Comparative analysis of various waste cooking oils for esterification and transesterification processes to produce biodiesel

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Abstract
This study is focused on the production of biodiesel from waste cooking oils to turn the waste into energy by biological means. Physicochemical analysis of the oil samples was performed by measuring their density, acid value and saponification value for evaluating their efficacy towards biodiesel synthesis. Metal catalyzed glycerolysis and acid catalyzed esterification was carried out to reduce the free fatty acid number of oils. Transesterification of treated oils was done by using different alkalis and lipase (free and immobilized) as catalysts. Furthermore, the impact of all the reaction parameters including time, temperature, catalysts and methanol concentration was observed. Biodiesel was purified by wet and dry washing. Maximum product formation was achieved with 1% potassium hydroxide (KOH) followed by 4% free lipase and 6% immobilized lipase. Chemical composition analysis by gas chromatography and mass spectrometry (GCMS) showed the presence of about 97 and 93% fatty acid methyl esters for KOH and lipase based biodiesel, respectively. Fuel properties including distillation temperature, kinematic viscosity, flash, fire, cloud and pour point of the final product were found in accordance with the standard American Society Testing and Materials biodiesel specifications.

1. Introduction
Rapid industrialization of the world has substantially increased the stipulation of petroleum fuels around the globe, whereas, the combined effect of excessive fuel consumption, its exorbitant cost and environmental concerns has attracted interest towards biofuels production (1). Biodiesel is one of the effective alternate to petroleum-based fuels to be utilized in automobile engines and energy generation (2). It is defined as a mixture of mono-alkyl esters of long chain fatty acids obtained from renewable vegetable oils and animal fats (3). Biodiesel generation by renewable feed stock is approaching a new high globally as it is better than natural diesel in terms of lubricity, better flash point, biodegradability, and insignificant sulfur content. Biodiesel consists of mono-alkyl esters of long chain fatty acids available as triacylglycerol (TAG) or free fatty acid (FA). In addition, its properties like non-toxicity, renewability and biodegradability makes it suitable as compared to petroleum diesel (4). Other major characteristics of biodiesel include higher cetane number and the presence of 10–11% oxygen by weight. Hence, the exhaust emissions of carbon monoxide, hydrocarbons and particulate matter in the exhaust gas are sufficiently reduced (5). Furthermore, biodiesel is preferred over...
other biofuels as it exhibits greater cetane ratio which leads to its increased lubricating characteristics. Consequently, the vehicle’s endurance improves when biodiesel is utilized as a combustion source (4). Although the biodiesel is feasible in many aspects of environmental friendliness and engine suitability, but still it does not compete petroleum fuels economically due to high food grade oils. Therefore, waste cooking or frying oil is considered to be a much better replacement as its price is 60% less than the pure oil (6). It also reduces disposal issues of used frying oils as tons of waste oil is disposed off annually which cause water pollution, soil pollution, health problems and disruption of an aquatic ecosystem (7). So, rather than dumping this oil without treatment, it can be utilized in biodiesel formation as a cheap and efficient alternative feedstock (8).

In various research studies, second-generation feedstock is being used for the synthesis of biodiesel. There are several feed stocks such as wastes from agricultural products, and food products. In the current study, waste cooking oil has been employed as the second generation feedstock and its impact on the significant production of biodiesel has been investigated (5).

Raw vegetable oil can be harmful to engines due to its increased viscosity and decreased volatility. It can escort severe engine deposition, injector cocking and ring sticking (6). So these problems are dealt by the transesterification to have maximum yield of methyl esters in the presence of catalyst and alcohol. Biodiesel production is mainly catalyzed by acids, bases and enzymes. Non-catalytic processes such as supercritical fluids can also be used for conversion, but this is not a commonly employed process due to high operational costs and conditions. Similarly, bio catalyzed process is also expensive for industrial purpose due to high enzyme cost, large reaction time and enzyme deactivation (8).

During pre-treatment methods, distinct parameters such as time, temperature, the concentration of glycerol, and different catalysts have been investigated to determine their impact on FFA in oil. Since waste oil contains high proportion of free fatty acids (FFA), as a result of repeated frying, it hinders its conversion into esters. Therefore, an initial pretreatment is carried out to lower FFA content of waste cooking oil (9). Most common pretreatment procedures include heat treatment, glycerol treatment, metal catalyzed glycerol treatment and acid catalyzed esterification. Re-esterification reaction in the presence of metal catalysts is preferred as it increases the reaction rate to double. It also eliminates extra purification step to remove catalysts, water and unreacted glycerol (10). Oil with the acid value less than 1% is selected for conversion into biodiesel as it gives higher biodiesel yield (11).

