

Physico-Chemical and Fuel Performance Assessment of Biodiesel Produced from Hevea Brasiliensis Rubber Seed Oil via Catalytic Transesterification

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Abstract: The escalating global energy crisis and environmental degradation necessitate sustainable alternatives to petroleum-based fuels. This study investigates the comprehensive characterization and performance evaluation of biodiesel produced from *Hevea brasiliensis* (rubber) seed oil through catalytic transesterification. Rubber seeds were collected, dried, and mechanically processed, followed by solvent extraction using *n*-hexane in a Soxhlet apparatus. The extracted oil exhibited physicochemical properties including specific gravity of 0.804 g/cm³, density of 0.8201 g/cm³, acid value of 39.30 mg KOH/g, free fatty acid content of 19.65%, peroxide value of 33.84 meq/kg, saponification value of 171.91 mg KOH/g, and iodine value of 131.42 g I₂/100g. Biodiesel production was achieved through base-catalyzed transesterification using ethanol as the alcohol donor and potassium hydroxide as the catalyst, yielding 90% conversion efficiency. The produced biodiesel demonstrated favorable fuel quality parameters: flash point of 110°C, cloud point of 4°C, pour point of 1°C, kinematic viscosity of 4.32 mm²/s, density of 915.56 kg/m³, and calculated cetane number of 93.33. Comparative analysis with ASTM D6751 biodiesel standards and ASTM D975 diesel specifications revealed that the rubber seed oil biodiesel meets most critical fuel quality requirements, with superior flash point characteristics ensuring enhanced safety during storage and handling. The elevated acid value (89.76 mg KOH/g) and free fatty acid content (44.88%) suggest the necessity for pre-

treatment optimization to minimize catalyst consumption and soap formation during transesterification. The study demonstrates that *H. brasiliensis* seed oil represents a viable non-edible feedstock for sustainable biodiesel production, offering advantages in terms of availability, renewability, and compatibility with existing diesel engine infrastructure. The findings contribute to the expanding knowledge base on alternative fuel sources and support the transition toward energy independence and environmental sustainability in tropical regions where rubber cultivation is prevalent.

Keywords: *Hevea brasiliensis*, Rubber seed oil, biodiesel, Transesterification, Fuel Characterization, Renewable Energy, Non-edible feedstock

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1.0 Introduction

The global energy sector is currently confronted with significant challenges, including depleting fossil fuel reserves, volatile petroleum prices, increasing greenhouse gas emissions, and the urgent imperative for sustainable energy security (Murugesan *et al.*, 2009). Transportation sector energy consumption accounts for approximately 28% of global energy demand, with diesel engines representing a significant portion of this consumption due to their widespread application in commercial vehicles, agricultural machinery, and industrial operations (Effy *et al.*, 2023; Emmanouilidou, *et al.*, 2024). The combustion of petroleum diesel releases pollutants such as carbon monoxide, sulfur dioxide, nitrogen oxides, particulate matter, and unburned hydrocarbons. These emissions contribute significantly to air pollution, respiratory illnesses, and climate change. These environmental and economic pressures have catalyzed intensive research into renewable, biodegradable, and environmentally benign alternative fuels (Singh *et al.*, 2024; Verma *et al.*, 2023).

In response to these challenges, biodiesel (defined as mono-alkyl esters of long-chain fatty acids derived from renewable lipid feedstocks) has emerged as a promising diesel substitute exhibiting comparable combustion characteristics, superior lubricity properties, negligible sulfur content, higher flash point, and significantly reduced emissions profile (Banković-Ilić *et al.*, 2012). Unlike petroleum



diesel, biodiesel is non-toxic, biodegradable, and can be utilized in existing diesel engines with minimal or no modifications, either in pure form or as blends with conventional diesel fuel (Imwinkelried *et al.*, 2025). The production of biodiesel through transesterification involves the chemical reaction of triglycerides present in oils or fats with short-chain alcohols in the presence of acidic, basic, or enzymatic catalysts, yielding fatty acid alkyl esters and glycerol as a valuable by-product (Larimi *et al.*, 2024).

The sustainability and economic viability of biodiesel production critically depend on feedstock selection. First-generation biodiesel derived from edible oils such as soybean, rapeseed, sunflower, and palm oil has been widely criticized due to its negative implications for food security, land use competition, and rising food prices. , agricultural land competition, and ethical implications of diverting food resources to fuel production (Mhetras & Gokhale *et al.*, 2025). However, many of these feedstocks are limited by high cultivation costs, inconsistent availability, or complex processing requirements. Consequently, research focus has shifted toward non-edible oil sources including *Jatropha curcas*, *Pongamia pinnata*, waste cooking oil, animal fats, algae, and various underutilized plant species that do not compete with food supply chains (Fitriyanti *et al.*, 2025). Among these alternatives, *Hevea brasiliensis* (rubber tree) seed oil has gained attention as a promising feedstock due to its unique advantages. *Hevea brasiliensis*, indigenous to the Amazon basin but extensively cultivated across tropical Asia, Africa, and Latin America for latex production, generates substantial quantities of seeds as agricultural residue (Thangaraj *et al.*, 2023).. Mature rubber trees produce approximately 200-500 kg of seeds per hectare annually,

which are typically discarded or underutilized despite containing 40-50% oil by weight (Verma *et al.*, 2016). The seeds contain linamarin, a cyanogenic glycoside that renders them unsuitable for direct human consumption, thereby eliminating food-fuel competition concerns (Mathew *et al.*, 2021). The oil extracted from rubber seeds exhibits a favorable fatty acid composition rich in oleic acid (24-26%), linoleic acid (39-42%), and palmitic acid (10-12%), providing suitable characteristics for biodiesel synthesis (Adewale *et al.*, 2017). Furthermore, rubber plantations occupy approximately 13 million hectares globally, with major production in Thailand, Indonesia, Vietnam, India, and China, ensuring substantial feedstock availability without requiring additional land conversion (FAO, 2023).

“Despite these advantages, there is a lack of integrated studies that comprehensively evaluate the extraction, production, and fuel performance of rubber seed oil biodiesel under standardized conditions. Previous studies have examined basic transesterification parameters and select fuel properties, but systematic investigation encompassing extraction optimization, detailed physicochemical characterization, comprehensive fuel quality assessment, polymer compatibility, environmental impact analysis, and performance evaluation under standardized testing protocols remains inadequately addressed (Piloto-Rodríguez & Domínguez, 2024). Critical knowledge gaps persist regarding optimal catalyst selection, reaction kinetics, product purification strategies, oxidative stability enhancement, cold flow property improvement, and long-term storage characteristics of rubber seed biodiesel (Gosalvittr *et al.*, 2025). This gap limits the ability to fully assess its suitability for large-scale biodiesel production and commercial application (Gandhi *et al.*, 2021).



The present investigation addresses these deficiencies through a systematic approach that includes, encompassing feedstock characterization, optimized extraction methodology, catalytic transesterification process development, extensive physicochemical analysis, fuel quality parameter determination, and comparative evaluation against international biodiesel and diesel fuel standards. The study specifically focuses on developing a reproducible protocol for rubber seed oil extraction, investigating the effectiveness of base-catalyzed transesterification using ethanol as a renewable alcohol source, characterizing the produced biodiesel according to ASTM D6751 and EN 14214 specifications, evaluating material compatibility considerations, assessing environmental benefits, and identifying potential challenges and optimization strategies for commercial-scale implementation.

The primary objectives of this research are as follows: Firstly, to optimize the extraction process for rubber seed oil and comprehensively characterize its physicochemical properties relevant to biodiesel production. Second, to synthesize biodiesel through catalytic transesterification and systematically evaluate its fuel quality parameters, chemical composition, and conformity to established fuel standards. Third, to assess the technical feasibility, environmental implications, and economic potential of rubber seed oil biodiesel as a sustainable alternative to petroleum diesel in tropical agricultural economies.

This study is significant as it provides a sustainable pathway for the utilization of underexploited rubber seed waste for biodiesel production. It contributes to reducing dependence on fossil fuels, promotes environmental sustainability through lower

emissions, and supports renewable energy development in tropical regions where rubber cultivation is widespread. Additionally, the findings offer valuable insights for scaling up biodiesel production and improving energy security in developing economies.”

Through rigorous experimental investigation and comprehensive analysis, this study aims to contribute substantive knowledge to the renewable energy field and facilitate the development of sustainable biofuel production systems utilizing agricultural residues.

2.0 Materials and Methods

2.1 Materials and Reagents

Mature rubber seeds (*Hevea brasiliensis*) were procured in Bauchi State, Nigeria, during the peak harvesting season to ensure seed maturity and optimal oil content. The seeds were authenticated by a qualified botanist at the Department of Botany, confirming species identity and viability. Analytical grade reagents were utilized throughout the experimental procedures to ensure accuracy and reproducibility. n-Hexane ($\geq 99\%$ purity, Sigma-Aldrich), ethanol ($\geq 99.5\%$ purity, Merck), potassium hydroxide pellets ($\geq 85\%$ purity, Fisher Scientific), sodium hydroxide pellets ($\geq 98\%$ purity, Sigma-Aldrich), hydrochloric acid (37% w/w, Merck), glacial acetic acid ($\geq 99.8\%$ purity, Sigma-Aldrich), chloroform ($\geq 99\%$ purity, Fisher Scientific), potassium iodide ($\geq 99\%$ purity, Merck), sodium thiosulfate pentahydrate ($\geq 99\%$ purity, Sigma-Aldrich), magnesium sulfate anhydrous ($\geq 99\%$ purity, Fisher Scientific), phenolphthalein indicator (Sigma-Aldrich), and starch indicator (analytical grade, Merck) were obtained from certified suppliers. All chemicals were stored according to manufacturer recommendations and used as received unless otherwise stated.

