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Original Research Article

Esterification of Handal Oil (*Citrullus Colocynthis L.*) Using Acid Catalyst Preparation for Biodiesel Production

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Abstract

Production of biodiesel represents a way to attain economic growth by increasing and securing energy supply for the developing countries, and it can also create job opportunities and an attractive source for the farmers. The research aims to utilize the Citrullus Colocynthis (L.) plant as a valuable alternative for producing biodiesel. The attention was drawn to the *Citrullus Colocynthis (L.)* due to the plant having the advantage of needing less water compared to other plants. Also, the Citrullus colocynthis plant can be planted in different climate conditions, so it has a good impact on the production capacity. Furthermore, the use of p-p-toluene-4-sulfonic acid monohydrate (PTSA) as an acid catalyst for pretreatment of *Citrullus Colocynthis (L.)* is the first time to use the acid as a catalyst with *Citrullus Colocynthis (L.)* oil to produce biodiesel. *Citrullus colocynthis (L.)* plant seeds were collected from western and northern Sudan; the oil was extracted through screw press extraction using an oil extractor; and the Citrullus colocynthis oil (CCO) was treated to reduce the free fatty acid (FFA) contents before starting the transesterification reaction using 0.3% p-toluene-4-sulfonic acid monohydrate (PTSA) as an acid catalyst. FFA was reduced to 0.3%, and FFA conversion was 91.76%. The final biodiesel produced was found to be 98.51%. The physiochemical properties of biodiesel were flash point 228°C, measured by the Seta Multiflash Cleveland Flash Point Tester; kinematic viscosity at 40°C, 5.094 cSt, measured by the viscometer (Petro Test Instrument Model TV400); pour point, -30°C, measured by the Cloud and Pour Point Test Cabinet (Norma Lab Analysis Instrument Model P592-France); and density at 150°C, 0.878 gm/cm³, measured by the Digital Density Meter (Petro Instrument Model DMA4500). The results attained in this study conformed to the international standard specifications for biodiesel fuel. The results of this study show that the Citrullus Colocynthis (L.) oil has the potential for use as an industrial feedstock for biodiesel production. PTSA can be used as a catalyst in the pretreatment of CCO via esterification reaction, where it showed very high catalytic activity to reduce the free fatty acids (FFA) content in the CCO; also, it has less environmental impact due to its ease of recoverability and reusability.

Keywords: Thumba Seed Oil (*Citrullus Colocynthis (L.)*), Transesterification, Biodiesel, Triglyceride, p- P-Toluene-4-Sulfonic Acid Monohydrate (PTSA).

1. INTRODUCTION

Biofuels are an energy fuel composed of organic materials (also known as biomass) produced by plants and other living organisms that may be constantly cultivated and harvested. (Al-Hwaiti *et al.*, 2025). Over 95% of the world's biodiesel is produced using edible vegetable-based oils, such as canola, corn, coconut, sunflower seed, palm, soybean, and peanut. Because the emissions from burning biodiesel are roughly equal to the CO_2 sequestered by the plants that serve as its primary source of feedstock, biodiesel is considered a carbon-neutral fuel. On the other hand, the usage of fossil fuels has increased emissions, especially of CO_2, which has caused the greenhouse gas effect. Reducing reliance on fossil fuels is difficult, but one useful negative emission technology (NET) for mitigating climate change is carbon sequestration from biomass. (Al-Hwaiti *et al*., 2020). *Citrullus Colocynthis (L.)* Schrad is a plant species in the Cucurbitaceae family. It is also referred to as Bitter Apple or Colocynth, Handal in Arabic, Hendevaneh-ye Aboujahl in Farsi, Acı kavun in Turkish, and Cocomero amara in Italian. This perennial vine is indigenous to the Middle East, North Africa, and portions of Asia and the Mediterranean region. The plant is well-known for its unusual look; its fruit is significantly smaller and tastes more bitter than a little yellow-green watermelon (Fallah *et al*., 2023). *Citrullus colocynthis (L.)* is a wild plant widely distributed in the northern, western, eastern, and central parts of Sudan. Due to the importance of this plant and its availability in Sudan, and due to the scarcity of scientific studies on *Citrullus colocynthis (L.)* oil usage, it was decided to study its characteristics and industrial usage (Abdalla, 1997). These plants are widely used in traditional medicine. Different parts of these plants serve as a therapy for diseases such as jaundice, diabetes, and asthma (Rao and Poonia, 2023). Transesterification is one of the accepted processes for the production of biodiesel from oils and fats with alcohols in the presence of homogeneous catalysts or heterogeneous catalysts. The homogeneous catalysts have been proven to be more practical in application. The alkaline catalyst is capable of producing higher yield and purity of biodiesel with a reaction time of between 30 and 60 min and limits the FFA content to not more than 1.0%. This has made it only suitable for the processed or refined vegetable oil to be used as the feed material due to its low FFA and purer triglycerides (TG) content compared to the crude vegetable oil (Manish *et al*., 2015).

