Effect of Fruit Maturity on Yield and Quality of Seed Oil and Biodiesel of Jatropha Curcas Found in Botswana.

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Abstract

This study was undertaken to investigate the influence of fruit maturity stage on yield and quality parameters of *Jatropha curcas* seed oil and derived biodiesel. The influence of fruit maturity on phorbol ester content (toxicity) in *Jatropha curcas* seed oil and seed cake was also investigated. *Jatropha curcas* (hereafter referred to as Jatropha) seeds used in this study were harvested from Jatropha plants adapted to Botswana climatic conditions. Biodiesel has received great attention as one of the renewable and clean burning fuels. This is so in an effort to reduce greenhouse gas emissions. Jatropha seed oil is considered as one of highly promising feedstock for biodiesel production. In order to meet the high demand of large scale biodiesel production, increment of seed oil output from Jatropha seeds is necessary. Harvesting Jatropha seeds/fruits when seed oil content is maximum is one of the factors than can help increase seed oil output from Jatropha seeds. Results from this investigation have shown that harvesting Jatropha fruits when they are yellow increases seed oil output by 6 to 9% as compared to harvesting the fruits on their final maturity stage (brown dry). Thus harvesting Jatropha seeds when they are yellow may increase feedstock (seed oil) availability in biodiesel production. The maximum oil content in Jatropha seeds is attained at yellow maturity stage, and these are 30.1%, 30.6%, 26.2% and 27.9% for Jatropha seeds harvested from Thamaga, Mmadinare, Shashe and Maun areas, respectively. It is pertinent to mention that accumulation of seed oil in Jatropha seeds harvested from the aforementioned geographical locations follow a similar trend. Seed oil in Jatropha seeds increases continuously during fruit maturation and reaches peak level when the fruit turns yellow, thereafter it starts to decline until the final maturity stage (brown dry). The quality of both feedstock and biodiesel are of paramount importance, and Jatropha seed oil and derived biodiesel are no exception. Results from this investigation have revealed that fruit maturity have an impact on the quality of the seed oil hence the derived biodiesel. Fruit maturity influences some quality parameters of the seed oil. For instance, Free Fatty Acids (FFA) content in Jatropha seed oil varies with fruit maturity. Free fatty acid content in Jatropha seed oil increases continuously with seed maturity and this applies for all feedstock collected from all the geographical locations under investigation. These FFA content in Jatropha seed oil ranges from 0.2 to 0.7% for the four different fruit maturity stages, namely green yellow, yellow, yellow brown and brown dry. Seeds from Mmadinare area (21.8811^{o}S latitude, 27.7514^{o}E longitude) recorded relatively highest FFA content from brown dry fruits (0.75%) whereas seeds from Thamaga area (24.72° S latitude, 25.53°

E longitude) recorded relatively lowest FFA content (0.44%). Fatty acid composition of Jatropha seed oil also varies with fruit maturity. Fractional composition of unsaturated fatty acids, which makes up more than 70% of the total lipid, declines continuously with each consecutive maturity stage. As Jatropha fruits matures from green yellow to brown dry, the fractional composition of linoleic acid decrease by 8 to 9%. There is a logical relationship between the trend in fatty acid composition in Jatropha seed oil and seed oil content during fruit maturation. Based on this trend of unsaturated fatty acids in Jatropha seed oil, particularly linoleic and oleic acid, it can be concluded that reduction of seed oil content during seed desiccation stage (from yellow brown to brown dry) is a result of breakdown of some of the unsaturated fatty acids. The decline in fractional composition of unsaturated fatty acids in Jatropha seed oil during fruit maturation causes the cetane number of derived biodiesel to increase continuously with each successive maturity stage. The Peroxide Value (PV) of both Jatropha seed oil and derived biodiesel increase gradually and linearly with fruit maturity. The PV of seed oils from all the investigated geographical locations in Botswana (Mmadinare, Thamaga, Shashe and Maun) follow the same trend and in the same range. Effect of fruit maturity on other quality parameters such as cloud point, pour point, density, viscosity and calorific value (energy content) is minimal. Jatropha seeds from selected geographical locations in Botswana contain some levels of Phorbol Esters (PE) hence toxic. The PEs content vary with fruit maturity. Phorbol ester content in Jatropha seed oil ranges from 3.4 to 4.2mg/g whereas in seed cake ranges from 1.7 to 2.0mg/g during the four different fruit maturity stages. Jatropha seed oil and seed cake from yellow brown maturity stage are relatively the most toxic since they contain highest concentration of PEs.