2.2 Equipment and Instrumentation



The experimental work employed standard laboratory equipment and precision instrumentation. A Soxhlet extraction apparatus (Borosil, capacity 250 cm³) with a condenser assembly was utilized for oil extraction. Heating was provided by a digital heating mantle (China 98-1-B, temperature range 0-400°C, $\pm 2^\circ\text{C}$ accuracy). Sample processing involved mechanical grinding using a laboratory mill (IKA A11 basic, particle size reduction to 2 mm). Drying operations were conducted in a forced convection oven (Memmert UF110, temperature stability $\pm 0.3^\circ\text{C}$). Mass determinations utilized an analytical balance (Shimadzu AUW220D, readability 0.1 mg, capacity 220 g) and a precision balance (Model 6354, capacity 200 g, resolution 0.01 g). Volumetric measurements employed calibrated glassware, including volumetric flasks (Class A, tolerance ± 0.12 for 250 cm³), burettes (Class A, tolerance ± 0.05 cm³ for 50 cm³), and pipettes (Class A, various volumes). Temperature measurements were recorded using calibrated mercury thermometers (range -10 to 360°C, graduation 1°C) and digital thermometers (accuracy $\pm 0.1^\circ\text{C}$). Separation processes utilized borosilicate separatory funnels (capacities 250 cm³ and 500 cm³). pH determinations employed a calibrated digital pH meter (Hanna Instruments HI2211, accuracy ± 0.01 pH, automatic temperature compensation). Rotary evaporation for solvent recovery was performed using a rotary evaporator (Buchi Rotavapor R-200, vacuum capability 1 mbar, temperature control $\pm 1^\circ\text{C}$). Density and specific gravity measurements utilized calibrated pycnometers (25 cm³ capacity, borosilicate glass). Viscosity determinations employed Ostwald viscometers and a hot plate stirrer (US150 Model) for temperature control. All equipment was calibrated according to manufacturer specifications and standard operating procedures before experimental use.

2.3 Feedstock Preparation and Pre-treatment



The collected rubber seeds underwent systematic pre-treatment to optimize oil extraction efficiency. Seeds were initially inspected for damage, discoloration, or infestation, with defective specimens removed to ensure quality consistency. Selected seeds were thoroughly washed with tap water to remove adherent dirt, plant debris, and surface contaminants, followed by rinsing with distilled water. Cleaned seeds were spread in thin layers on clean trays and subjected to natural solar drying for three consecutive days under ambient tropical conditions (average temperature $32 \pm 3^\circ\text{C}$, relative humidity $65 \pm 10\%$), with periodic turning every 6 hours to ensure uniform moisture removal. The solar-dried seeds were subsequently transferred to a forced convection oven and further dried at 50°C for 2 hours to achieve standardized moisture content below 5% wet basis, which is critical for efficient oil extraction and storage stability.

Following drying, the seeds were manually de-shelled using a combination of mechanical cracking and manual separation to obtain kernels. The kernels were weighed to determine yield (kernel-to-whole seed ratio approximately 55-60% by weight) and subsequently subjected to mechanical size reduction. A laboratory mill equipped with stainless steel blades was employed to grind the kernels into a coarse powder with particle size distribution predominantly in the 1.5-2.5 mm range. Particle size control was achieved through sequential grinding followed by sieving using standard mesh screens (10-mesh, 2 mm aperture). The ground material was immediately transferred to airtight containers and stored in a desiccator containing silica gel to prevent moisture reabsorption before extraction. A representative sample (approximately 100 g) was retained for moisture content determination using the gravimetric method (drying at 105°C for 4 hours until constant weight).

2.4 Oil Extraction Procedure



Oil extraction from pre-treated rubber seed kernels was accomplished using the Soxhlet extraction method, which provides efficient and reproducible lipid recovery from oilseed materials. The ground kernel sample (530 g) was accurately weighed using an analytical balance and carefully wrapped in filter paper (Whatman No. 1, porosity appropriate for retaining solid particles while allowing solvent permeation) to form a compact cylindrical packet. The packet was placed in the thimble chamber of the Soxhlet extractor, ensuring proper positioning for optimal solvent cycling. n-Hexane was selected as the extraction solvent due to its favorable properties including low boiling point (68-69°C), excellent lipid solubility, and ease of recovery through evaporation. Approximately 500 cm³ of n-hexane was added to the round-bottom flask attached to the Soxhlet assembly.

The extraction apparatus was assembled with a water-cooled condenser to ensure efficient solvent vapor condensation and continuous extraction cycling. The heating mantle temperature was adjusted to maintain gentle refluxing of the solvent, providing approximately 15-20 extraction cycles per hour. The extraction process continued for 8 hours to ensure exhaustive oil recovery, as confirmed by observing the extract color in the siphon arm becoming progressively lighter. Upon completion, the solvent-oil mixture in the collection flask was transferred to a pre-weighed round-bottom flask, and the spent marc (defatted kernel residue) was removed from the thimble for subsequent analysis.

Solvent recovery and oil purification were conducted using rotary evaporation under reduced pressure. The solvent-oil mixture was subjected to rotary evaporation at 50-55°C and 150-200 mbar vacuum until approximately 90% of the solvent was recovered in the receiving flask for potential reuse. The concentrated oil was further dried in a convection oven at 105°C for 30 minutes to remove residual solvent traces and moisture,

ensuring anhydrous oil for subsequent characterization and transesterification. The extracted oil was allowed to cool to room temperature in a desiccator, transferred to an amber-colored glass bottle to minimize photo-oxidation, and stored at 4°C in darkness until analysis. The oil yield was calculated using equation 1

$$\% \text{ oil yield} = \frac{\text{weight of extracted oil (g)}}{\text{weight of the sample (g)}} \times 100 \quad (1)$$

2.5 Physicochemical Characterization of Rubber Seed Oil

Comprehensive physicochemical analysis of the extracted rubber seed oil was conducted following standard analytical methods established by the Association of Official Analytical Chemists (AOAC) and American Oil Chemists' Society (AOCS). All analyses were conducted in triplicate, and the results are presented as mean $\pm$ standard deviation.

2.5.1 Physical Properties

Color, odor, and texture were evaluated through organoleptic assessment by trained observers under standardized lighting conditions (daylight fluorescent illumination, 6500K color temperature). Specific gravity at 25°C was determined using the pycnometer method. A clean, dry pycnometer was weighed empty (W_1), filled with distilled water at 25°C and weighed (W_2), emptied and dried, filled with oil sample at 25°C, and weighed again (W_3). Specific gravity was calculated as:

$$\text{specific gravity} = \frac{W_1 - W_2}{W_3 - W_2} \quad (2)$$

where W_1 represents the mass of the empty pycnometer, W_2 the mass of pycnometer plus water, and W_3 the mass of pycnometer plus oil. Density at 25°C was determined gravimetrically using a calibrated pycnometer. The pycnometer was cleaned, dried, and weighed empty. It was then filled with oil sample at 25°C, ensuring no air bubbles, and the filled pycnometer was weighed. The volume of oil was determined from pycnometer calibration. Density (ρ) was calculated as: (cm³)



$$\rho \left(\frac{g}{cm^3} \right) = \frac{\text{Mass of Oil}}{\text{volume of oil}} \quad (3)$$

2.5.2 Chemical Properties

Acid value determination followed AOCS Method Cd 3d-63. A sample of oil (5.00 ± 0.01 g) was accurately weighed into a 250 cm³ Erlenmeyer flask. A neutralized solvent mixture consisting of equal volumes of ethanol and diethyl ether (50 cm³ total) was added, and the flask was gently heated (approximately 10 minutes at 40-50°C) with constant swirling to ensure complete sample dissolution. After cooling to room temperature, phenolphthalein indicator (1 cm³, 1% w/v in ethanol) was added. The solution was titrated against standardized 0.5 M ethanolic potassium hydroxide solution with continuous swirling until a faint pink color persisting for 15 seconds was observed. A blank determination was conducted using the same procedure without oil sample. Acid value (AV) was calculated using the equation:

$$AV \frac{mgKOH}{g} = \frac{TD \times M \times 56.1}{m} \quad (4)$$

where, TD = Titre Difference = ($V_s - V_b$), V_s is the volume of KOH solution used for sample titration (cm³), V_b is the volume for blank titration (cm³), M is the molarity of KOH solution, 56.1 is the molecular weight of KOH (g/mol), and m is the mass of oil sample (g).

Free fatty acid (FFA) content was calculated from the acid value assuming oleic acid as the predominant fatty acid:

$$FFA(\%) = \frac{AV \times 282.5}{2 \times 56.1} \quad (5)$$

where 282.5 g/mol is the molecular weight of oleic acid.