During the transesterification of crude oils, FFA can easily react with an alkali catalyst, producing soaps and water, and, consequently, the downstream recovery and purification of the product becomes difficult. In this regard, several researchers have reported that for the use of an alkali catalyst, the oil should have lower FFA content (<1.0%). Therefore, acid-catalyzed processes are preferred to reduce the FFA (Yasir *et al*., 2014). Since glycerine is the main byproduct of biodiesel production, the high-acidity oils could be esterified with the glycerine surplus to transform the free fatty acids (FFA) into triglycerides before performing the transesterification (Juan *et al*., 2019). The process involves conducting the acid-catalyzed esterification in the first step for the purpose of lowering the FFA content to an accepted range, followed by the addition of an alkaline catalyst, after the removal of the acid catalyst, to complete the transesterification process. Nevertheless, this process too has drawbacks, where acidic wastewater is produced along with alkyl esters from the esterification reaction. The problem of managing the highly acidic effluent, the difficulty in catalyst recovery processes, and the high cost of stainless steel equipment needed for the acidic reaction media became the main limitations for applying this process more recently (Yasir *et al*., 2014). The two-step conversion process—an acid-catalyzed esterification pretreatment to lower the FFA content, followed by the traditional alkali-catalyzed transesterification is widely used in both industry and laboratory. The acid-catalyzed esterification requires additional acid and methanol usage, but the majority of methanol can be reclaimed through a methanol recovery system, which is now commonly installed by biodiesel manufacturers. A solid organic acid catalyst such as p-toluene sulfonic acid monohydrate (PTSA) can be used for the pretreatment of CCO to produce biodiesel (Adeeb *et al*., 2013). This study introduces CCO as a low-cost feedstock using PTSA to produce biodiesel. PTSA is one of the solid acid catalysts that can be used for many types of reactions, such as esterification of FFA. PTSA is easy to handle, transfer, and store due to its solid formation. Referring to the Advanced Biofuel Center, CJP In recent years there is more concentration on promoting new oilseeds that have a potential effect on producing biofuel and are used in different industrial processes. The main objective of this study is to study the potential of CCO as an industrial feedstock and to evaluate the activity of PTSA and characterize the final biodiesel product.

2. MATERIALS AND METHODS

2.1 Material

Citrullus Colocynthis (L.) seeds were collected from western and northern Sudan. Toluene-4-sulfonic acid monohydrate (PTSA), methanol (anhydrous, purity ≥ 99.8%), KOH (anhydrous, purity ≥ 98%), and analytical reagents (e.g., phenonaphthalen) were used. The seeds were carefully cleaned of dust and traces and dried until a constant weight was obtained. Seeds were extracted through screw press extraction using an oil extractor (TINYTECH RAJKOT INDIA). Then it was filtered.

2.2 Oil Analysis

2.2.1 Moisture Content for the Oil

The moisture content was determined according to the American Oil Chemistry Society (AOCS, 1993), where 5 g of the sample was weighed; after that, it was placed in an oven adjusted to 105°C for 2 hours. The sample was then removed, cooled in a desiccator at room temperature, and weighed. The moisture content was calculated according to the following equation.

$$M.C = \frac{W1 - W2}{W1} * 100 \quad (1)$$

W1: weight of original sample (g) W2: weight of dried sample (g) M.C.: Moisture content

2.2.2 Acid Value and Free Fatty Acid Content

The acid value is determined by directly titrating the oil in an alcoholic medium against a standard potassium hydroxide or sodium hydroxide solution. In this study, 5 grams of oil were weighed into a conical flask and added to 50 mL of neutralized ethanol. The solution was titrated with standardized sodium hydroxide using 2 ml of phenolphthalein as an indicator (with vigorous shaking) to the appearance of the pink color.

