



Article The Novel Approach of Catalytic Interesterification, Hydrolysis and Transesterification of *Pongamia pinnata* Oil

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Abstract: The properties of biodiesel are completely dependent on the fatty acid profile of feedstock oils. Several feedstocks are not in use for biodiesel production because of the presence of unsuitable fatty acids in their oils. The present study was conducted to overcome this problem by the utilization of interesterification and hydrolysis processes. The present study reports biodiesel with much better cold flow properties than previous studies. Fatty acids present in Pongamia pinnata oil were optimized via interesterification and hydrolysis treatment of feedstock prior to alkali-catalyzed transesterification. The physiochemical properties of fuel were evaluated by standard test methods and the results were compared with EN 14214 and ASTM D6751 standards. Biodiesel composition was analyzed by a gas chromatographic analysis. The density, saponification and iodine values of the biodiesel derived from treated and non-treated oil were found to be within the range recommended by the international fuel standards. The acid values of biodiesel produced from non-treated and treated fractions were high (0.7–0.8 mg of KOH/g of oil), as compared to the biodiesel produced from non-treated and treated pure oil. The cloud points and pour points of biodiesel produced from hydrolyzed and interesterified oil were in the range of (8.1 to -9.6 °C) and (2.03 to -12.5 °C), respectively, while those of non-treated oil were in the range of (13.37 to -1.53 °C). These results indicate that treatments of oil specifically improved the low-temperature properties of biodiesel.

Keywords: fatty acids; derivatives; physiochemical properties; interesterification; hydrolysis; transesterification

1. Introduction

Biodiesel derived from animal fats, and vegetable oils (non-edible and edible), used in frying oils, is considered a potential renewable and carbon-neutral alternative to petroleum fuels. Biodiesel is a multi-component mixture of long-chain mono-alkyl esters of fatty acids [1–5]. The Renewable Fuel Standard system, which relates to transportation fuel, now involves the worldwide blending of biodiesel with petroleum diesel [6]. In American and European countries, biodiesel is produced on a commercial scale. Biodiesel can be used in the diesel engine directly, without demanding any modifications in the engine [7]. The global production of biodiesel is expected to exceed 23 billion liters by 2025 [8]. Generally, biodiesel is produced by the transesterification process, where triglycerides react with alcohol in the presence of a catalyst to produce fatty acid alkyl esters [9]. Methanol is the most commonly used alcohol in the transesterification process, so the produced biodiesel is generally termed as fatty acid methyl esters (FAMEs) [1,10–13].

Biodiesel properties are significantly influenced by the feedstock's characteristic fatty acid (F.A.) composition. It is commonly assumed that the typical profile of fatty acid



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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). composition remains unchanged during the derivatization of feedstocks to biodiesel via transesterification [14]. This is why the properties of biodiesel are significantly related to the fatty acid profile of feedstocks [15]. The modifications in the composition of fatty acid profile can lead to the modification of biodiesel properties to guarantee its compatibility with quality standards such as EN 14214 and ASTM D6751 [16]. This can be achieved by mixing different vegetable oils in different proportions. However, as the sources of fatty acids are variable and the fatty acid chemical composition of each source is not always completely characterized, this variability of composition clarifies uncertainty for the production planner, location, harvest times, etc., and it can influence the maturity of the seed, crop, and the composition of fatty acids [14,16].

The fatty acid differs in the number of double bonds in the carbon chain and the length of carbon chain. The fatty acids can be unsaturated or saturated. The unsaturated fatty acids contain one or more double bonds, while saturated fatty acids have only single bonds [17]. The compositional properties of FAMEs have a significant effect on biodiesel's physical and chemical qualities. Modifications in one of the compositional characteristics such as chain length, chain branching, and unsaturation typically produce both desired and undesired changes in the FAME properties. Relationships of biodiesel properties are incompatible; for instance, the compositional characteristics that are suitable for good oxidative stability lead to poor low-temperature properties [14].

Most of the FAME compositional characteristics have a contradictory impact on the fuel properties. Thus, it is impossible to specify fatty acid composition that is optimum to other main fuel qualities. The biodiesel properties that elucidate the suitability of FAME as the engine fuel include viscosity, oxidative stability, cetane number, lubricity, and cold flow (low temperature) properties. The most critical properties include the cold flow and oxidative stability of the fuel. Therefore, FAMEs compositional features have been investigated by many researchers to optimize the performance of biodiesel with respect to cold flow properties and oxidative stability [14,18].

