Effect of Fruit Maturity Stage on Some Physicochemical Properties of Jatropha Seed Oil and Derived Biodiesel

Mbako Jonas,* Clever Ketlogetswe, and Jerekias Gandure

ABSTRACT: The quality of a feedstock in biodiesel production is of paramount importance, and Jatropha seed oil is no exception. This study investigates the influence of the fruit maturity stage on the physicochemical properties of Jatropha seed oil and the derived biodiesel. Free fatty acid content, peroxide value, moisture content, density, and kinematic viscosity are some of the important quality parameters of oil and biodiesel. Results from this investigation have revealed that free fatty acid content and peroxide value of seed oil varies as Jatropha fruits mature from green to brown dry. The free fatty acid content in Jatropha seed oil increases continuously with seed maturity following the three-order polynomial trend. The free fatty acid content in Jatropha seed oil from the investigated geographical locations in Botswana ranges from 0.2 to 0.7% for the four different fruit maturity stages. Similarly, the peroxide value of Jatropha seed oil increases gradually and linearly with fruit maturity. The peroxide value of Jatropha seed oil ranges from 1.2 to 3.7 mEq/kg oil, while that of derived biodiesel ranges from 2.1 to 4.4 mEq/kg oil during the four different fruit maturity stages. However, the variation of density and kinematic viscosity of both Jatropha seed oil and derived biodiesel with fruit maturity is insignificant. Moisture content in Jatropha seeds varies as fruits mature from green to brown dry.

1. INTRODUCTION

There is growing interest in biodiesel production in an effort to reduce greenhouse gas emissions generated from the combustion of fossil fuels. Biodiesel is environmentally friendly and has numerous advantages such as being renewable, biodegradable, and nontoxic and having reduced emissions by 53–70% when compared with petro-diesel. Several feedstocks can be used in the production of biodiesel. However, some of these feedstocks are of low quality and therefore require pretreatment prior to conversion to biodiesel. This pretreatment of the feedstock increases the overall production cost of biodiesel and hence the cost of biodiesel. Free fatty acid (FFA) content and peroxide value (PV) are some of the important quality parameters of oil and biodiesel. Free fatty acids (FFAs) are fatty acids liberated from their ester linkage, and they are unattached to any glycerol present in the oil. Peroxide value (PV) is a measure of primary oxidation (rancidification) of oils and biodiesel and is mainly influenced by factors such as degree of unsaturation of fatty acids. Quality of feedstock in terms of physical and chemical properties is an important factor in biodiesel production. Atadashi et al. revealed that the main determinants of feedstock quality in biodiesel production are water content, free fatty acid (FFA) content, and saturation level or fatty acid profile. In the present investigation, Jatropha seed oil is being considered as a feedstock for biodiesel production. Jatropha has numerous advantages as a feedstock for biodiesel production, one of which is that it is inedible and therefore has minimal competition with food demand as is the case with other feedstocks such as sunflower oil, rapeseed, and soybean oil. One of the challenges in producing biodiesel from Jatropha seed oil is the presence of high level of free fatty acids. In some parts of the world, several researchers who used Jatropha seed oil from the final maturity stage (brown dry) reported high levels of free fatty acids ranging from 4 to 14% in Jatropha seed oil. These free fatty acids react with the alkaline catalyst during biodiesel production to produce soaps that makes separation difficult. Side reactions are undesirable during biodiesel production because they reduce biodiesel yield. Therefore, Jatropha seed oil has to undergo pretreatment to reduce the level of FFA to allowable limits of below 3%. This pretreatment of seed oil comes at a cost, which then increases the overall cost of biodiesel production. This was also echoed by Jayasinghe, who also investigated the pretreatment of oil during biodiesel production. Density and viscosity are some of the important quality parameters of a biofuel. They influence the injection and atomization of biodiesel fuel in an engine. According to Boz et al. and Allah, higher density values of...
Figure 1. Variation of peroxide value in seed oil and derived biodiesel with fruit maturity of Jatropha harvested in (a) Thamaga, (b) Maun, (c) Mmadinare, and (d) Shashe areas.