The acid value was calculated from the following equation.

$$A.V = \frac{N * V * Mw}{W} \quad (2)$$

A.V: The acid value N: normality of sodium hydroxide
W: weight of oil (g)
V: volume (ml) of sodium hydroxide from titration.
Mw: molecular weight of sodium hydroxide
The percentage of free fatty acid (as oleic acid) was calculated from the following equation.

$$FFA\% = \frac{N * V * 28.2}{W} \quad (3)$$

FFA: The percentage of free fatty acid
28.2: (is the equivalent weight of oleic acid in mili equivalent (meq)

2.2.3 Saponification Value

The saponification value is the number of mg of potassium hydroxide required to saponify 1 gram of oil. The saponification value was determined according to the AOCS official method (cd 3.25, 2003). In this study, 5 grams of the oil sample were weighed into a volumetric flask. Then 50 mL of 1.0N alcoholic KOH was pipetted and allowed to drain for about 1 minute into the mixture. A condenser was connected to the flask, and the mixture sample was allowed to boil gently but steadily for 1 hour to complete saponification. The flask and the condenser were then cooled, but not sufficiently to form a gel; the inside of the condenser was washed down with about 1 ml of distilled water. The condenser was disconnected, and 1 ml of phenolphthalein indicator was added. The solution was titrated with 0.5 N hydrochloric acid (HCl) until the pink color just disappeared. A blank determination was conducted simultaneously with the sample. The saponification value was calculated using the formula below.

$$S.V = \frac{N * (B - S) * 56.11}{W} \quad (4)$$

Where:
56.11: molecular weight of KOH.
S.V: saponification value.
W = weight of sample, (g)
N = normality of hydrochloric acid (HCl)
B = Volume in ml of standard hydrochloric acid required for the blank.
S = Volume in ml of standard hydrochloric acid required for the sample,

2.2.4 Average Molecular Weight

The average molecular weight of oil was determined according to the following equation.

$$Mw = \frac{56.11 * 3 * 1000}{(S.V - A.V)} \quad (5)$$

Where:
56.11: molecular weight of KOH.
S.V: saponification value.
A.V: acid value.

2.3 Two-Step Catalytic Processes

2.3.1 Catalyzed Esterification of CCO

The objective of this step was to reduce the FFA contents of CCO. Before starting the transesterification reaction using p-toluene sulfonic acid monohydrate as an acid catalyst, the experiment was carried out in a 250 ml three-neck round flat-bottom flask equipped with a reflux condenser. The flask was placed on a hot plate equipped with a magnetic stirrer and temperature controller. The CCO was first preheated to remove the moisture (105°C) and then cooled to 60°C for the esterification process. Then, a fixed amount of freshly prepared methanol solution was added to the oil, and the reaction continued for the established times. After the proposed time (105 min), the product formed was separated carefully in a separating funnel, and the layer containing the treated CCO was subjected to alkali-catalyzed hydrolysis. In order to confirm the conversion of FFA during the esterification process, the sampling was done manually, and the acid value was calculated from the acidity reduction using an acid value equation. The acid value was then used to calculate FFA conversion as shown in the following equation.

$$FFA\ conversion = \frac{AVi - AVt}{AVi} \quad (6)$$

AVi : is initial acid value of the CCO
AVt: is the acid value at a "t" time

2.3.2 Base-Catalyzed Transesterification Reaction

The base-catalyzed transesterification reaction was carried out using similar procedures and different conditions; the treated CCO was heated up to 60°C. A solution of potassium methoxide was prepared in a 250 ml beaker using 1 wt.% potassium hydroxide pellets with the molar ratio of 1:6 of oil to methanol. The solution was stirred until the potassium hydroxide pellet was completely dissolved (the mixture was called potassium methoxide solution). The solution was then heated up to 600°C and slowly poured into the treated oil. The mixture was stirred (600 rpm) for 50 min (the complete reaction time). Finally, the mixture was allowed to settle for 24 hours in a separating funnel. Thereafter, the upper layer biodiesel was decanted into a separate beaker, while the lower layer, which comprised glycerol and soap, was collected from the bottom of the separating funnel. To remove any excess glycerol and soap from the biodiesel, hot water was used to wash it, and then it was allowed to remain in a separating funnel until clear water was seen below the biodiesel in the separating funnel. The washed biodiesel sample was then dried by placing it on a hot plate (1050°C) for 1 hour, and excess water was removed from the biodiesel. These batches were taken to achieve a higher yield and to study the effect of these parameters on the yield of methyl ester. Finally, the major properties of biodiesel, such as density at 15°C, kinematic viscosity at 40°C, acid value, flash point, cloud point, and pour point, were obtained according to the American Society for Testing and Materials methods (ASTM).

