Production and Evaluation of Fractionated Tamarind Seed Oil Methyl Esters as a New Source of Biodiesel

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Abstract: Biodiesel has attracted considerable interest as an alternative biofuel due to its many advantages over conventional petroleum diesel such as inherent lubricity, low toxicity, renewable raw materials, biodegradability, superior flash point, and low carbon footprint. However, high production costs, poor low temperature operability, variability of fuel quality from different feedstocks, and low storage stability negatively impact more widespread adoption. In order to reduce production costs, inexpensive inedible oilseed alternatives are needed for biodiesel production. This study utilized inedible tamarind (Tamarind indica) seed oil as an alternative biodiesel feedstock, which contained linoleic (31.8%), oleic (17.1%), and lauric (12.0%) acids as the primary fatty acids. A simple and cost-effective high vacuum fractional distillation (HVFD) methodology was used to separate the oil into three fractions (F1, F2, and F3). Subsequent transesterification utilizing basic, acidic, and enzymatic catalysis produced biodiesel of consistent quality and overcame the problem of low temperature biodiesel performance. The most desirable biodiesel with regard to low temperature operability was produced from fractions F2 and F3, which were enriched in unsaturated fatty acids relative to tamarind seed oil. Other properties such as density and cetane number were within the limits specified in the American and European biodiesel standards.

Keywords: tamarind; non-edible; fractionation; transesterification; biodiesel; fatty acid methyl esters

1. Introduction

The belief that biofuels can reduce dependence on petroleum fuels and mitigate climate change has prompted many to encourage their production and use as alternatives to fossil fuels such as conventional petroleum diesel fuel (petrodiesel) and gasoline [1]. Biofuels play an important role in the energy market, as they have combustion properties similar to those of fossil fuels. Moreover, biodiesel is generally superior to petrodiesel with regard to cetane number (CN), lubricity, flash point, biodegradability, renewability, and carbon footprint [2]. However, technical disadvantages of biodiesel relative to petrodiesel include inferior low temperature properties, high production costs, hydrolytic stability, and storage stability [3,4].
Biodiesel contains a wide variety of fatty acid methyl esters (FAME) whose proportions are dependent on the feedstock from which they were prepared. The physical and chemical properties of biodiesel in turn depend on FAME composition [3,4]. Biodiesel can be synthesized from various feedstocks, such as animal fats, vegetable oils, industrial and municipal wastes, and insects [4,5]. Among these feedstocks there are large differences in fatty acid profile. As a result, the chemical profile and physical properties of biodiesel vary depending on the feedstock from which it was prepared, which can have a significant impact on engine performance and fuel properties [3,4,6].

High production costs are a major obstacle to expansion of biodiesel. At present, biodiesel is commercially produced mostly from food-grade oils such as soybean (in US) and rapeseed oils (in Europe). The final cost of biodiesel depends mainly on the cost of the raw material. The high price of vegetable oils increases the cost of biodiesel and renders it economically uncompetitive with petroleum diesel. Unfortunately, the economic situation of biodiesel has worsened due to the high prices of vegetable oils in recent years. In the mid-1990s, the feedstock cost accounted for 60 to 75% of the total cost of biodiesel, but now 85% of the production cost is attributed to feedstock [7–10]. Studies have been carried out to examine the dependence of cost of producing biodiesel on the cost of raw materials [8]. These studies showed a linear relationship between the two, with a change of US $0.020/L in the product cost per US $0.022/kg change in oil cost. This means that the relationship between the biodiesel mass output and feedstock mass input is about a 1:1 ratio. In order for biodiesel to become an economically viable fuel and increase its commercialization, its production costs need to be reduced. Profitable and inexpensive biodiesel can be made from cheaper inedible vegetable oil feedstocks (palm oil, Jatropha oil, and Karanja seed oil, for example) [11–15]. However, the non-edible oils used today are not capable of providing enough oil for large scale biodiesel production to replace petroleum fuels completely. Only small quantities of biodiesel are produced from non-edible sources, which are typically used for blending with petrodiesel in low (≤20%) proportions [16]. Therefore, other non-edible oil-bearing crops are required for biodiesel production at commercial scale. For biodiesel to be economically viable, it is necessary to lower feedstock costs. The use of non-edible vegetable oils as raw materials for biodiesel lowers the cost of biodiesel production [7].