Figure 2. Variation of free fatty acids content in seed oil with fruit maturity of Jatropha harvested in (a) Mmadinare, (b) Thamaga, (c) Maun, and (d) Shashe areas.
fuel makes injection and atomization of the fuel into the engine difficult, resulting in decreased engine performance. High viscosity of vegetable oils or fats leads to operational problems such as blockage of pipes and fuel injectors, difficulty in pumping, and engine deposits when used in diesel engines.3,5,15,16 Therefore, it is quite essential to reduce the viscosity of vegetable oils before being used in diesel engines.

Harvesting Jatropha seeds at a prime maturity stage is one of the main factors that can improve the quality of seed oil in biodiesel production. This study, therefore, investigates how maturity of Jatropha fruit/seeds affect some quality parameters including free fatty acid content, peroxide value, density, and kinematic viscosity of Jatropha seed oil and derived biodiesel.

2. RESULTS AND DISCUSSIONS

2.1. Effect of Fruit Maturity on Peroxide Value of Seed Oil and Derived Biodiesel. The peroxide value (PV) of Jatropha seed oil ranges from 1.2 to 3.7 mEq/kg oil, while that of derived biodiesel ranges from 2.1 to 4.4 mEq/kg oil across the four different fruit maturity stages as depicted in Figure 1. The PV of biodiesel is 30% greater than that of seed oil. Analysis of variance test results revealed that the effect of fruit maturity on Jatropha seed oil and derived biodiesel is statistically significant since the significance values are less than 0.05, as shown in Table 1. Furthermore, the F-values are greater than 4.07, indicating that the influence of fruit maturity on peroxide value is statistically significant. The PV of Jatropha seed oil increases gradually and linearly as fruits mature from green yellow to brown dry. The PV of biodiesel derived from the seed oil follows the same trend. This trend is similar for all the four different geographical locations under investigation, as shown in Figure 1. These results revealed that Jatropha seed oil experience some level of oxidation during maturation of the fruit/seed. By the time the fruit reaches its final maturity stage (brown dry), the seed oil will have already reached a certain level of oxidation. This trend is similar to the one reported by Desouky et al.,19 who investigated the influence of fruit maturity stage on the peroxide values of Arbequina, Bouteillan, and Koroneiki cultivars oil. They found out that the peroxide values of extracted oils increased as fruits matured from green to purple and increased further in black fruits. Sohaemy et al.20 also reported similar findings while investigating the effect of olive fruit maturity on oil peroxide value. They observed a significant increase in peroxide value of olive oil (0.1−11.9 mEq/kg oil) as fruits matured from green to black. There is currently no regulation by international standards on maximum peroxide value of both seed oil and biodiesel. However, increase in PV of both seed oil and biodiesel results in deterioration of feedstock and fuel quality. Increase in the PV of seed oil and biodiesel results in increase in viscosity, increase in cetane number, and increase in corrosiveness of the fuel21−23. Therefore, using biodiesel with relatively high peroxide value may result in corrosion of both the fuel system and engine components. Seed oil and biodiesel from early stages of fruit maturity (green yellow and yellow) have relatively low PV as demonstrated in Figure 1, indicating a relatively better quality.