For transesterification, triglycerides in oil are converted into fatty acid methyl esters and glycerol using various acids, alkali and enzyme catalysts along with short chain alcohols (12). Multiple heterogeneous catalysts calcined animal bones and biocatalysts are being utilized to produce biodiesel, for reducing energy input and increasing feasibility of product, in modern day research. The advantage of homogenous catalysis over heterogeneous is that the process can occur even under slight operational parameters and thus have escalated specificity as well as reactivity. However, this specificity has proved to be one of its limitation particularly for FFA concentration (13). Methanol, as an acyl receptor, is preferred among other alcohols due to its low cost and fast reaction. Glycerol is a main byproduct of transesterification reaction which requires complete separation from biodiesel through different purification processes (14). There are multiple techniques for quantitative and qualitative characterization of biodiesel, but chemical composition analysis of the purified biodiesel is performed with gas chromatography mass spectrometry (GCMS), while the fuel characteristics are measured by ASTM protocols and compared with the standard ranges provided for biodiesel (15). The main objective of the paper is to provide comparison of different waste cooking oil samples processed through esterification and transesterification procedures along with parameter optimization for its efficient utilization in biodiesel production. The present work also focuses on the chemical characterization and fuel properties analysis of the final product by GCMS and ASTM standard protocols, respectively. The novelty of the current study is the economical biodiesel production from second generation feedstock i.e. waste cooking oil by employing cheap pretreatment of waste oil and reusability strategy of indigenously synthesized microbial lipase.

2. Materials and methods

Five waste cooking oil samples were collected from different industries, restaurant and roadside food stalls, of Lahore Pakistan, in dried canes and bottles. All the samples were filtered to remove food residues left in them after cooking or frying and heated at 100°C to remove moisture content. Different chemicals of analytical grade used for the research work were obtained from Fischer scientific, Sigma and Merck Ltd.

2.1. Physicochemical analysis

Physicochemical analysis of samples was performed by determining their properties such as acid value, density and saponification value. Density of waste oil
was measured using mass over volume formula. Acid value of samples was obtained by taking 2 g of oil, 25 mL titrant (1:1 isopropanol and toluene) along with 2–3 drops of phenolphthalein indicator in a 50 mL flask. Flask constituents were titrated against 0.02 N KOH until the appearance of light pink color. A blank was run in parallel which contained similar components except oil which was replaced by water. Acid value of oil was calculated by the following formula (16).

\[
\text{Acid value} = \frac{\text{KOH for sample} - \text{KOH for blank} \times N \text{ of KOH} \times 56.11}{\text{Weight of sample}}
\]

For saponification value, 2 g of oil was weighed and added with 25 mL of alcoholic KOH and 3 mL of ethanol and toluene mixture (1:1). A blank reaction was run in parallel which contained similar components except oil which was replaced by water. Acid value of oil was calculated by the given formula (17).

\[
\text{Saponification value} = \frac{\text{Vol of HCl for blank} - \text{vol of HCl for sample} \times 56.1 \times N \text{ of HCl}}{\text{Weight of sample}}
\]

2.2. Pretreatment

Two pretreatment procedures, metal catalyzed glycerolysis and acid treatment, were applied on all the waste oil samples to achieve the lowest free fatty acid (FFA) content. In metal catalyzed glycerolysis, the reaction mixture was augmented with metal catalysts (zinc dust, ferric chloride, manganese chloride and stannous chloride, separately) along with the supplementation of glycerol, heating and stirring and their effect on FFA content was observed by measuring the acid value of waste cooking oil by titration method (18). Esterification with the use of homogenous acids is a commonly employed procedure in which oil was added with methanol, acid catalyst (conc. H2SO4, HCl and H3PO4) and reaction mixture was transferred to round bottom three necked flask attached with water cooled condenser placed on hot plate. Physical and chemical parameters, temperature, time, amount of glycerol, alcohol and catalyst were optimized for both pretreatment methods (19).