There is an opinion that optimum FAMEs' compositional characteristics would have a low level of polyunsaturated fatty acids and a high level of monounsaturated fatty acids to minimize the oxidative instability, and a relatively low level of saturated fatty acids to minimize the problems related to cold flow properties. The palmitoleic acid (16:1) and oleic acid (18:1) provide a compromise between biodiesel properties such as cold flow properties and oxidative stability without affecting the cetane number. Various efforts are in progress in laboratories to improve the feasibility of biodiesel feedstocks via the chemical modification of fatty acids [14].

One of the methods to modify the chemical composition of the fatty acid is the chemical or enzymatic interesterification of oil or fat. In this common practice, the fatty acids are distributed among the triglycerides (TAG) under the influence of a catalyst. Fats that are interesterified have different functional physical properties and are modified for many purposes such as overall changing of the melting profile, improving plasticity by the change in the behavior of crystallization, thereby improving the compatibility of the triglycerides, and combining properties of mixed oils and fats [19]. Hydrolysis that is catalyzed by mineral acid is a highly efficient process with low energy, cost and time consumption. In the reaction of hydrolysis, triglycerides are split into carboxylic acids and free glycerol molecules. The effect of the breakage of triglycerides of pure oil and its fractions prior to esterification and transesterification has been studied in this work, as the hydrolysis process can notably reduce the viscosity of oil [20]. Many researchers have investigated the improvement in physiochemical properties of biodiesel through blending, additives, and winterization [21]. This research investigation's main goal was to optimize the fatty acids via their modification, in order to improve biodiesel's physiochemical properties (specifically low-temperature properties). In this work, we have chemically modified the chemical composition of the parent oil, as the composition of FAME is directly related to the composition of the parent oil. For this purpose, oil was treated by chemical interesterification and hydrolysis processes.

2. Materials and Methods

2.1. Materials

Pongamia pinnata seeds were collected from different areas of Faisalabad, Pakistan. Potassium hydroxide (KOH, 99.9%); sodium hydroxide (NaOH, 99%); sulphuric acid (H_2SO_4 , 95%); hydrochloric acid (HCl, 37%); methanol (99%); sodium metal (99%); Wijs solution; phenolphthalein; starch; anhydrous sodium sulphate; ethanol; and petroleum ether were of analytical grade, used as received and purchased from Merck, Hangzhou, China. Sodium methoxide was prepared in the Nano and Biomaterials lab, Department of Chemistry, University of Agriculture, Faisalabad, Pakistan.

2.2. Oil Extraction and Vacuum Fractionation

When the seeds were fully dried, their upper covering was removed, and oil was extracted by the screw press method. Oil was treated with charcoal to remove the color impurities, followed by vacuum filtration. After the purification, pure oil was subjected to fractionation. In the process of fractionation, components of the oil were separated based on their different boiling points. The digital thermometer was connected with the fractionation assembly for the measurement of temperature and vacuum pump to create the required and optimized pressure. Two fractions of oil were collected. The first fraction consisted of low molecular weight and short-chain fatty acids.

2.3. Treatments of Oil

Two treatments, interesterification and hydrolysis, were applied on pure oil, fraction of oil and residual oil.

2.3.1. Interesterification

Pure oil, fraction and the residue were subjected to the process of interesterification. The sodium methoxide (NaOCH₃) was used as a catalyst.

Preparation of Sodium Methoxide (CH₃ONa)

Sodium methoxide was prepared by the exothermic reaction of sodium metal with methanol. In the first step, methanol was distilled to remove the impurities. A total of 65 mL of distilled methanol was placed in a 300 mL flask and chilled in an ice bath. Once the methanol was cooled, 3 g of freshly cut sodium metal was added to the flask. The flask was attached to the condenser. The reaction was immediate, and it was refluxed until the sodium metal was fully dissolved into methanol. The reaction mixture was further refluxed for 15 min. The reaction mixture was heated on the hot plate to remove the excess methanol. The sodium methoxide was obtained in powder form.

