Research Article

Production of Biodiesel from Mixed Castor Seed and Microalgae Oils: Optimization of the Production and Fuel Quality Assessment

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The diminishing reserves and environmental consequences of the fossil fuel-based petrodiesel necessitate the exploration of an alternative fuel with better quality and minimum environmental impacts. The study explores the optimization of biodiesel production from nonfood and locally available mixed feedstocks as an effective and a sustainable approach to solve the insufficiency and high costs of single oil feedstock. The selection of suitable oil feedstocks and optimization of process variables are the prime issues for cost-effective industrial scale production of biodiesel from mixed feedstocks toward the industrial scale production of biodiesel. The objective of this study was to optimize process variables for the alkaline transesterification of mixed castor seed and microalgae oils to optimize the yield of biodiesel. Oils were extracted from dried microalgae (Chlorella vulgaris) biomass and castor seed kernel using methanol. The oils were purified, characterized, mixed in a 1:1 ratio, and converted to biodiesel. The transesterification experiments designed according to the central composite design (CCD) were used to optimize the yield of biodiesel through the response surface methodology (RSM). Experimental results were analyzed by response surface regression to produce a model for predicting biodiesel yield. Model significance, fitness, the effect of significant variables, and interactions between the variables on the yield of biodiesel were studied through the analysis of variance (ANOVA). The optimization of transesterification process variables revealed that the catalyst concentration of 1.23% (w/w), ethanol to mixed oil ratio of 5.94:1 (v/v), and reaction temperature of 51.0 °C were the optimum conditions to achieve an optimum biodiesel yield of 92.88%. Validation experiments conducted under the optimum conditions resulted in the biodiesel yield of 92.36%, which is very close to the model predicted value. Various standard methods were used to characterize the biodiesel produced under optimum conditions, and it was found compatible with ASTM 751 and EN14214 biodiesel standards.

1. Introduction

Nowadays, the global community heavily depends on nonrenewable and unsustainable fossil fuels, whose continued use has been linked to atmospheric pollution and global warming [1]. At the current utilization rate, fossil fuels will be out of stock within the next few decades [2, 3]. Hence, it is highly desirable to find an alternative energy source with appropriate fuel quality. Biodiesel is a renewable, nontoxic, ecofriendly, and carbon-neutral energy source that can be used in the existing engine without significant changes [4]. Biodiesel can also reduce air pollution because of sulfur oxides as its sulfur content is insignificant [5].

The feedstocks of biodiesel can be grouped into edible vegetable oils, nonedible vegetable oils, waste or recycled oils, animal fats and oils, and algae oils. Several studies have been conducted to use single oil feedstocks to make biodiesel [6]. Moreover, edible single vegetable oils have been used to produce biodiesel in higher income countries to reduce greenhouse gas (GHG) emissions [7] and to minimize the dependence on fossil fuel-based petrodiesel [8, 9]. The use of edible single oil feedstocks for biodiesel production compromises food security [5], and its economic viability cannot be effective [10]. A simultaneous use of edible oils as feedstocks of biodiesel and human food may lead to deforestation in creating more farmland for cultivation [9]. An
increase in global human population may lead to the peaking of the price of edible oils, which may affect the economic viability of biodiesel production. Therefore, a large-scale biodiesel production at a reasonable cost needs the use of nonedible mixed vegetable oils [7].

Castor plant (Ricinus communis L) is native to Ethiopia though it is widely distributed throughout the tropical, subtropical, and warmer temperate regions [11]. The oil content of castor seed varieties ranges from 45 to 55%, though the actual yield depends on the oil extraction method. Castor oil is nonedible because of the presence of a toxic protein [12]. The fatty acid composition of castor oil reveals that it contains a significant amount of ricinoleic acid (80–90%) [3], a small amount of linoleic acid (4.50%), oleic acid (3.50%), palmitic acid (1.50%), stearic acid (1.50%), and linolenic acid (0.10) [13, 14].

Recently, microalgal oils have become potential feedstock for the large-scale production of biodiesel [15]. Fast growth rate, significant oil content, and ability to grow using wastewater and marginalized lands make microalgae an excellent feedstock for biodiesel production [16]. Microalgal oil is mainly composed of a mixture of unsaturated fatty acids (e.g., palmitoleic, oleic, linoleic, linolenic acids, and eicosapentaenoic acid) and small amount of saturated fatty acids (e.g., palmitic, myristic acid, and stearic acids) [17].

Biodiesel can also be made from two or more oil mixtures. The use of mixed feedstocks may respond to the rising demand for biodiesel, reduce the costs of feedstocks, and improve the fuel quality of the resultant biodiesel [18]. The use of inexpensive nonedible oil mixtures reduces the costs of biodiesel production, dependency on edible oils, produces biodiesels with favorable physicochemical properties, and improves its engine performance and emission characteristics [19]. Since the cost of feedstocks accounts for more than 70% of the total costs of biodiesel production, the selection of appropriate feedstocks is critically important to make biodiesel production cost-effective [2]. This study investigates the transesterification of inexpensive nonedible and locally available oil mixture to produce biodiesel with suitable physicochemical properties in the presence of a base catalyst.

High viscosity and low ignition quality are the major constraints to use crude oils directly in engines [8]. The transesterification of crude oils is one of the most commonly used approaches to overcome these constraints [20]. Base-catalyzed transesterification is much faster than acid-catalyzed reactions, though it is sensitive to water and free fatty acid (FFA) contents of the feedstock [21]. Under alkaline conditions, excess water and FFAs may trigger saponification reactions [22]. A feedstock containing an excess FFA must be pretreated prior to base-catalyzed transesterification [23]. Furthermore, catalyst recovery and the production of unwanted wastewater are the major constraints of homogeneous base-catalyzed transesterification, which can increase the cost of biodiesel production [24]. On the other hand, homogeneous acid catalyzed transesterification is insensitive to FFA content as it catalyzes both esterification and transesterification reactions simultaneously [8]. However, homogeneous acid-catalyzed transesterification is corrosive to equipment, low in catalyst activity, and needs a higher reaction temperature, a longer reaction time, and higher alcohol to oil molar ratio to achieve a significant biodiesel yield [25]. Transesterification process can also be catalyzed by heterogeneous acid and heterogeneous base catalysts. Heterogeneous catalysts are reusable, recoverable, and easily separated from a product mixture and perform esterification and transesterification reactions simultaneously [26]. The limitation of heterogeneous catalysts is their low reaction rate associated with limited diffusion in the three-phase reaction mixture (oil–alcohol–catalyst) [27]. To overcome the constraints of homogeneous acid catalysts, Xie and Wang [28] developed a magnetically recyclable and reusable heterogeneous acid catalyst for the one-step conversion of low-cost oils to biodiesel in an efficient and ecofriendly manner. The use of heterogeneous base catalysts, which are shown to have high catalytic activities, can solve the pollution problem related to undesirable wastewater production and realize the production of biodiesel at industrial scale [24]. However, some heterogeneous base catalysts leach their active components during transesterification that may hinder the large-scale production of biodiesel as it leads to both homogeneous and heterogeneous reaction routes, in which the former needs to be removed from the product mixture. To improve the stability and recoverability of heterogeneous base catalysts, Xie and Wang [24] synthesized a magnetically recyclable composite material for the efficient transesterification of oils to biodiesel. A study was carried out by Xie and Wang [29] to solve the problem of the catalytic efficiency of heterogeneous acid catalysts by synthesizing a magnetically recoverable composite heterogeneous acid solid catalyst for the one-step conversion of oils to biodiesel in an ecofriendly manner.

