Variation of *Jatropha curcas* seed oil content and fatty acid composition with fruit maturity stage

Mbako Jonas*, Clever Ketlogetswe, Jerekias Gandure

Department of Mechanical Engineering, University of Botswana, Gaborone, Botswana

**ARTICLE INFO**

**Keywords:**
- Agricultural engineering
- Chemical engineering
- Biochemical engineering
- Biofuel
- Plant biology
- Jatropha
- Biodiesel
- Fruit maturity stage
- Seed oil content
- Fatty acid composition

**ABSTRACT**

Seed oil production in *Jatropha* seeds through different maturity stages have been investigated. In order to meet the high demand of oil (feedstock) for large scale biodiesel production, increasing oil content or output in *Jatropha* seeds is required. *Jatropha* fruits were harvested at four different maturity stages and the seeds were analysed for oil content. The seed oil was analysed for fatty acid profile. Results from four different geographical locations investigated namely; Mmadinare, Thamaga, Maun and Shashe, have shown a similar trend in lipid accumulation in *Jatropha* seeds as the fruits mature from green to brown dry. However, maximum oil content in seeds varies with geographical location. Accumulation of oil in *Jatropha* seeds during maturation follows a parabolic trend and reaches its peak when fruits are yellow. Oil yield in *Jatropha* seed kernels ranges from 38.7% to 45.8% for the four maturity stages investigated. Overall results have revealed that harvesting *Jatropha* fruits when they are yellow increases seed oil output by 6–9% when compared to harvesting the fruits when they are brown dry. There is a relationship between the trend in fatty acid composition in *Jatropha* seed oil and seed oil content trend during fruit maturation. Based on the trend of unsaturated fatty acids in *Jatropha* seed oil, particularly linoleic and oleic acids, it can be deduced that reduction of seed oil content from yellow brown to brown dry stage is a result of breakdown of some of the unsaturated fatty acids.

1. Introduction

Global energy demand is on the rise, and most of this energy (over 80%) comes from fossil fuels [1, 2]. However, fossil fuels are finite and face depletion soon. On the other hand, the combustion of fossil fuels such as petrol-diesel and many others such as coal and natural gas contributes significantly to greenhouse gas (GHG) emissions resulting in climate change (global warming). Therefore, alternative energy sources are needed, and renewable energy is the answer. Biodiesel is one of the renewable and clean-burning fuels, which can be used in diesel engines [3, 4, 5]. Among the seed oil producing plants, *Jatropha* seed oil has emerged as one of the promising feedstock for commercial biodiesel production. This is mainly due to the fact that *Jatropha curcas* oil is non-edible therefore has no competition with food demand as it is the case when food crops such as rapeseed and sunflower which are used as feedstock for production of biodiesel [5, 6]. Biodiesel has received growing interest in the past years in an effort to reduce greenhouse gas emissions. Availability of enough feedstock still remains a challenge in large scale biodiesel production. Increasing oil output from oil-bearing plants such as *Jatropha* can help meet the demands of large scale biodiesel production.

*Jatropha curcas* plant has a good adaptation to a large variety of soil and climatic conditions [7, 8, 9, 10]. It is a perennial plant that can grow in marginal land and a quick maturing plant species that starts bearing fruits within a year of its planting [11, 12]. It is for these reasons that previous researchers believe that *Jatropha curcas* is one of the best candidates for commercial biodiesel production. Increasing oil output from the seeds is one of the factors that can make commercial biodiesel production from the plant economically viable. Therefore, harvesting *Jatropha* fruits/seeds when oil content is maximum would increase overall oil output. Higher seed oil yield may increase the economic viability of *Jatropha* as a feedstock for biodiesel production, therefore harvesting fruits/seeds when oil content is maximum is necessary [13, 14]. Dranski, et al., (2010), investigated the effect of maturation of *Jatropha* fruits on oil content in seeds. The authors reported variation of oil yield with maturity of fruits and seeds undergo both physical and chemical changes [15]. However findings on yield trends with fruit maturity differ [11, 12, 16, 17].
According to previous investigations, there is no clear trend on accumulation of oil in Jatropha seeds during their various maturation stages, therefore more research is still required to establish the best phase/stage to harvest jatropha seeds for optimal oil production. This study investigates how lipid content in Jatropha seeds varies during various maturation stages of fruits/seeds. The seeds were harvested in four different geographical locations in Botswana. Consequently, maturity stage at which oil content in seeds is maximum has been identified.