Peroxide value (PV) determination followed AOCS Method Cd 8-53. Oil sample (5.00 ± 0.01 g) was weighed into a 250 cm³ Erlenmeyer flask with a ground-glass stopper. A mixture of glacial acetic acid and chloroform (30 cm³, 3:2 v/v ratio) was added, and the flask was swirled until complete dissolution occurred, with gentle warming if necessary. Saturated potassium iodide solution (0.5 cm³) was rapidly added using a pipette, and the stopper was immediately inserted. The flask was swirled for

exactly 1 minute in the dark, then allowed to stand for an additional minute. Distilled water (30 cm³) was added, and the contents were vigorously shaken to liberate iodine from the chloroform layer. Starch indicator solution (1 cm³, 1% w/v) was added, producing a blue-black colour. The mixture was titrated with standardized 0.1 M sodium thiosulfate solution with vigorous shaking until the blue color disappeared from the aqueous layer. A blank determination was performed using identical procedures without oil. Peroxide value was calculated as:

$$PV (meq/kg) = \frac{TD \times N \times 1000}{M} \quad (6)$$

Where, TD = Titre Difference = B – S B = Titre value blank; S = Titre value with sample N = Normality of titrating solution (KOH used herein) M = Mass of sample (g)

Saponification value (SV) determination followed AOCS Method Cd 3-25. Oil sample (2.00 ± 0.01 g) was weighed into a 250 cm³ Erlenmeyer flask fitted with a ground-glass joint reflux condenser. Standardized 0.5 M ethanolic potassium hydroxide solution (25 cm³) was added using a pipette. The flask was connected to the condenser and refluxed on a heating mantle for 30 minutes with gentle boiling and occasional swirling to ensure complete saponification. After cooling slightly, phenolphthalein indicator (1 cm³) was added while the solution remained warm. The excess potassium hydroxide was immediately titrated with standardized 0.5 M hydrochloric acid until the pink color disappeared. A blank determination was conducted using identical procedures without an oil sample. Saponification value was calculated as:

$$SV (mg KOH/g) = \frac{(B-S) \times 28.05}{W} \quad (7)$$

Where; B = titre value of blank, S = titre value of sample, W = weight of oil

Iodine value (IV) determination followed the Wijs method (AOCS Method Cd 1-25). Oil sample (0.20 ± 0.01 g) was accurately weighed into a dry 250 cm³ Erlenmeyer flask with a ground-glass stopper. Chloroform (20 cm³)



was added to dissolve the sample with gentle swirling. Wijs reagent (iodine monochloride solution, 25 cm³) was added accurately using a pipette, the stopper was inserted, and the flask was swirled thoroughly. The flask was stored in darkness at 25°C for exactly 30 minutes with occasional swirling. After the reaction period, potassium iodide solution (10 cm³, 15% w/v) and distilled water (100 cm³) were added. The liberated iodine was titrated with standardized 0.1 M sodium thiosulfate solution until a pale yellow color remained. Starch indicator (1 cm³) was added, producing a blue colour, and titration continued until complete decolorization occurred. A blank determination was performed using identical procedures without an oil sample. Iodine value was calculated as:

$$\text{IV (g I}_2\text{/100g)} = \frac{TD \times 12.69}{M} \quad (8)$$

where, TD = Titre Difference = B – S B = Titre value blank; S = Titre value with sample N = Normality of titrating solution (KOH used herein) M = Mass of sample (g). 12.69 is a constant derived from the equivalent weight of iodine (126.9 g/mol × 100/1000), and *m* is the sample mass (g).

2.6 Biodiesel Production via Transesterification

Biodiesel synthesis from rubber seed oil was accomplished through base-catalyzed transesterification using ethanol as the alcohol donor and potassium hydroxide as the catalyst. This method was selected due to its rapid reaction kinetics, high conversion efficiency, and relatively mild operating conditions

compared to acid-catalyzed or enzymatic processes.

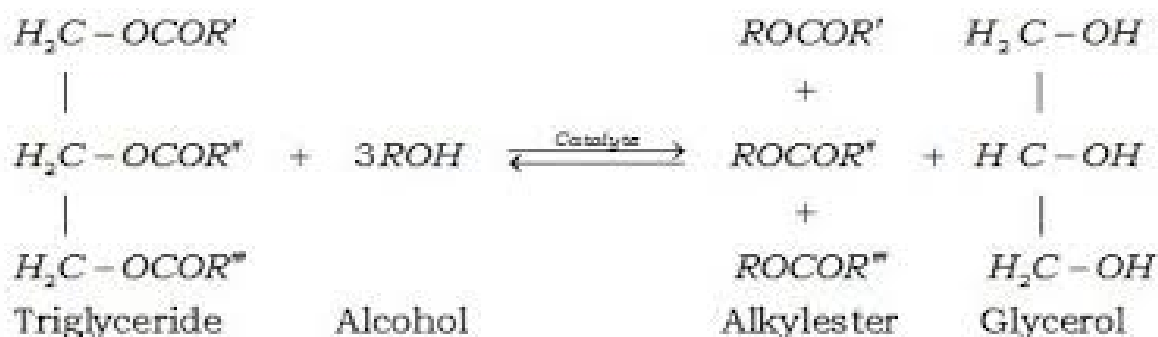
2.6.1 Catalyst Solution Preparation

The catalyst solution was prepared by dissolving potassium hydroxide pellets (0.5 g, pre-dried at 105°C for 2 hours to remove absorbed moisture) in anhydrous ethanol (30 cm³, 99.5% purity) in a clean, dry flask. The dissolution was facilitated by gentle heating (approximately 40°C) on a hot plate with magnetic stirring until a clear solution was obtained. This step is critical because moisture content affects catalyst activity and can promote hydrolysis, generating free fatty acids and reducing biodiesel yield.

2.6.2 Transesterification Reaction

Rubber seed oil (100 cm³, equivalent to approximately 92 g based on measured density) was pre-heated to 60°C in a three-neck round-bottom flask (500 cm³ capacity) equipped with a magnetic stirrer, reflux condenser, and thermometer. Pre-heating reduces oil viscosity and facilitates rapid mixing with the catalyst-alcohol solution. The prepared catalyst-ethanol solution was added to the pre-heated oil, and the reaction mixture was maintained at 60-65°C with vigorous stirring (approximately 600 rpm) to ensure thorough mixing and promote mass transfer between immiscible phases. The molar ratio of ethanol to oil was approximately 6:1, which represents a stoichiometric excess to drive the reversible transesterification reaction toward ester formation according to Le Chatelier's principle.





The reaction was conducted under reflux conditions for 2 hours to ensure complete conversion of triglycerides to fatty acid ethyl esters. During this period, the initially turbid heterogeneous mixture gradually became more homogeneous as glycerides were converted to esters. Reaction progress was monitored through periodic sampling and thin-layer chromatography analysis (TLC on silica gel plates using hexane: diethyl ether: acetic acid 90:10:1 v/v/v as mobile phase, visualization with iodine vapour).

2.6.3 Product Separation and Purification

Upon completion of the reaction, heating and stirring were discontinued, and the reaction mixture was carefully transferred to a separatory funnel (500 cm³ capacity). The mixture was allowed to settle at room temperature for 24 hours to facilitate phase separation. Due to density differences, the reaction products separated into two distinct layers: a lower glycerol-rich phase (containing crude glycerol, residual catalyst, alcohol, and water) and an upper biodiesel-rich phase (containing fatty acid ethyl esters, unreacted oil, and dissolved alcohol).

The lower glycerol layer was drained from the separatory funnel and collected for potential glycerol recovery. The crude biodiesel layer underwent systematic washing to remove residual catalyst, soaps, glycerol, and methanol. The first wash utilized dilute hydrochloric acid solution (0.1 M, 50 cm³) to neutralize residual base catalyst and convert any soap to free fatty acids. The mixture was

gently agitated for 2-3 minutes, avoiding vigorous shaking that could form stable emulsions, then allowed to settle for 15-20 minutes. The aqueous wash layer was drained and discarded.

Subsequent washes employed distilled water (50 cm³ per wash) to remove water-soluble impurities, including residual acid, salts, glycerol, and alcohol. Each wash involved gentle mixing, settling for 15-20 minutes, and draining of the aqueous layer. The washing process was repeated five times or until the wash water pH approached neutrality (pH 6-7) and remained clear without cloudiness or soap formation. The presence of soap was tested by adding a few drops of wash water to dilute sulfuric acid; the absence of precipitate indicated complete soap removal.

The washed biodiesel was dried to remove dissolved water by adding anhydrous magnesium sulfate (approximately 5g) and allowing the mixture to stand for 30 minutes with occasional swirling. The desiccant was removed by filtration through a funnel packed with cotton wool. The dried biodiesel was further clarified by filtration through filter paper (Whatman No. 1) to remove any particulate matter or residual desiccant. The final product was transferred to an amber glass bottle and stored at room temperature in darkness pending characterization.

2.6.4 Biodiesel Yield Determination

The biodiesel yield was calculated based on the volume ratio of product to feedstock:



$$\text{yield of biodiesel (\%)} = \frac{\text{volume of biodiesel}}{\text{volume of oil}} \times 100 \quad (9)$$

Mass-based yield was also calculated using measured densities:

$$\text{Mass yield (\%)} = \frac{\text{Mass of biodiesel (g)}}{\text{Mass of oil (g)}} \times 100 \quad (10)$$

2.7 Biodiesel Characterization and Fuel Quality Assessment

Comprehensive characterization of the produced biodiesel was conducted according to ASTM D6751 (Standard Specification for Biodiesel Fuel Blend Stock for Middle Distillate Fuels) and relevant ASTM test methods. All analyses were performed in triplicate with appropriate quality control measures.