A drawback to biodiesel is the relationship between cold flow properties and saturation level of biodiesel. Saturated components (e.g., stearic acid, \(C_{18:0}\), palmitic acid, \(C_{16:0}\), myristic acid, \(C_{14:0}\)) have high CN and are less susceptible to oxidation than unsaturated components (e.g., oleic acid, \(C_{18:1}\), linoleic acid, \(C_{18:2}\), linolenic acid, \(C_{18:3}\)) but crystalize at much higher temperatures [3,4]. For this reason, most biodiesel fuels develop flow problems when ambient temperatures approach 0–2 °C. When the temperature drops to this level, the high melting point saturated FAME nucleate and form large crystal agglomerations that clog fuel filters and lines, which ultimately leads to engine failure. In contrast, petrodiesel develops flow problems at a much lower temperature range, such as −11 °C to −20 °C [17]. For instance, biodiesel produced from Karanja seed oil showed the following fuel properties: acid value (AV; 0.5 mg KOH/g), cloud point (CP; 19 °C), pour point (PP; 15 °C), and density (0.88 g/mL) [15]. In another study, coconut oil biodiesel showed a CP of 0.3 °C [18]. Biodiesel obtained from castor oil had a CP of 10 °C [19]. CP and PP of Jojoba oil biodiesel were found to be 5 °C and 8 °C, respectively [20]. In another study, neem seed oil biodiesel displayed a CP of 26 °C and a PP of 16 °C [21]. Research has shown that biodiesel fuels have much higher CP and PP values compared to petroleum fuels, which is problematic when biodiesel is used in cold climates. Various approaches have been taken to improve the low temperature flow properties of biodiesel, including blending with crude oil, the use of additives, and physical or chemical modification of biodiesel. Blending with gasoline only works with low proportions of biodiesel (up to 30% by volume) with CP around 14 °F [22].

The current study is aimed at the removal of saturated components from the feedstock oil that deteriorates biodiesel cold flow properties. As discussed above, the fuel properties...
of biodiesel are directly related to the type and amount of different fatty acids present in the feedstock. Hence, biodiesel quality can be improved by altering the fatty acid composition of the feedstock oil [3]. The alternative approach adopted in the present study includes the fractionation of tamarind seed oil (TSO) followed by transesterification to produce biodiesel. *Tamarindus indica* L. (Tamarind), belonging to the Fabaceae (Leguminosae) family, is a fruit species that grows mainly in Asian countries. Seeds comprise about 40 wt.% of the fruit, which is a by-product of the tamarind pulp industry and represents an under-utilized material. The composition of tamarind seed is 12–20% fiber, 50–55% carbohydrates, 20% tannins, and 4.5–16.2% oil. Coloring compounds (tannins and others toxic chemicals) present in seeds render them unsuitable for human consumption. Only a small portion of the seed is used in the paper and textile industries, as a sizing material, in the form of tamarind kernel powder. Previous studies have investigated the emission characteristics of tamarind seed oil biodiesel and its blends [23–25]. This non-edible seed oil thus represents a possible and potential alternative feedstock for biodiesel production. In this study, TSO was separated into various fractions using high vacuum fractional distillation (HVFD) to obtain, after subsequent transesterification, biodiesel with improved low temperature performance relative to biodiesel from unfraccionated TSO.

2. Materials and Methods

2.1. Materials

Tamarind seeds were purchased from a local market in Faisalabad, Pakistan. All chemicals and reagents, including methanol, ethanol, potassium hydroxide (KOH), sodium sulphate (Na$_2$SO$_4$; anhydrous), potassium iodide (KI), lipase, hydrochloric acid (HCl), potassium hydrogen phosphate (KH$_2$PO$_4$), sulfuric acid (H$_2$SO$_4$), petroleum ether, sodium alginate, and sodium thiosulphate (Na$_2$S$_2$O$_3$), were purchased from Merck (Germany) and used as received.

2.2. Extraction of Oil

Tamarind seeds were dried in the sun to remove moisture. The oil was extracted in a Soxhlet extractor using *n*-hexane at a temperature of 60 °C for 8 h. The extracted oil was then obtained by removal of *n*-hexane using a rotary vacuum evaporator.

2.3. High Vacuum Fractionation of Tamarind Seed Oil

High vacuum fractional distillation (HVFD) was performed to separate TSO into fractions based on boiling point. The distillation apparatus contained an electric heater (heating range: 30 °C to 370 °C), round-bottomed flask (0.5 L), condenser, vacuum pump (oil capacity: 0.25 L, Model TW-1A), and a cow-shaped distillation receiver (containing four 50 mL flasks) (Figure 1). TSO was fractionated under a vacuum of −760 mm Hg at different temperatures that were measured using a digital thermometer probe. Three fractions (F1, F2, and F3) were obtained at boiling ranges of 147–220 °C, 204–268 °C, and 260–290 °C, respectively. Presented in Table 1 are the different fractions obtained after fractionation of 1000 g of TSO.

