0.9869	0.9728	0.9270

**Figure 1.** The relationship between the predicted and experimental rubber seeds *in situ* esterification: (a) yield and (b) acid number.

Consequently, only 2.06% of the variability in yield and 1.31% in acid number remain unexplained by the models. The adjusted R^2 values are 0.9572 for methyl ester yield and 0.9728 for acid number, which are in close agreement with the predicted R^2 values of 0.9245 and 0.9270, respectively. The differences between adjusted and predicted R^2 are 0.0327 for methyl ester yield and 0.0458 for acid number, both below 0.2. These findings indicate strong agreement between the models and the experimental data and demonstrate that the quadratic regression models provide an adequate and reliable fit.

3.2 Parameter influences

Figure 2 illustrates the interaction effect between the mixture volume-to-seed mass ratio and the methanol-to-mixture volume ratio on methyl ester yield at an H_2SO_4 catalyst concentration of 15 wt.% and a reaction time of 3 hours. When the mixture volume-to-seed mass ratio exceeds 13:1 mL/g and the methanol-to-mixture volume ratio is greater

than 0.6, the methyl ester yield falls below 70%. In contrast, when the mixture volume-to-seed mass ratio is between 7:1 and 9:1 mL/g and the methanol-to-mixture volume ratio is below 0.4, the yield exceeds 80%. Pereira reported a yield of 66.17 wt.% from the *in situ* transesterification of catolé coconut using a 5:1 methanol-to-oil ratio, which is comparable to the present methanol-to-seed mass ratio of 7:1 mL/g (Pereira et al., 2023).

The methyl ester yield increases as both the mixture volume-to-seed mass ratio and the methanol-to-mixture volume ratio decrease. However, the methanol-to-mixture volume ratio has a more pronounced effect on yield than the mixture volume-to-seed mass ratio. This trend is evident in the figure, where the yield begins to decline once the methanol-to-mixture ratio exceeds 0.4. Moreover, at a methanol-to-mixture ratio of 0.6, increasing the mixture-to-seed ratio from 7:1 mL/g to 15:1 mL/g results in yields within a similar range of 60–70%, further confirming the stronger influence of the

methanol fraction. This behavior can be explained by the composition of the liquid mixture in *in situ* esterification, which contains methanol and non-polar n-hexane used for oil extraction.

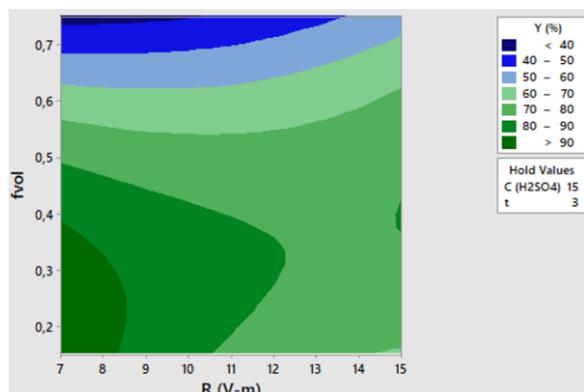


Figure 2. The correlation effects of the ratio of the mixed solution to the rubber seed mass and the blended solution volume fraction on rubber seed *in situ* esterification yield

Increasing the proportion of n-hexane enhances the extraction of rubber seed oil, thereby improving *in situ* esterification yield. In contrast, a higher methanol-to-mixture volume ratio reduces the relative amount of n-hexane, leading to lower oil extraction efficiency and reduced methyl ester yield. Methanol is polar, whereas vegetable oil is non-polar; consequently, methanol has limited capacity to extract rubber seed oil.

Nevertheless, methanol is essential for facilitating esterification and transesterification reactions in the presence of the H_2SO_4 catalyst. An excessive mixture volume-to-seed mass ratio may also reduce yield. As this ratio increases, the total reactant volume in the system increases. If the reactant volume becomes too large, the H_2SO_4 catalyst may become diluted and more widely dispersed within the mixture, reducing its effective concentration. This dilution can hinder esterification and transesterification reactions, ultimately resulting in a lower methyl ester yield.

Figure 3 illustrates the effects of H_2SO_4 catalyst mass percent ($C_{H_2SO_4}$) and the methanol volume fraction in the mixed solution (f_{vol}) on rubber seed *in situ* esterification yield (biodiesel yield), at a fixed mixed solution-to-seed mass ratio of 10.5:1 mL/g and a reaction time of 3.5 hours.