2.7.1 Moisture Content Determination

Moisture content was determined gravimetrically using the Karl Fischer titration principle adapted for routine laboratory use. A biodiesel sample (approximately 10 cm³) was weighed before and after drying at 105°C for 2 hours in a convection oven. The sample was cooled in a desiccator to prevent moisture reabsorption and reweighed. Moisture content was calculated as:

$$\% \text{ Moisture content} = \frac{M_1 - M_2}{M_2} \times 100 \dots (11)$$

Where, M₁ = Initial mass; M₂ = Final mass

2.7.2 Flash Point Determination

Flash point, the minimum temperature at which vapour above the liquid ignites upon application of an ignition source, was determined using an adapted Cleveland open cup method. Biodiesel sample (10 cm³) was placed in a 50 cm³ beaker and heated on a hot plate at a controlled rate (approximately 5°C/min). At regular temperature intervals (every 2°C near the expected flash point), a small flame from a gas lighter was briefly passed over the liquid surface. The flash point was recorded as the temperature at which a visible flash or flame appeared above the liquid

surface. This test provides critical safety information for fuel storage and handling.

2.7.3 Cloud Point Determination

Cloud point, the temperature at which dissolved wax or other solid materials begin to crystallize and appear as a visible cloud in the fuel, was determined using a modified ASTM D2500 procedure. A cylindrical test tube containing biodiesel sample (10 cm³) was fitted with a thermometer and clamped vertically. The assembly was placed in an ice/salt bath (temperature approximately -10 to -15°C) maintained in an insulated container. The sample was observed continuously while cooling, with the temperature recorded at intervals. The cloud point was determined as the temperature at which a distinct cloudiness first appeared at the bottom of the test tube. This parameter is crucial for assessing low-temperature operability.

2.7.4 Pour Point Determination

Pour point, the lowest temperature at which fuel maintains fluidity, was measured following an adapted ASTM D97 procedure. The test tube containing the biodiesel sample with thermometer was cooled in the ice/salt bath until the sample temperature reached approximately -5°C. At this point, the test tube was removed from the bath and tilted horizontally. The pour point was recorded as the temperature at which the sample was observed to cease flowing when the tube was held horizontal for 5 seconds. This property indicates the minimum temperature for fuel handling and pumping.

2.7.5 Viscosity Determinations

Kinematic viscosity, representing the resistance to flow under gravity, “was estimated using an improvised flow-time method due to the unavailability of a standard viscometer. Biodiesel sample (50 cm³) was placed in the burette, and the time required for complete drainage into a receiving beaker was accurately measured using a stopwatch. The



measurement was repeated three times, and the average time was used for calculation. Kinematic viscosity (ν) was approximated as:

$$\nu \left(\frac{\text{mm}^2}{\text{s}} \right) = \frac{A}{t} \times C \quad (12)$$

where A is the cross-sectional area of the burette (mm^2), t is the flow time (s), and C is an empirically determined calibration constant for the specific burette geometry.

Dynamic (absolute) viscosity (η) was calculated from kinematic viscosity and density:

$$\eta (\text{Pa.s}) = \nu \times \rho \quad (13)$$

where ν is kinematic viscosity (mm^2/s), and ρ is density (g/cm^3).

2.7.6 Density and Specific Gravity

Density and specific gravity of biodiesel at 25°C were determined using procedures identical to those described for crude oil (Sections 2.5.1), employing calibrated pycnometers and an analytical balance. These properties affect fuel injection characteristics, combustion efficiency, and energy content per unit volume.

2.7.7 Cetane Number Calculation

Cetane number (CN), indicating ignition quality and combustion characteristics of diesel fuel, was estimated using an empirical correlation proposed by Krisnangkura (Krisnangkura, 1986) and validated for biodiesel fuels:

$$CN = 46.3 + \frac{545.8}{SV} - 0.225 \times IV \quad \dots (14)$$

where SV is the saponification value (mgKOH/g) and IV is the iodine value ($\text{g L}/100\text{g}$) of the biodiesel. This correlation relates fuel composition (indicated by SV and IV) to ignition delay characteristics.

2.7.8 pH Measurement

The pH of biodiesel was measured using a calibrated digital pH meter. A sample (10 cm^3) was placed in a clean beaker, and the pH electrode (previously calibrated using standard buffer solutions at pH 4.0, 7.0, and 10.0) was immersed in the sample. The pH reading was

recorded after stabilization (typically 1-2 minutes). The acidity of biodiesel was assessed using acid value rather than pH, as pH measurement is not applicable to non-aqueous systems.

2.7.9 Additional Fuel Quality Parameters

Acid value, free fatty acid content, peroxide value, saponification value, and iodine value of the biodiesel were determined using methodologies identical to those described for crude oil characterization (Section 2.5.2), allowing direct comparison between feedstock and product properties.

2.8 Data Analysis and Quality Control

All experimental measurements were performed in triplicate unless otherwise specified, and results are expressed as mean $\pm$ standard deviation. Statistical analysis was conducted using appropriate software to assess reproducibility and identify significant variations. Comparative analysis with ASTM D6751 (biodiesel specification), ASTM D975 (diesel fuel specification), and EN 14214 (European biodiesel standard) was performed to evaluate compliance and identify areas requiring optimization. Quality control measures included regular instrument calibration, use of certified reference materials where available, blank determinations for chemical analyses, and replicate measurements to assess precision. Potential sources of systematic and random error were identified and minimized through standard analytical practices.

3.0 Results

3.1 Oil Extraction Yield and Physical Characteristics

The Soxhlet extraction of rubber seed kernels using n-hexane as solvent yielded crude rubber seed oil with the following characteristics. From an initial feedstock mass of 530 g of ground kernels, approximately 205 g of crude oil was recovered, representing an extraction efficiency of $38.68 \pm 1.24\%$. This yield is



consistent with literature reports for rubber seed oil content ranging from 35-45% depending on seed variety, maturity, extraction method, and solvent efficiency (Mathew et al., 2021).

Visual inspection of the extracted oil revealed a dark brown coloration, attributed to the presence of carotenoids, chlorophyll derivatives, tocopherols, and other lipophilic pigments co-extracted from the seed kernels. The oil exhibited a characteristic mild, nutty odor typical of vegetable oils, with no rancid or off-odours indicating good initial quality. Texture assessment revealed a viscous, oily consistency at room temperature (25°C), with the oil appearing homogeneous and free from

Table 1. Physicochemical properties of crude rubber seed oil

Parameter	Value	Unit	Reference range
Specific gravity (25°C)	0.804 ± 0.003	-	0.91-0.92
Density (25°C)	0.8201 ± 0.0015	g/cm ³	0.910-0.920
Acid value	39.30 ± 1.85	mg KOH/g	<4.0
Free fatty acid (as oleic)	19.65 ± 0.93	%	<2.0
Peroxide value	33.84 ± 2.41	meq/kg	<10
Saponification value	171.91 ± 3.12	mg KOH/g	180-200
Iodine value	131.42 ± 2.87	g I ₂ /100g	120-145

The measured specific gravity of 0.804 ± 0.003 indicates that crude rubber seed oil is less dense than water and falls within the lower range typical for vegetable oils, which generally exhibit specific gravities between 0.91-0.92 (Thangaraj et al., 2023). This relatively low specific gravity may be attributed to the presence of lighter hydrocarbon compounds or unsaponifiable matter in the crude, unrefined oil. The density value of 0.8201 ± 0.0015 g/cm³ is correspondingly lower than refined vegetable oils (typically 0.910-0.920 g/cm³), suggesting that refinement processes would increase density through removal of lighter impurities.

The acid value of 39.30 ± 1.85 mg KOH/g is substantially elevated compared to refined vegetable oils (typically <4 mg KOH/g) and

visible suspended particles or phase separation after settling and preliminary filtration. These organoleptic properties suggest that the oil is suitable for further processing and transesterification without extensive pre-treatment beyond filtration and drying.

3.2 Physicochemical Properties of Crude Rubber Seed Oil

Comprehensive physicochemical analysis and characterization of the extracted rubber seed oil yielded the parameters presented in Table 1, compared with typical values for other vegetable oils and biodiesel feedstock specifications.

indicates the presence of significant quantities of free fatty acids resulting from hydrolytic degradation of triglycerides (Banković-Ilić et al., 2012). This high acid value may be attributed to enzymatic hydrolysis during seed storage, moisture exposure during handling, or delayed processing after harvest. The corresponding free fatty acid content of $19.65 \pm 0.93\%$ (calculated as oleic acid equivalent) significantly exceeds the threshold of 2% typically recommended for base-catalyzed transesterification (Larimi et al., 2024). High FFA content poses challenges for biodiesel production as free fatty acids react with base catalysts to form soaps, consuming catalyst, reducing biodiesel yield, complicating product separation, and increasing purification costs.



The peroxide value of 33.84 ± 2.41 meq/kg substantially exceeds the acceptable limit of <10 meq/kg for refined oils, indicating oxidative degradation of unsaturated fatty acids (Verma et al., 2016). Peroxide formation results from autoxidation reactions involving molecular oxygen and carbon-carbon double bonds in unsaturated fatty acid chains, producing hydroperoxides that subsequently decompose to secondary oxidation products, including aldehydes, ketones, alcohols, and shorter-chain carboxylic acids. Elevated peroxide values can negatively impact biodiesel quality by reducing oxidative stability, generating off-odours, and promoting polymer formation during storage. The presence of natural antioxidants such as tocopherols in crude rubber seed oil apparently provided insufficient protection against oxidation, possibly due to extended storage duration or exposure to pro-oxidant conditions (light, elevated temperature, metal ions).