2.2. Effect of Fruit Maturity on Free Fatty Acid Content. Free fatty acid (FFA) content in Jatropha seed oil at four different fruit maturity stages are presented in Figure 2 for Jatropha harvested in Mmadinare, Thamaga, Maun, and Shashe areas. Analysis of variance revealed that the effect of fruit maturity on FFA content in all of the four geographical locations is statistically significant since the significance values are less than 0.05, as shown in Table 1. Furthermore, the F-values are greater than 4.07, indicating that the influence of fruit maturity on FFA content is statistically significant. The FFA content of Jatropha seed oil increases gradually and linearly as fruits mature from green yellow to brown dry. The FFA content of biodiesel derived from the seed oil follows the same trend. This trend is similar for all the four different geographical locations under investigation, as shown in Figure 1. These results revealed that Jatropha seed oil
are less than 0.05 as shown in Table 1. Furthermore, the F-values are greater than 4.07, indicating that the influence of fruit maturity is statistically significant. Free fatty acid content in Jatropha seed oil from the investigated geographical locations in Botswana ranges from 0.2 to 0.7% for the four different fruit maturity stages. Seed oil derived from green yellow and yellow fruits contained relatively low FFA content while seed oil derived from brown dry fruits contained relatively high FFA content. The results in Figure 2 suggest that free fatty acids in Jatropha seed oil increase as the fruit matures from green yellow to brown dry. This observation suggested that as the fruit matures, more fatty acids get liberated from their ester linkage with the parent triglyceride molecule. The fatty acids get liberated or freed from the glycerol molecule and exist as free fatty acids instead of triglycerides. Free fatty acid content in Jatropha seed oil follows a three-order polynomial trend, as shown in Figure 2. They increase continuously during the entire maturation stages. There are no international regulations on the maximum FFA content in the feedstock for biodiesel production. However, previous researchers have successfully converted oil with up to 3% free fatty acid content via homogeneous base transesterification. Therefore, Jatropha seed oil from all the four maturity stages in the present study can be converted to biodiesel using homogeneous base transesterification. Several authors including Berrchmans and Hirata, and Jayasinghe et al. reported that higher levels of free fatty acids reduce methyl ester yield during the transesterification process. Therefore, oil with the least FFA content will be preferred for biodiesel production. The level of free fatty acids in Jatropha seed oil increased with the increase in maturity stage. This particular trend of continuous increase of the FFA content during maturation of Jatropha fruits has not been reported in previous studies.

2.3. Effect of Fruit Maturity on Density of Seed Oil and Derived Biodiesel. Variation in density of both Jatropha seed oil and derived biodiesel with fruit maturity is insignificant as indicated in Figure 3. The densities seem to be not affected much by fruit maturity. Density of Jatropha seed oil is 0.91 g/cm³ and that of biodiesel is 0.87 g/cm³. After converting seed oil to biodiesel, the density drops by 0.04 g/cm³, which is equivalent to 4.4%. Analysis of variance indicated that the effect of fruit maturity on density of both seed oil and derived biodiesel in all the four geographical locations is statistically insignificant since the significance values are greater than 0.05 as shown in Table 1. The European standards (EN ISO 3675, EN ISO 12185) specifies density of 0.86–0.9 g/cm³ for biodiesel. The American Society for Testing and Material (ASTM) standard has no regulation on biodiesel density. Jatropha biodiesel density at all fruit maturity stages is well within the standards specifications as depicted in Figure 4. The density of seed oil from all stages of fruit maturity is above the maximum limit set by European standards. This implies that using seed oil in diesel engines might have a negative impact in the atomization of the fuel as reported elsewhere.