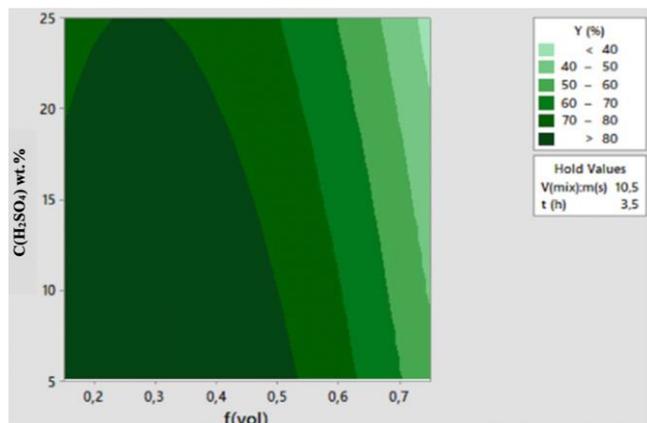


Figure 3. The effect of H_2SO_4 catalyst mass percentage and methanol in the blended solution volume fraction on rubber seeds *in situ* esterification yield.

The results show that yields exceeding 80% were obtained when the H_2SO_4 catalyst mass percent was below 25% and the

methanol volume fraction was below 0.55. The yield increased as the methanol fraction in the mixed solution increased and the H_2SO_4 catalyst mass percent decreased.

During *in situ* esterification, simultaneous extraction, esterification, and transesterification occurred (Ahmed et al., 2022). The extraction step strongly influences methyl ester yield, as nonpolar n-hexane facilitates the leaching of rubber seed oil. Small additions of methanol to the methanol-n-hexane mixture enhance methyl ester production by enabling esterification of free fatty acids and triglycerides. However, if the methanol fraction is too low, the process becomes less efficient because insufficient methanol is available to fully convert free fatty acids and triglycerides into methyl esters.

Increasing the H_2SO_4 catalyst mass percentage from 5 wt.% up to approximately 15 wt.% improved methyl ester yield. Acid catalysts accelerate esterification and transesterification reactions, so increasing catalyst concentration generally increases methyl ester formation. Thanh et al. noted that high methanol and H_2SO_4 concentrations combined with elevated temperatures can also enhance oil extraction from biomass (Thanh et al., 2022). However, excessive acid can reduce methyl ester yield because water is generated as a by-product of esterification. Water can hydrolyze methyl esters back into free fatty acids, increasing the acid number and lowering the effective biodiesel yield.

Figure 4 shows a contour plot of the acid number as a function of methanol fraction in the mixed solution and H_2SO_4 catalyst mass percentage, at a mixture volume-to-seed mass ratio of 11:1 mL/g and a 3-hour reaction time. The acid number remained below 1 mg KOH/g when the methanol-to-mixture volume fraction ranged from 0.4 to 0.6 and the H_2SO_4 catalyst concentration exceeded 12 wt.%. These conditions indicate that an adequate methanol fraction and sufficient catalyst are critical to minimizing residual free fatty acids while maintaining high methyl ester yield.

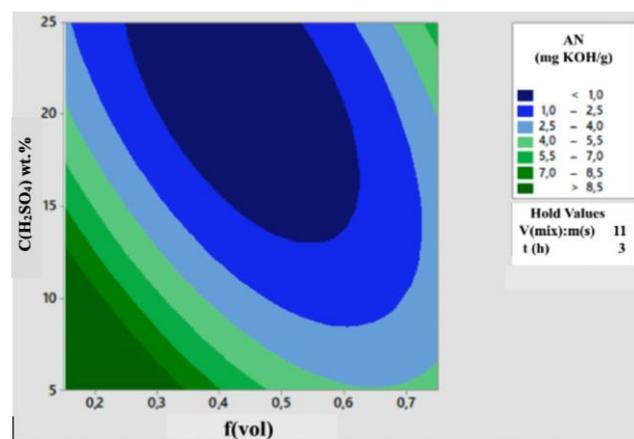


Figure 4. The influence of the interaction between the volume fraction of methanol in the mixed solution and the H_2SO_4 catalyst mass percentage on the acid number of the rubber seed *in situ* esterification product.

This graph demonstrates that the mass percentage of the H_2SO_4 catalyst and the methanol proportion in the combined solution substantially affect the acid number of the rubber seed *in situ* esterification product. The acid number decreased with increasing mass percentages of the H_2SO_4 catalyst and methanol in the combined solution. *In situ* esterification utilized more methanol when the methanol proportion within the combined solution increased. A specific mass percentage of the H_2SO_4 catalyst could drive the esterification and

transesterification reactions optimally. Under these conditions, the highest amount of free fatty acids and triglycerides was converted to methyl esters (biodiesel); consequently, the acid number of the resulting methyl ester was low.

