Nigerian Research Journal of Engineering and Environmental Sciences 7(1) 2022 pp. 147-155
p ISSN: 2635-3342; e ISSN: 2635-3350

Nigerian Research Journal of Engineering and Environmental Sciences
Journal homepage: www.rjees.com

Original Research Article

Prevention of Biodeterioration of Biodiesel Produced from Castor Seed Oil via the use of Antimicrobial Agents

Obeni, M.P., *Esenogho, G.O., Amenaghawon, N.A. and Obahiagbon, K.O.

Department of Chemical Engineering, Faculty of Engineering, University of Benin, Benin City, Nigeria.
*godstimeesenogho@gmail.com; obeniprecious24@gmail.com

http://doi.org/10.5281/zenodo.6722444

ARTICLE INFORMATION

Article history:
Received 16 May, 2022
Revised 13 Jun, 2022
Accepted 14 Jun, 2022
Available online 30 Jun, 2022

Keywords:
Castor oil
Biodeterioration
Biodiesel
Mitracarpus scaber
Ethanol

ABSTRACT

This research is based on the investigation of the possible use of antimicrobial agents such as ethanol and extracts from locally sourced Mitracarpus scaber to prevent biodeterioration of biodiesel. Four castor oil biodiesel samples under various treatment conditions (containing different volumes of antimicrobial agents) and a control (without antimicrobial agents) were monitored for five weeks to check for likely variation in some of the biodeterioration indicating parameters such as acid value (AV), flash point (FP) and cetane number (CN). Results obtained for the control experiment indicated biodeterioration of the biodiesel sample as the presence of microbial activity was noticed from the variation in the AV, FP, and CN. Results obtained also showed that microbial proliferation was mitigated when antimicrobial agents were introduced to the biodiesel samples as seen in the most stable values of acid value, flash point, and cetane number. Of all the samples analyzed, sample A containing the highest percentage of Mitracarpus scaber extract performed best as can be seen from the most stable values of acid value, flash point, and cetane number which are some of the most important biodeterioration indicating parameters of biodiesel, which therefore indicates the introduction of stability in the biodiesel samples.

1. INTRODUCTION

Since the beginning of civilization, the curiosity of men has guided men to always want to know and explore the diverse and numerous potentials inherent in the universe and harness them to produce unique products invaluable to humanity (Timmons *et al.*, 2014). One of these innovative ideas is the exploration and the use

of hydrocarbon compounds as a source of energy. Over the years, it has become so clear that the consumption and utilization of these hydrocarbon compounds, especially the long chain compounds, pose a great threat and danger to the ecosystem, as these compounds are known to be the bedrock of greenhouse gases, which contribute tremendously in air pollution, and so many other adverse effects (Ayoola *et al.*, 2016). Biodiesel, which is another innovation of man, was brought to the fore as an alternative source of energy because of its cleaner and biodegradable tendencies (Zimmer *et al.*, 2013). Biodiesel is a compound formed by the chemical reaction of vegetable oils or animal fats with an alcohol, usually methanol, in the presence of a catalyst, usually a strong base, such as potassium hydroxide (KOH) or sodium hydroxide (NaOH) (Ma and Hanna, 1999). Alcohols such as ethanol and propanol can also be used for biodiesel production, but methanol is preferable due to its relatively low cost and its physical and chemical advantages (polar and shortest chain alcohol) (Fukuda, Kondo and Noda, 2001). It can quickly react with triglycerides, and NaOH is easily dissolved in it. In the same vein, acid catalysts can still be employed, but oftentimes, the reaction is very slow compared to when alkali catalysts are used (Knothe et al., 2005).

However, just like hydrocarbon compounds, biodiesel comes with some of its disadvantages, albeit relatively lenient. It is known to be highly hygroscopic, as such, water tends to accumulate especially during storage. This causes the breeding and proliferation of microorganisms which could aid in lowering the quality and sustainability of its uses, a problem known as the biodeterioration of biodiesel (Santos *et al.,* 2016). In a broader context, biodeterioration is any undesirable change in the quality of a material caused by agents of microbiological origin. The change typically results from a breakdown in the structure of the material in question (Obahiagbon and Amenaghawon, 2014). This is a serious problem because, if neglected, it could favor the causation of corrosion, clogging, bio-sediments deposition, unpleasant smell, etc. (Santos et al., 2016).