2.3. Biodiesel conversion

Pretreated waste cooking oil samples were subjected to transesterification reaction in the presence of alkali (NaOH, NaOCH3 and KOH) and enzyme (free and immobilized lipase produced from Bacillus subtilus IIB-210 taken from the culture bank of Institute of Industrial Biotechnology, GC University Lahore Pakistan) catalysts along with the addition of methanol. For alkaline catalysis, 100 g of oil was taken in a conical flask. It was added with 1% catalyst, 6:1 methanol to oil molar ratio and incubated at 50°C for 1.5 h (20). However, in case of free lipase, 5% enzyme was added in the oil along with stepwise addition of methanol and incubated at 40°C for 72 h. For immobilized enzyme, lipase was first entrapped in calcium alginate beads and then used in transesterification reaction. The methanol Enzyme assay for lipase was performed after immobilization. Factors involved in all the reactions such as time and temperature. Methanol to oil molar ratio and catalyst to enzyme ratio of 4:1, 6:1 and 8:1 were employed and optimized to obtain maximum biodiesel yield. When transesterification was completed, the reaction mixture was transferred to separating funnel for overnight to completely separate the biodiesel from glycerol layer (21).

2.4. Purification

Biodiesel produced in transesterification reaction was purified to remove the unreacted catalysts, methanol and soaps. Wet wash and dry wash methods were applied to obtain a purified product. In wet wash, water was heated at 60°C to wash the biodiesel until a clearer product layer was obtained, then the purified layer was dried by heating at 100°C to remove water content (22). On the other hand, for dry washing silica and Amberlite were used as adsorbents to remove all the contaminants. About 5% adsorbent was added to oil and placed on the plate at 60°C along with mild stirring for 3 h. After that adsorbent was allowed to settle at the bottom and biodiesel layer was separated (23).

2.5. GCMS analysis

GCMS analysis of samples was done by dissolving 100 µL of sample in 900 µL of chloroform solvent in an Eppendorf. Gas chromatograph (GC-17A Shimadzu, USA) with a direct capillary column (TG-WAX MS) of 30 m × 0.25 mm × 0.25 µm thickness was used. Column temperature was initially held at 80°C and then increased by 5°C per min to 200°C withhold time of 2 min. Temperature was further increased to 280°C withhold time of 10°C per min. The column injector temperature was set up to 270°C. Helium acted as a carrier gas having a constant flow rate of 1 mL/min. The sample components
were identified by comparison of their retention times and mass spectra with a Supelco 37-component fatty acid methyl ester (FAME) mix purchased from Sigma Aldrich and used as standard (24).

2.6. Fuel properties analysis

Main fuel properties of biodiesel such as kinematic viscosity, distillation temperature, carbon residue content, cloud, pour, flash and fire point were determined according to the ASTM protocols provided for each characteristic such as ASTM D664, D88, D86, D189, D5853, D92 and D93, respectively (25–31). Carbon residue of samples was measured by using Conradson carbon residue apparatus, flash point by Pensky-Martens closed cup tester, distillation temperature by distillation assembly and fire point by Cleveland’s open cup tester (32).

2.7. Emission properties

Two different biodiesel blends, B20 and B40, were prepared by mixing 200 mL of biodiesel with 800 mL petroleum diesel, and 400 mL biodiesel with 600 mL petroleum diesel, respectively. Generator was run on these biodiesel blends and the emissions were measured with SOx, NOx and NO2 analyzers (33).

2.8. Statistical analysis

All the experiments were conducted in triplicates. Computer software, costat cs6204W.exe, was utilized for statistical analysis of the results.

3. Results and discussion

3.1. Physicochemical properties of waste oil samples

Waste cooking oil has different properties than the pure oil as it undergoes repeated frying at high temperatures. These characteristics tend to change in all the waste oils depending on the number of frying, temperature and the type of cooked in it (34). Waste canola oils collected from industry and roadside food stalls were found to have higher density, acid value as well as saponification value due to excessive hydrolysis and polymerization reactions as compared to local restaurant and in oil collected from a different industrial source (Table 1). Density and saponification value of samples were within the range, 0.91–0.924 g/cm³ density and 188–207 mg KOH/g saponification value, as provided by Carlinia et al. (35). Acid value (3.57 and 3.66 mg KOH/g) of sample collected from industry and local food restaurant resembled to the acid value (3 to 5 mg KOH/g) of samples used by Ding et al. who collected restaurant waste in their research work (36).