The production of biodiesel can be performed via noncatalytic supercritical reaction route. This route of biodiesel production results in a higher yield, however, it is energy intense as it requires high temperature and pressure, high alcohol to oil molar ratio, corrodes equipment, and is low in catalyst recovery, product separation, and purification [8]. Enzyme-catalyzed transesterification is considered to be efficient as it is insensitive to FFA and water contents of the feedstock, requires a simple procedure for product purification, generates no byproducts, and catalyzes both transesterification and esterification reactions simultaneously. Moreover, product separation and catalyst recovery are easy, and it produces higher yield under mild conditions [30]. However, enzyme-catalyzed transesterification is not a feasible and cost-effective method of biodiesel production because of high cost, requirement of a long reaction time, and deactivation of the enzyme in the presence of impurities [8]. Reaction temperature, reaction time, alcohol to oil molar ratio, catalyst concentration, mixing speed, and type of feedstock are the most important parameters that affect the transesterification of oils/fats into biodiesel [8]. Stoichiometrically, transesterification requires three moles of alcohol and one mole of triglyceride to produce three moles of biodiesel and one mole of glycerol. Excess alcohol must be used to promote the yield of biodiesel [21]. The lower yield of
biodiesel at low catalyst concentration might be related to the incomplete conversion of the triglyceride to biodiesel [31]. Although a higher temperature lowers viscosities, increases reaction rate, and reduces reaction time, reaction temperature above the optimum reduces biodiesel yield [22]. Also, reaction temperature must be lower than the boiling point of alcohol used for the transesterification process to avoid its loss to vaporization. The conversion rate of triglyceride increases with reaction time, however, reaction time above the optimum reduces the yield of biodiesel [22]. A maximum yield of biodiesel is achieved at a reaction time of ≤120 min for alkaline catalyzed transesterification [8]. Other studies show that catalyzed transesterification processes of nonedible oils require about 90–120 min of reaction time to complete, regardless of operating conditions [8]. Studies indicate that more than 95% of biodiesel production comes from the various edible single oil feedstocks. However, the use of edible oils for biodiesel production has been linked to food versus fuel crisis [9]. To overcome the economic viability and insufficiency of single edible oil feedstocks for large-scale biodiesel production, the use of two or more oil mixtures at suitable ratio has been suggested as an effective approach toward industrial scale biodiesel production [9]. The use of locally available oil mixtures can solve the insufficiency of single oil feedstocks to produce biodiesel at large scale and improve the quality of the biodiesel [27]. Also, it enhances the production of biodiesel at a large-scale to satisfy the future energy demand. Mixed oils derived from nonedible and/or edible sources have gained significant importance as a potential feedstock for large-scale biodiesel production. Studies have been conducted to maximize production and improve the fuel properties of biodiesels by mixing different feedstocks, such as mahua and simarouba oils [32], cottonseed, soybean, and castor oils [33], castor and soybean oils [34], soybean and rapeseed oils [35], and Jatropha curcas and Ceiba pentandra oils [27]. The success of this method depends on the optimization of biodiesel production and the fuel quality of the resulting biodiesel [9]. The properties of biodiesels depend on the fatty acid composition of the oil mixtures used as the feedstocks. For instance, it has been found that the specific density and viscosity of biodiesels produced by the transesterification of oil mixtures are midway between the specific density and viscosity of the biodiesels derived from individual oils [36]. Though there are many pieces of literature related to biodiesels, studies relevant to the optimization of biodiesel production from locally available and nonedible oil mixture are limited. Moreover, most studies focused on the search for new feedstocks and modeling tools to optimize biodiesel production parameters. The main objective of this study was to use nonedible and locally available mixed oils (castor seed and microalgal oils) to optimize the production of biodiesel, with the additional advantage of the low cost of castor oil.

2. Materials and Methods

Wet microalgal biomass was collected from wastewater stabilization ponds at the Jimma Institute of Technology, southwest Ethiopia, using a 60.0 μm filter screen and concentrated by centrifugation. The biomass was dried using a freeze drier and milled to a paste using a mortar and pestle. The castor seeds were collected from southwest Ethiopia and undergone various preparation steps, including the manual removal of foreign materials and seed shells, drying at 105°C for 6 h, and grinding into paste. The moisture contents (MC) of dried castor seed kernel and microalgal biomass were determined using equation (1).

\[
MC(\%) = \frac{W_0 - W_1}{W_0} \times 100\%,
\]

where \( w_0 \) and \( w_1 \) (in g) are weights before and after drying.

2.1. Oil Extraction and Purification. Oils were extracted from dried microalgal biomass and castor seed kernel using methanol as a solvent. 100.0 g of castor seed kernel was placed in a flask. 500.0 ml methanol was added, and the mixture was stirred at 300 revolutions per min (rpm) and heated to 60.0°C for 8 h. Similarly, methanol was added to 100.0 g of dried microalgal biomass, and the mixture was stirred at the same mixing speed and kept at the same temperature for castor seed kernel. Oil extraction was repeated several times until all masses of the castor seed paste and microalgal biomass were used up, and only average result was reported for both feedstocks. After completing oil extraction, the samples were centrifuged at 2000 rpm for 20 min to remove solid residues. To remove the residual solvent, the top layer of the mixture was filtered and evaporated. The residue was dried at 105°C, cooled to room temperature, and reweighed to determine the oil content (OC) of microalgal biomass and castor seed kernel using equation (2).

\[
OC(\%) = \frac{\text{Weight of oil extracted} (g)}{\text{Weight of sample used for oil extraction} (g)} \times 100\%.
\]

The extracted oil was filtered through a sieve and heated to 120°C for 30 min to remove water and residual solvents before it was transferred to a separatory funnel for the separation of oil and sludge. The sludge, which might contain phosphatides, pigments, and other impurities, was discarded, while the oil was washed repeatedly with distilled water until the wash water became clear and achieved neutral pH. The trace NaOH remained after neutralization was removed by washing the oil with distilled water. Any water remained was removed by drying the oil in the oven at 105°C for 1 h.

2.2. Characterization of Mixed Oil and Biodiesel. The fuel quality of biodiesel is influenced by factors, such as the type of conversion, purification processes, and fatty acid composition of the feedstock [1]. Before converting the crude oil to biodiesel, it is critical to characterize the oil to determine the need for pretreatment [23]. Properties, such as moisture content, saponification value, acid value, kinematic viscosity,
density, fatty acid composition, and ash content, were determined for mixed oil to ensure its appropriateness for alkaline transesterification. On the other hand, the biodiesel produced from mixed oil was analyzed according to ASTM D6751 to determine its physicochemical properties and fuel qualities. The results were compared to European Union (EN14214) and the United States ASTM D6751 biodiesel quality standards (Table 1).