A lot of interventions have been made to combat this major drawback of biodiesel some of which include the introduction of biocides, antioxidants, and other chemical substances (Karavalakis *et al.*, 2011). But research has shown that some of these chemicals are non-biodegradable and thus toxic to the environment. So, attention has shifted to other eco-friendly sources and means by which biodeterioration can be mitigated. The potential use of a locally sourced plant known as *Mitracarpus scaber*, on which this research work is based, is highly beneficial because of its antimicrobial and biodegradable tendencies (Abere *et al.*, 2007)

Mitracarpus scaber is a perennial, annual herb of about 30 cm tall or much smaller and possesses rough leaves. In Nigeria, it is known as Obuobwa in the Igbo language, Gududal in the Hausa language, and Irawo lle in the Yoruba Language (Abere, Onwukaeme and Eboka, 2007). It is claimed that the plant has both antibacterial and antifungal activities (Obahiagbon and Amenaghawon, 2014). In Nigeria, the juice from the crushed plant is known to be applied topically for the treatment of skin diseases such as ringworm, lice, itching, and other fungi diseases or applied to dressings for fresh cuts, wounds, and ulcers. It is also used as an ingredient in fish poison by some pagan tribes (Abere et al., 2007). Thus, the aim of this study is to investigate and compare the effects of locally sourced *Mitracarpus scaber* and commercially available ethanol as anti-microbial agents in preventing biodeterioration of biodiesel.

2. MATERIALS AND METHODS

2.1. Material Collection and Preparation

All the reagents used in this research work were of analytical grade and were used without further purification. Methanol, ethanol, and castor oil were obtained from a local chemical store in Benin City, Edo State of Nigeria. The plant was sourced locally in Benin City, Nigeria, identified and authenticated as *Mitracarpus scaber* by the plant curator of the Herbarium, Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City, Nigeria. The fresh plant in its entirety was sun-dried, and its surface area was increased by grinding into powder after which, it was stored in a dry and well-stoppered bottle. The Soxhlet extraction method was adopted to extract the contents of the milled plant using methanol

as the solvent. The solvent used in each batch was recovered under pressure until dry extracts were obtained and then stored in a well-stoppered bottle.

2.2. Methods

2.2.1. Biodeterioration inhibition studies

Characterization of the biodiesel produced was done on day zero (before the introduction of antimicrobial agents) and subsequently at intervals of seven days (one week) for a total of 35 days (five weeks). The antimicrobial agents (ethanol and *Mitracarpus scaber* extract) formulated were tested for the ability to control microbial contamination in biodiesel. Biodeterioration inhibition studies of the chosen antimicrobial agents were carried out in five closed vessels. The content of the vessels is listed in Table 1. The samples were monitored for five weeks for biodeterioration indicating parameters such as acid value, flashpoint, and cetane number.

Table 1: Composition of biodiesel samples for biodeterioration experiments

Biodiesel samples	Content (ml)		
	Biodiesel	Ethanol	*Mitracarpus scaber*
Control	115	0	0
Sample A	115	0	6
Sample B	115	6	0
Sample C	115	3	3
Sample D	115	2	4

2.2.2. Determination of specific gravity

A 50 ml pycnometer was washed thoroughly with detergent and water, dried, and then weighed (W_0). The bottle was then filled with the biodiesel sample and weighed (W_1), and finally, the bottle was emptied and washed thoroughly and was filled with water, and weighed (W_2). The specific gravity was then calculated from Equation 1, which is equal to the mass of a substance per mass of an equal volume of water (Fasina and Colley, 2008)

$$Specific\ \ gravity = \frac{(W_1 - W_0)}{(W_2 - W_0)} = \frac{mass\ of\ substance}{mass\ of\ an\ equal\ volume\ of\ water} \quad (1)$$