2.4. Effect of Fruit Maturity and Temperature on Kinematic Viscosity of Seed Oil and Derived Biodiesel. The kinematic viscosity of both seed oil and derived biodiesel of Jatropha at four different fruit maturity stages was measured at a temperature range from 22 °C (room temperature) to 48 °C. The data in Figure 4 indicate that viscosity of seed oil does not change much with fruit maturity. As Jatropha fruits mature from green to brown dry, viscosity of seed oil remains almost the same. Viscosity of Jatropha seed oil ranges from 8.8 to 9 mm²/s at 40 °C for all fruit maturity stages and for all the three geographical locations investigated, as shown in Figure 4a (NB: 1 mm²/s = 1 cSt). Viscosity of Jatropha biodiesel is in the range of 2.3–2.4 mm²/s at 40 °C in all fruit maturity stages and all the geographical locations investigated, as shown in Figure 4b. The results showed that there was a rapid drop in seed oil viscosity with temperature at relatively lower temperature (up to 30 °C) as shown in Figure 5. Thereafter, seed oil viscosity reduces gradually with an increase in temperature until it becomes almost constant after 40 °C. There is a 67% change in Jatropha seed oil viscosity as temperature increases from 22 to 48 °C. Biodiesel produced from the seed oil also changed in viscosity as temperature increased from 22 to 48 °C as demonstrated in Figure 6. However, biodiesel viscosity only changed slightly with a change in temperature as compared to seed oil. There was a 35% change in biodiesel viscosity as temperature increased from 22 to 48 °C. Both the ASTM and European biodiesel standards require that kinetic viscosity of biodiesel be specified at 40 °C. The American Society for Testing and Material standards require that biodiesel kinematic viscosity be within the range of 1.9 to 6 mm²/s, while European standards specify a narrower range of 3.5 to 5 mm²/s. Jatropha biodiesel viscosity from all the four maturity stages are within the specified range of ASTM standards. However, the viscosity is slightly below the minimum European standards regulations. Harvesting Jatropha seeds at different maturity stages does not impact the viscosity of the oil. However, temperature has a high influence on the viscosity of both Jatropha seed oil and derived biodiesel.
2.5. Variation of Moisture Content in Jatropha Seeds, Seed Oil, and Derived Biodiesel with Fruit Maturity. The data in Figure 7 depict that the moisture content in seeds decreases continuously as the fruit matures from green yellow to brown dry. The data further show that seeds extracted from brown dry fruits recorded the least moisture content of 4.7%. Before drying, seeds extracted from green yellow fruits have the highest moisture content. Drying seeds naturally do not eliminate all of the moisture from the seeds. According to some studies, there is always residual moisture in seeds after drying. After drying, the moisture content of seeds equilibrate with the relative humidity of the surrounding air, and this residual moisture in seeds is termed as equilibrium moisture content (EMC) of the dried seeds. After drying in oven, results have revealed that dried seeds (natural drying) from all the four different maturity stages contain 3.5% moisture. However, this is well below the 6% moisture content recommended by previous researchers who investigated the safe storage moisture content of Jatropha seeds. Therefore, it is appropriate to conclude that natural drying method is recommendable for drying Jatropha seeds from any maturity stage. Drying Jatropha seeds is essential to reduce the moisture content of fresh harvested seeds to a safe storage level. Moreover, processing techniques such as oil extraction requires dry seeds (moisture content below 6%) to maximize de-oiling of the seeds hence reduce residual oil left in the seed cake. Moisture content in biodiesel derived from Jatropha seed oil ranges from 0.03 to 0.05% in all of the fruit maturity stages investigated, as indicated in Figure 8b. Since seed oil and biodiesel are derived from dried seeds, the moisture content in seed oil and biodiesel is not influenced much by fruit maturity as is the case in seeds. According to ASTM biodiesel standards (D 2709), the recommended maximum moisture content in biodiesel is 0.05%. Therefore, results from this study are within the recommended limits. However, biodiesel has a tendency of absorbing moisture from the atmosphere during storage (hygroscopic) as echoed by some researchers in literature. This being the case, the moisture content in biodiesel is likely to vary with time. Therefore, it is advisable to store biodiesel in an air-tight container to avoid absorption of moisture. Available information indicates that the presence of water in oil triggers side reactions such as saponification during the transesterification process, resulting in a reduction in biodiesel yield. Therefore, it is necessary to eliminate water present in oil prior to conversion to biodiesel. A common method for removing moisture from the oil prior to conversion to biodiesel is by heating the oil at 100−105 °C for about an hour. For large-scale production, vacuum is applied in addition to heating to accelerate the moisture-removal process.

3. CONCLUSIONS

Analysis of the effect of Jatropha seed maturity stage on the physicochemical properties of Jatropha seed oil and derived biodiesel have been performed. The investigation has revealed that free fatty acid content in Jatropha seed oil increases...
continuously during the entire maturation stages following a
third-order polynomial trend. Jatropha seed oil from green
yellow and yellow fruits had the lowest FFA content whereas
seed oil from brown dry fruits had relatively high FFA content.
Seed oil and biodiesel from early stages of fruit maturity (green
yellow and yellow) had relatively low PV and were therefore of
relatively better quality. On average, there was almost a 100%
increase in both FFA content and peroxide value between
green yellow and brown dry maturity stages. Variation of FFA
content and peroxide value with investigated geographical
locations in Botswana was insigni-
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seed oil and derived biodiesel was insigni-
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cant. Moisture
content in Jatropha seeds decreased as fruits mature from
green to brown dry. However, the moisture content in seed oil
and derived biodiesel was not influenced by maturity stage
since they were derived from dried seeds. Results from this
investigation have revealed that using seed oil from early stages
of fruit maturity may improve the quality of both the seed oil
(feedstock) and hence of derived biodiesel in contrast to the
final maturity stage.