2.2.1. Moisture Content. The moisture content of an oil in excess of 0.050% lowers the yield of biodiesel by facilitating FFA hydrolysis [18] and soap formation, which hinders the purification of biodiesel [23]. In the current study, the moisture content of mixed oil and biodiesel was determined using the oven drying method. The preweighed mixed oil and biodiesel samples were dried at 105°C for 1 h, and the moisture content of each was determined using equation (1).

\[ \text{Moisture content} = \left( \frac{W_{w} - W_{o}}{W_{o}} \right) \times 100 \]  

where \( W_{w} \) is the mass of 50.0 ml of mixed oil at 20.0°C, and \( W_{o} \) is the mass of an equal volume of water at the same temperature.

2.2.2. Acid and Free Fatty Acid Values. The acid value (AV) quantifies the amount of KOH (in mg) required to neutralize 1.0 g of oil or biodiesel sample [23]. AV has a significant effect on alkali-catalyzed transesterification reactions [37]. In this study, ethanol was added to the mixed oil, and the mixture was boiled using a water bath. After cooling, two drops of phenolphthalein indicator were added to the mixture and then titrated against 0.50 mol L\(^{-1}\) HCl using phenolphthalein as indicator until the end point reached, and the volume of HCl used to bring the change was recorded. A blank (without mixed oil or biodiesel) was saponified following the same procedure. The SV of the mixed oil and biodiesel was determined using equation (5).

\[ \text{SV} = \left( \frac{B_{b} - B_{s}}{C} \right) \times C \times \frac{56.10}{W (g)} \]  

where \( B_{b} \) is the volume of HCl (ml) used for the titration of blank, \( B_{s} \) is the volume of HCl (ml) used for the titration of mixed oil/biodiesel, \( C \) is the concentration of HCl (mol L\(^{-1}\)), \( W \) is the mass of oil/biodiesel (g), and 56.10 is the molecular weight of KOH (g mol\(^{-1}\)).

2.2.3. Saponification Value (SV). Saponification value (SV) is the amount of alkali catalyst required to saponify 1.0 g of a triglyceride. In this study, 2.0 g of mixed oil was added to a flask containing 0.50 mol L\(^{-1}\) KOH solution in anhydrous ethanol and heated to 70.0°C for 30 min. After cooling, few drops of phenolphthalein indicator were added to the mixture. Excess KOH was titrated against 0.50 mol L\(^{-1}\) HCl using phenolphthalein as indicator until the end point reached, and the volume of HCl used to bring the change was recorded. A blank (without mixed oil or biodiesel) was saponified following the same procedure. The SV of the mixed oil and biodiesel was determined using equation (5).

\[ \text{SV} = \left( \frac{B_{b} - B_{s}}{C} \right) \times C \times \frac{56.10}{W (g)} \]  

where \( B_{b} \) is the volume of HCl (ml) used for the titration of blank, \( B_{s} \) is the volume of HCl (ml) used for the titration of mixed oil/biodiesel, \( C \) is the concentration of HCl (mol L\(^{-1}\)), \( W \) is the mass of oil/biodiesel (g), and 56.10 is the molecular weight of KOH (g mol\(^{-1}\)).

2.2.4. Density (ρ). The density of oil/biodiesel indicates the delay between fuel injection and combustion, as well as the energy per unit mass of oil/biodiesel [21]. The specific gravity (S\(_{p}\)) of the mixed oil was determined by taking the ratio of the mass of 50.0 ml of mixed oil at 20.0°C to the mass of an equal volume of water at the same temperature, as in equation (6).

\[ S_{p} = \frac{W_{o}}{W_{w}} \]  

where \( W_{o} \) is the mass of 50 ml of oil at 20.0°C, and \( W_{w} \) is the mass of 50.0 ml of water at the same temperature.

| Physicochemical property | Unit | Determined values | Biodiesel standards |
|-------------------------|------|-------------------|---------------------|
|                         |      | Mixed oil | Biodiesel | ASTM D751 | EN 14214 |
| Specific gravity at 20°C | —    | 0.920    | 0.89     | 0.86–0.90 | 0.86–0.90 |
| Density at 20/15°C      | (Kg/m³) | 920.0   | 890.0    | 860–900  | 860–900   |
| Kinematic viscosity at 40°C | (mm²/s) | 44.60  | 5.80     | 1.9–6.0  | 3.5–5.0   |
| Flash point (°C)        |       | 160.0    | 133.33   | ≥12.00   | ≥130.0    |
| Cetane number           |       | 57.44    | 58.12    | ≥47.0    | ≥51.0     |
| Acid value              | (mg KOH/g) | 20.56  | 0.10     | ≤0.50    | ≤0.50     |
| Free fatty acid value   | (%)   | 10.28    | 0.05     | ≤0.050   | ≤0.050    |
| Saponification value    | (mg KOH/g) | 180.90 | 1.80     | —        | —         |
| Iodine value            | (g I₂/100 g) | 83.82 | 82.22    | ≤120.0   | ≤140.0    |
| Moisture content        | (%)   | 0.060    | 0.027    | ≤0.03    | ≤0.05     |
| Ash content             | (%)   | 0.045    | 0.028    | ≤0.03    | ≤0.02     |
| Caloric value           | (MJ/kg) | 43.27  | 48.12    | ≥42.0    | ≥35.0     |
| Ethyl ester content     | —     | 98.64    | —        | >96.5    | —         |

Table 1: Properties of mixed oil and biodiesel produced under optimum conditions from the mixed oil.
density of mixed oil was determined by multiplying its specific gravity with the density of water (1000 kg/m³). On the other hand, the density of biodiesel was determined using ASTM D1298 Standard at the reference temperature of 15.0°C using a hydrometer [21].

2.2.5. Kinematic Viscosity (V). A digital Vibro viscometer (SV-10, Australia) was used to determine the viscosity of the mixed oil. After falling into the viscometer’s cup, the mixed oil sample was kept in a water bath at 40.0°C for 30 min. The viscometer tip was then inserted into the viscometer cup containing the mixed oil, and the reading was taken (in centistokes). The dynamic viscosity was corrected to kinematic viscosity, as in equation (7).

\[ R \frac{mm^2}{s} = \frac{\text{Dynamic viscosity}}{\rho \text{ of sample}}. \] (7)

The V of biodiesel was obtained by determining the flow time for a volume of biodiesel to pass between two marked points [38]. From the flow time (in sec), kinematic viscosity was calculated using equation (8).

\[ R = \text{Calb} \times t, \] (8)

where Calb is the calibration constant specified by manufacturer (0.040350 mm²/s² at 40°C), and t is the time required to travel between the marked points (in sec). Measurements were conducted in triplicate, and only the average value was reported.