Interesterification Reaction

The 100 g of pure oil, fraction, and the residue was placed in the side-arm flask, and 1% (w/w) of powdered catalyst was added. The interesterification reaction was carried out under a reduced pressure of 20 mmHg, with constant stirring at 400 rpm at 70 °C for 1 h. The reaction was interrupted by the addition of 5 mL of distilled water at 60 °C, then 5 g of sodium sulphate was added to remove the moisture contents. The oil was vacuum-filtered to remove the catalyst [22].

2.3.2. Hydrolysis

The 100 g of pure oil fraction and the residual oil were placed in the 250 mL two-neck flask. The condenser was attached to one neck, and the thermometer was inserted into the other neck of the flask. The oil was refluxed and heated on the heating mantle at 115 °C. When the temperature reached 115 °C, 4% of H₂SO₄ was added and again heated at 115 °C for 10 min. Then, it was cooled to 100 °C and 150 mL of distilled water was added. The temperature was kept at 100 °C, and the reaction mixture was also stirred continuously and

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refluxed for 2 h for the formation of the homogeneous emulsion. The obtained emulsion was washed with warm water; the washing was necessary to remove the excessive catalyst.

Esterification

The hydrolyzed pure oil fraction and the residual oil were esterified with sulphuric acid to convert the free fatty acids into the fatty acid methyl esters. The 3:1 of methanol and oil was placed in a two-neck flask, and 1% catalyst (sulphuric acid) was added. The reaction was refluxed for 1 h. After reaction completion, it was allowed to settle in the separatory funnel. Two layers were separated; the lower layer had by-products (glycerol and soap), while the upper layer had fatty acid methyl esters and unreacted free fatty acids. Then, it was washed with warm water to remove the excess methanol [23].

2.4. Production of Fatty Acid Methyl Esters (Biodiesel) Using Alkali Catalyzed Transesterification

The treated (interesterified and hydrolyzed) and non-treated pure fraction and the residual oil were converted into the fatty acid methyl esters via alkali catalyzed transesterification with methanol. Five concentration levels of KOH catalyst (0.6, 1.2, 1.8, 2.4, 3 (w/w) % of oil) and four concentration levels of methanol were selected to optimize the biodiesel yield. To carry out the transesterification reaction, the mixture of methanol, oil and the catalyst was stirred at 400 rpm at 40 °C for 2 h. When the reaction was completed, the mixture was transferred from the flask to the separatory funnel and allowed to stand under the gravity effect. Two distinct layers were observed: the upper layer consisted of fatty acid methyl esters and the lower layer consisted of glycerol and soap. Biodiesel was washed with warm water to remove the excess methanol and glycerol along with soap. The upper layer of FAME was collected into the bottles, and anhydrous sodium sulphate was added to remove the moisture contents, followed by syringe filtration. However, in the case of residue, it was centrifuged to remove the anhydrous sodium sulphate because of its high viscosity.

2.5. Determination of Physiochemical Properties of Biodiesel

The biodiesel composition was determined by a gas chromatographic analysis using Gas Chromatograph (capillary column), Biobase, Shandong Province, China, with a flow rate of 30 mL/min of nitrogen as a mobile phase using an FID detector. The density of all the biodiesel samples was determined by weighing 0.1 mL of each sample. The iodine value of the samples was determined by taking 0.01 g of the sample in a conical flask of 125 mL capacity. In the sample, 2 mL of petroleum ether and 2.5 mL of Wijs solution was added. The flask containing the reaction mixture was shaken vigorously and placed in the dark for 30 min. In the next step, 2 mL of 15% potassium iodide (K.I.) solution and 10 mL of distilled water were added to the reaction mixture. Then, it was titrated against the 0.1 N solution of sodium thiosulphate (Na₂S₂O₃·5H₂O) using starch as an indicator until the yellow color of the reaction mixture disappeared. The iodine value was calculated by using the following formula (Equation (1)).

Iodine Value =
$$\frac{(BT - ST) \times Normality \text{ of } Na_2S_2O_3 \cdot 5H_2O \times 12.69}{Weight \text{ of the sample in grams}}$$
(1)

where BT = Blank titration value; ST = Sample titration value.

The saponification value was determined by taking 0.05 g of the biodiesel sample. Then, 2 mL of 0.5 N alcoholic KOH was added and refluxed until the solution became clear, indicating reaction completion. The reaction mixture was cooled under room temperature and titrated against the 0.5N HCl solution using phenolphthalein as an indicator until the pink color of the solution disappeared. Saponification values were calculated using the following formula (Equation (2)).