Declaration

I declare that this dissertation is my own original work and has not been submitted before to any institution for assessment purposes. I further declare that I have acknowledged all sources used and have cited these in the reference section.

Signed: ……..

Date: ……..

Certification

This is to certify that the work contained in the thesis entitled “Effect of Fruit Maturity on Yield and Quality of Seed Oil and Biodiesel of Jatropha Curcas Found in Botswana”, submitted by Mbako Jonas (200901050) for the award of the degree of Doctor of Philosophy (PhD) in Engineering to the University of Botswana, is a research work carried out by him under my direct supervision and guidance.

I considered that the thesis has reached the standards and fulfilling the requirements of the rules and regulations relating to the nature of the degree. The contents embodied in the thesis have not been submitted for the award of any other degree in this or any other university.

Supervisors

Prof. Clever Ketlogetswe

Signed: …… Date: ……

Prof. Jerekias Gandure

Signed: …… Date: ……

Dedication

This thesis is dedicated to my late mother Nelia Jonas, my grandmother, Bakani Jonas and my father.

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I am tremendously indebted to a number of people who contributed significantly towards the completion of this thesis. Foremost, I would like to express my sincere gratitude to my supervisors, Prof. Clever Ketlogetswe and Prof. Jerekias Gandure for their advice, guidance, support and encouragement during my research at the University of Botswana.

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I thank God for giving me the strength, courage and perseverance during all the challenging moments in completing this thesis.

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Abbreviations

ANOVA	Analysis of variance
AOCS	American Oil Chemists' Society
ASTM	American Society for Testing and Materials
AV	Acid Value
CFPP	Cold filter plug point
CP	Cloud Point
CV	Calorific Value
FAME	Fatty Acid Methyl Ester
FFA	Free Fatty Acid
GC-MS	Gas Chromatography – Mass Spectrometry
GHG	Greenhouse Gas
HPLC-DAD	High-performance liquid chromatography with diode array detection
HPLC-MS	High-performance liquid chromatography with mass spectrometry
ICl	Iodine Mono-chloride
IV	Iodine value
PEs	Phorbol Esters
PMA	Phorbol-12-myristate 13-acetate
PP	Pour Point
PV	Peroxide Value
SE	Standard Error
SV	Saponification value

Chapter 1

1. Introduction

1.1. Background

Global energy demand is on the rise, and most of this energy (over 80%) comes from fossil fuels (REN21, 2019; Bice, 2010). However, fossil fuels are finite and face depletion in the near future. Therefore, alternative energy sources are needed, and renewable energy is the answer. Combustion of fossil fuels such as petro-diesel and many others such as coal and natural gas contributes significantly to greenhouse gas (GHG) emissions resulting in climate change (global warming). The Intergovernmental Panel on Climate Change (IPCC) project temperature increase due to the emissions mainly from the combustion gases around the world as shown in *Figure 1-1*. The models used three emission scenarios (lower, higher and even higher emission scenario) to estimate average global temperature increases from the year 1900 to 2100. Global fossil fuel and industrial emissions are projected to result in temperature increase of 5.2°C by year 2100 (Scherer, 2012). Therefore, there is growing interest for renewable energy sources in an effort to reduce GHG emissions. Biodiesel is one of the renewable and clean burning fuels, which can be used in diesel engines. Biodiesel is produced from animal fat and plant oils such as Jatropha seed oil. It has numerous advantages over petro-diesel such as reduce exhaust emissions, renewable, biodegradable, negligible sulphur content and it is nontoxic (Moser, 2009; Ma & Hanna, 1999; Xue, et al., 2011; Knothe & Steidley, 2005).

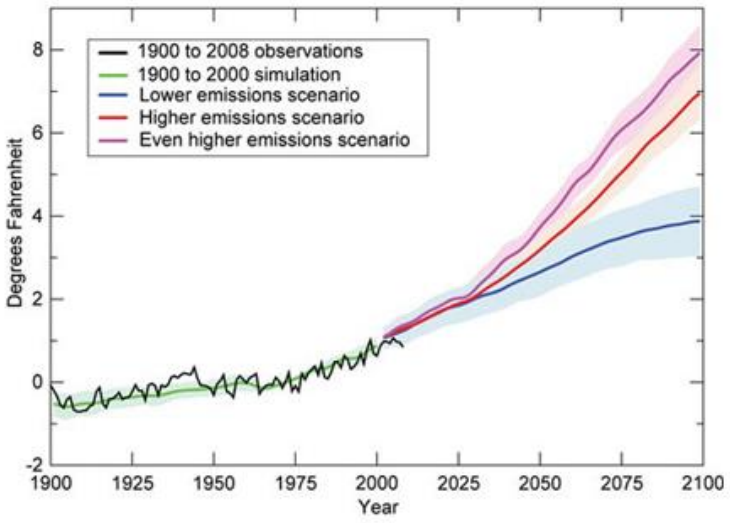


Figure 1-1. Projection of temperature change by Intergovernmental Panel on Climate Change (IPCC) (Scherer, 2012).