According to the graphs of methyl ester yield (Figure 3) and the acid number of the *in situ* rubber seed esterification product (Figure 4), an increase in yield could enhance the acid number within a specific range. When the methanol volume fraction in the blended solution was below 0.4 and the H₂SO₄ catalyst mass percentage was below 15 wt.%, both the yield and acid number increased. Under these conditions, the amount of methanol was insufficient to react with the unbound fatty acids and triglycerides in the rubber seed oil relative to the mass percentage of H₂SO₄ required to maximize the reaction rate. Excess methanol was needed for the *in situ* esterification reaction; consequently, unbound fatty acids and triglycerides could react to form methyl esters. A specific amount of H₂SO₄ catalyst was required to achieve both a low acid number and a high methyl ester yield.

Figure 5 depicts a contour graph of the methyl ester yield as a function of the methanol volume in the blended solution and the reaction duration, at a ratio of 11:1 mL/g of blended solution to rubber seed mass and an H₂SO₄ catalyst mass percentage of 15 wt.%.

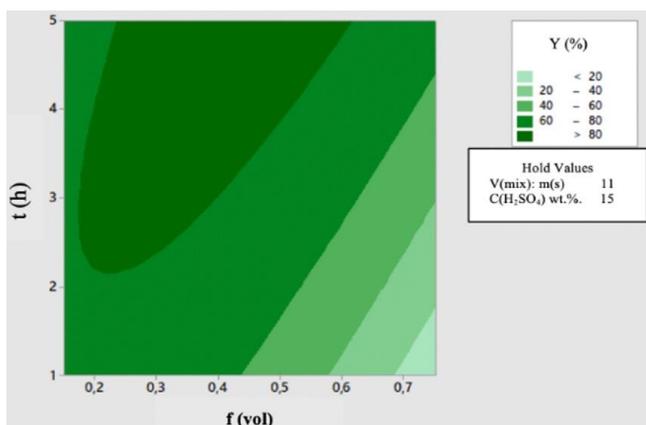


Figure 5. The impact of reaction duration and the methanol volume proportion in the combined solution on methyl ester yields.

The methyl ester yield exceeded 80% when the reaction duration was over two hours and the methanol proportion in the mixture ranged from 0.15 to 0.6. The yield ranged from 60% to 80% at a methanol proportion within 0.15 to 0.44 after two hours of reaction. When the methanol volume in the combined solution significantly exceeded the n-hexane volume, the methyl ester yield declined at methanol proportions above 0.44. Consequently, less rubber seed oil was extracted, lowering the methyl ester yield. A high yield could be achieved by extending the reaction duration while the methanol volume exceeded the n-hexane volume.

Figure 6 presents a contour graph illustrating the correlation between reaction duration and the methanol proportion in the blended solution, and the acid number of the methyl ester at an H₂SO₄ catalyst mass percentage of 15 wt.% and a solution-to-seed ratio of 11:1 mL/g. An acid number below 1 mg KOH/g was observed for reaction durations between 1 and 5 hours, with a methanol-to-combined-solution volume ratio ranging from 0.45 to 0.68.

The acid number decreased with increasing reaction duration and methanol proportion in the combined solution. Methanol was utilized to reduce the acid number through *in*

situ esterification, converting unbound fatty acids and triglycerides into methyl esters in an acid-catalyzed methanol–n-hexane mixture. Excess methanol was required to achieve effective esterification and transesterification of the rubber seeds. Therefore, both the reaction duration and the methanol volume in the combined solution were critical factors. Extending the reaction period decreased the acid number and increased the methyl ester yield. The reaction reached saturation at a specific time; beyond this point, the acid number no longer decreased, and the methyl ester yield did not increase further.

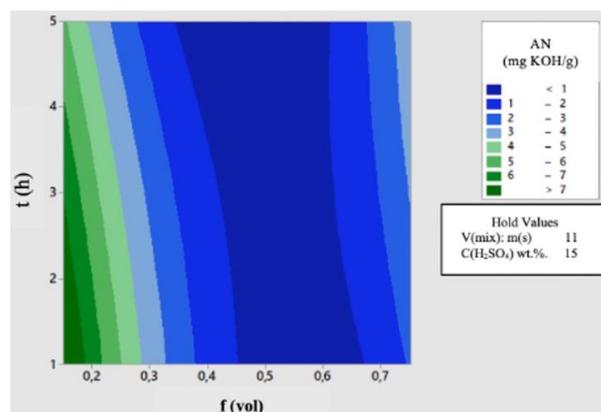


Figure 6. The effect of reaction duration and methanol volume fraction in the combined solution on the acid number of the rubber seed *in situ* esterification product.

3.3 Optimization of rubber seed *in situ* esterification

Rubber seed *in situ* esterification was performed using a methanol–n-hexane blend, achieving a maximal methyl ester yield with a low acid number. Optimization was conducted following statistical analysis of variance (ANOVA) and the derivation of predictive equations for both responses.