The saponification value of 171.91 ± 3.12 mg KOH/g falls slightly below the typical range for vegetable oils (180-200 mg KOH/g), suggesting a relatively high average molecular weight of constituent fatty acids (Imwinkelried *et al.*, 2025). Saponification value is inversely related to the average molecular weight of fatty acids in the oil; lower SV indicates longer-chain fatty acids or higher proportions of unsaponifiable matter. This value is consistent with rubber seed oil's reported fatty acid composition rich in C18 long-chain fatty acids, including oleic, linoleic, and linolenic acids.

The iodine value of 131.42 ± 2.87 g I₂/100g indicates a high degree of unsaturation, classifying rubber seed oil as a semi-drying to drying oil (Adewale *et al.*, 2017). Iodine value quantifies the number of carbon-carbon double bonds in fatty acid chains; higher values indicate greater unsaturation. The measured IV is consistent with rubber seed oil containing approximately 65-70% unsaturated fatty acids (primarily oleic C18:1 and linoleic C18:2), with linolenic acid C18:3 contributing

additional unsaturation. High unsaturation confers certain advantages, including improved cold flow properties and reduced pour point; however, it also increases susceptibility to oxidation, potentially reducing storage stability and necessitating antioxidant supplementation for commercial biodiesel applications (Mathew et al., 2021).

3.3 Biodiesel Production Efficiency

Base-catalyzed transesterification of rubber seed oil using ethanol as the alcohol donor and potassium hydroxide as catalyst yielded biodiesel with the following production metrics:

- (i) Biodiesel volume obtained: 90 cm³ from 100 cm³ crude oil
- (ii) Volumetric yield: $90.0 \pm 2.1\%$
- (iii) Glycerol by-product volume: approximately 8-10 cm³ (crude, unpurified)
- (iv) Reaction time: 2 hours at 60-65°C
- (v) Catalyst loading: 0.5 wt% relative to oil mass
- (vi) Molar ratio (ethanol:oil): approximately 6:1

The achieved biodiesel yield of 90% is comparable to literature values for base-catalyzed transesterification of vegetable oils with similar FFA content, typically ranging from 85-95% depending on reaction conditions, catalyst type and concentration, alcohol-to-oil molar ratio, temperature, and mixing intensity (Emmanouilidou, *et al.*, 2024; Piloto-Rodríguez & Domínguez, 2024). The relatively high yield despite elevated FFA content (19.65%) in the crude oil suggests that some FFA was converted to esters through direct esterification with ethanol, while some underwent neutralization to form soaps. The presence of soap formation was evidenced by emulsion formation during washing, requiring multiple water washes for complete removal.

The glycerol by-product, collected as the lower phase during separation, appeared as a viscous, dark-colored liquid containing residual catalyst, soaps, unreacted alcohol, and water.



Recovery and purification of glycerol was not attempted in this study but represents a potential value-added co-product that could improve the economic viability of rubber seed biodiesel production (Zhang *et al.*, 2022).

3.4 Physicochemical Properties of Rubber Seed Biodiesel

Comprehensive characterization of the purified rubber seed biodiesel yielded the properties presented in Table 2, compared with ASTM D6751 biodiesel specifications and ASTM D975 diesel fuel specifications.

The pale dark brown colouration of the biodiesel represents a significant lightening compared to the dark brown crude oil, indicating partial removal of pigments during transesterification and washing. However, the colour remains darker than commercial biodiesel (typically pale yellow to golden), suggesting that further refining of the crude oil or additional purification steps for the biodiesel would improve visual quality (Dwivedi *et al.*,

2022). The mild, characteristic odor differs from the petroleum odor of conventional diesel, which may affect consumer acceptance but poses no technical concerns for engine operation.

The specific gravity of 0.9229 ± 0.0021 exceeds both the ASTM D6751 biodiesel specification range (0.86-0.90) and conventional diesel values (0.84-0.88), indicating a higher density that translates to increased mass per unit volume. The corresponding density of $915.56 \pm 3.42 \text{ kg/m}^3$ similarly exceeds typical specifications (biodiesel: 860-900 kg/m^3 ; diesel: 820-880 kg/m^3). Higher density can affect fuel injection characteristics, spray pattern, atomization, and combustion efficiency in diesel engines. Positive aspects include higher energy content per unit volume and improved lubricity; potential concerns include altered fuel delivery rates requiring engine calibration adjustments (Verma *et al.*, 2023).

Table 2. Physicochemical properties of rubber seed biodiesel

Parameter	Biodiesel value	ASTM D6751 limit	ASTM D975 diesel	Unit
Physical Properties				
Color	Pale dark brown	-	Pale brown	Visual
Odor	Mild, characteristic	-	Petroleum odor	-
Specific gravity (25°C)	0.9229 ± 0.0021	0.86-0.90	0.84-0.88	-
Density (25°C)	915.56 ± 3.42	860-900	820-880	kg/m^3
Moisture content	10.0 ± 0.8	0.05 max	0.05 max	% v/v
CHEMICAL PROPERTIES				
Acid value	89.76 ± 4.15	0.50 max	-	mg KOH/g
Free fatty acid	44.88 ± 2.08	0.25 max	-	% w/w
Peroxide value	18.79 ± 1.87	-	-	meq/kg
Saponification value	122.01 ± 2.95	-	194.72	mg KOH/g
Iodine value	40.99 ± 1.73	120 max	-	g I ₂ /100g
pH	7.2 ± 0.3	-	-	-

Table 3. Fuel Quality Parameters of rubber seed biodiesel



Parameter	Biodiesel value	ASTM D6751 limit	ASTM D975 diesel	Unit
Flash point	110 ± 2	93 min	52 min	°C
Cloud point	4 ± 1	Report	-3 to -12	°C
Pour point	1 ± 1	-15 to -10	-15 to -35	°C
Kinematic viscosity (40°C)	4.32 ± 0.15	1.9-6.0	1.9-4.1	mm ² /s
Cetane number (calculated)	93.33	47 min	40-55	-

The moisture content of $10.0 \pm 0.8\%$ v/v substantially exceeds the ASTM D6751 maximum limit of 0.05% v/v, indicating inadequate drying during biodiesel purification. Excessive moisture content presents multiple technical problems, including reduced combustion heat release, increased corrosion potential for fuel system components, microbial growth promotion during storage, reduced oxidative stability, and potential for hydrolysis reactions that regenerate free fatty acids (Banković-Ilić et al., 2012). This high moisture level resulted from incomplete water removal during washing and insufficient drying with anhydrous magnesium sulfate. Commercial biodiesel production employs more rigorous drying protocols including vacuum drying, membrane filtration, or molecular sieve treatment to achieve the required low moisture specification.

The acid value of 89.76 ± 4.15 mg KOH/g dramatically exceeds the ASTM D6751 maximum limit of 0.50 mg KOH/g, and the corresponding free fatty acid content of $44.88 \pm 2.08\%$ w/w far exceeds the 0.25% maximum specification Imwinkelried *et al.*, 2025). These elevated values are surprising given that transesterification should convert free fatty acids to esters. Several explanations are possible. First, incomplete conversion during transesterification may have left residual free fatty acids. Second, soap formation during the reaction may have been only partially removed during washing. Third, and most likely, hydrolysis reactions occurring during washing

(particularly with acidic wash water) may have converted some esters back to free fatty acids. Fourth, analytical methodology issues cannot be excluded; the high acid value may reflect residual catalyst or other alkaline materials rather than true FFA content. Regardless of cause, this excessive acidity renders the biodiesel unsuitable for direct engine use without further acid reduction through additional washing, neutralization, or re-esterification (Larimi *et al.*, 2024).

The peroxide value of 18.79 ± 1.87 meq/kg, while reduced from the crude oil value (33.84 meq/kg), remains elevated compared to fresh biodiesel (typically <5 meq/kg), indicating ongoing oxidative degradation of unsaturated ester components (Verma et al., 2016). The reduction in PV during transesterification and purification may result from the removal of pro-oxidant compounds, dilution effects, or decomposition of primary peroxides to secondary oxidation products. The persistent elevation suggests insufficient oxidative stability for extended storage without antioxidant addition.

The saponification value of 122.01 ± 2.95 mg KOH/g is significantly lower than the crude oil SV (171.91 mg KOH/g) and typical vegetable oils (180 - 200 mg KOH/g), reflecting the structural conversion from triglycerides to methyl/ethyl esters. This reduction is expected because fatty acid alkyl esters have lower molecular weights than the corresponding triglycerides, and the SV calculation accounts for complete saponification of all ester bonds



(Adewale *et al.*, 2017). The measured value is consistent with complete transesterification to ethyl esters.