Production of biodiesel from vegetable oil was conducted as early as 1853, by scientist E. Duffy and J. Patrick (Abdalla & Oshaik, 2013). Available information indicates that there is growing interest in biodiesel in an effort to reduce greenhouse gas emissions generated from the combustion of fossil fuels. It is pertinent to emphasize that fossil fuels also face depletion in the future therefore there is need to search for an alternative such as biofuels whose emission potentials are relatively low due to the fact that these fuels are obtained from renewable energy sources such as biological materials. Several studies including (Gandure, et al., 2013; Hillion, et al., 2003; Datta & Mandal, 2012; Tesfa, et al., 2014) have shown that combustion of biofuels produce considerably less emissions than fossil fuels, therefore environmentally friendly. In fact the combustion of biodiesel has been reported to reduce emissions by 53–70% as compared to diesel fuel (Xue, et al., 2011; Palash, et al., 2013; Canakci & Gerpen, 2003). As mentioned earlier the main advantage of using biodiesel in internal compression ignition engines is that it is produced from renewable biological resources such as soybeans, rapeseed, palm, Jatropha, and many others. However, researchers have echoed that the major challenge in rapid development of biodiesel production at a relatively large scale is production cost and limited availability of raw materials in some parts of the world (Barua, 2011; Bouaid, et al., 2016; Talebian-Kiakalaieha, et al., 2013). The present study is focusing on *Jatropha curcas* plant seed oil from different fruit maturity stages as a feedstock for biodiesel production, due to the numerous advantages in has over other oil bearing plant species.

Jatropha curcas (hereafter referred to as Jatropha) plant belongs to the Euphorbiaceae family, and available evidence indicate that the plant originated in Central America (European-Food-Safety-Authority, 2015; Wink, et al., 2004). The plant bares seeds rich in crude oil. Several authors including (Openshaw, 2000; Barua, 2011; Arslan, et al., 2015) reported that Jatropha plant has a good adaptation to a large variety of soil and climatic conditions. In addition, the authors further report that the plant is well adapted to dry and semiarid conditions and it can be cultivated in areas unsuitable for food production (European-Food-Safety-Authority, 2015). The plant is drought resistant and has an economic life of up to 35 years and can live for 50 years and reach a height of 3 to 8 meters (Barua, 2011; Heller, 1996). It is a quick maturing plant species and starts bearing fruits within a year of its planting (Sowmya, et al., 2012). The relatively high percentage of oil in seeds which approximately ranges between 27 and 40% makes the Jatropha curcas a distinct potential for the oil industry (Nzikou, et al., 2009; Adebayo, et al., 2011; Achten, et al., 2008). Interest in Jatropha seed oil for biodiesel production has propelled a large-scale Jatropha curcas plantations across Asia, Africa, and South American countries (Devappa, et al., 2010; Soliman & He, 2015).

Despite having seeds rich in oil, Jatropha seeds including the oil are toxic, therefore unsuitable for human or animal consumption. By-products from Jatropha oil production such as seed cake cannot be used as animal feed due to the toxicity nature of Jatropha plant, unless detoxification processes have been applied. Presence of Phorbol Esters (PE) in Jatropha curcas plant have been identified as the primary substance responsible for the plant's toxicity. Previous studies have shown that PEs are distributed in different parts of Jatropha curcas plant and this include seeds, leaves, stem, flower, buds, roots, bark and wood, however they are mainly concentrated in the seed kernel (Devappa, et al., 2013; Pradhan, et al., 2010; Makkar & Becker, 2009; Xiao, et al., 2015). Information on variation of these PE's with fruit maturity is still lacking, therefore the need to be investigated and made available to the scientist community.