The optimization results, illustrated in Figure 7, indicated a methyl ester yield of 89.60% and an acid number of 0.45 mg KOH/g. These results were obtained at a methanol volume fraction of 0.44 in the blended solution, an H₂SO₄ catalyst mass percentage of 12.37 wt.%, a reaction duration of five hours, and a solution-to-seed volume ratio of 7:1 mL/g.

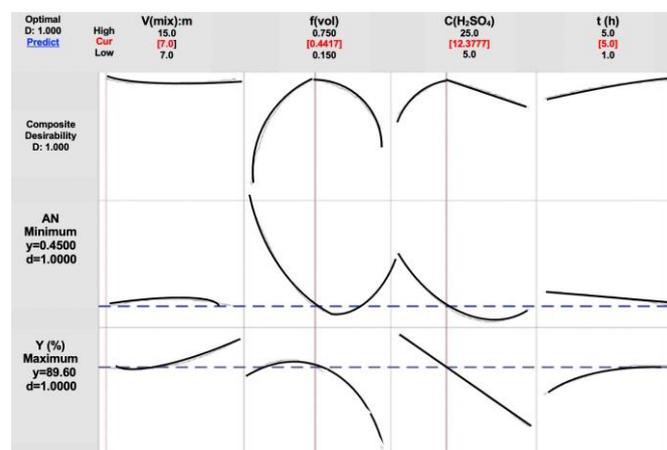


Figure 7. Rubber seed *in situ* esterification optimization results.

3.4 Validation of *in situ* esterification optimization results

Validation was conducted to assess the agreement between experimental data and the optimization results obtained using Response Surface Methodology in Minitab 18. Experimental

validation was performed after obtaining the optimization predictions by applying Response Surface Methodology based on the Central Composite Design.

The validation results indicated a methyl ester yield of $89.92 \pm 0.99\%$, which was 0.33% higher than the predicted value of 89.60%. The methyl ester acid number was 0.45 mg KOH/g. The discrepancy between the experimental outcomes and the predicted values was less than 5%. Therefore, the optimization of rubber seed *in situ* esterification using the Central Composite Design within the Response Surface Methodology framework, employing a methanol–n-hexane mixture with a sulfuric acid catalyst, was confirmed to be valid.

Table 5 presents a comparison of methyl ester yield and acid number obtained from rubber seeds via *in situ* esterification and heterogeneous catalyst esterification, in which the reaction occurs in both solid and liquid phases.

3.5 Impact of reaction duration variation on methyl ester yields and acid numbers

Methyl ester experiments were conducted at varying reaction durations using the optimized rubber seed *in situ* esterification conditions, which included an H_2SO_4 catalyst mass percentage of 12.37 wt.%, a methanol volume fraction of 0.44 in the blended solution, and a combined solution-to-rubber seed mass ratio of 7:1, with reaction times ranging from 1 to 12 hours.

The methyl ester yield at different reaction durations is presented in Figure 8. After the first hour, the biodiesel yield was 43.8%. One hour later, the methyl ester yield increased to 65.2%. The yield continued to rise with reaction time, reaching 87.6% after 5 hours. Extending the reaction time from 6 to 12 hours did not result in a significant increase in methyl ester yield, which remained comparable.

Figure 9 depicts the effect of reaction duration on the methyl ester acid number. Increasing the reaction period led to

a decrease in the methyl ester acid number, reaching less than 0.5 mg KOH/g within 5 hours. Beyond this duration, further extension of the reaction time did not significantly affect the acid number.

Figure 10 presents the methyl ester composition of rubber seed *in situ* esterification as determined by gas chromatography–mass spectrometry (GC-MS) analysis. The GC-MS results indicated that the methyl ester consisted of 10.93% methyl palmitate (C16:0), 39.56% methyl linoleate (C18:2), 37.49% methyl oleate (C18:1), and 12.02% methyl stearate (C18:0).

Characteristics analysis of the rubber seed *in situ* esterification methyl ester indicated a specific gravity of 0.8847 at 15°C, a kinematic viscosity of 5.74 mm^2/s at 40°C, and a water content of less than 0.01%. These methyl ester properties comply with SNI 7182:2015, the Indonesian biodiesel standard.

3.6 Scalability and environmental limitations of n-hexane

Although n-hexane was selected for this laboratory-scale study due to its high extraction efficiency and nonpolar properties, its scale-up for industrial production presents significant challenges. From an environmental perspective, n-hexane is a fossil fuel derivative classified as both a hazardous air pollutant and a neurotoxin. These characteristics necessitate stringent safety protocols and containment systems, increasing capital expenditures. Moreover, while solvent recovery via distillation is technically feasible, the process is energy-intensive and raises the overall carbon footprint. To align with the sustainability objectives of renewable energy production, future research should explore 'green' alternatives, such as bio-ethanol extraction. These alternatives offer lower toxicity and improved environmental compatibility, although they may exhibit different extraction efficiencies.

