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A Novel Route of Mixed Catalysis for Production of Fatty Acid Methyl Esters from Potential Seed Oil Sources

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Abstract: Depleting petroleum resources coupled with the environmental consequences of fossil fuel combustion have led to the search for renewable alternatives, such as biodiesel. In this study, sunflower (*Helianthus annus*), mustard (*Brassica compestres*) and pearl millet (*Pennisetum americanum*) seed oils were converted into biodiesel (fatty acid methyl esters) by acid-, base- and lipase-catalyzed transesterification, and the resultant fuel properties were determined. The methyl esters displayed superior iodine values (102–139), low densities, and a high cetane number (CN). The highest yield of biodiesel was obtained from mustard seed oil, which provided cloud (CP) and pour (PP) points of −3.5 and 5 °C, respectively, and a CN of 53. The sunflower seed oil methyl esters had a density of 0.81–0.86 kg/L at 16 °C, CP of 2 °C, PP of −8 °C, and a CN of 47. The pearl millet seed oil methyl esters yielded a density 0.87–0.89 kg/L, CP and PP of 4 °C and −5 °C, respectively, and a CN of 46. The major fatty acids identified in the sunflower, mustard, and pearl millet seed oils were linolenic (49.2%), oleic acid (82.2%), and linoleic acid (73.9%), respectively. The present study reports biodiesel with ideal values of CP and PP, to extend the use of biodiesel at the commercial level.

Keywords: immobilized lipase; transesterification; catalysis; fatty acid methyl esters; sunflower; mustard; pearl millet; biodiesel

1. Introduction

Fossil fuels are a major source of environmental pollution and anthropogenic emissions. Huge quantities of carbon dioxide, sulfur dioxide, nitrogen oxides, polyaromatic hydrocarbons, and particulate matter are released into the atmosphere due to the combustion of fossil fuels. Promising replacements for fossil fuels are biofuels, such as biodiesel. Biodiesel is a renewable alternative to petroleum diesel, and comes with a number of ecological and practical advantages. Many sources, including sunflower, rapeseed, soyabean, jatropha, pongamia, waste cooking oil, grease, and algal oils, can be converted into biodiesel (fatty acid methyl esters) by transesterification, which is normally conducted in the presence of methanol and a catalyst [1,2]. Transesterification consists of several consecutive, reversible reactions. In these reactions, the triglycerides are converted stepwise to

diglycerides and monoglycerides, with the release of fatty acid methyl esters (three per triglyceride) and glycerol.

Biodiesel is gaining more attention worldwide as an alternative clean fuel, due to the development of new methodologies and non-food feedstocks for low-cost production, and its renewable nature and lower emission relative to petroleum diesel [3,4]. The environmental benefits of biodiesel come from its chemical structure and sources of production. Biodiesel is mostly produced from plants that contain low quantities of sulfur, which results in negligible SOx emission. Biodiesel also produces 50% less NOx emissions than petroleum diesel. Another difference between fossil fuels and biofuels is the oxygen contents. Biodiesel has a much higher oxygen content (10–45%) than petroleum diesel, due to the presence of ester groups within the fuel [5]. Most importantly, fossil fuels such as coal, petroleum and natural gas are limited and non-renewable, and will get exhausted or phased out through policy changes. Thus, there is a dire need to develop new production processes and sources for renewable and sustainable biofuels [6–14].

Mustard (*Brassica compestres*) is an inexpensive feedstock for biodiesel production, as its seeds contain a high oil content (58–70%). The production costs associated with growing mustard are much lower than other crops such as soyabean, due to lower inputs such as less pesticides, fertilizes and irrigation, etc. [15,16]. Sunflower (*Helianthus annus*) seeds contain 22–36% oil and are a good source of monosaturated and polyunsaturated fatty acids, including oleic (omega-9) and linoleic (omega-6) acids [17]. Sunflower seed oil also contains appreciable quantities of sterols, vitamin E, squalene, aliphatic hydrocarbons, and terpenes [17]. Currently, sunflower is one of the leading oil seed crops cultivated for the production of biodiesel [18]. Pearl millet (*Pennisetum americanum*) is an important crop for developing countries in Asia and Africa, which collectively produces about 93% of the total world production [19]. The above-referred potential seed oil crops are not only important for developed countries, but these can also be explored to support the economies of developing countries. Commercially, biodiesel is produced by catalyzed chemical reactions and space still exists for improved production methodologies [7]. The objectives of the present study were to produce biodiesel from sunflower, mustard and pearl millet seed oils using acid-, base-, and lipase-catalyzed transesterification, and to measure the resultant fuel properties. A further objective was to compare the fuel properties against major international biodiesel fuel standards, such as ASTM D6751 and EN 14214.