Saponification Value =
$$\frac{(B-S) \times N \times 56.1}{W}$$
 (2)

where S = volume of titrant (mL) for sample; B = volume of titrant (mL) for blank; N = normality of HCl; 56.1 = molecular weight (MW) of KOH (mg/mmol); and W = sample mass (g).

The cetane number of the biodiesel samples was calculated by using the formula given in Equation (3) [24].

$$CN = \frac{46.3 + 5458}{SV} - 0.225 \times IV$$
(3)

where CN = cetane number; SV = saponification value; and IV = iodine value.

The acid value was determined by acid-base titration. A total of 0.01 g of biodiesel sample and 0.2 mL of ethanol was placed in the conical flask of 125 mL capacity. The reaction mixture was titrated against 0.01 N NaOH solution using phenolphthalein as an indicator until the appearance of a pink color. The free fatty acid percentage and the acid value were determined by using the following equations (Equations (4) and (5)) [24].

$$\% FFA = \frac{V \times N \times 28.2}{W}$$
(4)

Acid Value = $1.989 \times \%$ Free Fatty Acids (5)

where N = normality of titrant that is NaOH (mol/1000 mL); V = volume of titrant NaOH (mL); and W = mass of sample used (g).

The treated (interesterified and hydrolyzed) and non-treated pure fraction and the residual oil were converted into the fatty acid methyl esters via alkali catalyzed transesterification with sodium hydroxide (NaOH).

3. Results and Discussion

3.1. Mechanism of Interesterification, Hydrolysis and Alkali-Catalyzed Transesterification

In the interesterification reaction, acyl groups are exchanged and exchange can be within the same triglyceride or between different triglycerides. (Figure 1) shows the rearrangement of fatty acids (R_1 , R_2 , R_3) within the same triglyceride. This reaction requires a high temperature to proceed, but the use of a catalyst (CH₃ONa) can induce the reaction at a low temperature.



Figure 1. Plausible chemical interesterification reaction.

The hydrolysis of triglycerides (Figure 2) occurs in three steps. The addition of water molecule results in the formation of hydronium ion, which catalyze the breaking of covalent bonds via a nucleophilic replacement reaction. In the first step, one of the fatty acids is released from the triglyceride molecule, which results in the formation of diglyceride as a product. The diglyceride is further hydrolyzed in the next step, which results in the formation of one fatty acid and monoglyceride. Monoglyceride is hydrolyzed in the last step, which produces a molecule of fatty acid and glycerol. Overall, from the hydrolysis of one molecule of triglyceride, three fatty acids and one molecule of glycerol are produced.

The alkali-catalyzed transesterification reaction proceeds very fast. When the alkali catalyst is used in the presence of methanol, the first step of transesterification reaction is the formation of methoxide ion. The alkali (NaOH) breaks into Na⁺ and OH⁻ ions. OH⁻ captures the proton of alcohol and forms a water molecule. The second step is the attack of the methoxide ion, which is a strong nucleophile. It attacks the electrophilic carbon of the carbonyl group of

triglycerides, resulting in a tetrahedral intermediate formation. In the third step, tetrahedral carbon is removed from the intermediate and ester is produced (Figure 3).

R(OCOR) ₃ triglyceride	$\overset{H^+}{_{H_2O}}$	R(OH)(OCOR) ₂ - diglyceride	ł	RCOOH fatty acid
R(OCOR) ₂ diglyceride	$\overset{H^{+}}{{}_{H_{2}O}}$	R(OH) ₂ (OCOR) · monoglyceride	Ŧ	RCOOH fatty acid
R(OCOR) ₁ monoglyceride	H^+ H_2O	OH OH OH glycerol	÷	RCOOH fatty acid

Figure 2. Plausible hydrolysis reaction.

Step 1

$$H_3C$$
—OH + NaOH \Longrightarrow CH_3O^{-} + Na⁺ + H_2O

Step 2



Step 3



Step 4



Figure 3. Mechanism of the alkali-catalyzed transesterification reaction.