Table 5. Comparison of methyl ester yield and acid number produced from rubber seeds.

	Methyl ester yield	Acid number (mg KOH/g)
Samart, et al. (2019)	$83.10 \pm 0.39\%$	-
Dhawane, et al. (2018)	84.05%	0.13
Widayat, et al. (2013)	91.05%	48.42 to 66.33
Rahman, et al. (2022)	80.2% (CaO)	0.95
	94.12% (Zn-CaO)	-
Zamberia, et al. (2016)	86.44%	0.15
This study	$89.92 \pm 0.99\%$	0.45

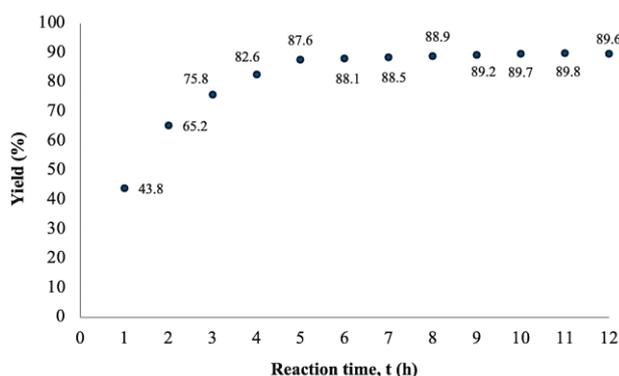


Figure 8. Reaction period impact on methyl ester yields (Y%)

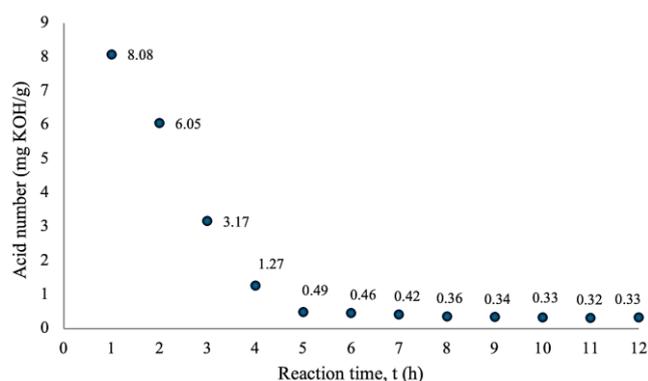


Figure 9. The effect of reaction duration on methyl ester acid number (AN)

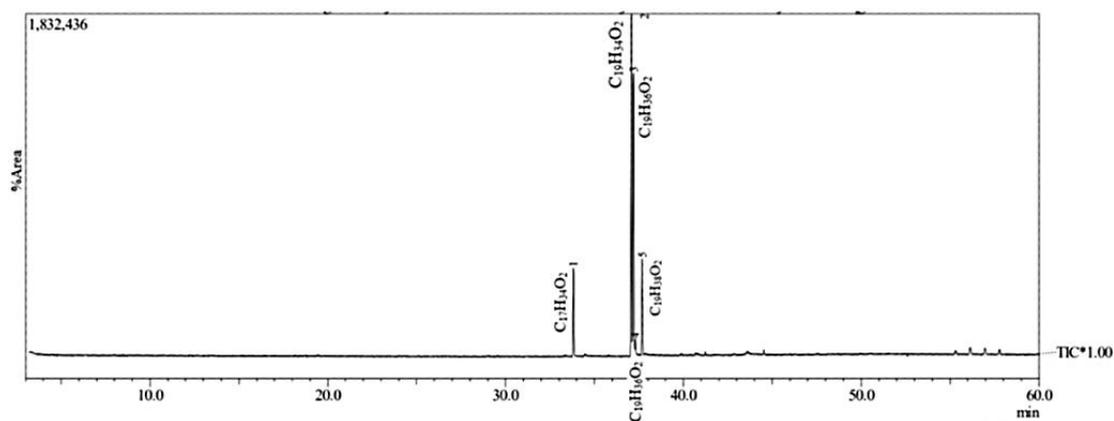


Figure 10. Methyl ester compositions of rubber seed *in situ* esterification

4. CONCLUSIONS

In situ esterification of rubber seeds using a methanol–n-hexane mixture catalyzed by sulfuric acid (H_2SO_4) was performed. A central composite design based on 2^4 full factorials was applied as the experimental design to determine the optimal process parameters that maximize methyl ester yield while minimizing the acid number. Optimal conditions for rubber seed *in situ* esterification were obtained at a mixed solution-to-seed mass ratio of 7:1, a methanol volume fraction of 0.44 in the blended solution, an H_2SO_4 catalyst mass percentage of 12.37 wt.%, and a reaction duration of 5 hours. Under these conditions, the observed methyl ester yield was $89.92 \pm 0.99\%$, and the minimum acid number was 0.45 mg KOH/g. Experiments conducted at various reaction durations showed that methyl ester yield increased and the acid number decreased with longer reaction times up to 5 hours. Beyond 5 hours, no significant changes in yield or acid number were observed. The resulting methyl ester characteristics met the requirements of SNI 7182:2015, the Indonesian biodiesel standard.