2.2.3. Determination of acid value

One gram of COME was measured and poured into a conical flask. Ten milliliters each of benzene and ethanol was measured each of benzene and ethanol was measured and poured into the beaker containing the oil sample. The mixture was shaken vigorously for some seconds. Two drops of phenolphthalein indicator were added to the sample and were titrated against 0.05M KOH till the color turned pink and persisted for 15 s. The acid value of the sample was determined using Equation 2.

$$Acid\ value = \frac{56.1 \times V \times N}{W} \quad (2)$$

Where AV= acid value, V= volume of standard alkali used, N= normality of standard alkali used and W= weight of biodiesel used.

2.2.4. Determination of saponification value

One gram (1 g) of biodiesel sample was weighed into a conical flask, and 25 ml of 0.1 N ethanoic potassium hydroxide was added. The mixture was constantly stirred and allowed to boil gently on a heating mantle for 60 min. A reflux condenser was placed on the flask containing the mixture. Few drops of phenolphthalein indicator were added to the warm solution and titrated with 0.5 M HCl to the endpoint until the pink color

of the indicator disappeared. The same procedure was used for other samples and blank (Al-harbawy and Al-mallah, 2014). The saponification value was calculated using Equation 3.

$$Saponification\ value = \frac{28.05\times(b-a)\times N}{W} \quad (3)$$

Where b = volume of standard ethanol potassium hydroxide used in blank titration, a = volume of standard ethanol potassium hydroxide used in titration with the biodiesel sample and W = weight of biodiesel sample.

2.2.5 Determination of iodine value

One gram (1 g) of the biodiesel sample was weighed and poured into a glass-stopper bottle of about 250 ml capacity. Ten millilitres of chloroform was added to the oil, and the mixture was warmed with a heating mantle for a few minutes and was allowed to cool down. Twenty-five millilitres (25 ml) of Wij's solution was added, and a stopper was inserted and allowed to stay in the dark for 30 mins. Ten millilitres (10 ml) of potassium iodide solution (10%) was introduced, and the mixture was thoroughly mixed and titrated with 0.1M sodium thiosulphate solution until the yellow color almost disappeared. Few drops of the starch indicator were then added and the titration continued by adding sodium thiosulphate dropwise until the blue coloration disappeared after vigorous shaking. The same procedure was used for the blank test and was carried out at the same time, starting with 10 ml of carbon tetrachloride (titration = 'B' ml) (Asmare and Gabbiye, 2014). The iodine value (IV) was calculated from Equation 4.

$$IV = \frac{0.1269\times(B-A)\times N\times 100}{W} \quad (4)$$

where B = volume of sodium thiosulphate used in the blank titration, A = volume of sodium thiosulfate used in titration with biodiesel sample, N= normality of sodium thiosulphate and W= weight of biodiesel sample used.

2.2.6. Determination of viscosity

A viscometer was used to determine the viscosity of the oil. Chloroform was poured first into the viscometer and the time at which the chloroform reached the bottom of the equipment was taken. The oil was then poured into the viscometer, and the viscosity of the sample was recorded in mm^2/s.

2.2.7. Flash point determination

The flashpoint of biodiesel was measured by a flash point tester, which consists of a 75 ml closed copper cup, heater, and a source that gives continuous sparks. The continuous sparks source consists of a battery connected to a small engine, disporater, coil, and spark plug. The engine is used to rotate a disporater, which is used to fractionate the current to electrical pulses. A coil is used to amplify the electrical pulses, and a spark plug is used to create sparks inside the cup. Biodiesel sample is heated and the vapor accumulated inside the cup, at the moment that the vapor was sufficient to ignite the flashlight is noticed, and the temperature is measured (Eman, 2015).