3.2. Effect of Catalyst Concentration on Biodiesel Yield

Catalyst concentration is one of the independent variables of the transesterification process. The selection of catalyst concentration is an important factor because of its significant effect on the yield of FAMEs. In this study, we optimized the catalyst concentration, and the effect of catalyst concentration on biodiesel yield (shown in Figures 4–6) was observed at five concentration levels (0.6, 1.2, 1.8, 2.4, and 3 w/w% of oil). In the case of fatty acid methyl esters produced from a non-treated pure oil and treated (interesterified and hydrolyzed) pure oil, a significant increase in the yield of FAME was observed up to certain catalyst concentrations. Beyond that concentration, biodiesel yield started to drop gradually. A possible reason could be that at high catalyst concentration, the saponification reaction was more favourable, resulting in the formation of soap and water (by-products), which caused difficulty in the separation and purification of FAMEs. Efavi et al. observed a similar effect of catalyst concentration on the yield of biodiesel [25].



Figure 4. Effect of catalyst concentration on the yield of biodiesel produced from the non-treated pure, fraction, residual oil.



Figure 5. Effect of catalyst concentration on the biodiesel yield produced from interesterified pure oil, fraction and residue.



Figure 6. Effect of catalyst concentration on the yield of biodiesel produced from hydrolyzed pure oil, fraction of oil, and residue.

In the case of FAME produced from non-treated, interesterified, and hydrolyzed fractions, the maximum yield was obtained at catalyst concentrations of 0.3%, 3%, and 1.8%, respectively. The non-treated fraction of oil produced maximum FAMEs at the lowest catalyst concentration (0.3%). The possible reason for this could be that the first fraction of oil consists of low molecular weight fatty acids and free fatty acids. Free fatty acids are quite sensitive to basic catalytic transesterification due to a higher possibility of saponification reaction instead of transesterification reaction, making purification of FAMEs difficult, and resulting in a poor yield at high concentrations. A maximum FAMEs yield from interesterified fraction was observed at the highest catalyst concentration. To be certain, the effect of a further increase in catalyst concentrations, a gradual decrease in biodiesel yield was observed. The maximum yield of FAMEs produced from non-treated, interesterified, and the hydrolyzed residue, was observed at the catalyst concentration of 1.2%, 1.2%, and 1.8%, respectively. It consists of high molecular weight triglycerides with high viscosity, and its handling and purification were difficult.

3.3. Effect of Methanol Concentration on Biodiesel Yield

Methanol concentration is one of the critical factors that influence biodiesel yield, reaction efficiency, and production cost [26]. The catalyst concentration which produced the maximum yield of biodiesel was kept constant, and the oil to methanol ratio was varied at four different levels (3, 6, 12, 24 g per 5 g of oil) to observe the effect of methanol concentration on the yield of biodiesel. The effect was studied at the restricted and excess supply of methanol. It was found that the yield of biodiesel increased with an increase in methanol concentration. The possible reason could be that as the methanol concentration increased, the reaction equilibrium favored the forward reaction, resulting in a greater production of alkyl esters [27]. The increase in the yield of biodiesel with an increase in methanol concentration was observed up to a certain level. Beyond this concentration, the biodiesel yield decreased (as shown in Figures 7–9). At a low methanol concentration, the yield was low because not enough methoxide ions were produced to break the glycerine-fatty acid linkages, and the reaction was not completed. A high methanol concentration

caused difficulty in separating methyl esters and glycerol due to the increased solubility of glycerol. When the solubility of glycerol increased, many methyl esters were lost due to the soap formation.



Figure 7. Effect of oil to methanol ratio on the yield of biodiesel produced from non-treated pure oil, fraction of oil, and residue.



Figure 8. Effect of methanol concentration on the yield of biodiesel produced from interesterified pure oil, fraction of oil, and residue.



Figure 9. Effect of methanol concentration on the yield of biodiesel produced from the hydrolyzed pure oil, fraction of oil, and residue.

3.4. Gas Chromatographic Analysis

The fatty acid profile of the prepared sample was determined through a GC analysis. Oleic acid (54.32%) was the major component in biodiesel of interesterified fraction. Linoleic acid (12.01%) was the second major component. Other components are mentioned in Table 1.