5. ACKNOWLEDGEMENT

The authors are highly grateful to the Department of Nuclear Engineering and Engineering Physics, Faculty of Engineering, Universitas Gadjah Mada, Yogyakarta, Indonesia, for satisfying the laboratory facility and research finances.

NOMENCLATURE

Y	Yield (%)
AN	Acid number (mg KOH/g)
R_{V-m}	Proportion of methanol and n-hexane solution to the seed mass (mL/g)
f_{vol}	fraction of methanol volume to methanol and n-hexane volume
$C_{H_2SO_4}$	Sulphuric acid catalyst mass percent (wt.%)
t	Reaction time (h)
H_2SO_4	Sulphuric acid
SNI	Standar Nasional Indonesia (Indonesian National Standard)
GCMS	Gas chromatography-mass spectrometer

REFERENCES

- Abdulkadir, B.A., Danbature, W., Yirankinyuki, F.Y., Magaji, B., & Muzakkir, M.M. (2014). *In Situ* Transesterification of Rubber Seeds (*Hevea brasiliensis*). *Greener Journal of Physical Sciences*, 4(3), 038-044. <https://gjournal.org/GJPS/archive/vol-43-april-2014/bashir-et-al.html>
- Abdulkadir, B.A., Uemura, Y., Ramli, A., Osman, N., Kusakabe, K., & Kai, T. (2015). Production of Biodiesel from Rubber Seeds (*Hevea Brasiliensis*) by *In situ* Transesterification Method. *Journal of Japan Institute of Energy*, 94(7), 763-768, <https://doi.org/10.3775/jie.94.763>
- Adenuga, A.A., Idowu, O.O., & Oyekunle, J.A.O. (2020). Synthesis of quality biodiesel from Calophyllum inophyllum kernels through reactive extraction method: Optimization of process parameters and characterization of the products. *Renewable Energy*, 145, 2530-2537. <https://doi.org/10.1016/j.renene.2019.08.035>
- Ahmed, M., Abdullah, A., Patle, D.S., Shahadat, M., Ahmad, Z., Aslam, M., & Vo, D. V. N. (2022). Feedstocks, catalysts, process variables and techniques for biodiesel production by one-pot extraction-transesterification: a review. *Environmental Chemistry Letters*, 20, 335–378. <https://doi.org/10.1007/s10311-021-01358-w>
- Al-Humairi, S.T., Lee, J.G.M., Salihu, M., & Harvey, A.P. (2022). Biodiesel Production through Acid Catalyst *In Situ* Reactive Extraction of *Chlorella vulgaris* Foamate. *Energies*, 15, 4482. <https://doi.org/10.3390/en15124482>
- Arumugam, A., Thulasidharan, D., & Jegadeesan, G.B. (2018). Process optimization of biodiesel production from *Hevea brasiliensis* oil using lipase immobilized on spherical silica aerogel. *Renewable Energy*, 116, 755-761. <https://doi.org/10.1016/j.renene.2017.10.021>
- Budiastuti, H., Hanifah, N.A., Mardiani, D.U., Haryadi, Rusdianasari, & Fudholi, A. (2022). Biodiesel Production from Rubber Seed Oil as Alternative Energy Source – A Review. *International Journal of Applied Technology Research*, 3(2), 120-134. <https://doi.org/10.35313/ijatr.v3i2.92>
- Dhawane, S.H., Kumar, T., & Halder, G. (2015). Central composite design approach towards optimization of flamboyant pods derived steam activated carbon for its use as heterogeneous catalyst in transesterification of *Hevea brasiliensis* oil. *Energy Conversion and Management*, 100, 277–287. <http://dx.doi.org/10.1016/j.enconman.2015.04.083>
- Dhawane, S. H., Kumar, T., & Halder, G. (2018). Process optimisation and parametric effects on synthesis of lipase immobilised carbonaceous catalyst for conversion of rubber seed oil to biodiesel. *Energy Conversion and Management*, 176, 55-68. <https://doi.org/10.1016/j.renene.2016.12.096>
- Gimbun, J., Ali, S., Kanwal, C. C. S. C., Shah, L., A., Ghazali, N.H.M., Cheng, C., K., & Nurdin, S. (2013). Biodiesel Production From Rubber Seed Oil Using Activated Cement Clinker As Catalyst. *Procedia Engineering*, 53, 13-19. <https://doi.org/10.1016/j.proeng.2013.02.003>
- Gomaa, M.A., Gombocz, N., Schild, D., Mjalli, F.S., Al-Harrasi, A., & Abed, R.M.M. (2021). Effect of organic solvents and acidic catalysts on biodiesel yields from primary sewage sludge, and characterization of fuel properties. *Biofuels*, 12(4), 405-413. <https://doi.org/10.1080/17597269.2018.1479137>
- Ibrahim, H. D., Siswanto, D., Saefulhak, Y., Suharyati, Pratiwi, N. I., Pambudi, S. H., Wibowo, J. L., Arifin, F. D., Sauqi, A., Damanik, J. T., Pangaribuan, D. B. T., & Kristanto, N. (2022). *Indonesia Energy Outlook 2022*. Bureau of Energy Policy and Assembly Facilitation Secretariate General of The National Energy Council. <https://www.scribd.com/document/656333181/Buku-Energi-Outlook-2022-Versi-Bhs-Inggris>