The Jatropha plant have received criticism in recent years as a feedstock for large scale biodiesel production. Some researchers have pointed out limitations that pose as a challenge in plantation of Jatropha for biodiesel production. These critics about the plant vary with geographic location around the world. Some researchers have reported that the plant is prone to pests and diseases which in turn affect production (Soto, et al., 2018; Anitha & Varaprasad, 2012). In other

geographical locations, the plant have been reported as potentially invasive, which is a negative environmental impact (Kashe, et al., 2018; Negussie, et al., 2013; Dai, et al., 2018). Information on the level of invasiveness of Jatropha plant is insufficient since there are no scientific investigations which involve controlled field experiments. Negussie et al (Negussie, et al., 2015; Negussie, et al., 2012) reported that there was insufficient evidence of Jatropha curcas invasiveness and more scientific investigations are required to verify this. One of the reported challenges was low seed oil yield which hinders its use in large scale biodiesel production. It is therefore necessary to investigate ways of increasing seed oil output in order to increase the economic viability of Jatropha as a feedstock for biodiesel production. Despite all the criticism and mixed responses towards the use of Jatropha plant for biodiesel production, there is still great potential in use of Jatropha seed oil for biodiesel production. The Jatropha plant should be studied at country-level since it responds differently in different geographical locations or countries.

Production of biodiesel from Jatropha plant involves plantation of the plant, harvesting the fruits, processing the fruits and seeds to extract oil and processing of the seed oil to produce biodiesel. Prior to oil extraction from Jatropha seeds, they have to be dried either naturally or oven dried at a temperature of about 105^{o}C (Achten, et al., 2008; Pradhan, et al., 2009; Cañadas-López, et al., 2018). There are two main methods used to extract oil from Jatropha seeds, namely; mechanical and solvent extraction. Mechanical expellers can be fed with either whole seeds or kernels or a mix of both. Jatropha fruits are usually harvested when they are dry brown. However, they can be harvested at different maturity stages then processed to extract the oil. It is critical to harvest Jatropha fruits when they contain maximum oil yield for economic reasons. It is also important to harvest Jatropha fruits when they can yield oil of better quality to ensure production of better quality biodiesel which is the main focus of the present study.

The main process used in the production of biodiesel is called transesterification. It is a chemical process of swapping the organic groups an ester with the organic group on an alcohol using a catalyst. After transesterification, the biodiesel is separated from glycerin. One of the challenges in producing biodiesel from the Jatropha curcas is the presence of high level of free fatty acids in some Jatropha curcas seed oil (Berchmans & Hirata, 2008; Bojan & Durairaj, 2012; Azhari, et al., 2008). Therefore the Jatropha curcas crude oil has to undergo pre-treatment before the actual transesterrification process such as reduction of free fatty acids. In addition some species of Jatropha curcas are toxic as mentioned earlier. Consequently, an increase in cultivation of the

Jatropha plant means that there is likely to be an increase in exposure to the plant by humans and other organisms. Therefore it is vital that risk factors and toxicity in exposure and contact with the plant and its products is understood and appropriate precautions be taken into account during handling.

The cost of biodiesel is currently higher than petroleum diesel due to the high cost of feedstock and processing cost (Moulana, et al., 2013; Tareen, et al., 2000; Le, et al., 2013; Kapilakarn & Peugtong, 2007). Some of the ways for reducing the cost of biodiesel is to use less expensive feedstock of better quality (physical and chemical properties) that require less or no pre-treatment hence reduce production costs. For example, using Jatropha seed oil with low free fatty acid content require less or no pre-treatment hence reduce production costs. Relatively high seed oil yield on the other hand may increase the economic viability of Jatropha as a feedstock for biodiesel production, therefore harvesting fruits (seeds) when oil content is maximum is necessary (Canakci & Gerpen, 2003).

During the maturation process of fruits, seeds undergo physical and chemical changes (Dranski, et al., 2010). Knowledge in maturity of Jatropha fruits in terms of chemical composition and oil yield is very important to the biodiesel production industry which considers Jatropha as a feedstock. It helps in determining the appropriate fruit maturity stage of harvest for sustaining better quality oil from the seeds and highest possible oil yield. Increased oil quality and yield from the seeds will ensure more biodiesel of better quality is produced.