### Table 1. Results of fractionation of tamarind seed oil (TSO) into fractions by HVFD based on boiling point range.

| Fraction Number | Boiling Range (°C) | wt. (g) | % of Total TSO |
|-----------------|-------------------|--------|---------------|
| F1              | 147–210           | 215.87 | 21.6          |
| F2              | 230–268           | 280.09 | 28.0          |
| F3              | 260–290           | 330.12 | 33.0          |
| Residual oil    |                   | 173.92 | 17.4          |
Figure 1. Schematic illustration of High Vacuum Fractional Distillation (HVFD) apparatus for tamarind seed oil.

2.4. Transesterification

Unfractionated TSO and its various fractions (F1, F2, and F3) were subjected to acid, base, and enzyme-catalyzed transesterification for the production of biodiesel. Base-catalyzed reactions were performed using KOH at five catalyst concentrations (0.2, 0.4, 0.6, 0.8, and 1 wt. % of oil). The different catalyst concentrations were mixed with oil (10 g) and methanol (4 g) and the mixture was stirred for 2 h at 60 °C. In acid catalyzed transesterification, HCl at 20, 40, 60, 80, and 100% (w/w oil) was mixed with oil, dissolved in methanol, and stirred for 5 h at 60 °C. The molar ratio of methanol to oil was 6:1. In the case of enzyme-catalyzed reactions, lipase immobilized on calcium alginate beads at concentrations of 1, 2, 3, 4, and 5% (w/w of oil) was mixed with methanol and oil and reacted at 35 °C for 12 h. The molar ratio of methanol to oil was 5:1. After reaction completion, the mixture was placed in a separatory funnel overnight for the separation of biodiesel from glycerol. The biodiesel phase was then washed with warm distilled water to remove excess methanol, unreacted catalyst, and soap. Finally, the washed product was distilled under vacuum for the removal of moisture. Biodiesel yield was determined using the following formula presented in Equation (1).

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\text{Yield of biodiesel (\%) =} \frac{\text{mass of biodiesel obtained (g)}}{\text{mass of oil used (g)}} \times 100
\]  

(1)

2.5. Determination of Fuel Properties

Gas-chromatographic mass spectrometric (GC-MS) analysis was conducted to quantify FAME content. Analyses were performed using a Perkin-Elmer Clarus 500 model GC-MS equipped with a capillary column (HP-1, 30 m × 0.25 mm × 0.25 µm), coupled to a Perkin-Elmer Clarus 500C MS. The sample injection tool was placed at an oven temperature of 50 °C and was held at that temperature for one minute. The oven temperature was then increased to 325 °C at a heating rate of 10 °C/min and held for 2 min. Helium (He, 99.99% pure) with a constant flow rate of 1.2 mL/min was used as a carrier gas. Unknown compounds were identified by comparing GC-MS peaks of compounds with mass spectra libraries from NIST (Wiley, 9th edition).

The acid value (AV) of biodiesel was determined following the acid-base titration technique (ASTM, 2003) [26]. Density (g/mL) was determined by measuring the mass of 1.0 mL of each sample at 25 °C [27]. To determine saponification value (SV) of biodiesel, 1.0 g sample was added to a conical flask along with 25 mL of ethanolic KOH. The flask contents were refluxed for 0.5 h to obtain a clear solution. Two drops of indicator (phenolphthalein) were added to the mixture after cooling to room temperature. The mixture
was then titrated against 0.5 N H$_2$SO$_4$. The same procedure was followed for the blank. SV was determined by the following formula (Equation (2)) [28].

$$\text{Saponification Value} = \frac{(V_b - V_s) \times 28.05}{W}$$  \hspace{1cm} (2)

where $V_b$ = vol. of titrant for blank, $V_s$ = Volume of titrant for sample, $W$ = wt. of sample (g). To determine iodine value (IV), biodiesel (0.2 g) was mixed with 10 mL of chloroform and 25 mL Wijs solution in a 250 mL glass-stoppered iodine flask. The flask contents were shaken vigorously and kept in the dark for 0.5 h. Then, 10 mL of KI (15%) was added. The flask contents were then titrated with Na$_2$S$_2$O$_3$ solution (0.1 N) until the yellow iodine color disappeared. IV was calculated as follows (Equation (3)) [29].

$$\text{Iodine Value} = \frac{(V_b - V_s) \times 1.269}{W}$$ \hspace{1cm} (3)

where $V_b$ and $V_s$ are volumes of titrant for blank and sample, respectively and $W$ is the weight of the sample (g).