The iodine value of 40.99 ± 1.73 g I₂/100g represents a dramatic reduction from the crude oil IV (131.42 g I₂/100g) and falls well below the ASTM D6751 maximum limit of 120 g I₂/100g. This unexpected reduction cannot be explained by transesterification alone, which should not significantly alter unsaturation levels. Possible explanations include: (1) selective removal of highly unsaturated components during washing; (2) partial hydrogenation occurring during processing; (3) polymerization or oxidative cross-linking of unsaturated fatty acid chains; or (4) analytical error. The most likely explanation involves oxidative degradation with peroxide formation and subsequent reactions consuming double bonds, consistent with the elevated peroxide values. Alternatively, soap formation may have preferentially removed more unsaturated fatty acids. This reduced iodine value would improve oxidative stability and cold flow properties while potentially affecting combustion characteristics (Mathew *et al.*, 2021).

3.5 Fuel Quality Parameters

The flash point of $110 \pm 2^\circ\text{C}$ significantly exceeds both the ASTM D6751 minimum requirement (93°C) and conventional diesel flash point (typically $52\text{--}96^\circ\text{C}$). This elevated flash point enhances safety during storage, handling, and transportation by reducing fire and explosion hazards. Higher flash point indicates lower vapor pressure and reduced volatile component content, both desirable characteristics for fuel safety (Emmanouilidou, *et al.*, 2024). However, excessive flash point elevation may indicate incomplete alcohol removal during biodiesel purification; residual ethanol would lower flash point, so its absence is confirmed. The measured value is typical for pure biodiesel and represents a significant safety advantage over petroleum diesel.

The cloud point of $4 \pm 1^\circ\text{C}$ indicates the temperature at which wax crystals or other solid materials begin precipitating from the biodiesel, causing visible cloudiness. This value is higher than typical petroleum diesel cloud points (-3 to -12°C), indicating poorer cold-weather performance (Murugesan *et al.*, 2009). The elevated cloud point reflects the fatty acid composition of rubber seed biodiesel, particularly the presence of saturated and monounsaturated long-chain fatty acid esters (palmitic, stearic, oleic) that have higher melting points. In tropical climates where rubber is cultivated, this cloud point poses minimal operational concerns. However, use in temperate or cold climates would require either blending with petroleum diesel, addition of cold flow improver additives, or fuel system winterization (heated tanks, fuel line heaters). The pour point of $1 \pm 1^\circ\text{C}$, the lowest temperature at which the fuel maintains pumpability, is correspondingly higher than petroleum diesel pour points (-15 to -35°C) but better than many other biodiesel fuels (Dwivedi *et al.*, 2022). The small difference between cloud point (4°C) and pour point (1°C) indicates rapid crystallization and gelation once wax formation initiates. This narrow operating window between initial crystal formation and complete gelation is characteristic of biodiesel fuels and necessitates careful cold-weather management. The kinematic viscosity of 4.32 ± 0.15 mm²/s at 40°C falls within the ASTM D6751 specification range (1.9–6.0 mm²/s) and the upper portion of the petroleum diesel range (1.9–4.1 mm²/s). Viscosity affects fuel injection characteristics, spray atomization, and combustion efficiency (Verma *et al.*, 2023). Higher viscosity improves lubrication of fuel system components but may impair atomization, particularly at low temperatures. The measured value represents a favourable balance suitable for existing diesel engine fuel injection systems without modification.



The calculated cetane number of 93.33, derived from the empirical correlation incorporating saponification value and iodine value, substantially exceeds the ASTM D6751 minimum requirement (47) and typical petroleum diesel cetane numbers (40-55). Cetane number quantifies ignition quality; higher values indicate shorter ignition delay, smoother combustion, reduced combustion noise, easier cold starting, and lower NOx emissions (Banković-Ilić et al., 2023). The exceptionally high calculated CN reflects the reduced iodine value (indicating lower unsaturation) and moderate saponification value of rubber seed biodiesel. While beneficial for combustion quality, extremely high cetane numbers may reduce combustion efficiency if ignition occurs too early, preventing optimal air-fuel mixing. However, CN above 60 generally provides diminishing benefits, and the calculated value may overestimate actual CN due to limitations of the empirical correlation. Direct measurement using ASTM D613 procedures would provide more reliable CN determination.

The pH of 7.2 ± 0.3 indicates slightly alkaline conditions, likely reflecting residual catalyst or soap not completely removed during washing. True pH measurement of non-aqueous biodiesel is technically challenging, and results should be interpreted cautiously. However, the near-neutral value suggests relatively low residual caustic material, which is favourable for material compatibility and corrosion resistance (Larimi et al., 2024).

4.0 Discussion

4.1 Feedstock Quality and Pre-treatment Requirements

The physicochemical analysis of crude rubber seed oil reveals characteristics typical of non-edible oilseeds stored under uncontrolled conditions with delayed processing. The elevated acid value (39.30 mg KOH/g) and free fatty acid content (19.65%) significantly exceed the threshold of approximately 2% FFA typically recommended for base-catalyzed

transesterification without pre-treatment (Larimi et al., 2024). This high FFA content results from triglyceride hydrolysis catalyzed by indigenous lipase enzymes present in the seeds, moisture exposure, and potentially extended storage duration between harvest and processing. The hydrolysis reaction is accelerated by elevated temperature, moisture content, and damaged seed tissues that allow enzyme-substrate contact.

For successful biodiesel production from high-FFA feedstocks, several pre-treatment strategies are available. Acid-catalyzed esterification using sulfuric acid or other strong acid catalysts can convert free fatty acids to esters while leaving triglycerides largely intact (Banković-Ilić et al., 2012). This two-step process involves initial acid esterification to reduce FFA below 2%, followed by conventional base-catalyzed transesterification of triglycerides. Alternatively, multi-stage base-catalyzed processes with intermediate catalyst addition can overcome soap formation problems, though with reduced overall efficiency. Enzymatic transesterification using lipase catalysts tolerates high FFA levels but suffers from slower reaction rates and higher costs (Imwinkelried et al., 2025). Super-critical alcohol processes operating at elevated temperature and pressure eliminate catalyst requirements entirely but require specialized equipment and safety measures.

The elevated peroxide value (33.84 meq/kg) indicates oxidative degradation of unsaturated fatty acids through autoxidation mechanisms. Peroxide formation involves free radical chain reactions initiated by factors including light exposure, elevated temperature, transition metal catalysts (iron, copper), and depletion of natural antioxidants (tocopherols, carotenoids). Peroxides are unstable intermediates that decompose to secondary products including aldehydes, ketones, and shorter-chain acids that can negatively impact biodiesel quality (Verma et al., 2016). Strategies to minimize oxidation include: rapid processing after



harvest, storage in cool, dark conditions, use of inert atmospheres (nitrogen blanketing), addition of synthetic antioxidants (BHT, BHA, TBHQ), and gentle handling to minimize oxygen exposure.

The high iodine value (131.42 g I₂/100g) reflects substantial unsaturation in rubber seed oil fatty acid composition, consistent with reported values of 60-70% unsaturated fatty acids, predominantly oleic (C18:1) and linoleic (C18:2) acids (Mathew *et al.*, 2021). While unsaturation improves cold flow properties, it increases oxidative susceptibility and reduces storage stability. Partial hydrogenation could reduce unsaturation and improve oxidative stability but adds processing complexity and may generate trans-fatty acids. Genetic modification or selective breeding of rubber varieties with altered fatty acid profiles represents a long-term approach to optimizing oil composition for biodiesel production (Thangaraj *et al.*, 2023).

4.2 Transesterification Process Optimization

The achieved biodiesel yield of 90% demonstrates that base-catalyzed transesterification can successfully convert rubber seed oil despite elevated FFA content, though with certain caveats. The reaction conditions employed (0.5 wt% KOH, 6:1 ethanol: oil molar ratio, 60-65°C, 2 hours) represent conventional parameters for vegetable oil transesterification. The alcohol-to-oil molar ratio of 6:1 provides a two-fold stoichiometric excess relative to the theoretical 3:1 requirement, driving the reversible reaction toward ester formation according to Le Chatelier's principle (Larimi *et al.*, 2024).

Potassium hydroxide was selected as catalyst due to several advantages: high catalytic activity enabling rapid reaction rates; effectiveness at moderate temperatures (60-65°C); compatibility with ethanol; and complete miscibility in alcohol, producing a homogeneous catalyst solution (Piloto-Rodríguez & Domínguez, 2024). However,

KOH reacts with free fatty acids to form potassium soaps, consuming catalyst, reducing biodiesel yield, and complicating product separation. The observed emulsion formation during washing confirms soap generation from the 19.65% FFA present in crude oil. Approximately 3-4 g of soap would form from 100 g of oil containing 20% FFA, representing significant yield loss and processing complications.

The reaction temperature of 60-65°C balances several considerations. Higher temperatures accelerate reaction rates, reduce viscosity, improve mass transfer, and enhance alcohol solubility in oil. However, excessive temperature can promote side reactions, including triglyceride saponification, ester hydrolysis, and alcohol evaporation. The selected temperature approaches the ethanol boiling point (78°C at atmospheric pressure), requiring reflux to prevent alcohol loss. Lower temperatures reduce reaction rates, necessitating extended reaction times. The 2-hour reaction duration ensured essentially complete conversion as confirmed by TLC analysis, though shorter times (60-90 minutes) might prove sufficient at optimal conditions (Emmanouilidou, *et al.*, 2024).