13. Ikwuagwu, O.E., Ononogbu, I.C., & Njoku, O.U. (2000). Production of biodiesel using rubber [*Hevea brasiliensis* (Kunth. Muell.)] seed oil. *Industrial Crops and Products*, 12, 57-62. [https://doi.org/10.1016/S0926-6690\(99\)00068-0](https://doi.org/10.1016/S0926-6690(99)00068-0)
14. Jain, S. (2023). Biodiesel production from food waste using *in situ* transesterification method. *Sustainable Energy Technologies and Assessments*, 58, 103380. <https://doi.org/10.1016/j.seta.2023.103380>
15. Jain, S. (2026). Optimized In-Situ Transesterification of Spirogyra Using KOH-Activated Wheat Straw Biochar Catalyst. *Results in Engineering*, 29. <https://doi.org/10.1016/j.rineng.2025.108896>
16. Khazaai, S. N. M., Maniama, G. P., Rahima, M. H. A., Yusoff, M. M., & Matsumura, Y. (2017). Review on methyl ester production from inedible rubber seed oil under various catalysts. *Industrial Crops and Products*, 97, 191-195. <https://doi.org/10.1016/j.indcrop.2016.11.052>
17. Lakshmi, S.B.A.V.S., Pillai, N.S., Mohamed, M.S.B.K., & Narayanan, A. (2020). Biodiesel production from rubber seed oil using calcined eggshells impregnated with Al₂O₃ as heterogeneous catalyst: A comparative study of RSM and ANN optimization. *Brazilian Journal of Chemical Engineering*, 37, 351-368. <https://doi.org/10.1007/s43153-020-00027-9>
18. Lüneburger, S., Gallina, A. L., Soares, L. C., & Benvegnù, D., M. (2022). Biodiesel production from *Hevea Brasiliensis* seed oil. *Fuel*, 324, 124639. <https://doi.org/10.1016/j.fuel.2022.124639>
19. Mani, Y., Devaraj, T., Devaraj, K., AbdurRawoof, S.A., & Subramanian, S. (2020). Experimental investigation of biodiesel production from *Madhuca longifolia* seed through *in situ* transesterification and its kinetics and thermodynamic studies. *Environmental Science and Pollution Research*, 27, 36450-36462. <https://doi.org/10.1007/s11356-020-09626-y>
20. Milano, J., Ong, M.Y., Tiong, S.K., Ideris, F., Silitonga, A.S., Sebayang, A.H., Tana, C.H., Fattah, I.M.R., Fonad, Z., & Hossaine, N. (2025). A comparative study of the production of methyl esters from non-edible oils as potential feedstocks: Process optimization and two-step biodiesel characterization. *Results in Engineering*, 25, 104285. <https://doi.org/10.1016/j.rineng.2025.104285>
21. Myers, R. H., & Montgomery, D. C. (2009). *Response Surface Methodology: Process and Product Optimization Using Designed Experiments*. Third Edition. A Wiley-Interscience Publication. John Wiley & Sons, INC. New York. <https://books.google.co.id/books?id=YFSzCgAAQBAJ>
22. Oladipoa, B., & Betiku, E. (2020). Optimization and kinetic studies on conversion of rubber seed (*Hevea brasiliensis*) oil to methyl esters over a green biowaste catalyst. *Journal of Environmental Management*, 268, 110705. <https://doi.org/10.1016/j.jenvman.2020.110705>
23. Othman, M. F., Adam, A., Najafi, G., & Mamat, R. (2017). Green fuel as alternative fuel for diesel engine: A review. *Renewable and Sustainable Energy Reviews*, 80, 694-709. <https://doi.org/10.1016/j.rser.2017.05.140>
24. Oyekunle, D.T., Gendy, E.A., Barasa, M., Oyekunle, D.O., Oni, B., & Tiong, S.K. (2024). Review on utilization of rubber seed oil for biodiesel production: Oil extraction, biodiesel conversion, merits, and challenges. *Cleaner Engineering and Technology*, 21, 100773. <https://doi.org/10.1016/j.clet.2024.100773>