3.2. Pretreatment

During pre-treatment experiments, distinct parameters such as time, temperature, concentration of glycerol, and different catalysts have been investigated to determine their impact on FFA in oil.

3.2.1. Impact of time on FFAs

Pretreatment reactions were carried out for different time periods, 2, 4, 6, 8 and 10 h, to find out the optimum reaction duration. For metal catalyzed glycerolysis the reaction time was reduced to 6 h as the addition of metal catalyst (zinc dust) accelerated the reaction rate and reduced the acid value of oil to 1.34 ± 0.14 mg KOH/g (Figure 1). Metal catalysts are preferred because they can be utilized in multiple treatments without causing change in properties of oil (37). On the other hand, acid catalyzed esterification of waste cooking oil was done for 30, 60, 90 and 120 min where minimum level of acid value (0.48 ± 0.03 mg KOH/g) was obtained after 90 min of reaction using HCl and followed by H2SO4 (0.70 ± 0.11 mg KOH/g) as illustrated in Figure 1(b). H2SO4 was selected for further studies as it gave lower soap formation in side reactions. Increase in time beyond the optimum level may cause fat splitting, hydrolysis and change in composition of oil which further increases the free fatty acid content. Saponification reaction may also occur in case of acid catalyzed process which decreases the quantity of substrate and requires extra purification as well (38). Felizardo et al. employed zinc dust, stannous chloride, manganese chloride and ferric chloride catalysts and maximum re-

Table 1. Physicochemical properties of various waste cooking oils.

| Sr.No. | Waste cooking oil samples | Density (g/m³) | Acid value (mg KOH/g) | Saponification value (mg KOH/g) |
|--------|---------------------------|----------------|-----------------------|-------------------------------|
| 1      | Industrial waste oil sample | 0.928 ± 0.20 | 7.24 ± 0.11 | 196.04 ± 0.15 |
| 2      | Industrial waste oil sample | 0.912 ± 0.13 | 3.57 ± 0.07 | 184.32 ± 0.11 |
| 3      | Local restaurant | 0.910 ± 0.23 | 3.66 ± 0.21 | 187.83 ± 0.05 |
| 4      | Roadside food stall | 0.912 ± 0.17 | 5.39 ± 0.18 | 189.81 ± 0.22 |
| 5      | Roadside food stall | 0.936 ± 0.11 | 9.01 ± 0.21 | 199.11 ± 0.09 |

Note: Standard deviation (±S.D) among replicates is represented in table by Y-error bars, differing at p ≤ 0.05.
esterification (50–5%) was achieved by using zinc dust and MnCl₂ along with 5 w/w% glycerol at 180°C after 6 h of reaction followed by SnCl₂; whereas, ferric chloride was not found to have a significant effect (39). Dhannasekaran and Dharmendirakumar performed esterification of used frying oil and the results were similar to the present finding as maximum reduction in acid value of oil was obtained after 90 min, at 60°C, 1% H₂SO₄ concentration and 10% methanol concentration (1:7 methanol/oil molar ratio) (40).

3.2.2. Impact of temperature on FFAs
Temperature acts as an important physical parameter to make the oil suitable for transesterification reaction. To observe the effect of temperature waste oil sample were treated at different temperatures and a suitable temperature, giving lowest acid value, was selected for carrying out further reactions. For metal catalyzed glycerolysis minimum FFA number was obtained at 160 and 180°C, respectively (1.22 ± 0.17 and 3.41 ± 0.12 mg KOH/g, respectively) as shown in Figure 2(a). Further increase in temperature may cause polymerization, oxidation and hydrolysis reactions in oil. For acid catalyzed esterification, temperature was optimized at 65°C as the reaction involves the addition of methanol whose hydroxyl group for FFA conversion to mono-, di-, and tri-glycerides; therefore, its minimum quantity that could convert maximum FFA to glycerides was determined to make the process appropriate. Incorporation of large amount of glycerol splits the glycerides and is left unreacted at the end of reaction (18).