2. Results and Discussion

This research was conducted to evaluate the possibility of using sunflower, mustard, and pearl millet seed oils as potential sources for biodiesel production, through the transesterification process. Three catalysts, including lipase, base and acid, were used for the transesterification of the oils. The fuel properties of the resultant fatty acid methyl esters, such as density, saponification value (SV), iodine value (IV), cloud point (CP), pour point (PP), and cetane number (CN), were then measured and compared against the international biodiesel standards ASTM D6751 and EN 14214.

2.1. Effect of Catalyst on Yield of Biodiesel

The effect of different catalysts on the yield of biodiesel are presented in Figures 1–4. Alkali-catalyzed transesterification was performed using three different concentrations of NaOH and KOH (0.5, 1.0 and 2.0 wt.% relative to oil). The maximum yield of biodiesel was attained from mustard seed oil using 0.5% KOH alkali-catalyzed transesterification. A further increase in KOH quantity resulted in the decreased biodiesel yield, due to the formation of soaps at higher alkali concentrations (Figure 1). A similar trend was noted for base-catalyzed transesterification using NaOH (Figure 2). However, the yields were higher using KOH because of less soap formation. Three concentrations of HCl (25%, 50% and 100% w/w of oil) were used for the acid transesterification of sunflower, mustard, and pearl millet seed oils (Figure 3). The maximum yield of biodiesel was obtained at the highest concentration of HCl. Immobilized enzyme transesterification was conducted at

three concentrations (3%, 4% and 5% *w/w* of oil) of immobilized lipase. The highest yield of biodiesel was obtained at a lipase concentration of 4% (Figure 4). The direct link that exists between the number of active sites and the amount of the substrate was noted [20]. Of all the catalysts (HCl, KOH, NaOH and lipase) and oil samples (sunflower, mustard, and pearl millet seed oils), a maximum yield of the biodiesel of 98% was achieved from mustard seed oil, using 100% HCl.

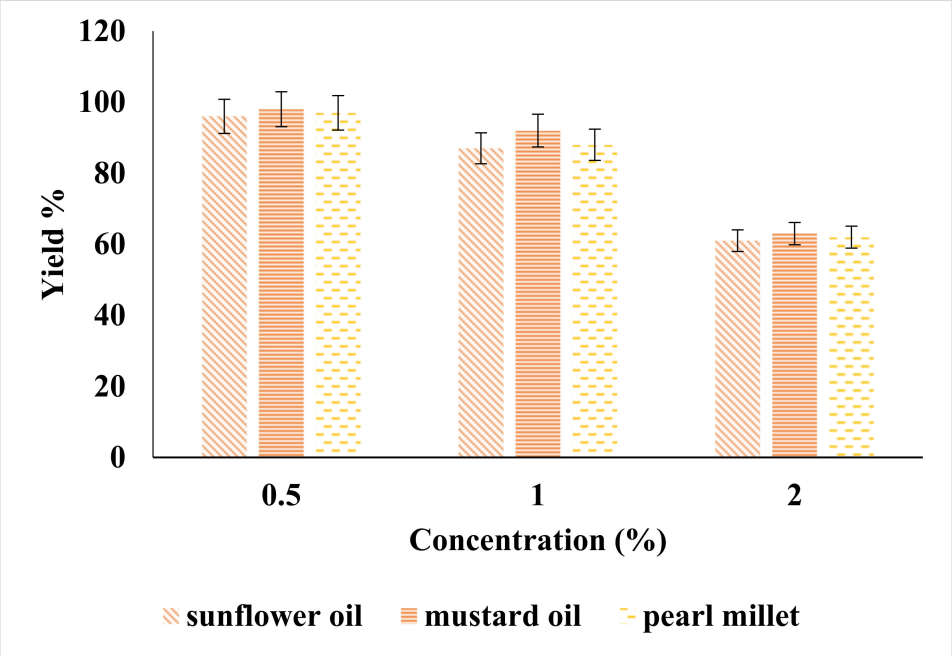

Figure 1. Percentage yield of biodiesel using KOH (wt.%) as a base catalyst.