This study further investigates the influence of fruit maturity stage on fatty acid composition of Jatropha seed oil.

2. Materials and methods

Jatropha fruits and seeds used in this study were harvested at different maturity stages (Green yellow, yellow, yellow brown and brown dry), Figure 1 (b), from four different geographical locations in Botswana, namely; Thamaga (24.72° S latitude, 25.53° E longitude), Maun (19.98° S latitude, 23.42° E longitude), Mmadinare (21.8811°S latitude, 27.7514°E longitude) and Shashe (21.433°S latitude, 27.450°E longitude), Figure 1 (a). The fruits were hand-picked from the parent plant. Hand picking allows accurate maturity selection since Jatropha fruits do not mature at the same time. Jatropha fruits show different maturity degrees within the same plant and bunch, therefore using time factor to measure Jatropha fruit maturity is unreliable and inaccurate.

2.1. Drying of seeds

Seeds were removed from the fruits within 24 h of harvesting and dried naturally for 10 consecutive 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 °C. A sample of 100g of seeds from each batch was monitored for change in weight on daily basis. Weighing of seeds was carried out using Shimadzu AW320 analytical balance with a precision of ±0.0001g. The seeds were considered dry when the reduction in weight remained constant. After drying, Jatropha seeds from each maturation stage was analysed for oil content.

2.2. Determination of seed oil content

Seed kernels were ground into powder using Mellerware Aromatic Grinder (29105). About 2g of grounded seed kernel was placed in ANK/XT4 filter bag and the filter bag was sealed. The samples in filter bags were dried in an oven at 105 °C for 3 h. The samples were then allowed to cool to room temperature for 15 min in a desiccator. After drying, the samples in filter bags were weighed using Adam Equipment Analytical Balance (AAA 250L). Oil was extracted from the samples using Ankom XT15 extractor. Petroleum ether (chromatography grade) was used as a solvent for the equipment. Sample bags were placed onto the bag holder then put into the Teflon insert. The Teflon insert was put back into the extraction vessel and locked in place into the instrument. The extraction temperature was set at 90 °C and extraction time at 60 min following the manufacturer’s recommendation for the use of the instrument. The extraction process was started with the instrument in automatic operation such that after the set time had elapsed, the extraction process stopped automatically. The extraction vessel was removed together with the Teflon insert. The filter bags were then dried in an oven at 105 °C for 30 min to remove residual solvent. The samples were placed in a desiccator for 15 min to cool to room temperature. Each filter bag was re-weighed. The percentage oil content in Jatropha seeds was determined by recording the weight of dried seeds before extraction of the oil then re-weighing the seeds (seed cake) after oil extraction. The procedure was

Figure 1. (a) Geographical locations where Jatropha fruits were harvested in Botswana and (b) Jatropha fruits harvested at different maturity stages.
repeated three times for each sample then calculated the average value. Oil yield was then calculated using Eq. (1).

\[
\text{% Oil yield} = \frac{W_2 - W_3}{W_1} \times 100%
\]  

(1)

Where:

\(W_1\) = Original weight of sample
\(W_2\) = Weight of pre-extraction dried sample + filter bag
\(W_3\) = Weight of dried sample + filter bag after extraction

2.3. Determination of fatty acid composition

Fatty acid composition of Jatropha seed oil and derived biodiesel from four different fruit maturity stages was determined using Agilent Technologies GC System 7890A gas chromatograph (GC) according to test method ASTM D6584. The instrument was equipped with an automated injector and HP-5MS capillary column (30 m x 250 μm x 0.25μm). The gas chromatograph was connected to a mass spectrometer (Agilent Technologies 5975C). The fatty acids in the seed oil were converted to methyl esters (biodiesel) by transesterification process [18] before they were injected into the gas chromatograph to improve their volatility. Helium was used as carrier gas. The carrier gas was set at a flow rate of 64 mL/min and pressure of 72kPa and according to manufacturers’ specification. The automated injector was set to inject 1μL of sample. The injector and detector were set to operate at a temperatures of 325 °C. The process was set to proceed as follows, initial oven temperature was 100 °C for 4 min. Thereafter, it was increased at a rate of 6 °C per minute to 235 °C, then 10 °C per minute to 300 °C for 8 min. The average run time was about 40 min.

2.4. Statistical analysis

The effect of fruit maturity on Jatropha seed oil content was tested using 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% (\(\alpha = 0.05\)).