2.2.6. Iodine Value (IV). Iodine value (IV) specifies the amount of iodine (in g) consumed by 100.0 g of a biodiesel sample. In this study, the IV of biodiesel was determined by placing 0.10 g of biodiesel sample in an Erlenmeyer flask, adding 10.0 ml of anhydrous chloroform, followed by the addition of 30.0 ml of Hanus iodine solution. The entire content was titrated against 0.140 M Na₂S₂O₃ until the solution turned light yellow. After adding 2.0 ml of 1.0% starch solution, titration was continued until the blue color disappeared. Blank titration was carried out without a biodiesel sample. The IV of the biodiesel was determined, as shown in equation (9).

\[ \text{IV} \left( \frac{gI_2}{100g} \right) = \frac{(V_b - V_r) \times N \times 12.69}{m(g)}. \] (9)

where \( m \) is the mass of biodiesel used (in g), \( N \) is the normality of Na₂S₂O₃, \( V_b \) is the volume (ml) of Na₂S₂O₃ used for the titration of blank, and \( V_r \) is the volume (ml) of Na₂S₂O₃ used for the titration of sample. The same procedure was used to determine the IV of the mixed oil.

2.2.7. Cetane Number (CN). The results of SV and IV were used to estimate the Cetane number (CN) of the biodiesel produced from mixed castor and microalgae oils using an empirical formula suggested by [39], as shown in equation (10).

\[ \text{CN} = 46.30 + \frac{5458.0}{\text{SV}} - 0.225 \times \text{IV}. \] (10)

2.2.8. Caloric Value. Caloric value (higher heating value) is the net heat content of biodiesel, which affects the fuel consumption of engine [32]. A higher calorific value is important as it improves engine performance during fuel combustion. Several mathematical models exist in the literature for estimating the heat value of oils and biodiesel. In this study, an empirical formula suggested by [40] was used to estimate the higher heat value (HHV) of the mixed oil and biodiesel as a function of IV and SV, as shown in equation (11).

\[ \text{HHV} \frac{MJ}{kg} = 49.43 - [(0.041 \times \text{SV}) + (0.015 \times \text{IV})]. \] (11)

2.2.9. Flash Point (FP). Flash point is the lowest temperature at which a fuel emits enough vapors upon exposure to a flame to ignite. It is inversely related to fuel volatility [41]. In this study, Pensky-Martens closed cup tester (FP-261, Germany) was used to measure the FP of the biodiesel. The cup was filled with the biodiesel sample and heated while being stirred. The stirring was stopped periodically, and the frame pivoted down to check if the vapor ignites. The temperature at which a small flash observed was recorded as FP and corrected to the atmospheric pressure (760 mmHg) using the correction factor, as shown in equation (12).

\[ \text{FP (corrected)} = T + 0.033(760 - P), \] (12)

where \( T \) is the observed temperature (°C), and \( P \) is the atmospheric pressure (mmHg) at the time of test.

2.2.10. Ash Content (AC). The furnace method was used to determine the ash content (AC) of the mixed oil and biodiesel. A cup containing 20.0 g of mixed oil was placed in a furnace at 550°C for 4 h. After burning, the residue was weighted, and the ash content was determined, as shown in equation (13).

\[ \text{AC} \% = \frac{W_2}{W_1} \times 100\%, \] (13)

where \( W_1 \) and \( W_2 \) are the mass of oil/biodiesel before and after burning (in g), respectively.

2.2.11. Compositions of Biodiesel. The conversion of mixed oil to its corresponding fatty acid ethyl esters was analyzed using gas chromatography (Shimadzu, Tokyo, Japan). The samples of biodiesel and n-hexane were added to the flask, shaken, and let to be idle for 1 min. After shaking, the solution was let to be idle for 5 min until sedimentation occurred. An anhydrous Na₂SO₄ was used to dry the solution. The solution was analyzed by gas chromatography (GC) equipped with a flame ionization detector with a Supelco-wax10 capillary column (30 mm × 0.25 mm × 0.25 μm). The
injection temperature was set at 250°C, while column temperature was programmed to increase from 50°C to 250°C at 4°C/min. 1.0 μL biodiesel sample was injected at a split ratio of 1:20. Standard fatty acids were injected into GC, and the identification of each fatty acid ethyl ester component was carried out by comparing the retention times of samples and references. The percentage composition of each fatty acid ethyl ester was obtained from the corresponding peak areas [13]. The same method was used to determine the fatty acid profiles of the castor oil and microalgae oil.

All experiments were conducted in triplicate using analytical grade reagents and solvents, such as n-hexane (99% purity), methanol (99% purity), anhydrous ethanol (99.5% purity), KOH (95% purity), anhydrous NaOH (98% purity), HCl (98% purity), H2SO4 (98% purity), anhydrous KI (98% purity), phenolphthalein (analytical grade), starch solution (analytical grade), chloroform (analytical grade), and sodium thiosulfate (analytical grade).

2.3. Design of Experiment (DOE). The conversion efficiency of feedstock to biodiesel depends on process variables [27]. Conventional process optimization (i.e., the optimization is carried out by varying a single variable, while all the other variables hold constant at a specific set of conditions) is not only time consuming and costly but also requires a large number of experiments [39]. Response surface methodology (RSM) is an effective statistical and mathematical technique to study the effects of individual factors and their interaction on a response [8]. In this study, the RSM-based central composite design (CCD) was used to evaluate the effects of individual process variables and their interactions on the yield of biodiesel. CCD was used to design transesterification experiments. Transesterification experiments were conducted according to 2^n complete factorial for the three independent variables with a total of 17 experimental runs (2^n + 2n + Cp), where Cp is the number of experiments at center points (Table 2).

| Variable (factor) | Symbol | Unit     | Coded level | Actual level |
|-------------------|--------|----------|-------------|--------------|
| Catalyst concentration | A      | W/w (%)  | −1.68, −1, 0, +1, +1.68 | 0.50, 0.70, 1.0, 1.3, 1.50 |
| Ethanol to mixed oil ratio | B      | (v/v)    | 3.0, 5.0, 7.5, 10.0, 12.0 | 1.0, 1.0, 1.0, 1.0, 1.0 |
| Reaction temperature | C      | (°C)     | 25.0, 35.0, 50.0, 65.0, 75.0 | 25.0, 35.0, 50.0, 65.0, 75.0 |

Each factor was varied over five levels. Eight factorial (2^n) and six axial (2n) experimental runs were conducted, while three experiments were carried out at the Cp to evaluate the pure error [39]. The distance of axial points (±α) from the Cp was calculated, as shown in equation (14).

\[ ± \alpha = \sqrt[4]{2^n}, \quad (14) \]

where \( \alpha \) is the distance of axial point from Cp.

Accordingly, the lowest and highest levels were −1.68 and +1.68 for coded levels of factors. The axial points were fixed at (±α, 0, 0), (0, ±α, 0), and (0, 0, ±α) for the three independent variables. Mixed oil transesterification was carried out at catalyst concentrations (0.50% ≤ A ≤ 1.5%), ethanol to mixed oil molar ratios (3:1 ≤ B ≤ 12:1), and reaction temperatures (25.0°C ≤ C ≤ 75.0°C). The mixing speed and reaction time were set at optimum points for all experimental runs based on previous studies [8]. Base-catalyzed transesterification reactions are basically complete within one hour [18]. The actual levels of independent variables were selected based on the operating limit of the transesterification process and literature data [31]. The highest reaction temperature (75.0°C) was chosen just below the boiling point of ethanol (78.0°C), whereas a temperature bit above room temperature (25.0°C) was taken as the lowest reaction temperature. The highest (1.50%) and lowest (0.50%) levels of catalyst concentrations were chosen based on a previous study [27]. The lowest level of ethanol to mixed oil molar ratio (3:1) was chosen based on the minimum stoichiometry for the transesterification reaction, whereas the highest level (12:1) was chosen based on literature data [42]. The catalyst concentration of 1.0 w/w (%), ethanol to mixed oil molar ratio of 7.5:1, and reaction temperature of 50.0°C were selected as actual Cp levels.