2.2.8. Determination of higher heating value (calorific value)

The higher heating value (HHV) of the castor oil and its biodiesel was determined using the empirical formula in equation suggested by Demirbas (1998).

$$HHV = 0.021 \times FP + 32.12 \quad (5)$$

where FP = Flashpoint

2.2.9. Cetane number estimation

The cetane number of the biodiesel was determined using the empirical formula suggested by Kalayasiri et al. (1996), using the result of saponification number and iodine value of the biodiesel in Equation 6.

$$CN = 46.3 + \frac{5458}{SN} - 0.225 \times IV \quad (6)$$

2.2.10. Production of Biodiesel from Castor Oil

The method adopted by Abdulkareem et al. (2016) was employed in the production of biodiesel from castor oil by the trans-esterification reaction. A reaction temperature of 65 ℃ was used throughout the reaction process. An alcohol/oil molar ratio of 6:1 was used for optimum yield. The agitation speed was between 600-1000 rpm and the reaction time was 1 h. A 1wt. % of KOH catalyst was used to catalyse the biodiesel oil production process (Akhabue and Okwundu, 2017). The transesterification reaction of castor oil was carried out in a 1000 ml round-bottom flask with the aid of a constant-temperature magnetic stirrer (Model No. HJ – 3D). Briefly, 740 g of the castor oil was weighed into the round-bottom flask and placed on the constant-temperature magnetic stirrer, and the oil was heated to 65 ºC. The methoxide mixture containing a known amount of KOH in methanol was added to the oil when the oil had attained the desired temperature, and the magnetic stirrer was switched on. At the end of the reaction, the mixture was transferred to a separating funnel and allowed to separate. Two layers were formed after separation; the lower part, which contains glycerol, was drained off, while the upper biodiesel (COME) layer was purified by washing with warm water to remove traces of soap, KOH, glycerol, or methanol that may still be present in the biodiesel. The washing process was repeated until the pH of the used wash water was almost neutral. The washed COME was thereafter heated in an oven at 100 ºC to remove traces of water that might still be present. The resulting COME recovered was weighed, and the COME yield was calculated using Equation 7 (Akhabue and Okwundu, 2017).

$$\text{COME yield} = \frac{Mass\ of\ COME\ recovered\ (g)}{Mass\ of\ oil\ used} \times 100 \quad (7)$$

3. RESULTS AND DISCUSSION

3.1. Base Properties of Castor Oil and Castor Oil Biodiesel

The properties of the castor oil and produced biodiesel are presented in Table 2. The acid value of the biodiesel was found to be 0.55 mgKOH/g. The result indicates that the acid value of the castor oil (1.683 mgKOH/g) decreased significantly after the transesterification reaction. Higher acid value results in a low yield of biodiesel and affects the storage stability of biodiesel by contact with air and water. The biodiesel has acid value within the standard specification limit of max 0.8 in ASTM D664 (Asmare and Gabbiye, 2014). Transesterification slightly reduced the iodine value of the castor oil as is seen in Table 2. The measured iodine value for both the biodiesel and castor oil fell within the EN14124 standard, which is a maximum of 120 mgI_2/100g of oil. This low iodine values indicate that there are less or few double bonds present in the castor oil and this affects the quality of the biodiesel by reducing the potential to polymerize in an engine and hence higher stability (Kotb et al, 2016; Taghizade, 2016). The flashpoint of the biodiesel was 196 ºC which is significantly higher than the limit of the ASTM standard at 130 ºC. This indicates that biodiesel is safe during handling and storage. The value of flash point depends on the boiling point which increases as the molecular weight increases; hence the flash point of biodiesel is higher than diesel as a result of increase in the molecular weight of the biodiesel (Sivaramakrishnan and Ravikumar, 2014). Biodiesel has lower energy content (heating value) than conventional diesel fuel. The result obtained (36.74 MJ/kg) is nearly in the range of ASTMD6751 for diesel oil. The viscosity and the density of the biodiesel were noted to be within acceptable limits in comparison to that of diesel fuel and ASTM standards. The cetane number was found in the range of the accepted standard of EN 14214. A higher cetane number causes shorter ignition delays, and thus, higher efficiency in the engine (Al-harbawy and Al-mallah, 2014).