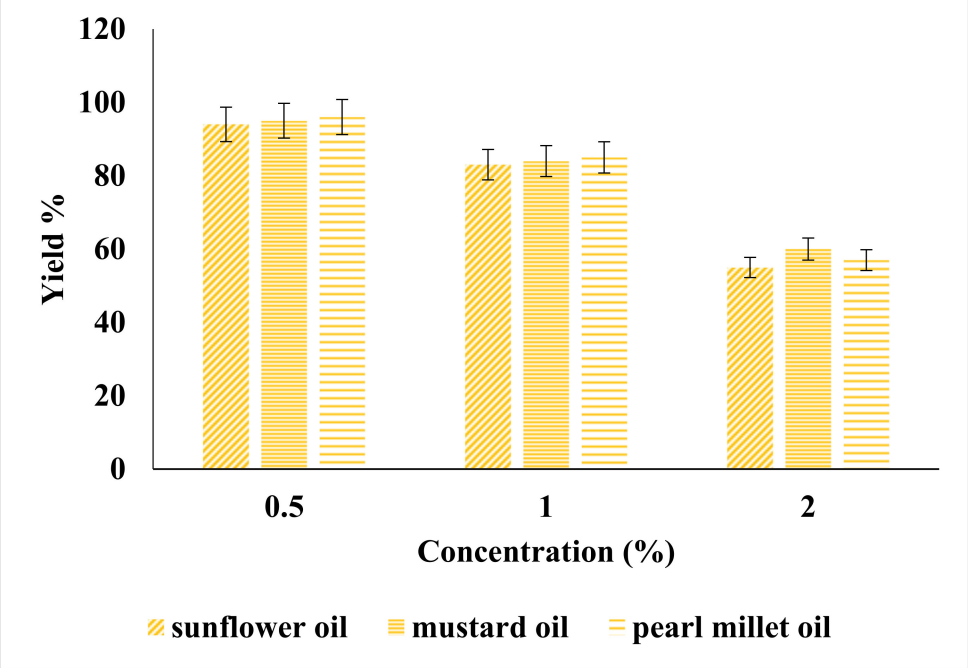

Figure 2. Percentage yield of biodiesel using NaOH (wt.%) as a base catalyst.

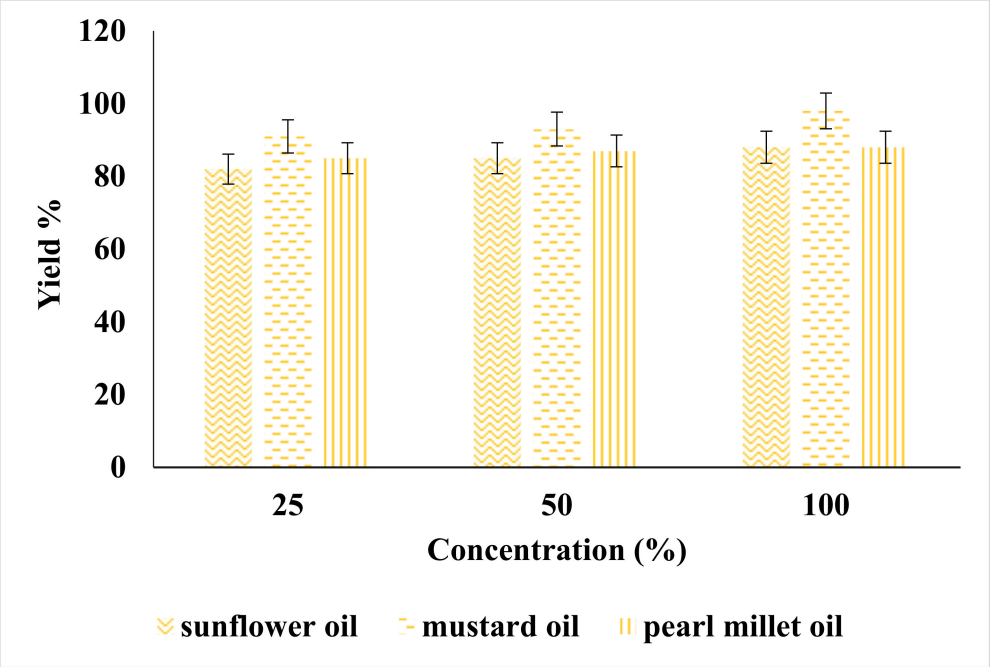

Figure 3. Percentage yield of biodiesel using HCl (wt.%) as acid catalyst.