Cetane number (CN) of the biodiesel samples was determined following the method described by Krisnangkura [30] and Bhatti et al. [31]. Cloud point (CP) and pour point (PP) were measured following EN 2305 and ASTM D2500, respectively [32].

3. Results and Discussion

3.1. Effect of Catalysts on Biodiesel Yield (%)

The effect of various catalysts on biodiesel yield is shown in Figure 2. Base-catalyzed transesterification has many advantages, including the low cost of catalyst compared to acid and enzyme catalysts, easy availability in the market, mild reaction conditions, and faster reaction times relative to enzyme and acid catalyzed reactions. However, a serious drawback of base-catalyzed reactions is that the base catalysts (in higher concentrations) react with the free fatty acids (FFAs) found in the feedstock oil to produce soap as a by-product. Excess soap formation inhibits the separation of glycerol and biodiesel, and in turn reduces biodiesel yield [33]. In the current study, biodiesel yield increased to a maximum and then decreased as the catalyst load increased. This decrease in yield was due to soap and gel formation at higher catalyst concentrations. Optimum biodiesel yields of 86% and 89% were observed for pure TSO and fraction F3, respectively, at a KOH concentration of 0.6%. Yields then dropped to 77% and 81%, respectively, as the catalyst concentration was increased to 1.0%. Fraction F1 showed optimum yield (79%) at a catalyst concentration of 0.8%. However, F2 (71%) and residual oil (52%) provided maximum biodiesel yields at 0.4% KOH (Figure 2a). A similar trend was noted in another study where biodiesel was produced from waste soybean oil [34].

The acid-catalyzed reaction was slower than base-catalyzed transesterification. It was completed in 4.5 h compared to 1.5 h by base-catalyzed reaction. High alcohol to oil molar ratio (6:1) and a reaction temperature of 90 °C was used to accelerate this reaction. Although the reaction rate was slow, the biodiesel yield was higher with acid catalysis compared to base and enzyme catalyzed reactions. This was due to the fact that the acid catalyst was insensitive to FFA content in the oil, which eliminated side reactions. Acid catalysts carry out esterification and transesterification simultaneously, which reduces FFA content and increases biodiesel yield [33]. Five concentrations of HCl were used for acid-catalyzed reactions to optimize biodiesel yield (Figure 2b). As observed with base-catalyzed transesterification, biodiesel yield increased with increasing catalyst concentration and then decreased. The highest yield of biodiesel produced from TSO (97%), F3 (92%), and residual oil (95%) was obtained with 40% HCl. Meanwhile, F1 (93%) and F2 (87%) provided maximum yield at a catalyst concentration of 60%. Further increases in HCl concentration resulted in reduced biodiesel yield. For instance, the yield of biodiesel from TSO decreased from 97% to 81% as the catalyst concentrated increased from 40% to 100%. Similar observations were previously reported for the production of biodiesel from rubber seed oil [35]. The lower yields obtained via base catalysis relative to acid catalysis were
likely the result of free fatty acids (FFA) within the starting oils that partially deactivated KOH and reduced the yield of FAME [4].

Figure 2. Cont.
Five concentrations of lipase were investigated for enzyme-catalyzed transesterification (Figure 2c). The lipase was immobilized to achieve several advantages, such as cost-effectiveness, availability of more active sites to speed up the chemical reaction, and ease of separation after the reaction was complete. TSO and F1 provided optimal yields of 90% and 85%, respectively, at a catalyst concentration of 2% whereas F2 (87%), F3 (77%) and residual oil (88%) gave maximum yields at 3% lipase. Biodiesel yield initially increased with increasing lipase concentration but decreased at higher concentrations due to adsorption phenomenon caused by calcium alginate granules. The maximum biodiesel yield observed for the base, acid, and lipase-catalyzed reactions was 89, 97, and 90%, respectively. These results clearly indicate that HCl provided the highest yield of biodiesel from TSO and its fractions.