3. RESULTS AND DISCUSSION

3.1 Physicochemical Properties of CCO

The quality of Citrullus colocynthis oil (CCO) was expressed in terms of selected physicochemical properties, such as moisture content, free fatty acid value, saponification value, etc., as shown in Table 1. The above mentioned properties were measured according to AOCS official methods, and the results were compared with different vegetable oil properties.

3.1.1 Moisture Content of Oil

The moisture content of the extracted CCO was 0.5% (see Table 1). The favorable level of moisture content for base-catalyzed transesterification is below 0.05% wt. However, the moisture content is more than 0.05% wt. will cause the formation of soap or a saponification reaction process, resulting in the consumption of the catalyst, which further will cause emulsion problems during post-processing through water washing, which is required for removing excess catalyst, alcohol, mono-, diglycerol, and free glycerol, and also reduce the biodiesel yield and purity significantly.

3.1.2 Acid Value and Free Fatty Acid

The acid value of the extracted oil was 5.196 mg KOH/g oil (see Table 1). This value was lower than the value (9.6 mg KOH/g oil) reported for CCO by Joledo *et al*., (2015) and higher if compared to the result of Chavan *et al*., (2014), which was 2.30 mg KOH/g oil. The high acid value indicates that a greater quantity of the base will be required to neutralize the acidity of the oil to be transesterified. High acid value also indicates high free fatty acid content. The oil had a significantly high content of FFA (3.6%) evaluated by the AOCS standard titration method, which is higher than the content (>1.0%) permitted in oils to be catalyzed directly by alkali catalysts; therefore, FFA was reduced to 0.3% by acid catalyst esterification, and the final FFA conversion was 91.76%.

3.1.3 Saponification Value

The saponification value of the oil was 186.4 mg KOH/g oil (see Table (1)). However, there are some vegetables with high saponification values, such as coconut oil (253 mg KOH/g), palm kernel oil (247 mg KOH/g), and butterfat (225 mg KOH/g). That oil with high saponification values contains a high proportion of short-chain fatty acids. The high saponification value of Citrullus colocynthis (L.) Shard. Suggests that the oils could be good for soap making and in the manufacture of lather shaving cream (Nzikou *et al*., 2007).

Table 1: Properties of CCO

Properties	Unit	Result
Oil moisture content	%	.617
FFA	%	.6
Acid value		.196
Average molecular weight	g/mol	92.05
Saponification value	mg KOH/g oil	86.4

3.2 Physicochemical Properties of Biodiesel Compared to ASTM and EN Standards

3.2.1 The Yield of Biodiesel

From Fig. 1, the biodiesel produced after washing and drying was found to be 98.51%. The biodiesel yield in this study is generally higher when compared to 98% recorded by Yasir *et al.,* (2014) for Citrullus colocynthis oil with a different catalyst (1,3-disulfonic acid imidazolium hydrogen sulfate (DSIMHSO4)) and also higher than 76.62% recorded by Adeeb *et al.,* (2010) when using the same catalyst (PTSA) for palm oil with high FFA; see Fig. 1. Although the high free fatty acid of oil in this study (see Table 1) The yield of biodiesel was quite high because the use of an acid catalyst for the esterification reaction reduced the free fatty acid to a minimum value before the alkaline transesterification reaction was done. Canakci (2007) has investigated that the conversion rate of soybean oil to biodiesel dropped from 90.84% to 58.77% as the FFA level increased from 3% to 33%. Biodiesel should be dried after water washing to get the water specification below 500 ppm (0.050%). Water in biodiesel could make the fuel go rancid and alter the chemical structure of biodiesel. If moisture is allowed to accumulate for a long time, it will increase the free fatty acid level of biodiesel. Free fatty acids may corrode metal parts in fuel lines. They can also react to make monoglycerides.