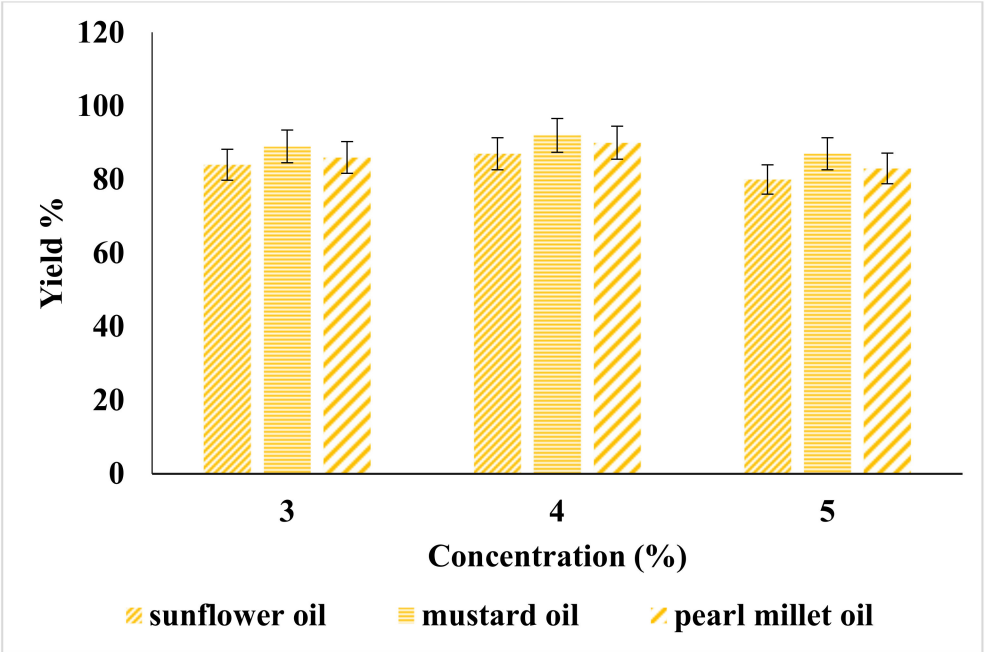

Figure 4. Percentage yield of biodiesel using lipase (%) as enzymatic catalysis.

2.2. Physiochemical Properties of Biodiesel

The density of the biodiesel samples was determined following standard methods (EN ISO 3675/12185 and ASTM standard D1298), as described previously [20]. In the present study, the densities of all the samples were in the range of 0.80 to 0.89 kg/L (Table 1). Pearl millet biodiesel, prepared from 2 wt.% KOH, provided the lowest density (0.80 g/mL). The maximum value (0.89 kg/L) was obtained from pearl millet seed oil methyl esters, using HCl (100% *w/w* oil-to-HCl) and a 6:1 methanol-to-oil molar ratio. The density of biodiesel plays an important role in the flow proprieties during different seasons. Higher fuel densities lead to lower flow rates of fuel injected into the combustion cylinder. If the

density is too low, then the power is reduced, because higher density fuels have more energy content. However, the weather conditions of a particular country play an important role in deciding which biodiesel to choose, due to the differences in low-temperature performance. In a previous study, biodiesel produced from beef tallow and chicken fat had densities of 0.856–0.867 kg/L [21]. The European biodiesel standard (EN 14214) requires density to be in the optimal range of 0.86–0.90 kg/L [1]. In another previous study, the biodiesel made from date seed oil had a density of 0.887 kg/L.

Table 1. Density (kg/L) of biodiesel from sunflower, mustard, and pearl millet seed oils.

Catalyst	Catalyst Conc. (%)	Sunflower	Mustard	Pearl Millet	American Standard ASTM	European Standards EN
KOH	0.5	0.85 ± 0.003	0.87 ± 0.005	0.82 ± 0.004	Not specified	0.86–0.90
	1	0.83 ± 0.004	0.85 ± 0.006	0.81 ± 0.003		
	2	0.81 ± 0.005	0.84 ± 0.004	0.80 ± 0.005		
NaOH	0.5	0.85 ± 0.003	0.85 ± 0.005	0.83 ± 0.005		
	0.1	0.83 ± 0.004	0.83 ± 0.003	0.81 ± 0.003		
	2	0.82 ± 0.006	0.83 ± 0.004	0.81 ± 0.006		
HCl	25	0.81 ± 0.002	0.85 ± 0.005	0.86 ± 0.004		
	50	0.81 ± 0.007	0.86 ± 0.002	0.87 ± 0.003		
	100	0.83 ± 0.006	0.88 ± 0.006	0.89 ± 0.004		
Immobilized lipase	3	0.81 ± 0.004	0.84 ± 0.007	0.85 ± 0.007		
	4	0.82 ± 0.003	0.85 ± 0.003	0.86 ± 0.003		
	5	0.81 ± 0.004	0.82 ± 0.004	0.84 ± 0.004		

An approximate estimate of the MW of the methyl ester components of biodiesel can be obtained from the SV, as SV is an indicator of the extent of short-chain alkyl groups attached to the fatty acid methyl esters [22]. A higher SV indicates the presence of more carboxylic groups per unit mass [1]. The SV of the biodiesel produced in the present study ranged from 174 to 193 mgKOH/g (Table 2). Thus, the SV indicated that pearl millet seed oil had more short alkyl chains as compared to other oil sources, as it yielded a maximum saponification value of 193 at 2% NaOH. Sunflower oil had higher quantities of long-chain alkyl groups. Previous studies determined SVs for biodiesel produced from various sources, such as 187.02 for castor oil [23], 259 for coconut oil [24], 201 for palm oil [25], and 323 for date seed oil [20]. The greater the value of saponification, the higher the density and the lower the volatility. Highly volatile biodiesel avoids misfire and burns smoothly in the engine. The lowest SV was observed in the current study for biodiesel prepared from pearl millet seed oil.

Table 2. Saponification value (mg KOH/1 g) of biodiesel from sunflower, mustard and pearl millet seed oils.