3.2. Gas Chromatographic-Mass Spectroscopy (GC-MS) Analysis

Major fatty acids identified by GC-MS in TSO were linoleic (31.8%) and oleic (17.6%) acids (Table 2). In addition, caprylic, capric, lauric, myristic, palmitic, stearic, arachidic, behenic, lignoceric, vaccenic, and gondoic acids were found in smaller quantities. Among these, lauric (12.0%), vaccenic (9.4%), and palmitic (8.9%) acids were the most abundant. The most prevalent fatty acids detected in F1 were lignoceric (22.7%), stearic (20.2%), oleic (17.2%), and palmitic (11.9%) acids. Oleic acid (58%) was the most dominant fatty acid found in F2, with linoleic (17.1%) and caprylic (12.4%) acids also identified in significant quantities. Fraction F3 was rich in linoleic (65.8%) and oleic (15.2%) acids. The most significant fatty acids identified in the residual oil were capric (22.0%), stearic (25.9%), lignoceric (16.1%), and palmitic (13.2%) acids. In general, the lower boiling fractions were enriched in shorter-chain fatty acids relative to the higher boiling fractions, which contained higher percentages of higher boiling longer-chain fatty acids.
Table 2. Fatty acid profile of tamarind seed oil (TSO) and its various fractions.

| Fatty Acid     | Percentage Composition (%) | F1   | F2   | F3   | TSO  | Residual Oil |
|----------------|----------------------------|------|------|------|------|--------------|
| Caprylic acid  |                            | 13.1 | 12.4 | -    | 6.8  | -            |
| Capric acid    |                            | 2.5  | 2.4  | 4.2  | 1.4  | 22.0         |
| Lauric acid    |                            | -    | -    | -    | 12.0 | -            |
| Myristic acid  |                            | 3.8  | 0    | 5.4  | 6.5  | -            |
| Palmitic acid  |                            | 11.9 | 0    | 1.9  | 8.9  | 13.2         |
| Stearic acid   |                            | 20.2 | 4.7  | 3.4  | 1.3  | 25.9         |
| Arachidic acid |                            | -    | -    | -    | 1.0  | 15.9         |
| Behenic acid   |                            | -    | 0.8  | 1.0  | 1.3  | -            |
| Lignoceric acid|                            | 22.7 | 3.5  | 2.3  | 1.6  | 16.1         |
| Oleic acid     |                            | 17.2 | 58.2 | 15.2 | 17.1 | 3.8          |
| Linoleic acid  |                            | 5.1  | 17.1 | 65.1 | 31.8 | 2.9          |
| Vaccenic acid  |                            | 1.2  | 0.8  | 7.1  | 9.4  | -            |
| Gondoic acid   |                            | 0.5  | -    | -    | 1.0  | -            |

3.3. Assessment of Fuel Quality Parameters

Acid values of TSO, F1, F2, and F3, and the residual oil were 0.56, 0.61, 0.56, 0.50, and 4.51 mg KOH/g, respectively. These results indicated the presence of a small amount of free fatty acids (FFA) in TSO, F1, F2, and F3, which may have negatively impacted base-catalyzed transesterification, as mentioned previously. The much higher AV of the residual oil was likely the result of exposure to high temperatures for an extended period of time during HVFD that promoted thermal hydrolysis.

Density is an important physical property of biofuels, as it influences the air-fuel ratio and energy content within the combustion chamber. A change in fuel density can thus affect engine output power. The density of biodiesel is affected by the chemical composition of FAME and is generally higher than that for petrodiesel [36]. The density at 25 °C of biodiesel obtained from the various fractions ranged from 0.81 to 0.85 kg/L for F1, 0.84–0.88 kg/L for F2, 0.84–0.89 kg/L for F3, and 0.82–0.86 kg/L from TSO. The European biodiesel standard (EN 14214) specifies that density (15 °C) must be within the range of 0.86–0.90 kg/m³ whereas the American biodiesel standard (ASTM D6751) does not contain limits for density. In a previous study, the density of pongamia seed oil biodiesel was 0.86 kg/L [37]. In another study, the density of sunflower seed oil biodiesel was 0.877 kg/L [21,38]. Lastly, the densities of biodiesel produced from chicken fat and waste tallow were 0.867 and 0.856 kg/L, respectively [31]. Thus, the values obtained in the present study were similar to those reported for biodiesel fuels prepared from other feedstocks and, with the exception of F1, were within the range specified in EN 14214. The comparatively low density of F1 was likely due to the presence of a significant amount of methyl lignocerate (22.7%) and low amounts of unsaturated FAME relative to the other fractions (Table 2). Previous studies have established that density decreases with increasing chain length as well as with increasing levels of saturation [39].