3.2.4. Impact of concentration of catalyst on FFAs
Concentration of catalyst is a crucial parameter in pretreatment processes. In acid catalyzed esterification the addition of 2% H₂SO₄ was found to provide a huge decline in acid number (0.70 ± 0.23 mg KOH/g) as compared to HCl and H₃PO₄ (Figure 4(a)). Sulfuric acid does not cause formation of soaps and water like the other two catalysts which compromise the overall substrate amount (41). Whereas, metal catalyst (zinc dust) was applied in different concentrations ranging from 0.25 to 1%. The effect of each catalyst concentration was observed on the acid value of waste oil. Among all, 0.5% zinc dust gave the maximum decline in acid value (1.02 ± 0.11 mg KOH/g) at 160°C and 6 h reaction time (Figure 4(b)). Metal catalysts are preferred because they do not react with oil to give side products and are easily separated as compared to other acid basic catalysts.

3.3. Comparison of different pretreatment methods and their effect on acid value of different waste oil samples
Waste cooking oil samples were treated with different pretreatment procedures and the acid values of oils after each method are given in Table 2. One industrial sample gave the lowest acid value with acid treatment (1.19 ± 0.25 mg KOH/g) followed by metal catalyzed glycerolysis (1.59 ± 0.11 mg KOH/g) while the second
industrial sample gave the minimum acid value of 0.88 mg KOH/g with acid pre-treatment. Sample from local food restaurant gave the lowest acid value of 0.41 ± 0.06 mg KOH/g among other oil samples following 0.71 ± 0.11 mg KOH/g acid value of oil sample from roadside food stall. Sample with appropriate characteristics was selected for biodiesel conversion.

### 3.4. Transesterification

Transesterification procedure was catalyzed by different alkali and enzyme catalysts and the effect of physical and chemical parameters was observed on final biodiesel yield.

#### 3.4.1. Impact of time on product yield

Biodiesel conversion using alkali catalysts, KOH, NaOH and NaOCH₃ was done for different time intervals of

![Figure 2](image)

**Figure 2.** Effect of temperature on acid value of waste cooking oil during (a): Metal catalyzed glycerolysis (b): Acid catalyzed esterification.

![Figure 3](image)

**Figure 3.** Effect of glycerol concentration on acid value of waste oil in metal catalyzed glycerolysis.

![Figure 4](image)

**Figure 4.** Effect of acid and metal concentration on acid value (a): acid catalyzed esterification (b): metal catalyzed esterification, respectively.
Table 2. Acid value of waste cooking oil samples after pretreatment procedures.

| Sr. No. | Oil Samples            | Acid value (mg KOH/g) |
|---------|------------------------|-----------------------|
|         | Metal catalyzed glycerolysis | Acid treatment        |
| 1       | Industrial             | 1.59 ± 0.11           | 1.19 ± 0.25          |
| 2       | Industrial             | 1.14 ± 0.18           | 0.88 ± 0.12          |
| 3       | Local restaurant       | 1.25 ± 0.08           | 0.41 ± 0.06          |
| 4       | Roadside food stall    | 1.87 ± 0.16           | 0.71 ± 0.11          |
| 5       | Roadside food stall    | 2.18 ± 0.21           | 0.41 ± 0.06          |

30, 60, 90 and 120 min and the best product yield (96.91%) was achieved by carrying out the reaction for 90 min at 55°C using potassium hydroxide (Figure 5(a)). With increasing the reaction time side product formation also starts increasing and the separated glycerol layer may also dissolve in the ester layer which becomes difficult to remove. However, for free and immobilized lipase catalyzed processes maximum product formation (89.64% and 80.14%, respectively) occurs after 72 h of reaction as the enzyme maximally converted glycerides into their respective methyl esters in the given time (Figure 5(b)). Reaction kept for longer durations provides poor quality product as the process is reversed and enzyme forms secondary metabolic components as well (42). Kamran et al. utilized 60°C for 1 h to get maximum biodiesel yield using potassium hydroxide (42).

3.4.2. Impact of temperature on product yield
For alkali catalytic systems, reaction mixture was subjected to different temperatures such as 45, 55, 65 and 75°C to analyze its impact on final product formation. The optimum temperature was obtained as 55°C at which highest biodiesel yield of 97.09% was achieved (Figure 6(a)). Further temperature escalation did not let the proper interaction of methanol and glycerides happen and the ester production was ultimately reduced with increased saponification (43, 44). In case of lipase catalytic transesterification temperature acts as a key factor as the slight change in temperature causes inactivation of lipase. Bacterial lipase efficiently converted glycerides to FAME at 40°C after 72 h of reaction time (90.01% with free lipase). Immobilized lipase was found stable at 40 and 42°C as the almost similar amount of product formation occurred at both temperatures as indicated in Figure 6(b) (79.9 and 79.22%, respectively). Ying and Chen reported similar parameters, 72 h, 40°C, 4:1 methanol and 4% enzyme, to attain 88–90% production formation (45).