25. Parthiban, K.S., Pandian, S., & Subramanian, D. (2021). Conventional and *in-situ* transesterification of *Annona squamosa* seed oil for biodiesel production: Performance and emission analysis. *Environmental Technology & Innovation*, 23, 101593. <https://doi.org/10.1016/j.eti.2021.101593>
26. Pereira, S.A., dos Santos, S.B.F., Arruda, T.B.M.G., Ferreira, E.N., Chaves, P.O.B., de Oliveira, D.L.V., de Almeida Sousa, J.E., Rodrigues, F.E.A., & Ricardo, N.M.P.S. (2023). *In situ* transesterification of the catole coconut (*Syagrus Cearensis*) utilizing experimental design by Taguchi method. *Industrial Crops & Products*, 205, 117490. <https://doi.org/10.1016/j.indcrop.2023.117490>
27. Rahman, W.U., Khan, A.M., Anwer, A.H., Hasan, U., Karmakar, B., & Halder, G. (2022). Parametric optimization of calcined and Zn-doped waste egg-shell catalyzed biodiesel synthesis from *Hevea brasiliensis* oil. *Energy Nexus*, 6, 100073. <https://doi.org/10.1016/j.nexus.2022.100073>
28. Ridwan I., Budiastuti, H., Indarti R., Wahyuni N. L. E., Safitri, H. M., & Ramadhan, R. L. (2023). The optimization of tetrahydrofuran as a co-solvent on biodiesel production from rubber seeds using response surface methodology. *Materials Science for Energy Technologies*, 6, 15-20. <https://doi.org/10.1016/j.msct.2022.11.002>
29. Sakthivel, R., Ramesh, K., Purnachandran, R., & Shameer, P. M. (2018). A review on the properties, performance and emission aspects of the third generation biodiesels. *Renewable and Sustainable Energy Reviews*, 82(3), 2970-2992. <https://doi.org/10.1016/j.rser.2017.10.037>
30. Samart, C., Karnjanakom, S., Chaiya, C., Reubroycharoen, P., Sawangkeaw, R., & Charoenpanich, M. (2019). Statistical optimization of biodiesel production from para rubber seed oil by SO₃H-MCM-41 catalyst. *Arabian Journal of Chemistry*, 12, 2028-2036. <https://doi.org/10.1016/j.arabj.2014.12.034>
31. Sugebo, B., Demrew, Z., Feleke, S., & Biazen, M. (2021). Evaluation and characterization of rubber seed oil for biodiesel production. *Biomass Conversion and Biorefinery*, 15, 22125-22135. <https://doi.org/10.1007/s13399-021-01900-4>
32. Thakkar, K., Shah, K., Kodgire, P., & Kachhwaha, S.S. (2019). In-situ reactive extraction of castor seeds for biodiesel production using the T coordinated ultrasound-microwave irradiation: Process optimization and kinetic modeling. *Ultrasonics-Sonochemistry*, 50, 6-14. <https://doi.org/10.1016/j.ultsonch.2018.08.007>
33. Thanh, N.T., Mostapha, M., Lama, M.K., Ishak, S., Dasan, Y.K., Lim, J.W., Tan, I.S., Lau, S.Y., Chin, B.L.F., & Hadibarata, T. (2022). Fundamental understanding of *in-situ* transesterification of microalgae biomass to biodiesel: A critical review. *Energy Conversion and Management*, 270, 116212. <https://doi.org/10.1016/j.enconman.2022.116212>
34. Widayat, Wibowo, A. D. K., & Hadiyanto. (2013). Study on production process of biodiesel from rubber seed (*hevea brasiliensis*) by *in situ* (trans)esterification method with acid catalyst. *Energy Procedia*, 32, 64-73. <https://doi.org/10.1016/j.egypro.2013.05.009>
35. Zamberia, M.M., Ani, F.N., & Abdollah, M.F. (2016). Heterogeneous Transesterification Of Rubber Seed Oil Biodiesel Production. *Jurnal Teknologi (Sciences & Engineering)*, 78: 6-10, 115-110. <https://journals.utm.my/jurnalteknologi/article/view/9196/5484>
36. Zhu, Y., Xu, J., Li, Q., & Mortimer, P. E. (2014). Investigation of rubber seed yield in Xishuangbanna and estimation of rubber seed oil based biodiesel potential in Southeast Asia. *Energy*, 69, 837-842. <https://doi.org/10.1016/j.energy.2014.03.079>