2.4. Transesterification of Mixed Oil under Different Conditions. The esterification and transesterification of mixed oil were conducted in a 500.0 ml three-neck round bottom flask equipped with a reflux condenser. A rubber stand was used to immerse a thermometer into one neck of the flask. The mixed oil was added to the preheated (60°C) three-necked round bottom flask submerged in a water bath placed on a hot plate with a magnetic stirrer. A freshly prepared KOH-ethanol solution was added to the preheated flask under constant mixing speed. Experiments were conducted at CCD designed actual values of reaction temperatures, catalyst concentrations, and ethanol to mixed oil molar ratios to find the optimum yield of biodiesel. At the end of the transesterification, a separator funnel was used to separate the mixture into fatty acid ethyl esters (biodiesel) and glycerol. Excess ethanol was removed using rotary evaporator, and the product was filtered and analyzed to determine the percent yield of biodiesel using equation (15).

\[ BY(\%) = \frac{\text{Biodiesel produced (g)}}{\text{Oil used (g)}} \times 100. \quad (15) \]

2.5. Purification of the Crude Biodiesel. A crude biodiesel may contain impurities, such as soap, water, glycerides,
excess catalyst, and unreacted alcohol [39]. To comply with fuel quality standards, impurities should be reduced to an acceptable level as they influence biodiesel stability and combustion system [22]. In this study, the biodiesel produced from mixed oil was purified by washing with hot distilled water at 60.0°C until the wash water achieved a neutral pH. Any water remaining after the washing operation was removed by drying the biodiesel at 80°C under a vacuum rotary evaporator and passing it over anhydrous Na₂SO₃.

2.6. Data Analysis. Experimental results were fitted to a polynomial model equation to correlate biodiesel yield and independent variables using regression analysis. The contribution and significance of each variable, as well as the optimum conditions for biodiesel production as a function of coded variables were determined using equation (16) [39].

\[
Z = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \sum_{j=1}^{n} \beta_{ij} X_i X_j,
\]

(16)

where \(Z\) is the biodiesel yield (%), \(\beta_0\) is the intercept, \(\beta_i\) is the first-order model coefficient, \(\beta_{ij}\) is the quadratic coefficient of the \(i\)th variable, \(\beta_{ij}\) is the linear coefficient of the model for the interaction between the \(i\)th and \(j\)th variables, \(X_i\) and \(X_j\) are the independent variables, and \(n\) is the number of variables studied and optimized in the experiment. Confirmatory experiments were conducted under the optimum conditions to validate the result predicted by the model equation. Design Expert® version 12 was used to carry out regression and graphical analyses. The quality of the model was evaluated using the coefficients of determination (\(R^2\)) and analysis of variance (ANOVA). Response surface plots were drawn by varying two variables, while the third variable was kept at the center point to show the effect of interactions between independent variables on the yield of biodiesel.

3. Results and Discussion

To determine the oil content of microalgal biomass, 2.54 kg of dried biomass was used to extract 800.40 g of oil with methanol as solvent. About 46.20 g of castor oil was extracted from 100.0 g of castor seed kernel using methanol as solvent. The oil content of dry microalgal biomass and castor seed kernel was determined using equation (2), and it was found to be 31.512% for microalgae and 46.21% for the castor seed kernel. The results obtained agree with what has been reported in the literature for castor seed kernel oil content (46–55%) [38] and microalgal oil content (20–50%) [27]. The acid value of the mixed oil was found to be 20.56 mg KOH/g, while its corresponding free fatty acid value was 10.28 mg KOH/g. A higher acid value may result in severe corrosion of equipment. The reduction of acid value of mixed oil was achieved through a two-step pretreatment process prior to the alkaline transesterification of the mixed oil to biodiesel. The acidity of mixed oil was titrated with 0.50 N NaOH until it becomes <0.050%. The mixed oil was also pretreated with H₂SO₄ to adjust the FFA of the mixed oil to an acceptable level. The acid value of the biodiesel was found to be 0.10 mg KOH/g. The acid value of petrodiesel (i.e., 0.017 mg KOH/g) is lower than the acid value of biodiesel. The density of mixed oil at 20°C was found to be 920.0 kg/m³, while it was 890.0 kg/m³ for the biodiesel produced from it. The density of biodiesel agrees with ASTM D751- and EN14214-specified standards (860–900 kg/m³). According to [40], vegetable oils have HHVs that range from 24.29 to 41.20 MJ/kg.

ASTM D751 and EN14214 test methods and literature-based empirical formulae were used to assess the fuel quality of biodiesel produced from mixed oils under the optimal conditions to comply its use in the current engine. Cetane number (CN) and calorific value were estimated as in equation (11) and equation (12) using the results of IV and SV. The CN of the mixed oil was found to be 57.44, while it was 58.12 for the biodiesel produced from the mixed oil. CN measures the ignition quality of a fuel [21]. The higher the CN value, the easier the biodiesel ignites when injected into an engine. Biodiesel has a higher CN value (46–60) as its oxygen content is higher. In this study, the SV of mixed oil was found to be 180.90, which is within the acceptable range of standards. The higher SV of an oil decreases the yield of biodiesel by saponification reaction if transesterification is conducted under alkaline conditions. A lower SV is preferred to obtain a better yield of biodiesel. The IV measures the degree of the unsaturation of the oil. The IV of biodiesel was found to be 82.22 g I₂/100 g, a value that agrees with the acceptable range of ASTM D751 standard. A higher IV may lead to deposit formation in the engine [43]. The physicochemical properties of mixed oil and fuel quality of biodiesel were compared to ASTM D751 and EN 14214 standards (Table 1).