3.4.3. Impact of methanol to oil molar ratio on product yield
Methanol to oil molar ratio is a crucial parameter in transesterification reaction as it directly affects the formation of fatty acid methyl esters (FAME). To attain its precise amount, methanol was added in different molar ratios varying from 2:1 to 12:1 MeOH. For catalytic reaction methanol reacted with glycerides at a rapid pace in 90 min at 55°C to produce methyl esters. Highest biodiesel production (96.86%) occurred upon using 6:1 methanol to oil molar ratio (Figure 7(a)). Further increase in its quantity caused the formation of water and soaps upon interaction with the base. Lipases are sensitive to methanol therefore a relatively small amount of methanol was added in intervals to prevent the inactivation of enzyme (46). About 4:1 methanol/oil was added in three equal intervals to generate larger product yield; and both free and immobilized enzyme gave maximum product formation, of 90.02 and 82.13%, respectively, in the presence of 4:1 methanol to oil and after that the overall yield declined (Figure 7(b)).

3.4.4. Impact of catalysts on product yield
The type and concentration of catalysts is important in determining the rate of reaction and the quality of final product. For alkali catalysts, maximum product formation occurred by using 1% KOH (97.01%). High

![Figure 5](image_url). Effect of time duration on biodiesel production (yield) during (a): alkali catalyzed, and (b): lipase (free and immobilized) catalyzed transesterification.
catalyst concentration causes soap formation which decreases the FAME yield (47) as shown in Figure 8(a). KOH provided minimum amount of soaps formation which were easy to remove, whereas NaOH and NaOCH₃ produced hard soaps which required intensive purification procedures and the certain amount of product is lost during purification step (48). For free lipase catalysis, 4%w/w enzyme (9 U/mL/min) yielded maximum product (91.61%) followed by the application of 6%w/w immobilized lipase (85.22%) as depicted in Figure 8(b). Presence of large amount of water in the reaction mixture inhibits the enzyme activity and dissolves the glycerol and ester layers (49).

3.5. Reusability of immobilized lipase

Immobilization of lipase was performed to make the process economically compatible as the immobilized enzyme can be employed in multiple reactions. Enzyme entrapped in calcium alginate beads was reused to check its efficiency in successive reactions where it was observed that the product yield decreased by 30%, from 84.64 to 51.27%, after 3rd cycle (Figure 9). The possible reason could be the release of enzyme from the matrix or its deactivation (50).

3.6. Purification

The produced biodiesel was then purified using wet and dry wash method which removed all the soaps, catalysts and alcohol residues. The absence of these impurities was estimated by the absence of extra peaks during GCMS analysis of samples.

3.7. GCMS analysis

Internal standards measure the process’s capacity to extract required analytes from the sample matrix, and

Figure 6. Effect of temperature on biodiesel yield after (a): alkali (KOH) catalyzed and (b): lipase catalyzed transesterification.

Figure 7. Effect of different methanol/oil molar ratios on product yield in (a): KOH and (b): lipase catalyzed transesterification.
are used to monitor and regulate instrument fluctuations. Chloroform is added in a specific amount to samples, the blank and calibration samples in a chemical analysis. It is used to correct for the damage of any analyte during sample preparation, injection or ionization. Higher ester amount indicates the good quality biodiesel formation with lower side products. The largest peak in Figure 10(a) showed the presence of high amount of esters of octadecanoic acid (62.02%) followed by octadecadienoic (10.85%), hexadecadienoic acid (8.91%) along with eicosadienoic acid (6.45%), pentadecanoic acid (4.23%), tetradecanoic acid (4.10%) and heptacosanoic acid (3.92%) in alkali catalyzed process.

The chemical composition of lipase catalyzed biodiesel Figure 10(b) gave the presence of high amount of octadecenoic methyl esters (60.09%) in the final product followed by hexadecadienoic acid methyl esters (7.38%), octadecadienoic acid methyl esters (12.31%) (51).