3. Results and discussions

3.1. Oil accumulation in Jatropha seeds

Oil yield in Jatropha seed kernel ranges from 38.7 to 45.8% for the four maturity stages and four different geographical locations investigated as shown in Figure 2. Jatropha seeds harvested in Mmadinare area recorded relatively highest oil yield of 45.8% from yellow fruits. Seeds from Shashe area recorded least oil yield of 40.4% from the same maturity stage, thus 3.3% less. For the four areas under review, variation of seed oil yield with fruit maturity appears to follow the same trend. Oil yield in Jatropha seeds reaches its peak when the fruit turns yellow, and this applies for all the four areas under review as depicted in Figure 3. Overall, the results in Figure 2 suggest that as the fruit turns brown dry oil content in seeds reduces by about 6–9% which is a significant difference. Therefore, harvesting Jatropha fruits when they are yellow increases oil output by 6–9% as compared to harvesting the fruits on their final maturity stage (brown dry).

Generally, accumulation of oil in Jatropha seeds from the four geographical locations in the present investigation follows the trend of a cubic equation of the form \(y = Ax^3 - Bx^2 - Cx + D\), where A, B, C and D are constants. Solving these equations results in \(x = 1, 2, 3, 4\) which represents green yellow, yellow, yellow brown and brown dry maturity stages, respectively. When oil yield in seeds is zero (\(y = 0\)), the cubic equations gives \(x\)-values between -1.5 and -2. Therefore, accumulation of oil in Jatropha seeds during fruit maturation can be measured on a scale of -2 to 4 where -2 indicates the beginning of oil synthesis. Oil accumulation trend in Jatropha seeds from the beginning of oil synthesis up to the final maturity stage can therefore be predicted according to the trend lines in Figure 3.

Seed oil content increases as Jatropha fruits matures and reaches its peak when the fruit turns yellow. Thereafter, as the fruit matures further seed oil content start to decline gradually till the final maturation stage of the seed. This decline in seed oil content start as the fruit turns yellow brown and declines further as the fruit turns brown dry. Accumulation of seed oil in Jatropha seeds during maturation follows a parabolic trend as clearly shown in Figure 3. Baud and Lepiniec [19] observed a similar trend when they were studying oil accumulation in maturing seeds of Arabidopsis thaliana. They found out that there was a slight fall of the seed oil content in Arabidopsis thaliana seeds at the very end of the maturation process. A similar trend was again reported by Eastmond and Rawsthorne [20] who studied oil accumulation in maturing rape seeds. The phenomenon of decline in seed oil content during the final maturation of seeds is still unclear. However, Chia, Pike and Rawsthorne [21] made an effort to investigate this phenomenon after they discovered that there was a loss of at least 10% of storage lipid from Brassica napus embryos during the final maturation stage of the seeds. They reported that during the final maturation stage of the seeds, some of the triglycerides undergo \(\beta\)-oxidation (degradation) producing acetyl-CoA (acetyl coenzyme A) hence reduction in overall lipid content. The maturation stage at which lipid content in seeds start to decline is termed ‘Seed desiccation’ [22].
the present investigation it is appropriate to conclude that Jatropha seed desiccation occurs when the fruit turns yellow brown to brown dry as depicted in Figure 3.

3.2. Influence of fruit maturity stage on fatty acid composition

Fractional compositions of fatty acids found in Jatropha seed oil at different fruit maturity stages of Jatropha harvested in Thamaga, Maun, Shashe and Mmadinare areas are shown in Table 1. Linoleic and oleic acids (unsaturated fatty acids) make a significant portion of Jatropha seed oil across all maturity stages of the fruit. For all maturity stages, unsaturated fatty acids make up more than 70% of total fatty acids in Jatropha seed oil. Therefore Jatropha seed oil is more unsaturated. Similar findings were reported by [23, 24, 25] who found that fractional composition of linoleic acid reduces by 8–9%. Based on this trend of linoleic and oleic acid in Jatropha seed oil, it can be concluded that reduction of seed oil content during seed desiccation stage (from yellow brown to brown dry), as reported earlier in Section 3.2, is a result of breakdown of some of the unsaturated fatty acids. Therefore, there is a relationship between the trend in fatty acid composition in Jatropha seed oil and oil content during the different fruit maturation stages. According to Chia, Pike and Rawsthorne [21] at least 10% of the triglycerides in seed oil undergo β-oxidation (degradation) producing acetyl-CoA (acetyl coenzyme A) during the final maturation stage of seeds hence reduction in overall lipid content in seeds. Unsaturated fatty acids are chemically unstable (easily oxidized), therefore high concentration of such fatty acids in Jatropha seed oil make it susceptible to oxidation and degradation of the oil. Linoleic acid is a polyunsaturated fatty acid (with two double bonds), therefore it is easily oxidized since it