3.1. Fuel Quality of the Biodiesel. A biodiesel could be used successfully in the existing engine if its physicochemical properties conform to ASTM D751 and EN 14214 standards. In this study, physicochemical properties, such as kinematic viscosity at 40.0°C, density at 15.0°C, flash point, calorific value, iodine value, acid value, ash content, fatty acid ethyl ester content, and Cetane number of the biodiesel produced by the transesterification of mixed oils under optimum conditions, were determined and compared with ASTM 751 and EN14214 standards. The main objective of transesterification is to reduce the viscosity of the crude oil as it results in deposit formation in the engine and affects fuel atomization when injected into the combustion chamber [38]. The viscosity of biodiesel increases as the fatty acid chain increases. A higher kinematic viscosity is undesirable as it lowers fluidity, slows fuel injection, and delays the mixing of air with fuel in the combustion chamber. The kinematic viscosity at 40°C of the biodiesel was found to be 5.80 mm²/s, a value within the range specified in ASTM D6751 (1.90–6.0 mm²/s) standard. The petrodiesel has kinematic viscosity at 40°C = 2.96 mm²/s, which indicates that it has better fluidity than biodiesel. The density of the biodiesel produced under optimum conditions falls within the range specified in the ASTM D6751 and EN14214 standards. The density of biodiesel produced from mixed oil
TABLE 3: Fatty acid profiles of castor seed oil and microalgal oil.

| Feedstock (oil content) | Palmitic acid | Stearic acid | Oleic acid | Linoleic acid | Linolenic acid | Erucic acid | Eicosapentaenoic acid |
|-------------------------|---------------|--------------|------------|---------------|---------------|-------------|-----------------------|
| Castor oil (45–55%)     | 3.15          | 1.89         | 3.5d1      | 3.44          | 0.10          | 86.50       | 0.55                  | 0.73                  |
| Microalgae oil (28–53)  | 7.10          | 22.7         | 21.5       | 11.30         | 7.81          | 6.90        | 16.11                 | 6.10                  |

was higher than the density of petrodiesel (846.10 kg/m³). Flash point is an important fuel quality parameter to assess fire risk at the time of transportation, storage, and use of biodiesel. Petrodiesels have flash points of 50.0–80.0°C, while biodiesel has a flash point over 120.0°C. The flash point of the biodiesel from mixed oil falls within EN 14214 and ASTM D751 standards. The calorific value of the biodiesel was found to be 48.12 MJ/kg, a value much higher than that of petrodiesel, indicating that the biodiesel has a better engine performance. The higher calorific value of the biodiesel might be because of a decrease in the mass fraction of oxygen as the fatty acid carbon chain length increases without a change in the saturation level. A high calorific value is an indication of energy content of the biodiesel. The calorific value increases as molecular weight increases but decreases as the number of double bonds increase. The fatty acid ethyl ester composition of biodiesel was analyzed with GC. The analysis of fatty acid ethyl ester composition revealed that ethyl ester groups make up 98.64% of the biodiesel, a value much higher than that of petrodiesel, indicating that the biodiesel has a better engine performance.

3.2. Fatty Acid Profiles of Castor Seed Oil and Microalgae Oil. Fatty acid composition of castor oil reveals that it mainly consists of linoleic acid (86.5%), stearic acid (3.44%), oleic acid (3.51%), palmitic acid (3.15%), a small amount of stearic acid (1.89%), eicosapentaenoic acid (0.73%), erucic acid (0.55%), and linolenic acid (0.10%). The oil content and fatty acid profile are the most important criteria to assess the potential of microalgae for biodiesel production [44]. Microalgal oil contains both saturated and unsaturated fatty acids (Table 3). In microalgal biomass used for the current study, the dominant fatty acid profiles were palmitic acid (16:0), stearic acid, oleic acid (18:1), linoleic acid (18:2), and linolenic acid (18:3). The content of particular fatty acid categories is important for the fuel quality of the biodiesel. A higher content of saturated and monounsaturated fatty acids (16:0, 18:1, 18:2, 18:3) is important for the fuel quality of biodiesel. The high concentration of saturated fatty acids (SFAs) ensures good oxidation stability for the biodiesel. Monounsaturated fatty acids (MUFAs) render the biodiesel a good oxidation stability and liquidity at low temperatures. A higher content of polyunsaturated fatty acids (PUFAs) may increase NOx emission from the biodiesel. However, the low content of PUFA profiles have a positive effect on the low temperature flow properties of the biodiesel.

3.3. Optimization of Biodiesel Production and Transesterification Variables Using RSM. The RSM was used to optimize conditions for the transesterification of mixed castor and microalgal oils. The three independent variables (catalyst concentration (A), ethanol to mixed oil molar ratio (B), and reaction temperature (C)) were varied over five levels to optimize the yield of biodiesel. The 17 experiments designed using CCD were conducted to investigate the effects of the three independent variables and the interaction between these variables on the yield of biodiesel (Table 4). A multiple regression analysis was carried on the experimental data to generate a model equation, which gives the yield of biodiesel (Z) as a function of coded variables [catalyst concentration (A), mixed oil to ethanol molar ratio (B), and reaction temperature (C)]. The polynomial model equation that consists of a center point, three linear coefficients (A, B, C), three quadratic coefficients (A², B², C²), and three interaction coefficients (AB, AC, BC) is shown in equation (17).

\[ Z = 91.28 + 11.69A + 4.76B - 0.83C - 5.15AB + 1.84AC - 1.63BC - 8.20A^2 - 3.54B^2 - 0.07C^2. \]  

(17)

The positive sign of regression coefficients in equation (17) shows the synergistic effect of variables and their interactions on the yield of biodiesel, while the negative sign indicates an antagonistic effect of the variables on the yield of biodiesel. The magnitude of regression coefficients indicates the degree of significance of each independent variable on the yield of biodiesel [43]. Accordingly, A (catalyst concentration linear coefficient) is the most significant independent variable. A final quadratic regression equation model, which contains only the significant variables, was produced after the significance of each regression coefficient
was determined at 5% significance level. Only terms with a \( p \) value < 0.05 were included in the final model equation \( I \) (equation (18)). Ignoring the insignificant model terms, the final model equation is shown in equation (18).

\[
Z = 91.28 + 11.69A + 4.76B + 5.15AB + 8.20A^2 + 3.54B^2.
\]  
(18)

The reduced model equation (18) can be used to obtain the percent yield of biodiesel from the alkaline transesterification of mixed oil over the entire level of the three independent variables studied.

### 3.4. Statistical Analysis of Transesterification Process Variables

Analysis of variance (ANOVA) was carried out to verify the significance and fitness of the model and the effects of the significant individual variables and their interactions on the yields of biodiesel. The significance of each regression coefficient was evaluated using \( p \) values. The \( p \) value signifies the probability of error and is used to confirm the significance of each regression coefficient. \( p \) values less than 0.05 indicate that the model terms are significant. Accordingly, A (catalyst concentration linear term, \( p < 0.0001 \)), B (ethanol to mixed oil molar ratio linear term, \( p < 0.0003 \)), \( A^2 \) (catalyst concentration quadratic term, \( p < 0.0001 \)), \( B^2 \) (ethanol to mixed oil molar ratio quadratic term, \( p < 0.0028 \)), and AB (interaction of catalyst concentration and ethanol to oil molar ratio, \( p < 0.0011 \)) were significant. All regression coefficients containing temperature terms (linear, interactions, and quadratic) were not significant at the \( p \) value less than 0.05 (Table 5).