Jatropha seed oil content has been related to maturity of the fruits, however findings from previous studies were inconsistent. For example, (Brito, et al., 2015) conducted their study in Bahia, Brazil and their findings indicate that seeds from brown fruits on the tree (not yet dry) yielded maximum oil content. On the other hand (Saragih, et al., 2007) conducted their study in Malinau District, Indonesia, and their findings indicate that seeds from yellow fruits yielded maximum oil content. Further on, (Negasu, 2015) and (Sowmya, et al., 2012) conducted their studies in Adami Tulu, Ethiopia and Bangalore, India respectively. They both reported a similar trend in oil content per maturity stage. They reported that green fruits had least oil content and brown dry fruits had maximum oil content, which is a different trend from the results reported by Brito et al (2015) and Saragih et al (2007). Based on the above observations, it is appropriate to conclude that seed oil content at various fruit maturity stage varied with location around the globe. Therefore the present

study investigate various maturity stages of Jatropha fruits found in selected locations in Botswana for oil content, physiochemical properties (quality) and toxicity as a feedstock for biodiesel production. The study was motivated by a number of factors which are discussed in the following Section 1.2.

1.2. Motivation

The move towards low carbon fuels and energy security appears to have stimulated research and development in the area of biofuels in a number of countries including Botswana. The government of Botswana, through the Ministry of Minerals, Green Technology and Energy Security started in 2007 with a feasibility study on the production and use of biofuels in the country. According to the report, *Jatropha curcas* was identified as one of the promising crops for biodiesel production in Botswana. The Botswana government is yet to approve national energy policy and guidelines on production and use of biofuels in an effort to promote biofuel production in the country. Commercial biodiesel production in Botswana will be of great benefit to the country and the world. These benefits include reduction in greenhouse gas emissions, energy security and employment creation. In Botswana, Jatropha seed oil is one of the feedstock with potential in biodiesel production. In recent years, Jatropha plant has received a number of criticism as to having limitations to be considered as a feedstock in commercial biodiesel production. One of the reported challenges was low seed oil yield which hinders large scale biodiesel production. Despite the criticism on Jatropha curcas in recent years, the plant still have a huge potential as a feedstock in biodiesel production. There are some geographical locations where the plant has proven to do well. For example, in Botswana, there are areas where the Jatropha plant still grows and produces fruits and seeds of considerable quantities. Therefore, Jatropha seed oil can be used alongside other feedstock in large scale biodiesel production. Increasing seed oil output from Jatropha seeds and of good quality was the primary motivation of this investigation. Increasing seed oil output and of high quality may increase the economic viability of Jatropha as a feedstock for biodiesel production. It is hypothesized that harvesting Jatropha fruits at other maturity stages other than the final maturity stage (brown dry) may improve seed oil output (yield) and its quality. This being the case, this study investigates the influence of fruit maturity stage on yield and quality of seed oil and derived biodiesel of Jatropha curcas found in Botswana. The research problem is described in the following Section 1.3.

1.3. Research Problem

In the case of Jatropha as a feedstock for biodiesel production, brown dry fruits are usually harvested and little attention is given to maturing or ripening fruits. Little effort has been made in studying oil derived from Jatropha fruits at different stages of maturity and production of biodiesel at those various fruit maturity stages. For example, several authors including (Abdullah, et al., 2013; Adebowale & Adedire, 2006; joshi, et al., 2011; Akbar, et al., 2009; Lu, et al., 2009), performed investigation on biodiesel yield levels, quality and toxic analysis using Jatropha seed oil from brown dry fruits. For the existing studies on Jatropha fruits at different maturity stages, findings vary with location on oil content and physiochemical properties at various maturity stages studied. Therefore the present investigations will focus on various maturity stages of Jatropha fruits found in selected regions in Botswana.

Previous studies on Jatropha in Botswana were on potential social, economic and environmental impacts (Kgathi, et al., 2012; Kgathi, et al., 2017; Kashe, et al., 2018; Mmopelwa, et al., 2017) and cultivation of the plant (Ishimoto, et al., 2017; Masukujane, et al., 2018) for biofuel production purposes. Information on the effect of fruit maturity on yield and quality of seed oil and derived biodiesel of *Jatropha curcas* in Botswana is lacking.

Furthermore, the price of biodiesel is currently higher than petroleum diesel due to the high cost of raw materials (feedstock) and processing cost (Moulana, et al., 2013; Tareen, et al., 2000; Le, et al., 2013; Kapilakarn & Peugtong, 2007). Feedstock of low quality in terms of physical and chemical properties require to undergo pretreatment prior to conversion to biodiesel which increases costs of production. For example, feedstock with more than 3% of free fatty acids (as a quality parameter) require to undergo pretreatment to reduce the level of FFA which are undesirable during transesterification process. Pretreatment of feedstock come at a cost which then increases the overall cost of biodiesel production. Therefore, feedstock of relatively high quality (physical and chemical properties) is among factors which may reduce cost of production since they require less or no pretreatment prior to transesterification process hence reduction in overall production costs. Relatively high oil yield from seeds on the other hand is likely to increase the economic viability of Jatropha as a feedstock for biodiesel production.