![Figure 3](image-url)

**Figure 3.** Accumulation of oil in Jatropha seeds from various geographical locations, (a) Mmadinare, (b) Maun, (c) Thamaga and (d) Shashe, from zero up to the final maturity stage (brown dry).
is the most unstable fatty acid in Jatropha seed oil. Fractional composition of Palmitic acid, Stearic acid, 11-Hexadecenoic acid and 10-Octadecenoic acid in Jatropha seed oil remain almost constant throughout the maturation stages. Palmitic and Stearic acids are the most stable as compared to the other acids in the seed oil. Their fractional composition remain almost unchanged throughout the maturation of Jatropha seeds.

### 3.3. Statistical analysis

Results of analysis of variance (ANOVA) on effect of fruit maturity on Jatropha seed oil content are presented in Table 2. The effect of fruit maturity on Jatropha seed oil yield is statistically significant because the significance values are less than 0.05 and the calculated F-values are greater than the critical F-value, 4.07. In other words, there is a significant influence of fruit maturity on Jatropha seed oil content. Results of analysis of ANOVA on influence of geographical location on Jatropha seed oil content are presented in Table 3. Influence of geographical location on Jatropha seed oil content is statistically significant. Post Hoc Tukey test results have indicated that variation of seed oil content with location is statistically significant because the significance values are less than 0.05 and the calculated F-values are greater than the critical F-value, 4.07. In other words, there is a significant influence of geographical location on Jatropha seed oil content.

### 4. Conclusions

Accumulation of seed oil in Jatropha seeds during maturation follows a parabolic trend. Oil yield/content in Jatropha seeds is maximum when fruits are yellow. Harvesting Jatropha fruits when they are yellow increases oil output by 6–9% as compared to harvesting the fruits on their final maturity stage (brown dry). This may significantly increase availability of feedstock for biodiesel production. There is a significant influence of fruit maturity on Jatropha seed oil content. Geographical location also influences seed oil content. Despite having a similar oil accumulation trend for all the investigated geographical locations, Jatropha seed oil content varies from one location to the other. There is a relationship between the trend in fatty acid composition in Jatropha seed oil and oil content trend during the different fruit maturation stages. Fractional composition of unsaturated fatty acids in Jatropha seed oil tend to decrease continuously as fruits mature from green yellow to brown dry resulting in a decline of seed oil content.

### Declarations

**Author contribution statement**

Mbako Jonas: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Clever Ketlogetswe & Jerekias Gandure: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

### Funding statement

This work was supported by the Ministry of Minerals, Green Technology and Energy Security in Botswana.

### Competing interest statement

The authors declare no conflict of interest.

### Additional information

No additional information is available for this paper.

### Acknowledgements

The authors would like to thank Mechanical engineering and Chemistry department laboratory technicians at University of Botswana for providing assistance during laboratory experiments.
References

[1] REN21, Renewables 2019 Global Status Report, 2019. Paris.

[2] D. Rice, Global Energy Sources, 2010 [Online]. Available: https://www.e-education.psu.edu/earth104/node/1345. (Accessed 6 September 2019).

[3] F.H. Kasim, In Situ Transesterification of Jatropha Curcas for Biodiesel Production, Newcastle University, United Kingdom, Newcastle, 2012.

[4] J. Xue, T.E. Grift, A.C. Hansen, Effect of biodiesel on engine performances and emissions, Renew. Sustain. Energy Rev. 15 (2011) 1098–1116.

[5] B.R. Moser, Biodiesel production, properties and feedstocks, In Vitro Cell. Dev. Biol. Plant 45 (2009) 229–266.

[6] A. Elbehri, A. Segerstedt, P. Liu, Biofuels and the Sustainability challenge: A Global Assessment of Sustainability Issues, Trends and Policies for Biofuels and Related Feedstocks, first ed., Food and agriculture organization of the united nations, 2013. Rome: TRADE AND MARKETS DIVISION.

[7] K. Openshaw, A review of Jatropha curcas: an oil plant of unfulfilled, Biomass Bioenergy 19 (2000) 1–15.