No.	Molecular Formula	Fatty Acids	Percentage Concentration (%)
1	C ₁₈ H ₃₄ O ₂	Oleic acid	54.32
2	$C_{18}H_{32}O_2$	Linoleic acid	12.01
3	$C_{16}H_{32}O_2$	Palmitic acid	9.83
4	$C_{20}H_{40}O_2$	Eicosanoic acid	7.43
5	$C_{22}H_{44}O_2$	Behenic acid	3.54
6	$C_{24}H_{48}O_2$	Lignoceric acid	3.23
7	C ₁₈ H ₃₀ O ₂	Linolenic acid	3.39
8	$C_{18}H_{36}O_2$	Stearic acid	2.95
9	$C_{14}H_{28}O_2$	Myristic acid	0.88
10	$C_{20}H_{40}O_2$	Arachidic acid	0.72
11	$C_{12}H_{24}O_2$	Lauric acid	0.64
12	$C_{10}H_{20}O_2$	Capric acid	0.25
13	C ₁₇ H ₃₄ O ₂	Margaric acid	0.12
14	C ₁₈ H ₃₄ O ₃	Ricinoleic acid	0.06
15	$C_{22}H_{42}O_2$	Erucic acid	0.02

Table 1. Fatty acid profile of biodiesel.

3.5. Physiochemical Properties of Biodiesel

3.5.1. Density

The fuel density is critical to measure the quantity of injected mass and consequently, the ratio of air-fuel and total energy contents inside the combustion chamber [16]. Density is only specified in EN 14214 standard with a range of 860–900 kg/m³ [28]; in the ASTM 6571 there is no specification for density. The degree of unsaturation and carbon chain length have a significant influence on the density of biodiesel; esters with a high chain length lead to low density, and a high degree of unsaturation results in high density [16].

Densities of biodiesel derived from treated and non-treated oil (fraction and pure oil) were in the range of recommended fuel standards. The biodiesel samples produced from the pure oil showed low densities, as compared to that of the fraction of oil. The possible reason for this could be that pure oil was the mixture of long and short-chain fatty acids, while the first fraction of oil consisted of only short-chain fatty acids.

3.5.2. Cold Flow Properties

A critical problem related to the commercial utilization of biodiesel is low-temperature properties. These properties are cloud point, pour point, and cold filter plugging point (CFPP). Cloud point (CP) is the temperature of the FAME sample at which the first visible crystal occurs when the temperature is lowered. Pour point (PP) is the lowest temperature at which fuel experiences great hindrance in flow but can flow, nonetheless. Cold filter plugging point (CFPP) is the lowest temperature at which the fuel starts to solidify and prevents the flow of fuel through the filter. The cold flow properties FAMEs (biodiesel) depend on the fatty acid profile of the parent oil. Generally, all the factors that disrupt the close packing of highly ordered molecules are influenced by cold flow properties. These factors include the degree of unsaturation, carbon chain length, and the extent of chain branching. The longer chain length results in high melting points, leading to the poorer cold flow properties. The well-organized packing of molecules is significantly disrupted by a double bond. It causes bending in the structure, and results in a low temperature of crystallization and better cold flow properties. Moreover, changes in double bond configuration have been observed: the cis configuration provides better fuel performance at low-temperatures than the trans configuration. The presence of branching molecules results in improved cold flow properties. Poor cold flow properties of FAME can cause the wax formation and molecule crystallization, which badly affect the fuel injection, pump, and flow [16]. At lower temperatures, molecules of FAME come closer and form the close packing structure, which results in the formation of crystal nucleation, leading towards the formation of wax. A further decrease in temperature results in the freezing of the whole sample. These crystals start to accumulate in the engine and significantly affect its efficiency.

The cloud point and pour point of FAME were investigated. The average value of the pour point and cloud point of biodiesel produced from non-treated and treated (interesterified and hydrolyzed) oils are presented in Table 2. The lowest cloud point and pour point value was recorded at -12.5 °C and 2.03 °C for FAME derived from the hydrolyzed fraction of oil. However, the maximum cloud point and pour point were found to be 16.03 °C and 0.68 °C, respectively, for FAME produced from non-treated residue. The treated samples showed improved low-temperature properties as compared to the non-treated samples. Treatments of oil before the transesterification process significantly improved the cold flow properties.

Table 2. Physiochemical properties of biodiesel produced from non-treated/treated pure oil, fractio	n,
and residue.	