Catalyst	Catalyst Conc. (%)	Sunflower	Mustard	Pearl Millet	American Standard ASTM	European Standards EN
KOH	0.5	175 ± 0.5	187 ± 0.4	190 ± 0.6	Not specified	Not specified
	1	177 ± 0.5	189 ± 0.5	192 ± 0.6		
	2	179 ± 0.6	191 ± 0.4	193 ± 0.6		
NaOH	0.5	174 ± 0.6	186 ± 0.4	190 ± 0.6		
	0.1	176 ± 0.5	189 ± 0.4	192 ± 0.6		
	2	177 ± 0.6	190 ± 0.5	193 ± 0.6		
HCl	25	177 ± 0.5	182 ± 0.4	188 ± 0.6		
	50	175 ± 0.5	178 ± 0.4	187 ± 0.6		
	100	174 ± 0.5	177 ± 0.4	186 ± 0.6		
Immobilized lipase	3	176 ± 0.6	181 ± 0.4	186 ± 0.6		
	4	174 ± 0.5	178 ± 0.5	184 ± 0.6		
	5	175 ± 0.5	180 ± 0.4	185 ± 0.6		

The degree of unsaturation is determined by the IV. It is defined as "the number of grams of iodine added to 100 g of oil or fat". Thus, biodiesel with no double bonds has as an IV of zero. This parameter greatly affects the oxidative stability of biodiesel, as oxidation is initiated at sites allylic to unsaturation. The IVs recorded during the present study ranged from 102 to 139 (Table 3). The minimum IV (102) was obtained from mustard seed oil methyl esters at 2% NaOH, by using a 10:3 methanol-to-oil molar ratio for 90 min at 60 °C. The maximum IV (139) was observed for sunflower oil methyl esters, which was because they contained the highest percentage of polyunsaturated fatty acids such as ὰ-linolenic acid. The upper limit for IV in the European biodiesel standard (EN 14214) is 120, but the American standards (ASTM D6751) do not specify a limit for the IV. Because oxidative stability and melt temperature are related to the degree of unsaturation, the IV gives an estimation of these parameters. The greater the IV, the higher the susceptibility to oxidation, due to the presence of more unsaturation. Peanut oil (IV 82–107) is more saturated than corn (IV 103–128), cottonseed (IV 99–113), or linseed (IV 155–205) oils; however, it is considerably less saturated than coconut (IV 7.7–10.5), palm (IV 44–54), or butter (IV 25–42) oils [26]. The CP and freezing points of biodiesel are dependent on the IV. The higher the degree of unsaturation, the greater the IVs and the lower CPs. However, greater unsaturation may cause the formation of engine deposits, due to polymerization at high engine temperatures. To avoid solidification of the fuel, at low storage temperatures, unsaturation is necessary, to a degree [1]. Thus, a balance must be maintained between the oxidative stability and low-temperature performance. However, the lower CP and PP values observed for biodiesel from mustard seed oil were due to the presence of short-chain fatty acids containing less van der Waals forces [20]. Overall, the IV was minimally affected by the production method, as acid-, base- and lipase-catalyzed transesterifications generally yielded similar results within a given biodiesel.

Table 3. Iodine value (g I_2/100 g) of biodiesel from sunflower, mustard, and pearl millet seed oils.

Catalyst	Catalyst Conc. (%)	Sunflower	Mustard	Pearl Millet	American Standard ASTM	European Standards EN
KOH	0.5	134 ± 0.2	106 ± 0.2	129 ± 0.3	Not specified	≤120
	1	132 ± 0.2	105 ± 0.2	127 ± 0.3		
	2	131 ± 0.2	103 ± 0.2	126 ± 0.3		
NaOH	0.5	133 ± 0.3	107 ± 0.2	128 ± 0.3		
	0.1	132 ± 0.2	105 ± 0.2	126 ± 0.3		
	2	130 ± 0.3	102 ± 0.3	124 ± 0.3		
HCl	25	134 ± 0.2	105 ± 0.2	127 ± 0.3		
	50	135 ± 0.2	107 ± 0.2	128 ± 0.3		
	100	139 ± 0.2	108 ± 0.2	129 ± 0.3		
Immobilized lipase	3	134 ± 0.2	106 ± 0.3	126 ± 0.3		
	4	136 ± 0.2	108 ± 0.2	128 ± 0.2		
	5	132 ± 0.2	105 ± 0.2	125 ± 0.5		