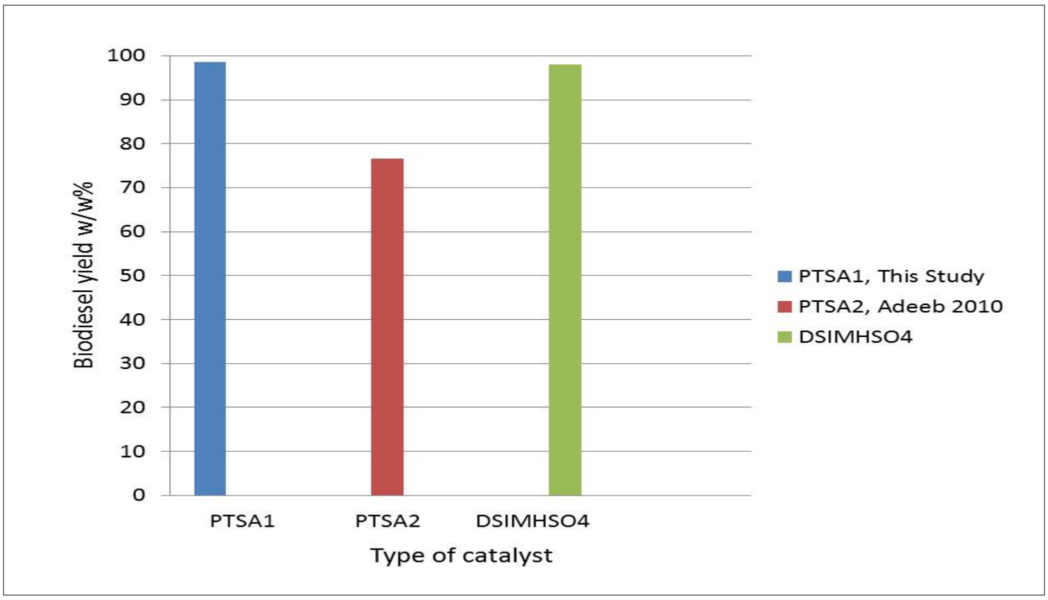

Fig. 1: yield of biodiesel using acid catalyst esterification for CCO. PTSA:p-toluene sulfonic acid monohydrate and DSIMHSO4:1,3-disulfonic acid imidazolium hydrogen sulfate.

4.2.2 Flash Point

From Fig. 2, the flash point of biodiesel yield was 228°C, which is higher than Yasir *et al*., (2014) (174°C) and Ashish *et al*., (2010) (187.5°C). See Fig. 2. The result is good if the biodiesel is compared with the standard in the United States, American ASTMD6751, and the European standard, EN14214. See Table 2. The flash point of biodiesel is higher, which makes it a safer fuel in storage, transport, and handling. The flash point of pure biodiesel is considered higher than prescribed limits, but it can decrease rapidly with increased residual alcohol. As these two aspects are strictly correlated, flash point is used as a regulation for categorizing the transport and storage of fuels with different thresholds from region to region, so aligning the standards would possibly require a corresponding alignment of regulation (Amit *et al*., 2009). The value of the flash point depends on the boiling point, which increases as the molecular weight increases, so the flash point of biodiesel is higher than diesel as a result of increases in the molecular weight of the biodiesel.