Table 2: Properties of castor oil and produced biodiesel

Property	Value	
	Biodiesel	Castor oil
Density	860 kg/m^3	925 kg/m^3
Acid value	0.55 mgKOH/g	1.683 mgKOH/g
Kinematic viscosity @ 250C	4.2 mm^2/s	7.6 mm^2/s
Saponification value	230.01 mgKOH/g	173.91 mgKOH/g
Iodine value	36 mgI_2/100g	38 mgI_2/100g
Flash point	200 ^{o}C	-
Higher heating value	36.74 MJ/kg	-
Cetane Number	61.93	-
Percentage yield	71.88%	-

3.2. Stability Studies

3.2.1. Acid value

The acid value is a measure of the number of acidic substances in fuel. During storage, micro-organisms used the moisture present in the biodiesel tank to attack the methyl esters which results in the hydrolysis of methyl esters and eventually leads to an increase in acid value as seen in Figure 1 (Dodos *et al.*, 2012). Another reason for the acid value increment is the fact that micro-organisms used the oxygen present in the biodiesel to react with the double bonds of unsaturated methyl esters to form hydroperoxide compounds (ROOH) through an oxidation process. The hydro-peroxide produced from the oxidative deterioration can undergo complex secondary reactions such as splitting into more reactive aldehydes, which further oxidize into acids, leading to an increase in acid value (Dodos *et al.*, 2012). It can be seen Figure 1 that the greatest degree of variation of acid value was recorded for the control experiment in which no antimicrobial agent was added. This shows that the indigenous micro-organisms present in the biodiesel were actively degrading the organic content of the biodiesel as reported by Obahiagbon et al. (2014). When antimicrobial agents were added to the biodiesel sample, some level of stability was observed as seen in Figure 1. However, the greatest level of stability was observed for sample A (containing 6 ml of *Mitracarpus scaber* extract only indicating that the *Mitracarpus scaber* extract was able to inhibit the action of the indigenous microbes initially present in the biodiesel sample which corresponds to the work done by Obahiagbon et al. (2014). This is also in line with the work of Abere et al. (2007) who reported that *Mitracarpus scaber* extract possesses antimicrobial activity that enables it to inhibit the action of micro-organisms such as bacteria and fungi which are typically found in biodiesel.

3.2.2. Flash point

The flashpoint temperature of biodiesel fuel is the minimum temperature at which the fuel will ignite on the application of an ignition source. This property is mostly considered important during storage and handling. The higher the flashpoint values, the safer it is to handle and store biodiesel. Figure 2 represents the variation of a flashpoint with time. It can be seen that the flash point showed a decrease in value due to fuel aging by microorganisms (Wahyudi *et al.*, 2020). It can also be seen that sample B which contains 6 ml of ethanol showed the largest decrease in flashpoint value from a value of 200 ^{o}C in the first week to a value of 68 ^{o}C in the fifth week which is entirely off in a range of standard values. This is so because the flashpoint of pure biodiesel is considered higher than prescribed limits but can decrease rapidly with increase in residual alcohol. Sample C, which contains 3 ml of ethanol and 3 ml of *Mitracarpus scaber*, also showed a decrease in values from 180 ^{o}C in the first week to a value of 98 ^{o}C in the fifth week. Sample D, which contains 2 ml of ethanol and 4 ml of *Mitracarpus scaber*, also showed a reduction in flashpoint point value from 182 ^{o}C in the first week to a value of 112 ^{o}C in the fifth week. There was also a slight decrease in value in the Control sample, but the flashpoint value is almost constant with time as can be seen with Sample A, which contains

6 ml of *Mitracarpus scaber*. This means that *Mitracarpus scaber* is more effective and maintains a greater degree of stability of flashpoint than ethanol.