To test the low-temperature performance of biodiesel, CP and PP are important parameters. Fuel solidification in engines filters and pipelines may cause many problems, such as damage to the engine and delayed ignition due to crystal formation. The CP is measured as the temperature at which the growing crystal within the biodiesel becomes visible and causes the fuel to turn cloudy. The temperature at which the fuel turns into a gel and no longer pours, due to continued crystal growth and agglomeration, is referred to as the PP [27]. Usually, vegetable oils have a higher CP and PP than the corresponding biodiesel fuels [5]. The delayed startup, misfire, and poor flow rates to the combustion chamber, and engine failure, are the main drawbacks of a high CP and PP. The CP decreases with unsaturation and increases with fatty acid chain length. The CP range of the biodiesel produced in this study was between 2.2 and 5.1 °C (Table 4) for sunflower, while the PP ranged from −7.5 °C to −8.1 °C. The significantly lower CP and PP relative to the other methyl esters indicated that sunflower biodiesel could also be used in cold weather

climates. Biodiesel from sunflower oil also exhibited a lower PP value when produced in the following reaction conditions: 0.5% NaOH, a 10:3 methanol-to-oil molar ratio, for 90 min at 60 °C. On the other hand, the maximum CP was exhibited by mustard biodiesel produced by using 2% NaOH, a 10:3 methanol-to-oil molar ratio, for 90 min at 60 °C. The European and American biodiesel standards report no specific CP limit. The climatic conditions usually define the CP limit. The values obtained for PP were between −3.7 °C and −8.1 °C. The minimum value was observed for biodiesel from sunflower seed oil, by using 100% HCl at a 6:1 methanol-to-oil molar ratio and 90 min of reaction at 65 °C. For comparison, the CP and PP reported previously were 4 °C and −1 °C for date seeds oil methyl esters, respectively [1].

Table 4. Pour point (°C) and cloud point (°C) of biodiesel from sunflower, mustard and pearl millet seed oils.

Catalyst	Catalyst Conc. %	Sunflower		Mustard		Pearl Millet		Biodiesel Standards	
		Cloud Point (°C)	Pour Point (°C)	Cloud Point (°C)	Pour Point (°C)	Cloud Point (°C)	Pour Point (°C)	Cloud Point	Pour Point
KOH	0.5	2.4 ± 0.093	−7.8 ± 0.086	4.7 ± 0.084	−4.3 ± 0.090	3.9 ± 0.084	−4.8 ± 0.088	Not specific depends on climatic condition	Not specific depends on climatic condition
	1	2.5 ± 0.089	−7.7 ± 0.082	4.8 ± 0.082	−4.1 ± 0.082	4.4 ± 0.079	−5.1 ± 0.082		
	2	2.7 ± 0.080	−7.5 ± 0.090	5.0 ± 0.093	−3.8 ± 0.093	4.2 ± 0.076	−5.0 ± 0.093		
NaOH	0.5	2.2 ± 0.080	−8.0 ± 0.090	4.8 ± 0.089	−4.3 ± 0.090	4.0 ± 0.069	−4.7 ± 0.090		
	1	2.4 ± 0.079	−7.9 ± 0.086	4.9 ± 0.060	−4.2 ± 0.060	4.3 ± 0.066	−4.6 ± 0.060		
	2	2.6 ± 0.083	−7.6 ± 0.087	5.1 ± 0.080	−3.9 ± 0.089	4.3 ± 0.082	−4.9 ± 0.089		
HCl	25	2.4 ± 0.093	−7.7 ± 0.071	5.0 ± 0.079	−4.3 ± 0.087	4.4 ± 0.087	−4.8 ± 0.077		
	50	2.5 ± 0.079	−7.9 ± 0.83	5.0 ± 0.83	−4.2 ± 0.83	4.4 ± 0.076	−5.2 ± 0.83		
	100	2.4 ± 0.082	−8.1 ± 0.093	4.8 ± 0.093	−4.1 ± 0.093	4.3 ± 0.085	−5.1 ± 0.093		
Immobilized Lipase	3	2.5 ± 0.081	−7.8 ± 0.079	5.0 ± 0.079	−4.0 ± 0.079	4.5 ± 0.070	−5.6 ± 0.079		
	4	2.3 ± 0.085	−8.0 ± 0.082	4.8 ± 0.084	−4.1 ± 0.087	4.3 ± 0.082	−6.1 ± 0.074		
	5	2.3 ± 0.082	−7.9 ± 0.085	4.9 ± 0.088	−3.7 ± 0.087	4.4 ± 0.079	−6.0 ± 0.085		

The measure of ignition delay between fuel injection and injection in the combustion chamber is referred to as CN. Fuels with a higher CN have shorter ignition delays, run more smoothly, and produce less engine knocking. CN is thus helpful in deciding what feedstock should be selected for biodiesel production [28]. As the chain length and degree of saturation increases, the CN also increases. Petroleum diesel has a lower CN than biodiesel, due to the presence of branching and aromaticity, both of which also lower the CN. The American and European biodiesel standards contain lower limits for CN, which are 47 and 51, respectively [20]. In the present study, the CN of the three samples ranged from 47 to 53 (Table 5). The maximum CN of 53 was observed for mustard seed oil methyl esters. Overall, CN was minimally affected by the production method, as acid-, base- and lipase-catalyzed transesterifications generally yielded similar results within a given biodiesel.