Figure 6. Variation of Jatropha biodiesel viscosity from four different fruit maturity stages with temperature, harvested from different geographical locations in Botswana: (a) Thamaga area, (b) Mmadinare, and (c) Maun.

Figure 7. Variation in moisture content in Jatropha seeds harvested at four different fruit maturity stages from four different geographical locations.

Figure 8. Variation in moisture content in (a) Jatropha seed oil and (b) biodiesel derived from seeds harvested at four different fruit maturity stages from four different geographical locations.
Thereafter, the oil was then separated from the slurry to allow the slurry to settle to the bottom through gravity. The oil was fed into the feeding hopper while the screw was rotating before oil extraction commenced to enhance oil extraction. Oil was converted to methyl esters (biodiesel) according to the procedure described by Jonas et al.\textsuperscript{17} Biodiesel was produced via transesterification of oil with methanol in the presence of potassium hydroxide as a catalyst.

### 4. MATERIALS AND METHODS

Jatropha fruits/seeds used in this study were harvested at four different maturity stages (green yellow, yellow, yellow brown, and brown dry) as shown in Figure 9, from Thamaga (24.72° S latitude, 25.53° E longitude), Maun (19.98° S latitude, 23.42° E longitude), Mmadinare, and Shashe areas in Botswana. Seeds were removed from the fruits within 24 h of harvesting and dried naturally for 6 days. Seeds were dried in open shade (not direct sunlight to avoid possibility of degradation) in a well-ventilated area at an average temperature of 25 \( ^\circ \)C. Thereafter, 75 mL of deionized water was added to the solution and 1 mL of starch was added as an indicator. The mixture was then titrated without the oil sample to obtain a blank value (control value). The peroxide value of the solution was then calculated using eq 1. The experiment was repeated five times for each sample and then the average was calculated.

\[
PV = \frac{1000 M_{ST} (V_A - V_B)}{m}
\]

where PV is peroxide value, \( m \) is weight of the sample (g), \( V_A \) is volume of sodium thiosulphate solution consumed for the sample (mL), \( V_B \) is volume of sodium thiosulphate solution consumed for the blank value (mL), and \( M_{ST} \) is concentration of sodium thiosulphate solution (mole/L).

#### 4.1. Seed Oil Extraction

An electric powered Kern Kraft KK40 mechanical screw press was used to extract oil from Jatropha seeds. The screw was first heated to about 85 \( ^\circ \)C before oil extraction commenced to enhance oil extraction, thus increasing the amount of oil produced. Whole seeds were then fed into the feeding hopper while the screw was rotating at a speed of 20 rpm. The extracted oil was discharged through the oil outlet holes, while the seed cake was ejected through a nozzle of 15 mm opening. The oil was left in a container for 24 h to allow the slurry to settle to the bottom through gravity. Thereafter, the oil was then separated from the slurry.

#### 4.2. Conversion of Seed Oil to Biodiesel

Jatropha seed oil was converted to methyl esters (biodiesel) according to the procedure described by Jonas et al.\textsuperscript{17} Biodiesel was produced via transesterification of oil with methanol in the presence of potassium hydroxide as a catalyst.

#### 4.3. Determination of Peroxide Value

Peroxide value of Jatropha seed oil and derived biodiesel from four different fruit maturity stages was determined according to the AOCS official method Cd 8–53 test standard for peroxide value. One and a half milliliters of oil sample was weighed into a 250 mL conical flask. Ten milliliters of chloroform was added into the conical flask to dissolve the sample and then 10 mL of glacial acetic acid was added to the solution. One milliliter of saturated potassium iodide solution was added to the solution in a conical flask. The conical flask was stoppered then shaken gently to mix the solution. The mixture was then stored in a dark room for 5 min at room temperature (25 \( ^\circ \)C). Thereafter, 75 mL of deionized water was added to the solution and 1 mL of starch was added as an indicator. The mixture was then titrated with 0.01 N sodium thiosulphate solution until the blue color disappeared (turns colorless). The experiment was repeated without the oil sample to obtain a blank value (control value). The peroxide value of the solution was then calculated using eq 1. The experiment was repeated five times for each sample and then the average was calculated.

\[
PV = \frac{1000 M_{ST} (V_A - V_B)}{m}
\]