3.2.3. Cetane number

The cetane number is a measure of the ignition performance of a biodiesel fuel obtained by comparing it to reference fuels in a standardized engine test. A fuel with good ignition quality has a high cetane number, in which the ignition delay period between the start of fuel injection and the outset of auto-ignition is short. From the different cetane values shown in Figure 3, it can be seen that there was a decrease for all samples during the five weeks, with Sample A which contains 6 ml of *Mitracarpus scaber* showing the slightest decrease in value. It can also be observed that both *Mitracarpus scaber* and ethanol maintain some levels of stability in the cetane number as can be noticed from samples A, B, C, and D. Although, Sample A which contains 100% of *Mitracarpus scaber* maintained the cetane number at almost a constant value throughout the five weeks and shows the highest level of stability. This confirms the fact that *Mitracarpus scaber* is a good antimicrobial agent compared to ethanol. The high level of stability observed in the cetane number of sample A could be attributed to the inactivity of micro-organisms which could have resulted from the introduction of the antimicrobial *Mitracarpus scaber* extract (Obahiagbon et al., 2014).

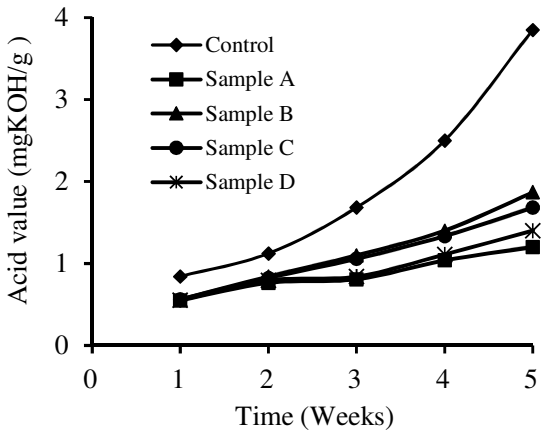

Figure 1: Variation of acid values with time

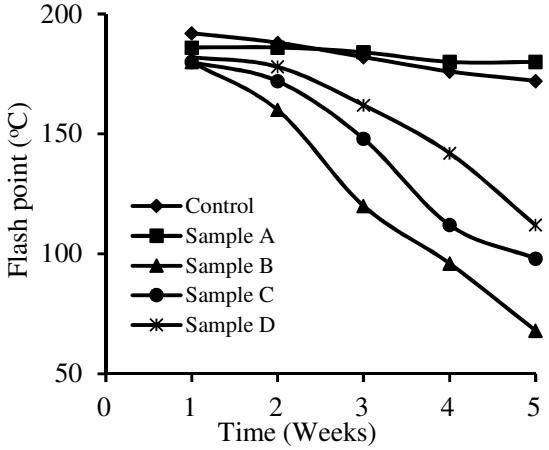

Figure 2: Flash point variation with time

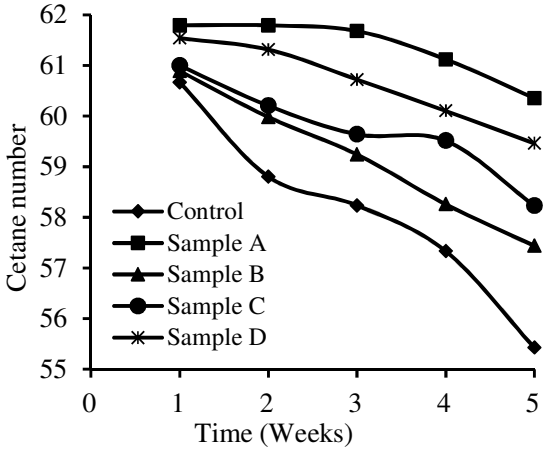

Figure 3: Cetane number variation with time

4. CONCLUSION

This study investigated the effect of two antimicrobial agents (ethanol and extract from *Mitracarpus scaber*) on castor oil biodiesel. This was done by checking the changes in the acid value, cetane number, and flashpoint properties of the biodiesel weekly for five weeks. For best performance, 6 ml of *Mitracarpus scaber* extract corresponding to sample A is recommended based on the results obtained. This study has shown that extract from locally sourced *Mitracarpus scaber* can be used to mitigate biodeterioration in biodiesel.

5. ACKNOWLEDGMENT

The authors wish to acknowledge the assistance and contributions of the laboratory staff of Department of Chemical Engineering and Department of Plant Biology and Biotechnology (PBB), University of Benin, Benin City toward the success of this work.

6. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

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