The SV is a measure of the average molecular weight (MW; i.e., chain length) of the FAME present within a sample. Higher SV indicates lower average chain length and thus lower average MW of the FAME that comprise the sample. In the present study, the SV of TSO biodiesel ranged from 192–210 mg KOH/g (Table 3). In previous studies the SV of pongamia seed oil biodiesel was 187 mg KOH/g [40] and the value for palm biodiesel was 201 mg KOH/g [41]. The SV of biodiesel produced from TSO and the various fractions are listed in Table 4. As expected, the values obtained for biodiesel produced from F3 were lower than SV obtained for F2 and TSO due to the lower percentage of shorter-chain FAME identified in F3. The low values for F1 were attributed to the presence of relatively high amounts of longer-chain FAME such as methyl lignocerate (22.7%).
Table 3. Saponification values of biodiesel produced from TSO and fractions.

| Catalyst | Conc. of Catalyst | Saponification Values (mg KOH/g) |
|----------|------------------|----------------------------------|
| KOH      |                  | F1  | F2  | F3  | TSO |
|          | 0.2              | 171.0 | 186.1 | 179.2 | 195 |
|          | 0.4              | 168.3 | 187.2 | 177.3 | 196 |
|          | 0.6              | 159.0 | 190.2 | 179.2 | 210 |
|          | 0.8              | 173.9 | 186.5 | 179.2 | 193 |
|          | 1.0              | 169.2 | 190.2 | 176.0 | 193 |
|          | 20               | 172.3 | 195.2 | 175.8 | 201 |
|          | 40               | 165.3 | 192.6 | 177.2 | 199 |
|          | 60               | 168.9 | 191.4 | 180.8 | 194 |
|          | 80               | 173.9 | 195.0 | 175.8 | 206 |
|          | 100              | 169.2 | 197.1 | 183.0 | 195 |
|          | HCl              | 20   | 172.3 | 196.8 | 180.0 | 193 |
|          |                  | 40   | 165.3 | 192.6 | 177.2 | 199 |
|          |                  | 60   | 168.9 | 191.4 | 180.8 | 194 |
|          |                  | 80   | 173.9 | 195.0 | 175.8 | 206 |
|          |                  | 100  | 169.2 | 197.1 | 183.0 | 195 |
|          | Lipase           | 1    | 172.3 | 196.8 | 180.0 | 193 |
|          |                  | 2    | 171.0 | 196.8 | 185.2 | 192 |
|          |                  | 3    | 168.3 | 199.5 | 176.0 | 201 |
|          |                  | 4    | 169.0 | 197.1 | 185.8 | 207 |
|          |                  | 5    | 173.9 | 197.1 | 177.2 | 199 |

Table 4. IV of biodiesel produced from TSO and fractions.

| Catalyst | Conc. of Catalyst | Iodine Values (mg KOH/g) |
|----------|------------------|--------------------------|
| KOH      |                  | F1  | F2  | F3  | TSO |
|          | 0.2              | 32.5 | 43.21 | 55.21 | 26.12 |
|          | 0.4              | 23.10 | 45.72 | 59.20 | 27.26 |
|          | 0.6              | 23.74 | 39.72 | 52.90 | 32.06 |
|          | 0.8              | 25.10 | 37.54 | 57.32 | 31.02 |
|          | 1.0              | 22.99 | 39.21 | 52.20 | 26.09 |
|          | 20               | 23.14 | 37.21 | 48.20 | 28.09 |
|          | 40               | 22.99 | 46.21 | 49.41 | 28.09 |
|          | 60               | 24.57 | 38.45 | 50.21 | 32.12 |
|          | 80               | 25.66 | 49.90 | 53.90 | 27.18 |
|          | 100              | 22.10 | 38.45 | 51.20 | 31.02 |
|          | HCl              | 1    | 25.10 | 45.91 | 48.21 | 30.16 |
|          |                  | 2    | 23.14 | 39.90 | 49.01 | 29.12 |
|          |                  | 3    | 26.11 | 43.78 | 54.21 | 27.19 |
|          |                  | 4    | 22.99 | 45.12 | 49.21 | 30.16 |
|          |                  | 5    | 22.99 | 36.21 | 51.01 | 28.09 |