Figure 9. Jatropha fruits harvested at different maturity stages.
a pink color. Free fatty acid content was then calculated using eq 2. The procedure was repeated three times for each sample, and an average value was then calculated.

\[
\text{\% free fatty acids} = \left( \frac{V \times N \times MW_{C_{18}:1}}{W \times 1000} \right)
\]

where \(V\) is volume of KOH solution consumed in the titration (mL), \(N\) is concentration of the KOH solution, \(W\) is weight of oil sample (g), \(MW_{\text{mol}} = \text{molecular weight (g/mol)}, MW_{\text{KOH}} = 56.1\) g/mol, and \(MW_{C_{18}:1} = 282.5\) g/mol (expressed as oleic acid).

4.5. Determination of Density. The density of seed oil and derived biodiesel was determined using a KEM Kyoto Electronics density meter, in accordance with the test method ASTM D1298. Before measuring the sample, the density of pure water was measured to ensure the accuracy of the instrument. The precision of the instrument is 1 ± 0.001 g/cm³. Factor calibration was carried out whenever the difference was greater than ±0.001 g/cm³. Density measurement was carried out by filling the cell with sample, then recording the reading from the display screen. Five measurements were carried out for each sample and then the average was calculated. The cell was cleaned before measuring a different sample using ethanol and then allowed to dry for 30 min according to the manufacturer’s specifications.

4.6. Determination of Kinematic Viscosity. Viscosity of seed oil and biodiesel was determined using a digital electronic viscometer (Fungilab Premium Series) in accordance with ASTM D445. The viscometer was connected to a Thermo-Scientific (Hake AC 150) water heater, which heats and circulates water around the test cup to control the temperature of the sample. The viscometer rotates a spindle, which is immersed in a test sample through a calibrated spring. The test cup/sample chamber was filled with 15 mL of seed oil/biodiesel such that the spindle was fully immersed in the test fluid. The depth of the test fluid was kept the same for all of the tests to ensure accurate results. A low-viscosity adapter (LCP spindle) was used for both seed oil and biodiesel since it can be used for fluids with viscosities as low as 1cp. The torque was maintained between 65 and 100% by varying the rotation speed of the spindle from 2 to 60 rpm and from 50 to 200 rpm for seed oil and biodiesel, respectively, depending on the viscosity of the test sample. Heated water was circulated around the test cup to control the temperature of the sample. The sample was heated gradually from room temperature (about 20 °C) to 50 °C while recording the corresponding viscosity at each temperature increment. Fifty degrees Celsius was set as the maximum temperature to avoid thermal decomposition of the oil due to excessive heat. Viscosity at 40 °C was noted, as specified by the American Society for Testing and Materials (ASTM D445). Viscosity recording of each sample was repeated five times and then the average was calculated.

4.7. Determination of Moisture Content. The moisture contents of Jatropha seeds, seed oil, and derived biodiesel were determined using an electronic moisture analyzer (KERN DBS version 1.3) in accordance with ASTM D6304. The principle of this equipment is such that the sample is weighed before and after heating, determining the material moisture by subtracting the final weight from the initial weight. A halogen radiator, which emits infrared heat, is used as the heating source. About 10 g of the sample was weighed. The sample was evenly distributed on the sample dish and then placed on the weighing chamber. The lid was closed and then the sample drying was automatically started. A drying temperature of 120 °C was set to ensure that all of the water content in the sample was evaporated. Rapid drying mode (RDM) was used since the samples did not form skin during the drying process. The drying process ended when the preset weight loss (\(\Delta M\)) of 0.01% remained constant for 30 s. The drying time varied between 2 min to 1 h depending on the moisture content of each sample. The weight loss was then recorded as the percentage of moisture content.

4.8. Statistical Analysis. The influence of fruit maturity on the free fatty acid content and peroxide value of seed oil and derived biodiesel was tested using a one-way analysis of variance (ANOVA). The statistical analysis was performed using SPSS version 20 software. The means were compared at a significance level of 5% (\(\alpha = 0.05\)). According to the critical F-values at 0.05 significance level provided by a previous study, the critical F-value for this test is 4.07.

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Notes
The authors declare no competing financial interest.

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