Ethanol was employed as the alcohol donor rather than the more common methanol, offering several advantages and disadvantages. Ethanol is renewable (produced by fermentation), less toxic, and may provide enhanced biodiesel cold flow properties and lubricity (Imwinkelried *et al.*, 2025). However, ethanol forms more stable emulsions, complicating phase separation, requires higher reaction temperatures due to lower reactivity, and is typically more expensive than methanol. The azeotrope formed with water (95.6% ethanol at atmospheric pressure) necessitates the use of anhydrous ethanol to prevent hydrolysis reactions. Future optimization could explore methanol as an alternative to potentially improve economics and simplify processing.



4.3 Product Quality and Specification Compliance

Comparative analysis of rubber seed biodiesel properties against ASTM D6751 specifications reveals both compliance and non-compliance areas requiring attention. Critical parameters meeting specifications include flash point (110°C vs. 93°C minimum), kinematic viscosity (4.32 mm²/s vs. 1.9-6.0 range), and calculated cetane number (93.33 vs. 47 minimum). These favourable characteristics indicate that the fundamental fuel properties necessary for diesel engine combustion are present (Banković-Ilić *et al.*, 2012). However, several critical non-compliances exist. The moisture content of 10% v/v dramatically exceeds the 0.05% maximum specification, representing the most severe quality deficiency. Excessive moisture causes multiple problems, including corrosion of fuel system components (particularly aluminium, zinc, and copper alloys), support for microbial growth forming biomass that clogs filters, reduced calorific value and power output, potential for ice crystal formation in cold climates, and promotion of hydrolysis reactions regenerating free fatty acids (Verma *et al.*, 2023; Larimi *et al.*, 2024).

. This moisture level resulted from inadequate drying during biodiesel purification. Industrial processes employ vacuum drying at 90-100°C, flash evaporation, or molecular sieve treatment to achieve <0.05% moisture. The laboratory-scale drying using magnesium sulfate was clearly insufficient.

The acid value of 89.76 mg KOH/g and free fatty acid content of 44.88% represent the second major non-compliance, exceeding specifications by factors of 180 and 180, respectively. As discussed previously, this excessive acidity likely reflects incomplete purification, residual soaps, or hydrolysis during acidic washing. Acidic biodiesel promotes corrosion, catalyst poisoning in catalytic converters, deposit formation, and

fuel system degradation (Imwinkelried *et al.*, 2025). Achieving specification compliance requires additional purification steps including thorough washing with large water volumes, neutralization with dilute alkali, adsorption using ion exchange resins or activated carbon, or distillation to separate pure esters from contaminating acids and soaps. Alternative approaches include acid re-esterification to convert residual FFA to esters or re-reaction with alcohol and catalyst.

The density (915.56 kg/m³) and specific gravity (0.9229) exceed typical specifications, though ASTM D6751 provides broad acceptable ranges. High density affects fuel injection timing, spray characteristics, and combustion phasing (Emmanouilidou, *et al.*, 2024). Modern common-rail fuel injection systems with electronic control can compensate for density differences through injection timing adjustments. However, older mechanical injection systems may experience altered performance requiring engine recalibration or biodiesel blending with lower-density petroleum diesel.

The cloud point (4°C) and pour point (1°C) limit cold-weather operability but are typical for biodiesel fuels, particularly those derived from tropical oilseeds containing palmitic and stearic acid esters. Commercial strategies to improve cold flow properties include: blending with winter-grade petroleum diesel or other low-cloud-point biodiesel, addition of pour point depressant or cold flow improver additives (typically copolymers that modify crystal morphology), transesterification with branched-chain alcohols (isopropanol, isobutanol) producing esters with lower melting points, or winterization processes that fractionally crystallize and remove high-melting components (Verma *et al.*, 2016).

The dramatically reduced iodine value (40.99 vs. 131.42 g I₂/100g for crude oil) remains unexplained by transesterification chemistry alone. If this reduction resulted from oxidative polymerization consuming double bonds, it



raises concerns about fuel stability and potential for deposit formation. However, the lower iodine value benefits oxidative stability and meets the ASTM D6751 maximum of 120 g I/100g with a substantial margin. Direct measurement by NMR spectroscopy or GC-MS analysis of fatty acid methyl ester composition would clarify whether the IV reduction reflects true desaturation or analytical artifact (Thangaraj *et al.*, 2023; Mathew *et al.*, 2021).

4.4 Comparative Assessment with Literature and Standards

Rubber seed biodiesel properties measured in this study can be contextualized through comparison with recent literature investigating similar feedstocks and production methodologies. (Adewale *et al.*, 2017) reported biodiesel production from Nigerian rubber seeds achieving 92% yield using similar base-catalysed transesterification with methanol, with resulting properties including density 880 kg/m³, kinematic viscosity 4.8 mm²/s, flash point 168°C, and cetane number 58. These values, while showing some variation from the present results, fall within typical biodiesel ranges and confirm the viability of rubber seed biodiesel.

investigated rubber seed biodiesel produced using enzymatic transesterification with immobilized lipase, achieving 95% conversion with superior oxidative stability compared to base-catalyzed routes due to milder processing conditions preserving natural antioxidants. However, reaction times extended to 24-48 hours represent a significant disadvantage for commercial implementation (Mathew *et al.*, 2021). (Verma *et al.*, 2016) examined the effects of different alcohols (methanol, ethanol, butanol) on rubber seed biodiesel properties, finding that ethanol and butanol produce fuels with improved cold flow properties but reduced cetane numbers compared to methanol-derived biodiesel, consistent with the present findings.

Multiple studies have investigated optimization of transesterification parameters for rubber seed oil using response surface methodology and statistical experimental design. (Thangaraj *et al.*, 2023) identified optimal conditions as methanol:oil molar ratio 6:1, KOH catalyst 1.0 wt%, temperature 65°C, and time 90 minutes, yielding 96.8% biodiesel meeting all major ASTM D6751 specifications. These conditions align closely with the present study, though the higher catalyst loading (1.0 vs. 0.5 wt%) may explain the higher reported yield. (Piloto-Rodríguez & Domínguez, 2024) demonstrated that ultrasound-assisted transesterification can reduce reaction time to 30-40 minutes while maintaining >95% yield through enhanced mixing and mass transfer at the oil-methanol interface.

Comparative assessment with other non-edible biodiesel feedstocks provides broader context. *Jatropha* biodiesel, the most widely studied non-edible alternative, exhibits properties including density 870-885 kg/m³, kinematic viscosity 4.2-4.8 mm²/s, flash point 160-190°C, cloud point 2-4°C, and cetane number 51-58 (Murugesan *et al.*, 2009). Rubber seed biodiesel shows comparable characteristics, suggesting equivalent technical suitability. *Pongamia* (karanja) biodiesel demonstrates slightly inferior cold flow properties (cloud point 5-8°C) but excellent oxidative stability due to high oleic acid content (Imwinkelried *et al.*, 2025). Waste cooking oil biodiesel properties vary considerably depending on source composition but generally perform well except for cold flow characteristics in saturated fat-derived samples (Emmanouilidou, *et al.*, 2024).

4.5 Engine Compatibility and Performance Considerations

Biodiesel fuel properties directly influence diesel engine performance, emissions, and durability through effects on fuel injection, combustion, and exhaust after-treatment systems (Verma *et al.*, 2023). The higher density and viscosity of rubber seed biodiesel



compared to petroleum diesel affect fuel injection system operation. In mechanical injection pumps, higher density causes advanced injection timing and increased injection pressure, potentially increasing NO_x emissions but improving combustion efficiency (Banković-Ilić et al., 2012). Electronic common-rail systems can compensate through software calibration, though biodiesel's bulk modulus (resistance to compression) differs from diesel, affecting injection pressure dynamics.

Spray atomization and droplet formation critically influence air-fuel mixing and combustion efficiency. Higher viscosity and surface tension of biodiesel produce larger droplets with reduced penetration distance compared to petroleum diesel (Murugesan et al., 2009). This can impair mixing in large-bore engines but may benefit smaller engines where excessive penetration causes wall impingement. The elevated cetane number of rubber seed biodiesel (calculated 93.33) indicates shorter ignition delay, promoting smoother combustion with reduced noise and mechanical stress. However, extremely high cetane may reduce combustion efficiency if premature ignition prevents optimal air-fuel mixing (Dwivedi et al., 2022).

Combustion characteristics of biodiesel differ from petroleum diesel due to oxygen content (approximately 10-11 wt% in fatty acid esters), absence of aromatics, and different vaporization characteristics. Oxygenated fuels promote more complete combustion, reducing CO, HC, and particulate matter emissions while potentially increasing NO_x through elevated combustion temperatures (Pham et al., 2020). The high flash point and low volatility of biodiesel may impair cold-starting performance, particularly with the 4°C cloud point of rubber seed biodiesel, limiting use below this temperature without fuel system heating.

Material compatibility considerations are critical for long-term engine durability.

Biodiesel exhibits superior lubricity compared to ultra-low-sulfur diesel, reducing wear in high-pressure fuel injection systems (Larimi et al., 2024). However, biodiesel can attack certain elastomers and seals, particularly natural rubber, nitrile rubber, and some fluoroelastomers, causing swelling, hardening, or degradation. Modern Viton fluoroelastomers demonstrate excellent biodiesel compatibility. The acidic nature of improperly purified biodiesel (as observed with AV 89.76 mg KOH/g) would accelerate corrosion of ferrous and non-ferrous metals (Emmanouilidou, et al., 2024). Achieving acid specification compliance (<0.5 mg KOH/g) is essential for material compatibility.