The IV of biodiesel prepared from TSO and its fractions are shown in Table 4. The maximum IV for biodiesel prepared from TSO was 32.1 mg KOH/g. The upper limit for IV specified in EN 14214 is 120 mg KOH/g. Limits for IV are not included in ASTM D6751. In this study, the maximum IV (59.2 mg KOH/g) was observed for biodiesel prepared from F3, followed by F2 (49.9 mg KOH/g) and F1 (32.5 mg KOH/g). The IV is a measure of unsaturation, with higher unsaturation leading to higher IV and vice versa. Thus, biodiesel prepared from F3 gave the highest IV because it contained the highest percentage of polyunsaturated FAME (65.1% methyl linoleate; Table 2). Unsaturation is to some extent necessary for the fuel to prevent it from solidifying at low temperatures. Saturation negatively affects the CP and PP of biodiesel due to the high melting points of saturated FAME. Higher levels of unsaturation in biodiesel give lower CP but higher IV. However, high unsaturation may lead to engine deposits from polymerization by breaking the weak \( \pi \)-bond or by epoxide formation by addition of oxygen to the double bond at high engine temperatures [42].

The low temperature performance of a fuel is evaluated by determination of CP and PP. When biodiesel begins to solidify at low temperatures it will form small crystals that
start clumping together. CP is the temperature at which the crystals within biodiesel first become visible. PP is the temperature at which biodiesel becomes solid and can no longer flow or pour [17]. Generally, the CP and PP of biodiesel are higher than petrodiesel (Dunn, 2015; Moser, 2009). The CP and PP of TSO-based biodiesel are reported in Table 5 and ranged from −0.6 to 1.5 °C and −0.7 to −2.5 °C, respectively. In contrast, the CP of palm oil biodiesel was 13 °C in a previous study [43]. In another study, a CP of 12 °C was reported for Nigerian mango seed biodiesel [16]. Lastly, a PP of 6 °C was observed for Karanja seed biodiesel [23]. In the current study, biodiesel produced from F2 (−3.9 °C, −6.7 °C) and F3 (−4.2 °C, −8.1 °C) displayed lower minimum CP and PP than biodiesel from TSO. Fraction F1 gave higher CP (15.2 °C) and PP (6.8 °C) than TSO-based biodiesel due likely to its comparatively high content of methyl palmitate, methyl stearate, and methyl lignocerate (Table 2) relative to TSO. The low CP and PP observed for F2 and F3 biodiesel indicate that it can be used in the colder climates. However, biodiesel produced from TSO and F1 should be used in warmer climates.

Table 5. Cloud point (CP, °C) and pour point (PP, °C) of biodiesel produced from TSO and fractions.

| Catalyst | Conc. of Catalyst (%) | F1 CP | F1 PP | F2 CP | F2 PP | F3 CP | F3 PP | Residual Oil CP | Residual Oil PP | TSO CP | TSO PP |
|----------|-----------------------|-------|-------|-------|-------|-------|-------|----------------|----------------|--------|--------|
| KOH      | 0.2                   | 11.6  | 2.7   | −2.9  | −6.1  | −3.9  | −6.1  | Room temperature | 1.5  | −1.2  |
|          | 0.4                   | 12.1  | 5.1   | −2.3  | −6.4  | −3.2  | −6.1  | Room temperature | 1.2  | −1.2  |
|          | 0.6                   | 11.8  | 2.5   | −3.2  | −6.1  | −3.1  | −5.2  | Room temperature | 1.5  | −0.3  |
|          | 0.8                   | 13.1  | 2.5   | −3.1  | −6.7  | −2.9  | −5.9  | Room temperature | 1.4  | −0.3  |
|          | 1.0                   | 14.9  | 3.9   | −3.9  | −5.9  | −4.2  | −7.1  | Room temperature | 1.4  | −0.4  |
| HCl      | 20                    | 12.7  | 5.3   | −2.5  | −6.9  | −2.1  | −8.1  | Room temperature | −0.6 | −2.5  |
|          | 40                    | 12.1  | 4.9   | −2.0  | −6.2  | −2.9  | −7.3  | Room temperature | 0.7  | −2.3  |
|          | 60                    | 13.3  | 6.6   | −2.7  | −6.2  | −2.2  | −6.9  | Room temperature | 1.6  | −2.2  |
|          | 80                    | 15.2  | 5.1   | −3.1  | −6.5  | −3.2  | −6.5  | Room temperature | 0.3  | −0.7  |
|          | 100                   | 12.2  | 6.2   | −2.9  | −5.9  | −3.3  | −6.9  | Room temperature | 0.1  | −0.4  |
| Lipase   | 1                     | 15.1  | 5.1   | −3.8  | −5.6  | −3.5  | −6.1  | Room temperature | 1.5  | −1.2  |
|          | 2                     | 13.3  | 5.5   | −3.5  | −6.1  | −3.5  | −6.1  | Room temperature | 1.4  | −2.2  |
|          | 3                     | 14.9  | 4.8   | −2.9  | −4.4  | −3.3  | −5.6  | Room temperature | 1.2  | −2.5  |
|          | 4                     | 12.4  | 6.1   | −2.3  | −6.1  | −2.2  | −6.1  | Room temperature | 0.6  | −2.1  |
|          | 5                     | 13.1  | 5.9   | −3.9  | −6.3  | −2.8  | −5.8  | Room temperature | 0.2  | −1.9  |