[8] P.K. Barua, Biodiesel from seeds of jatropha found in Assam, India, Int. J. Energy Inf. Commun. 2 (1) (2011) 53–65.

[9] S. Ahmed, S.M. Sultan, Physiological changes in the seeds of jatropha curcas L. At different stages of fruit maturity, Braz. Arch. Biol. Technol. 58 (2015) 118–123.

[10] A. Cañasadas-López, D.Y. Rade-Loor, M. Siegmund-Schultze, M. Iriarte-Vera, J.M. Domínguez-Andrade, J. Vargas-Hernández, C. Wegenkel, Productivity and oil content in relation to jatropha fruit ripening under tropical dry-forest conditions, Forests 9 (2018) 1–13.

[11] B. Saragih, Syahrinudin, B. Saragih, G. Siloy, The relation of Jatropha curcas L. fruit maturity level with the compound and oil content, Nat. Life 2 (1) (2000) 93–100.

[12] K.J. Sowmya, R. Gowda, P. Balakrishna, R.M.R. Gururaja, Effect of fruit maturity stages on seed quality parameters in jatropha (Jatropha curcas L.), Indian J. Plant Sci. 1 (1) (2012) 85–90.

[13] M. Canakci, J.H.V. Gerpen, Comparison of engine performance and emissions for petroleum diesel fuel, yellow grease biodiesel, and soybean oil biodiesel, Am. Soc. Agric. Eng. 46 (4) (2003) 937–944.

[14] W.M.J. Achten, L. Verchot, Y.J. Franken, E. Mathijs, V.P. Singh, R. Aerts, B. Muys, Jatropha bio-diesel productionanduse, Biomass Bioenergy 32 (2008) 1063–1084.

[15] J.A.L. Dranski, A.S.P. Junior, F. Steiner, T. Zee, U.C. Malavasi, M.D.M. Malavasi, V.F. Guimarães, Physiological maturity of seeds and colorimetry of fruits of Jatropha curcas L. Rev. Bras. Sementes 32 (4) (2010) 158–165.

[16] C.D.d. Brito, M.B. Loureiro, A.P.d.S. Junior, L.G. Fernandez, R.D.d. Castro, Morphophysiologica profile of Jatropha curcas L. fruits and seeds maturation, Ciência Agrárias, Londrina 36 (6) (2015) 3615–3628.

[17] G.B. Negau, Effect of harvesting Jatropha curcas L. seeds at different fruit maturity levels on germination, oil content and seed weight, Net J. Agric. Sci. 3 (3) (2015) 74–80.

[18] M. Jonas, C. Kelogetsewe, J. Gandure, Influence of jatropha fruit maturity on seed oil yield, composition and heat of combustion of derived biodiesel, Energy Power Eng. 10 (3) (2018).

[19] S. Baul, I. Lepiniec, Regulation of de novo fatty acid synthesis in maturing oilseeds of Arabidopsis, Plant Physiol. Biochem. 47 (6) (2009) 448–455.

[20] P.J. Eastmond, S. Rawsthorne, Coordinate changes in carbon partitioning and plastidal metabolism during the development of oilseed rape embryos, Plant Physiol. 122 (2000) 767–777.

[21] T.Y.P. Chia, M.J. Pike, S. Rawsthorne, Storage oil breakdown during embryo development of Brassica napus (L.), J. Exp. Bot. 56 (415) (2005) 1285–1296.

[22] R. Angelovici, G. Galili, A.R. Fernie, A. Fait, Seed desiccation: a bridge between maturation and germination, Trends Plant Sci. 15 (4) (2010) 211–218.

[23] B.M. Abdullah, R.M. Yusop, J. Salim, E. Youisif, N. Salih, Physical and chemical properties analysis of jatropha curcas seed oil for industrial applications, Int. J. Chem. Mol. Nucl Mater. Metall. Met. Eng. 7 (12) (2013) 893–896.

[24] J.M. Nzikou, L. Matos, F. Mbemba, C.B. Ndangui, N.P.G. Pambou-Tobi, A. Kimbonguila, T. Silou, M. Linder, S. Desobry, Characteristics and composition of jatropha curcas oils, variety Congo-brazzaville, Res. J. Appl. Sci. Eng. Technol. 1 (3) (2009) 154–159.

[25] A. Joshi, P.K. Singhal, R.K. Bachheti, Variation in oil content and physico-chemical properties of jatropha curcas seed collected from different areas of garwhal, uttarakhand India, Int. J. ChemTech Res. 5 (6) (2013) 2993–2999.