Table 5. Cetane number (CN) of biodiesel from sunflower, mustard, and pearl millet seed oils.

Catalyst	Catalyst Conc. (%)	Sunflower	Mustard	Pearl Millet	American Standard ASTM	European Standards EN
KOH	0.5	47.4	51.6	46.0	47	51
	1	47.4	51.5	46.2		
	2	47.3	52.5	46.2		
NaOH	0.5	47.7	51.6	46.2		
	0.1	47.6	51.5	46.4		
	2	47.9	52.1	46.7		
HCl	25	47.0	52.7	46.8		
	50	47.1	52.9	46.7		
	100	46.8	52.8	46.6		
Immobilized lipase	3	47.2	52.6	47.3		
	4	47.1	52.7	47.2		
	5	47.8	53.0	47.7		

2.3. Fatty Acid Profile of Biodiesel

Gas chromatography mass spectrometric analysis (GC-MS) determined the fatty acid profiles of the biodiesel samples produced from mustard, sunflower and pearl millet seed oils. The most abundant fatty acid in mustard seed oil was oleic acid (82.2%), followed by palmitic acid (7.9%). The major fatty acids identified in sunflower oil were $\grave{\alpha}$-linolenic (49.9%), erucic (27.7%) and gondoic (15.3%) acids. The dominant fatty acid in pearl millet seeds oil was linolenic acid (73.9%), followed by palmitic acid (21.9%). The total fatty acids identified by GC-MS of the three oils was 99.99–100% (Table 6). A careful review of the literature revealed that superior-quality biodiesel is produced from feedstocks having a major amount of fifteen carbon atoms fatty acids [29]. The biodiesel produced from sunflower, mustard and pearl millet seed oils was of good quality, as it did not have any major fatty acids with less than fifteen carbon atoms [30,31].

Table 6. Fatty acid profile of biodiesel from sunflower, mustard, and pearl millet seed oils.

Peak No.	Fatty Acid	Relative Contents %		
		Sunflower	Mustard	Pearl Millet
2	Palmitoleic acid (C16:1)	-	0.2	0.1
1	Palmitic acid (C16:0)	3.5	7.9	21.9
6	Linolenic acid (C18:3)	49.2	-	-
5	Linoleic acid (C18:2)	-	-	73.9
4	Oleic acid (C18:1)	-	82.2	-
3	Stearic acid (C18:0)	1.6	3.6	3.4
8	Gondoic acid (C20:1)	15.3	1.5	0.3
7	Arachidic acid (C20:0)	0.6	0.4	0.3
9	Erucic acid (C22:1)	27.7	4.1	-
11	Nervonic acid (C24:1)	1.8	-	-
10	Lignoceric acid (C24:0)	0.2	-	0.1
	Total	99.99	99.99	100.0

3. Materials and Methods

3.1. Material

Sunflower, mustard, and pearl millet seeds were purchased from a market in Jhang, Pakistan and were unfit for food use. Sodium hydroxide (NaOH), potassium hydroxide (KOH), hydrochloric acid (HCl), lipase, and methanol were purchased from Merck, Pvt. Ltd., Karachi, Pakistan (distributor). All chemicals and solvents were used as received and were of analytical grade.

3.2. Transesterification

The extracted sunflower, mustard and pearl millet seed oils were converted into fatty acid methyl esters by catalytic transesterification with methanol. Transesterification was performed in a round-bottom flask fitted with a reflux condenser heated on a magnetic stir plate. Three catalysts including lipase, base and acid were used for transesterification of the oils. Acid-catalyzed transesterification was conducted with HCl at three levels (25, 50 and 100%) using a methanol-to-oil molar ratio of 6:1 for 90 min at 65 °C. Base-catalyzed transesterification was performed using KOH or NaOH (0.5, 1 and 2 wt.%) with a 10:3 ratio of methanol-to-oil at 60 °C for 90 min. Lipase-catalyzed transesterification was performed at concentrations of 3, 4 and 5 wt.% at a methanol-to-oil molar ratio of 5:1 with a shaking time of 12 h at 40 °C. The lipase used in this study was obtained from microbes. Biodiesel and glycerol were separated using a separatory funnel after the two layers were allowed to settle. Soap was removed by washing biodiesel with hot (80 °C) distilled water.

3.3. Immobilization of Lipase

The lipase enzyme was immobilized in the form of beads by sodium alginate. Exactly 3 g of sodium alginate was dissolved in 100 mL of distilled water to produce a 3% solution.

Then, 0.25 g of lipase was mixed with 45 mL of the sodium alginate solution. This procedure was executed in sterilized surroundings. The beads were made by dropping the biopolymer solution through a syringe and needling it into an excess of 0.2 M $CaCl_2$ (100 mL) solution with continuous stirring at room temperature. A potassium phosphate buffer (3 mL) of pH 7.4 was added to the solution to maintain the pH of the lipase [32].