3.8. Fuel properties analysis

Density and kinematic viscosity are significant in engine operation as highly viscous fuel causes engine deposition and breakup of fuel spray from injector; both these properties were found in ASTM ranges (density 8.78 and 8.91 g/m³; viscosity 5.70 and 6.01 mm²/s).
Cloud and pour point are also important as they provide lowest temperature at which fuel ignition can occur properly and the values below this temperature indicate crystal formation (52, 53). These temperatures (cloud point $-5.6$ and $-7.6\,^\circ C$; pour point $-10$ and $-12.5\,^\circ C$) were also obtained within recommended ranges for both products. Similarly, the standard ranges of flash and fire point indicate the safety of product storage and transportation. The other important fuel properties such as distillation temperature (at which it gets converted into vapors), acid value (derivation of fatty acid content) and carbon residue (tendency of fuels to form carbon deposits) of both KOH and lipase catalyzed biodiesel were under the standard limits (Table 3). Elkady et al. reported almost similar results to this study i.e. 55.81% octadecanoic acid ME, 24.37% oleic acid ME and 5.88% stearic acid ME along with the presence of little amount of other methyl esters (54). These specific amounts are indicative of the good quality of biodiesel.

3.9. Emission properties

B20 (20% biodiesel in 80% conventional diesel) and B40 (40% biodiesel in 60% conventional diesel) of KOH and lipase catalyzed biodiesel were prepared and their emission properties were measured (with SOx and NOx analyzers) compared to petro-diesel. Sulfur oxides and total nitrogen oxides (sum of NO and NO$_2$) were as 0.033% and 1.95%, respectively for KOH biodiesel blend B20. However, SOx (0.022%) and total NOx (2.00%) were obtained for KOH biodiesel (B40), as shown in Table 4. On the other hand, B20 and B40 of lipase biodiesel blends gave 1.91 and 2.00% NOx, and 0.031 and 0.020% SOx, per year respectively. Biodiesel blends

Table 3. Fuel properties of alkali and lipase catalyzed biodiesel.

| Properties            | KOH biodiesel (B100) | Lipase biodiesel (B100) | Standard quantities (ASTM) |
|-----------------------|----------------------|------------------------|----------------------------|
| Acid value (mg KOH/g) | 0.04                 | 0.04                   | 0.2                        |
| Kinematic viscosity at 40°C | 5.70 mm²/s | 6.00 mm²/s | 6.00 mm²/s |
| Density               | 8.78 g/m³            | 8.91 g/m³              | 8.6-9.0 g/m³               |
| Flash point           | 165°C                | 190°C                  | 130min                     |
| Fire point            | 210°C                | 255°C                  | 150min                     |
| Distillation temperature | 300°C              | 320°C                  | 360max                     |
| Pour Point            | $-10.1\,^\circ C$    | $-12.5\,^\circ C$     | $-9\,^\circ C$             |
| Cloud Point           | $-5.6\,^\circ C$    | $-7.6\,^\circ C$      | 0°C                        |
| Carbon residue        | 0.01%                | 0.02%                  | 0.06%                      |
reduce emissions of SOx but NOx are found equal or higher than petroleum diesel (55, 56). Teixeira et al. obtained similar results of NOx (1.90–1.98%) and SOx (0.05–0.07%) as the current finding, 1.95% SOx and 0.03% SOx for B20 as they employed similar production methods and reaction conditions for biodiesel production (57).

4. Conclusions

The research demonstrated that waste cooking oil works as an inexpensive and efficient feedstock for biodiesel production. Reaction parameters i.e. time, temperature, methanol and catalyst concentration markedly affected the properties and yield of the final product. Metal catalyzed glycerolysis and acid pretreatments were found promising as they converted most of the free fatty acids into their respective glycerides. Higher product yield was obtained by using potassium hydroxide and free lipase as catalysts but the emissions and overall quality of lipase catalyzed biodiesel are quite improved. GCMS analysis of biodiesel depicted the presence of high amount of fatty acid methyl esters. Furthermore, biodiesel exhibited good fuel and emission properties according to ASTM specifications. The results of the experiments using biodiesel have been compared and examined with standard diesel which showed substantial improvement in the performance constraints as well as exhaust emissions associated with biodiesels. Hence, the biodiesel production can be made more economical and eco-friendly to fulfill the rising demands of fuel in the future by selecting the industrial waste oils as substrate. By using such wastes, the cost of buying feedstock can be minimized which ultimately leads to the production of cost effective biodiesel. Moreover, in future, attempts can be made to produce such biodiesels that have better lubricating features and negligible impact on the environment.

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