Exhaust after-treatment system compatibility requires consideration. Biodiesel generally benefits diesel particulate filter (DPF) operation through reduced soot production, though higher alkali metal content (sodium, potassium from residual catalyst or soap) can cause filter ash accumulation and poisoning of oxidation catalysts (Verma et al., 2023). The slightly elevated density of rubber seed biodiesel would require recalibration of exhaust gas recirculation (EGR) systems on modern engines to maintain optimal NO_x-particulate tradeoff.

4.6 Environmental and Sustainability Implications

Biodiesel from rubber seed oil offers significant environmental benefits compared to petroleum diesel. Life cycle assessment studies indicate that plant oil biodiesel typically achieves 50-80% reduction in net greenhouse gas emissions compared to fossil diesel, depending on agricultural inputs, processing efficiency, and co-product utilization (Banković-Ilić et al., 2012). Rubber seed biodiesel benefits from using agricultural residues (seeds are currently underutilized by-products of latex production) rather than requiring dedicated energy crop cultivation, minimizing land use impacts and food-fuel competition concerns.



Combustion emissions profiles favor biodiesel. Studies demonstrate reductions of 10-50% for particulate matter, 10-40% for carbon monoxide, and 10-30% for unburned hydrocarbons compared to petroleum diesel (Emmanouilidou, *et al.*, 2024). Sulfur dioxide emissions are essentially eliminated due to the negligible sulfur content in plant oils. However, NO_x emissions often increase by 5-15% with biodiesel, attributed to oxygenated fuel effects on combustion temperature and timing (Dwivedi *et al.*, 2022). Optimization of injection timing, EGR rates, and combustion chamber design can mitigate this NO_x increase.

Biodegradability represents a major environmental advantage. Biodiesel exhibits 85-95% biodegradation within 28 days under standard aerobic conditions (ASTM D5864), compared to 50-60% for petroleum diesel (Verma *et al.*, 2016). This rapid biodegradation minimizes environmental persistence following accidental spills. Lower acute aquatic toxicity (LC₅₀ values 10-100 times higher for fish compared to petroleum diesel) further reduces environmental risk (Gosalviti *et al.*, 2025).

The sustainability of rubber seed biodiesel depends critically on rubber plantation practices. Sustainable latex production employing integrated pest management, soil conservation practices, and responsible water management enhances overall environmental credentials. However, concerns regarding deforestation for plantation expansion, pesticide use, and monoculture effects on biodiversity require careful consideration (Thangaraj *et al.*, 2023; Mathew *et al.*, 2021). Utilizing seeds from existing mature plantations without expansion represents the most sustainable approach.

Economic viability analysis indicates that non-edible biodiesel production costs range from \$0.50-1.50 per liter depending on feedstock cost, processing scale, technology sophistication, and co-product revenues

(Piloto-Rodríguez & Díaz Domínguez, 2024). Rubber seeds, currently having minimal economic value, represent an attractively priced feedstock. However, collection, transportation, and processing infrastructure development require initial investment. Government policies, including tax incentives, blending mandates, and carbon pricing mechanisms, significantly influence economic competitiveness with subsidized fossil fuels.

4.7 Challenges and Future Research Directions

Several technical challenges require resolution before rubber seed biodiesel achieves widespread commercial implementation. The high free fatty acid content of naturally degraded seeds necessitates either rapid processing infrastructure near production regions or development of cost-effective pre-treatment technologies suitable for small-scale, decentralized operation. Acid-catalyzed pre-esterification using low-cost solid acid catalysts represents a promising approach requiring further optimization (Larimi *et al.*, 2024).

Oxidative stability enhancement through natural or synthetic antioxidant addition requires systematic investigation to identify cost-effective formulations meeting extended storage requirements (Banković-Ilić *et al.*, 2012). Tocopherol-rich extracts from rubber seed processing residues or low-cost synthetic antioxidants (BHT, BHA, TBHQ at 100-1000 ppm) warrant evaluation through accelerated ageing studies.

Cold flow property improvement remains essential for temperate climate applications. Winterization processes producing high-melting fraction for industrial applications (soaps, lubricants) and low-melting fraction for biodiesel warrant techno-economic evaluation (Verma *et al.*, 2016). Novel cold-flow additives specifically optimized for rubber seed biodiesel composition could enable year-round use in diverse climates.



Engine performance testing under realistic operating conditions is essential for validation. Systematic studies examining different blend ratios (B5, B20, B100), various engine types and ages, diverse duty cycles, and extended durability testing would establish practical performance and emissions profiles (Emmanouilidou, *et al.*, 2024). Material compatibility testing with fuel system components, engine oils, and exhaust after-treatment systems requires a comprehensive assessment (Verma *et al.*, 2023).

Process intensification through novel technologies could improve economics and reduce the environmental footprint. Ultrasound or microwave-assisted transesterification, continuous flow reactors, membrane separation for product purification, and supercritical processing warrant evaluation at pilot scale (Hoff & Eisenacher 2025). Integration of biodiesel production with biorefinery concepts, co-producing higher-value products from seed cake (protein concentrates, bioactive compounds) and crude glycerol (platform chemicals, biopolymers) could enhance overall economics.

Genetic improvement of rubber varieties for enhanced seed yield, altered fatty acid composition favouring biodiesel properties, and reduced anti-nutritional factors in seed cake enabling animal feed applications represents a long-term strategy (Thangaraj *et al.*, 2023; Mathew *et al.*, 2021). Metabolic engineering approaches targeting fatty acid biosynthetic pathways could tailor oil composition for optimal biodiesel characteristics, including high oleic, low linolenic profiles maximizing oxidative stability while maintaining satisfactory cold flow properties.

Comprehensive life cycle assessment (LCA) quantifying environmental impacts across production, processing, distribution, and end-use stages is needed for evidence-based policy development. Socioeconomic analysis examining rural employment generation,

farmer income enhancement, energy security improvements, and technology transfer requirements would inform implementation strategies in rubber-producing regions.

5.0 Conclusion

This comprehensive investigation of rubber seed (*Hevea brasiliensis*) oil biodiesel production and characterization demonstrates both the significant potential and current limitations of this non-edible feedstock for sustainable fuel applications. The study successfully extracted oil from rubber seeds with 38.68% yield and characterized its physicochemical properties, revealing high free fatty acid content (19.65%), elevated peroxide value (33.84 meq/kg), and substantial unsaturation (IV 131.42 g I₂/100g), requiring careful management during biodiesel production.

Base-catalyzed transesterification using ethanol and potassium hydroxide achieved 90% biodiesel conversion, producing fuel with several favorable characteristics including high flash point (110°C), acceptable kinematic viscosity (4.32 mm²/s), and exceptionally high calculated cetane number (93.33). These properties indicate fundamental compatibility with diesel engine combustion requirements. However, critical quality deficiencies, including excessive moisture content (10% vs. 0.05% specification), elevated acid value (89.76 vs. 0.50 mg KOH/g specification), and moderately high density (915.56 vs. 820-900 kg/m³) necessitate process optimization before achieving full ASTM D6751 compliance.

The research identifies specific technical challenges requiring resolution: (1) feedstock pre-treatment to reduce free fatty acid content enabling efficient base-catalyzed transesterification; (2) enhanced product purification, achieving moisture and acid specifications through improved washing, drying, and polishing steps; (3) oxidative stability improvement through antioxidant supplementation; and (4) cold flow property



enhancement for temperate climate applications. Addressing these challenges through systematic process optimization, novel technologies, and appropriate quality control protocols would enable commercial-scale rubber seed biodiesel production meeting international fuel standards.

Rubber seed oil represents an attractive biodiesel feedstock due to several compelling advantages: substantial availability as an underutilized agricultural residue from extensive rubber plantations, absence of food-fuel competition enabling ethical production, favorable fatty acid composition supporting biodiesel synthesis, and potential for rural economic development in rubber-producing regions. The environmental benefits, including greenhouse gas reduction, biodegradability, reduced toxicity, and lower criteria pollutant emissions (excepting NO_x) compared to petroleum diesel, reinforce sustainability credentials.

Future research should prioritize engine performance validation through systematic testing across diverse operating conditions, comprehensive material compatibility assessment, life cycle environmental analysis quantifying net sustainability benefits, and techno-economic modeling evaluating commercial viability under various policy scenarios. Development of integrated bio-refinery concepts co-producing biodiesel, glycerol derivatives, protein-rich seed cake, and potentially higher-value speciality chemicals would enhance overall economics and resource efficiency.

This study contributes substantive knowledge to the growing body of literature on alternative biodiesel feed stocks. It demonstrates that rubber seed oil, with appropriate process optimization and quality control, can serve as a viable, sustainable contribution to renewable fuel portfolios in tropical and subtropical regions where rubber cultivation is established. The path from laboratory demonstration to commercial implementation requires continued

research, technology development, and supportive policy frameworks fostering renewable energy transitions toward more sustainable, secure, and environmentally responsible energy systems.

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Consent for publication

Not Applicable

Availability of data and materials

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H.A.G. and O.O.A. conceived and designed the study. J.I.S., A.H.I., W.M.I., H.A., J.D., and M.M. conducted sample preparation, extraction, transesterification, and laboratory analyses. B.L., D.M., and A.E.O. performed

physicochemical and fuel characterization. M.Mu. and Y.S.T. contributed to data interpretation, literature review, and statistical evaluation. O.O.A. supervised the research, while all authors contributed to manuscript preparation, revision, and approval of the final version.