3.4. Determination of Physiochemical Characteristics

Gas chromatography mass spectrometry (GC-MS) of the fatty acid methyl esters (0.1 µL) was performed on an Agilent Technologies GC system 7890A that was fitted with an HP-5MS capillary column (30 m × 250 µm i.d × 0.25 µm film thickness, maximum temperature, 450 °C), coupled to a model 5975C MS with a split ratio of 50:1. Carrier gas used in the column was helium (99.99%) at a constant flow of 0.8 mL/min. The ion source, injection and line transfer temperatures were 250, 240 and 200 °C, respectively. The oven temperature program was held at 60 °C for 10 min, ramped to 310 °C at 10 °C/min and held at 310 °C for 5 min. The identification of peaks was done by comparing retention times with reference standards as well as comparison of the spectra with the included NIST05 library.

The pH of all of the biodiesel samples was determined by a model HI 8010 pH meter. Density (kg/L) was determined by measuring the mass of 1.0 mL of each sample. Specific gravity (SG) was measured by a specific gravity bottle. The following formula (Equation (1)) was used to calculate the SG by taking water as the standard:

$$\mathrm{SGtrue} = \frac{\rho\mathrm{Sample}}{\rho\mathrm{H2O}} \quad (1)$$

where ρSample is the density of the biodiesel sample and ρH_2O is the density of water. For measurement of iodine value (IV), 0.1 g of biodiesel was added to a 250 mL iodine flask, followed by the addition of 20 mL CCl_4 and 25 mL Wijs solution. The mixture was shaken vigorously and then left in the dark for 30 min. Then 20 mL of 15% KI solution and 100 mL of distilled water were added and mixed. The solution was titrated with 0.1 N $Na_2S_2O_3{\cdot}5H_2O$ until the color disappeared using starch as an indicator. The same procedure was followed for a blank solution. IV was calculated by using the following formula (Equation (2)) [33]:

$$\mathrm{IV} = \frac{(\mathrm{B} - \mathrm{S}) \times \mathrm{N} \times 12.6}{\mathrm{Sample\ (gm)}} \quad (2)$$

where B = titration volume of titrant used for the blank, S = volume of titrant used for sample and N = normality of $Na_2S_2O_3{\cdot}5H_2O$. For determination of the saponification value (SV), 0.5 g of biodiesel sample and 20 mL alcoholic KOH solution were added to a 250 mL round-bottom flask. The flask was then attached to a condenser and heated moderately until a clear solution was obtained (an indication of completion of saponification reaction). After cooling the solution to room temperature, it was titrated with 0.5 N HCl using phenolphthalein as an indicator until disappearance of the pink color. The same procedure was followed for a blank sample. The SV of the samples was calculated by the following formula (Equation (3)) [34]:

$$\mathrm{SV} = \frac{(\mathrm{B} - \mathrm{S}) \times \mathrm{N} \times 56.1}{\mathrm{W}} \quad (3)$$

where SV = g KOH per g of sample, B = volume of titrant (mL) for blank, S = volume of titrant (mL) for sample, N = normality of HCl (mmol/mL), 56.1 = molecular weight (MW) of KOH (mg/mmol) and W = sample mass (g). Cetane number (CN) was calculated by using following formula (Equation (4)) [35]:

$$\mathrm{CN} = 46.3 + \frac{5458}{\mathrm{SV}} - 0.225 \times \mathrm{IV} \quad (4)$$

Pour point (PP) and cloud point (CP) were determined simultaneously by using Tanaka mini pour/cloud point tester model MPC-101A. All measurements and calculations were performed in triplicate and mean values reported.

4. Conclusions

In the present study, the densities of all the prepared biodiesel samples were in the range of 0.80 to 0.89 kg/L, the CP ranged between 2.2 and 5.1 °C, and the CN was measured from 47 to 53. These values were generally within the ranges specified in ASTM D6751 and EN 14214, where appropriate. The biodiesel produced from sunflower, mustard and pearl millet seed oils had low viscosity and a high CN. The present study reports biodiesel with ideal values of CP and PP, to extend the use of biodiesel at the commercial level to low-temperature climates. Gas chromatographic mass spectrometric (GC-MS) analysis showed the most abundant fatty acids in mustard seed oil were oleic (82.2%) and palmitic (7.9%) acids. The major fatty acids in sunflower seed oil were ὰ-linolenic (49.2%), erucic (27.7%), and gondoic (15.3%) acids. The biodiesel from pearl millet seed oil was dominated by linolenic acid (73.9%), followed by palmitic acid (21.9%). Overall, this study demonstrated that fatty acid methyl esters, prepared by a variety of methods from a number of seed oils, yields biodiesel with favorable fuel properties. The production method (acid-, base- and lipase-catalyzed transesterification) had little effect on the resultant fuel properties.

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