The \( p \) value of less than 0.0001 indicates that the regression model is suitable and significant in predicting the yield of biodiesel at the 95% confidence level. The model has an \( F \)-value of 54.96, which indicates that it is significant in predicting the yield of biodiesel at 95% confidence level. Also, a \( p \) value of 0.0001 of the model indicates that the probability of obtaining a large \( F \)-value because of noise is

### Table 4: CCD-based experimental design and results of mixed oil transesterification.

| Design Run No. | Actual levels | Coded levels | Biodiesel yield (%) | Residue | Catalyst conc. (%) | Molar ratio | Temperature (°C) | A | B | C | Actual | Predicted |
|----------------|---------------|--------------|---------------------|---------|-------------------|------------|-----------------|---|---|---|---------|-----------|
| Factorial points | | | | | | | | | | | | | | |
| 1 | 1.30 | 10.0 | 35.0 | 1 | 1 | –1 | 90.87 | 91.67 | –0.80 |
| 2 | 0.70 | 10.0 | 35.0 | –1 | 1 | –1 | 82.22 | 81.50 | 0.72 |
| 3 | 1.30 | 5.0 | 65.0 | 1 | –1 | 1 | 91.94 | 94.53 | –2.59 |
| 4 | 1.30 | 10.0 | 65.0 | 1 | 1 | 1 | 89.44 | 90.66 | –1.22 |
| 5 | 0.70 | 5.0 | 35.0 | –1 | –1 | –1 | 59.19 | 59.83 | –0.64 |
| 6 | 1.30 | 5.0 | 35.0 | –1 | 1 | –1 | 88.49 | 89.43 | –0.94 |
| 7 | 0.70 | 10.0 | 65.0 | –1 | 1 | 1 | 72.13 | 73.06 | –0.93 |
| 8 | 0.70 | 5.0 | 65.0 | –1 | –1 | 1 | 56.44 | 57.51 | –1.07 |
| Axial points | | | | | | | | | | | | | | |
| 9 | 1.50 | 7.5 | 50.0 | 50.0 | 2 | 0 | 0 | 90.19 | 87.76 | 2.43 |
| 10 | 1.00 | 12.0 | 50.0 | 50.0 | 0 | 2 | 0 | 89.59 | 89.28 | 0.31 |
| 11 | 0.50 | 7.5 | 50.0 | 50.0 | –2 | 0 | 0 | 48.68 | 48.43 | 0.25 |
| 12 | 1.0 | 3.0 | 50.0 | 50.0 | 0 | –2 | 0 | 75.25 | 73.26 | 1.99 |
| 13 | 1.0 | 1.5 | 75.0 | 75.0 | 0 | 0 | 2 | 92.23 | 89.68 | 2.55 |
| 14 | 1.0 | 7.5 | 25.0 | 25.0 | 0 | 0 | –2 | 92.57 | 92.48 | 0.09 |
| Center points | | | | | | | | | | | | | | |
| 15 | 1.0 | 7.5 | 50.0 | 50.0 | 0 | 0 | 0 | 93.34 | 91.28 | 2.06 |
| 16 | 1.0 | 7.5 | 50.0 | 50.0 | 0 | 0 | 0 | 92.43 | 91.28 | 1.15 |
| 17 | 1.0 | 7.5 | 50.0 | 50.0 | 0 | 0 | 0 | 87.89 | 91.28 | –3.39 |

### Table 5: ANOVA for the fitting response surface quadratic model.

| Variation source | Sum of squares | DF | Mean square | F-value | \( p \) value | Remarks |
|------------------|---------------|----|-------------|---------|-------------|---------|
| Model            | 3268.62       | 9  | 363.18      | 54.96   | <0.0001     |         |
| A-Catalyst       | 1887.19       | 1  | 1887.19     | 285.61  | <0.0001     |         |
| B-Molar ratio    | 286.53        | 1  | 286.53      | 43.36   | 0.0003      |         |
| C-Temperature    | 9.50          | 1  | 9.50        | 1.44    | 0.2695      |         |
| AB               | 188.57        | 1  | 188.57      | 28.54   | 0.0011      | Significant |
| AC               | 27.60         | 1  | 27.60       | 4.18    | 0.0803      |         |
| BC               | 18.67         | 1  | 18.67       | 2.82    | 0.1367      |         |
| \( A^2 \)        | 750.30        | 1  | 750.30      | 113.55  | <0.0001     |         |
| \( B^2 \)        | 134.19        | 1  | 134.19      | 20.31   | 0.0028      |         |
| C\(^2\)         | 0.0522        | 1  | 0.0522      | 0.0079  | 0.9317      |         |
| Residual         | 46.25         | 7  | 6.61        | —       | —           | Not significant |
| Lack of fit      | 29.21         | 5  | 5.84        | 0.6853  | 0.6832      |         |
| Pure error       | 17.05         | 2  | 8.52        | —       | —           |         |
| Total            | 3314.88       | 16 | —           | —       | —           |         |

\( CV = 5.39\% \); \( R^2 = 0.9860 \); adjusted \( R^2 = 0.9681 \); predicted \( R^2 = 0.9214 \); DF = degree of freedom.
less than 0.01%. Hence, only model terms A, B, AB, A², and B² are significant. On the other hand, $p$ values greater than 0.10 indicate that the model terms are not significant.

The insignificance of the lack of fit of the regression model indicates that it describes sufficiently the relationship between independent variables (ethanol to oil molar ratio, reaction temperature, and catalyst concentration) and dependent variable (yield of biodiesel). The F-value of the lack of fit for this study is found to be 0.6953, while the $p$ value of the lack of fit is found to be 0.6932. The $p$ value of the lack of fit is > 0.05, indicating that there is a good fit between the model and experimental data. A small coefficient of variance (CV = 5.39%) indicates the reliability of the regression model. The low value of CV also indicates that the experimental data are accurate and reliable [27].

The quality of the model fit was evaluated based on the value of the coefficient of determination ($R^2$). The $R^2$ value shows the variability of the dependent variable with the independent variables [39]. A higher value of $R^2$ indicates that there is a good fit between the model and experimental data over the selected range of process variables. The value of $R^2$ for this study is 0.9860, which indicates that 98.60% of the experimental results are compatible with the model data (Table 5). In other words, only 1.41% of the total variability cannot be explained by the model. The adjusted coefficient of determination (adjusted $R^2$) indicates how well the model fits with the experimental data and the number of significant independent variables.

The adjusted $R^2$ increases if desirable independent variables are included in the model while decreases upon the addition of undesirable independent variables into the regression model [27]. A very high value of adjusted $R^2$ indicates that the model is very significant. The value of adjusted $R^2$ for this study is found to be 0.9681, which indicates that the model accounts for 96.81% of the variability.
in the biodiesel yield. A reasonable precision of model fitness was deduced from a higher value of the predicted coefficient of determination (predicted $R^2 = 0.9214$). The small difference between the $R^2$ and adjusted $R^2$ (1.80%) implies that there is a least chance that insignificant terms have been included in the model.

The plot profile of experimental versus predicted biodiesel yield indicates that the predicted values closely fit with the experimental values, showing that there is adequate correlation between the predicted and experimental data (Figure 1). The differences between the experimental and predicted values of biodiesel yield are less than 20%, indicating that there is a good agreement between the model and experimental data. It conforms to the coefficient of determination ($R^2$) and adjusted $R^2$ of the model with a value close to unity. It can be noted that the regression model gives a good estimate of the biodiesel yield with variations in the reaction temperature, ethanol to mixed oil molar ratio, and catalyst concentration.