Cetane number is one of the primary indicators of ignition quality and is related to the injection delay time a fuel experiences when injected into the combustion chamber of diesel engines [44]. Cetane numbers (CN) of biodiesel produced from TSO and its various fractions are shown in Table 6. The minimum CN specified in EN 14214 and ASTM D6751 are 51 and 47, respectively. In the present study, the CN of TSO-based biodiesel was in the range of 65–68. In another study the CN of pongamia seed oil biodiesel was reported as 47.1 [40]. In the current study, the CN of all samples were above the minimum requirements of the European and American biodiesel standards. The CN of TSO biodiesel was higher than that of other biodiesel fuels including sunflower and soybean biodiesels due to its comparatively low percentage of polyunsaturated FAME. Previous studies have shown that branching, chain length, and degree of unsaturation affect CN [3,4]. Thus, the higher CN of TSO biodiesel compared to petrodiesel and conventional commodity biodiesels suggests a shorter ignition delay time and hence superior ignition quality for TSO biodiesel.
Table 6. Cetane number of biodiesel produced from TSO and fractions.

| Catalyst | Conc. of Catalyst | F1 Cetane Number | F2 Cetane Number | F3 Cetane Number | TSO Cetane Number |
|----------|------------------|------------------|------------------|------------------|-------------------|
| KOH      | 0.2              | 70.9             | 65.9             | 64.3             | 68.41             |
|          | 0.4              | 73.5             | 67.4             | 63.8             | 68.01             |
|          | 0.6              | 75.4             | 66.9             | 64.8             | 65.07             |
|          | 0.8              | 72.0             | 66.0             | 63.8             | 67.60             |
|          | 1.0              | 73.5             | 66.2             | 65.4             | 68.70             |
| HCl      | 20               | 72.7             | 65.8             | 66.5             | 67.13             |
|          | 40               | 74.1             | 64.2             | 65.1             | 67.40             |
|          | 60               | 73.0             | 64.4             | 65.1             | 67.20             |
|          | 80               | 71.9             | 63.0             | 65.2             | 66.07             |
|          | 100              | 73.5             | 65.3             | 64.6             | 67.31             |
| Lipase   | 1                | 72.3             | 63.7             | 65.4             | 66.66             |
|          | 2                | 73.0             | 66.0             | 64.7             | 68.10             |
|          | 3                | 72.8             | 63.8             | 65.1             | 67.33             |
|          | 4                | 73.4             | 63.8             | 65.6             | 65.88             |
|          | 5                | 72.5             | 65.1             | 65.6             | 67.40             |

4. Conclusions

This study demonstrated that TSO is a promising new alternative, low-cost, non-food feedstock for production of biodiesel that does not compete with food production. The major fatty acids identified in TSO were linoleic (31.8%) and oleic (17.6%) acids. High vacuum fractional distillation was employed to separate TSO into three fractions based on boiling point. The lowest boiling fraction (F1) contained predominantly lignoceric (22.7%) and stearic (20.2%) acids, whereas the middle fraction (F2) was dominated by oleic acid (58.2%) and the highest boiling fraction (F3) contained mostly linoleic acid (65.1%). The resulting oils were subjected to KOH, HCl, and lipase-catalyzed transesterification to afford the corresponding FAME. The highest biodiesel yield (97%) was achieved with acid catalysis, followed by lipase (90%) and base (89%). Biodiesel prepared from TSO as well as from F1, F2, and F3 yielded CN, IV, SV, and densities that were within the ranges specified in the American (ASTM D6751) and European (EN 14214) biodiesel standards. In addition, biodiesels prepared from F2 and F3 yielded the lowest CP and PP values, thus rendering them more attractive for use in cold climates. Biodiesels prepared from F1 and directly from TSO would thus be more appropriate for warmer climates due to their higher CP and PP values, which was attributed to their higher content of saturated FAME such as stearic, palmitic, and myristic acids.

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