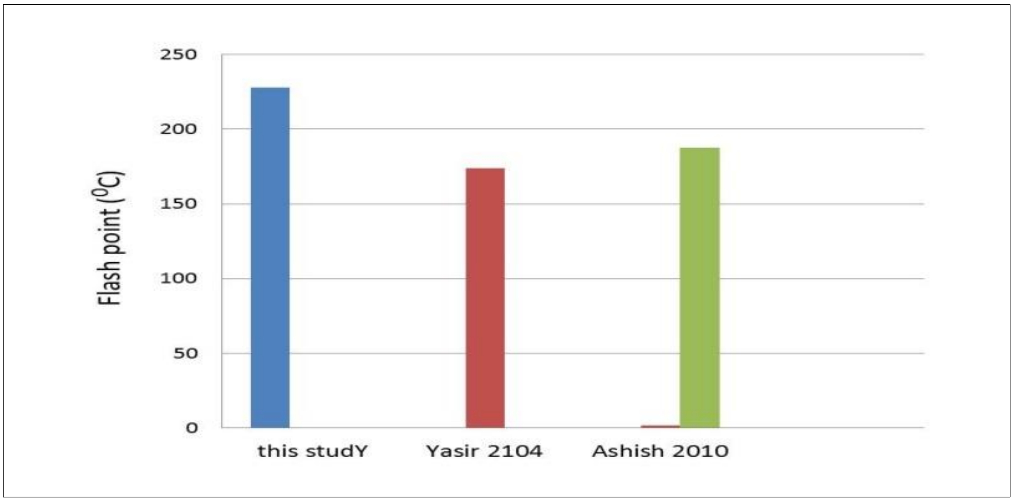

Fig. 2: Flash point of biodiesel produced from CCO

4.2.3 Kinematic Viscosity

From fig. 3, the kinematic viscosity of biodiesel yield was measured at 40°C, and the result was found to be 5.094 cSt, a result within acceptable limits for the ASTM biodiesel standard but slightly higher than the EN specification standard (see Table 2). The result is also slightly higher than biodiesel produced from some vegetable oils recorded by some researchers (Abdel Rahman, 2017, Jatropha oil methyl ester, 4.38 cSt; Chevan, 2014, Citrullus colocynthis methyl ester, 4.78 cSt; Aliyu *et al*., 2013, melon oil methyl ester, 3.83 cSt; Aliyu, 2013, moringa oil methyl ester, 3.68 cSt; see Fig. 3). If a batch of biodiesel yield does not meet this specification, the viscosity can be corrected by blending it with a fuel that has a lower or higher viscosity. Viscosity is greatly affected by temperature; many of the problems resulting from high viscosity are most noticeable under low ambient temperature and cold start engine conditions (Amit *et al*., 2009). Viscosity increases with the chain length of either the fatty acid or alcohol moiety in a fatty ester or in an aliphatic hydrocarbon. The increment in viscosity over a certain number of carbons is smaller in aliphatic hydrocarbons than in fatty compounds (Knothe *et al.,* 2005).

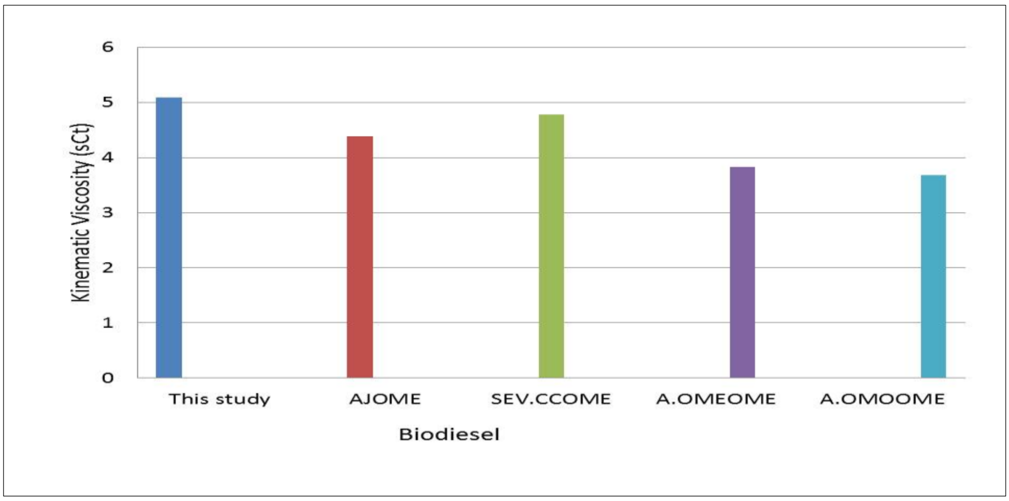

Fig. 3: Kinematic viscosity of biodiesel from some vegetable oils.

4.2.4 Pour Point

From Fig. 4, the pour point of biodiesel yield was found to be -30°C, which is higher than the -80°C of Ashish *et al*., (2010) and lower than the 30°C recorded by Aliyu *et al*., (2013); see Fig. 4. Biodiesel tends to freeze at higher temperatures than petro-diesel. This is one of the major factors hindering the use of biodiesel in cold climatic conditions; the pour point depends mostly on the fatty acid profile of the feedstock and the type and quantity of impurities in the fuel. Impurities such as monoglycerides can greatly increase the pour point. Biodiesel made from saturated fats has a higher pour point than biodiesel made from unsaturated fats. The pour point of biodiesel can be reduced by using a branched-chain alcohol instead of methanol during processing, for example, isopropyl alcohol (Lee *et al*., 1995). However, the use of isopropyl alcohol is more expensive, and the reaction is harder to complete than with methanol. Another way to improve the cold temperature performance of biodiesel is to blend it with another biodiesel with a lower pour point. This is an effective technique for reducing the pour point. (Moser, 2008).