### 3.5. Effects of Transesterification Process Variables on the Yield of Biodiesel

The statistical software package Design Expert® 12 was used to draw the response surface plots using the regression model equation. As the individual plot does not show the interactions between the process variables, three-dimensional surface plots were drawn to show the effects of variable interactions on the yield of biodiesel. To investigate the interaction effect of two independent variables on the yield of biodiesel, the third variable was kept at Cp. Based on the 95% confidence limit, only those terms with a $p$ value < 0.05 were considered for the investigation of variables effects. Accordingly, the linear and quadratic terms of ethanol to mixed oil molar ratio and catalyst concentration ($A$, $A^2$ and $B$, $B^2$) were significant. On the other hand, all the interaction terms were not significant, except for ethanol to mixed oil molar ratio and catalyst concentration (AB) (Figure 2).

The relationship between the percent yield of biodiesel and catalyst concentration is curvilinear with a positive linear coefficient and a negative quadratic coefficient, indicating that biodiesel yield is inhibited beyond the optimum catalyst concentration as the reverse reaction is favored at a higher catalyst concentration. At low ethanol to mixed oil ratio, the yield of biodiesel was improved with an increase in catalyst concentration. Increasing both mixed oil molar ratio and catalyst concentration at the same time also improved the yield of biodiesel, indicating that there is significant interaction between mixed oil molar ratio and catalyst concentration. The effect of reaction temperature and catalyst concentration on biodiesel yield at constant ethanol to oil molar ratio of 7.5:1 is shown in Figure 3.

The effect of reaction temperature ($^\circ$C) and its interactions with catalyst concentration was not significant at $p$ value < 0.05, indicating that temperature does not significantly affect biodiesel yield. However, the yield of biodiesel was improved with an increase in catalyst concentration toward 1.23% at room temperature, and after that, there was a substantial decrease in the yield with a further increase in catalyst concentration. It is because of the fact that excess catalyst might lead to soap formation. The formation of soap occurs at higher catalytic concentration beyond 1.25%.

The effect of ethanol to mixed oil molar ratio, reaction temperature, and their combined interaction at constant catalyst concentration (1.0 w/w%) is shown in Figure 4. The effect of reaction temperature and its interactions with mixed oil to ethanol molar ratio (BC) was not significant at $p$ value < 0.05, indicating that temperature does not significantly affect biodiesel yield because the mixed oil is soluble in ethanol at room temperature. At low catalyst concentration, the increasing temperature had minimal effect on the yield of biodiesel. Hence, increasing the molar ratio beyond the optimum may increase the cost for alcohol recovery rather than increasing the yield [22].

### 3.6. Validation of the Optimum Biodiesel Yield

The regression equation was solved using the Design-Expert® 12 software to obtain the optimum values for the three independent variables studied. To this end, the goal of the three independent variables was set to “in range,” while the response (percent of biodiesel yield) was set to “maximum.” In addition, the lower and upper weights were set to 1, while the importance was set at 3 (Table 6).
The predicted optimum values from the model were the catalyst concentration of 1.23%, ethanol to mixed oil molar ratio of 5.94:1.0, and reaction temperature of 51.0°C. The model predicts that the maximum biodiesel yield is 92.88%. To validate the result predicted by the model, confirmatory experiments were conducted under the optimum conditions of process parameters. As a result, the yield of biodiesel from the validation experiment is found to be 92.36%, which is very close to the model predicted value (Table 7).

3.7. Future Prospects. The optimization of biodiesel production from mixed castor and microalgae oil requires the improvement of feedstocks oil yield and oil conversion processes. The use of microalgae as a feedstock for biodiesel production needs to manipulate microalgal lipid and mass production of biomass. To make biodiesel production process economical, cost-effective, and energy efficient, a better microalgal biomass production, harvesting, and dewatering method must be investigated. Wastewater treatment can be integrated with nutrient removal and the mass production of microalgal biomass for large-scale biodiesel production. Castor oil contains a high amount of ricinoleic acid (monounsaturated fatty acid) and small amount of saturated fatty acids (linoleic acid, oleic acid, palmitic acid, and stearic acid). The conversion of oleic acid to ricinoleic acid can be muted by genetic modification to enhance the oleic acid content of castor seed to make it a better feedstock for biodiesel production. This study could inspire scientists toward further study aimed at the optimization of biodiesel production from mixed castor and microalgae oils using different oil mix ratios, homogeneous and heterogeneous catalysts, and alcohols. The results of this study could also elicit the interests of Ethiopian government, farmers, local and international investors, and other stakeholders to engage in the cultivation of higher oil yielding varieties of castor plants for biodiesel production. Ethiopia is located in an ideal climatic zone to cultivate castor plants and microalgae for biodiesel production.

4. Conclusions

The study was conducted to optimize the yield of biodiesel from mixed castor and microalgae oils by the optimization of transesterification process variables using RSM. Experimental results were analyzed to develop a regression model equation for predicting the conversion of mixed oil to biodiesel. It was found that ethanol to mixed oil molar ratio of 5.94:1.0, catalyst concentration of 1.23%, and reaction temperature of 51.0°C were the optimum conditions to achieve an optimum biodiesel yield of 92.88%. Validation experiments conducted under optimum conditions confirmed that the model predicted biodiesel yield agrees with the experimental results. The characterization of biodiesel using various standard methods revealed that the fuel properties of the biodiesel are compatible with standards. It can be concluded that biodiesel production from mixed castor and microalgae oil has the physicochemical properties close to commercial grade petrodiesel, indicating that it has a potential to replace either partially or fully substitute the fossil fuel-based petrodiesel.

Notation

Symbols

| Symbol | Definition                                      |
|--------|------------------------------------------------|
| C      | Concentration (mg L⁻¹)                          |
| M      | Molarity (mol L⁻¹)                              |
| MJ     | Mega joule                                      |
| N      | Normality (L/mol)                              |
| P      | Pressure (mmHg)                                 |
| n      | Number of variables                            |
| R      | Coefficients of variation (%)                  |
| T      | Temperature (°C)                               |
| V      | Volume (ml)                                     |
| W      | Mass (weight) (g)                              |

Greek letters

| Letter | Definition                                      |
|--------|------------------------------------------------|
| α      | Distance of axial point                        |
| β      | Regression coefficient                         |
| µ      | Micro                                          |
| ρ      | Density (kg/m³)                                |
| Σ      | Summation                                      |
| V      | Kinematic viscosity (mm²/s)                    |

Abbreviations

| Abbreviation | Definition                                      |
|--------------|------------------------------------------------|
| AC           | Ash content                                     |
| ASTM         | American Society for Testing and Materials     |
| ANOVA        | Analysis of variance                            |
| AV           | Acid Value                                      |
| BY           | Biodiesel yield                                 |
| CCD          | Central composite design                       |
| CN           | Cetane number                                   |
| CV           | Coefficient of variation                        |
| DOE          | Design of Experiment                            |
| FFA          | Free fatty acid                                 |
| FP           | Flashpoint                                      |
| EN           | European Biodiesel Standard                     |
| GC           | Gas chromatography                              |
| GHG          | Greenhouse gas                                  |
| HHV          | Higher heat value                               |
| IV           | Iodine value                                    |
| MC           | Moisture content                                |
| SV           | Saponification value                            |

Data Availability

All data, models, and code generated or used during the study appear in the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.
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