Test Property	Non-Treated		Interesterified		Hydrolyzed				
	Pure Oil	Fraction	Residue	Pure Oil	Fraction	Residue	Pure Oil	Fraction	Residue
Density (g/mL)	0.85	0.86	1.08	0.86	0.86	0.88	0.85	0.86	0.89
Cloud point (°C)	12.55	13.37	16.03	6.7	8.1	9.58	2.96	2.03	3.63
Pour point (°C)	-1.35	-1.53	0.68	-9.6	-8.8	-2.07	-11.4	-12.5	-8.03
Saponification value (mg KOH/g)	171.41	269.63	295.43	289.2	222.9	209.8	267.3	297.9	171.85
Iodine value	50.8	73.6	71.1	63.08	62.05	119.9	67.7	64.0	103.0
Cetane number	66.71	50.54	49.1	50.92	53.61	45.37	51.75	50.15	39.4
Acid value	0.5	0.8	0.6	0.4	0.7	0.8	0.6	0.7	0.7

3.5.3. Saponification Value

The saponification value is the milligrams (mg) of potassium hydroxide (KOH) needed to break the ester bonds between glycerol and fatty acids present in one gram of fat or oil, converting them into soap and glycerol. Basically, it is the determination of the chain length and average molecular weight of all the fatty acids present in oil or fat. The long-chain fatty acids have low saponification values due to the large number of carboxylic acid groups per unit mass of the oil, compared to short-chain fatty acids [29]. Pure oil was a mixture of low and high molecular weight fatty acids. However, the fraction of oil had short-chain hydrocarbons compared to pure oil and residue, resulting in a higher saponification number of its biodiesel, as presented in Table 2.

3.5.4. Iodine Value

The iodine value is the grams of iodine consumed by double bonds present in 100 g of oil. It estimates the degree of unsaturation, and it provides useful information about the degree of unsaturation in the oil, which has a direct impact on its oxidation stability. Iodine value and carbon chain length have a positive linear relationship. The iodine value decreases with carbon chain length and increases with the degree of unsaturation. The higher the carbon chain length, the higher the iodine value [30]. Biodiesel with a low iodine value is usually an efficient and more combustible fuel. The average iodine value of biodiesel from treated and non-treated oil was in the range of recommended standards. Biodiesel produced from non-treated pure oil showed the minimum iodine value.

3.5.5. Cetane Number

The cetane number (CN) indicates the ignition quality of the fuel. When the cetane number of a fuel is high, there is less delay time between the fuel injection and the start of ignition, which promotes the smooth engine operation. It has been reported in previous studies that fuels with a higher CN emit lower CO and NOx [15,31]. The biodiesel cetane number is influenced by the three parameters, i.e., the number of double bonds, degree of unsaturation, and chain length. The high degree of unsaturation leads to low CN, and a high chain length leads to a high cetane number [32,33]. A cetane number of a minimum 51 value is recommended by European standards, while the United States recommends 47 minimum value (Table 2). The cetane number of biodiesels produced from residue and non-treated pure oil was beyond the limits of recommended fuel standards. However, the cetane number of biodiesels produced from non-treated pure oil and fraction was found to be in the range of standards (Table 2).

3.5.6. Acid Value

The acid value or acid number is the measure of the mass (mg) of potassium hydroxide that is needed to neutralize the free fatty acids present in 1 g of the sample. The acid number of biodiesel should be less to ensure that no unreacted free fatty acids or processing acids are present in the fuel. This property of biodiesel is also used to monitor its stability [34]. A high level of free fatty acids affects biodiesel stability, and it can lead to corrosion and deposits in the fuel engine. The average acid values of FAME produced from treated and non-treated pure oil were in the limits of biodiesel standards. These results were analogous to the results reported in the literature [35].

4. Conclusions

Based on the collected observations, the following conclusions can be drawn. The independent variables of the transesterification reaction, catalyst and methanol concentrations must be optimized to obtain a better yield of biodiesel. Most of the fuel properties of non-treated oil and treated oil meet the standard limits, indicating its suitability as a fuel. Interesterification and hydrolysis of feedstock prior to the transesterification reaction improves the cloud point, pour point, and acid value of biodiesel. The overall results show

that modifications in the fatty acids composition of the oil can induce changes in the final product (biodiesel).

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