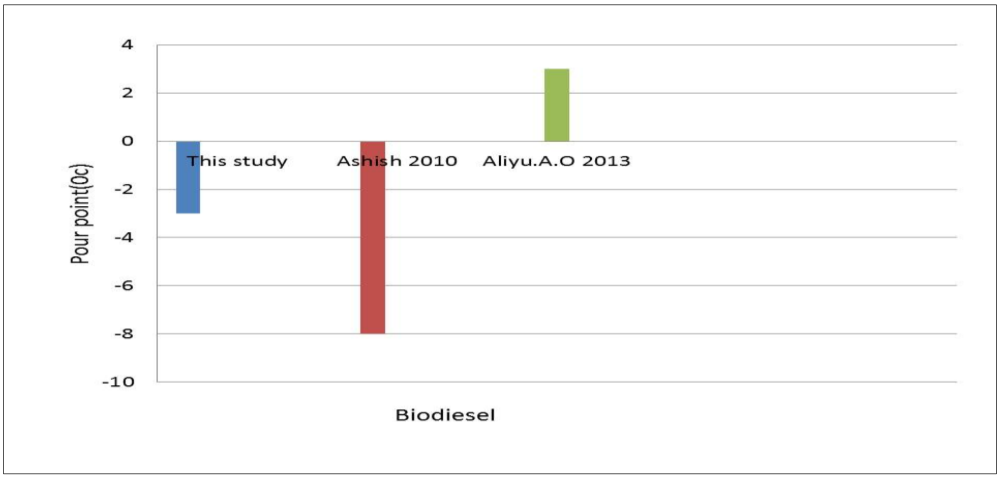

Fig. 4: Pour point of biodiesel from the same feedstock (Citrullus colocynthis).

4.2.5 Density

From table 2, the density of biodiesel yield at 15°C was 0.878 g/cm³, and the standard value of biodiesel density at 15°C (ASTM) is in the range of 0.860–0.900 gm/cm³. See Table 2. The measured value of biodiesel density agrees with the range of standard values of density of biodiesel. The density of biodiesel varies depending on its feedstock; biodiesel is denser than diesel because biodiesel has a higher degree of unsaturation. The degree of unsaturation of the molecule is an indicator of the number of double bonds present in its fatty acid chain; a higher number of double bonds represents a higher degree of unsaturation. Density increases as the degree of unsaturation increases.

Table 2: Physicochemical properties of biodiesel compared to ASTM and EN standards

Test	Result	Limits		Test methods	
		ASTM D6751	EN 14214	ASTM D6751	EN 14214
Flash point (^{0}C)	228	130 min	101 min	D93	ISO CD3679e
Kinematic Viscosity @ 40 ^{0}C (cSt)	5.094	1.9-6	3.5-5	D445	EN ISO 3204
Pour point (^{0}C)	-3	-	-	D 97	-
Density @15^{0}C (g/cm^2)	0.8874	-	0.860 – 0.900	D4052	ENISO3675

5. CONCLUSIONS

The results of the research showed that the Citrullus colocynthis oil has potential for use as an industrial feedstock for biodiesel production. P-toluene sulfonic acid monohydrate can be used as a catalyst in the pretreatment of Citrullus colocynthis oil via the esterification reaction, where it showed very high catalytic activity to reduce the free fatty acids (FFA) content in the CCO; also, it has less of an environmental impact due to its ease of recoverability and reusability. The results showed that the FFA content of CCO was reduced from 3.6% to 0.3%. The highest yield of biodiesel was recorded after the transesterification reaction and purification process (98.51%), with a 228°C flash point, a -3°C pour point, a 0.8874 g/cm² density at 15°C, and a 5.094°C st kinematic viscosity. Finally, the results attained in this study conformed to the international standard specifications for biodiesel fuel.

Highligts

- Use *the Citrullus Colocynthis (L.)* plant as an alternative, valuable choice in producing biodiesel.
- The attention was drawn to the *Citrullus Colocynthis (L.)* due to the plant having the advantage of needing less water compared to other plants.
- The use of p-p-toluene-4-sulfonic acid monohydrate (PTSA) as an acid catalyst for pretreatment of *Citrullus Colocynthis (L.)* is the first time to use the acid as a catalyst with *Citrullus Colocynthis (L.)* oil to produce biodiesel.

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