There is also a gap in physicochemical properties (quality) of Jatropha seed oil and derived biodiesel from different fruit maturity stages, particularly Jatropha adapted to Botswana's climatic

conditions (found in Botswana). Therefore this study seeks to identify the optimum maturity stage at which Jatropha fruits found in Botswana yield relatively high oil level of better quality (physical and chemical properties). The aim of the study is outlined in Section 1.4.

1.4. Aim

The aim of this study is to evaluate the effect of fruit maturity on seed oil and biodiesel yield and quality (physical and chemical properties) of Jatropha curcas adapted to Botswana's climatic conditions. The objectives of the study are outlined in Section 1.5.

1.5. Objectives

1. To quantify the oil yield of Jatropha curcas seeds at different stages of fruit maturity found in selected regions in Botswana.
2. To characterize the physical and chemical properties (quality) of Jatropha seed oil and derived biodiesel produced from seeds harvested at different fruit maturity stages of Jatropha found in Botswana.
3. To perform toxicity characterization of Jatropha seed cake and seed oil obtained from Jatropha fruits (seeds) at different maturity stages.

1.6. Research Scope

The study investigates various maturity stages of Jatropha fruits (mature green, green yellow, yellow, yellow brown and brown dry) found in Botswana for oil content, physiochemical properties and toxicity. Jatropha fruits at various maturity stages were harvested from selected regions in Botswana (this include Thamaga, Maun, Mmadinare and Shashe). Currently Jatropha plants are largely found in aforementioned areas in Botswana hence reason for selection as areas for harvesting Jatropha fruits.

1.7. Justification

The findings of this study will be of great benefit to the biodiesel production industry which considers Jatropha as a feedstock and to farmers who plant Jatropha for fruits and seeds. The

right time of harvest of Jatropha fruits will be identified for highest possible oil yield and best quality seed oil hence better quality biodiesel.

1.8. Limitations

During this research, one of the main challenges encountered was the attack of pests such as Mealy bugs resulting in some Jatropha plants being unproductive. Attack by Mealy bugs was mostly severe during the flowering season up until harvesting period. As a result, the quantity of Jatropha fruits harvested in some of the research areas was significantly reduced.

The following Chapter two, provides a comprehensive literature review on the use of Jatropha seed oil as a feedstock for biodiesel production. It covers work from previous research on production and characterisation of biodiesel particularly from Jatropha seed oil.

Chapter 2

2. Literature Review

2.1. Introduction

This chapter provide comprehensive review on biodiesel production, maturity of Jatropha fruits, as well as physicochemical properties of Jatropha seed oil and derived biodiesel from previous studies. It also covers areas on toxicity of Jatropha seeds as well as seed oil. As it will appear later in the chapter, previous researchers focused more on brown dry fruits and little attention given to other fruit maturity stages.

2.2. Maturation of Jatropha Fruits and Seeds

The quality (physicochemical properties) of fruits or seeds have been found to be associated with their maturity stage. The maturity of fruits is usually marked with colour change once they have reached their maximum growth size, and Jatropha fruits are no exception. During the maturation of Jatropha fruits and seeds, fruits change colour from green, green yellow, yellow, yellow brown and finally brown dry (Silva, et al., 2012; Brito, et al., 2015; Silip, et al., 2011; Negasu, 2015; Saragih, et al., 2007). 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. Previous studies have shown that visually inspecting Jatropha fruit colour is more accurate when determining maturity stage (Cañadas-López, et al., 2018; Saragih, et al., 2007; Sowmya, et al., 2012; Ahmad & Sultan, 2015). Different maturity stages of Jatropha fruits in terms of colour change are shown in *Figure 2-1*. In the case of Jatropha, brown dry fruits are usually harvested and little attention given to maturing or ripening fruits. However, little effort has been made in studying the effect of maturity of Jatropha fruits on seed oil and derived biodiesel yield and quality. Brito et al (Brito, et al., 2015) carried out a study to characterise the physiological profile during the development and maturation of Jatropha curcas fruits and seeds to identify the best maturation stage for harvesting the fruits. They reported that there is a relationship between fruit maturity in terms of colour change of the exocarp and maturity of the seeds.

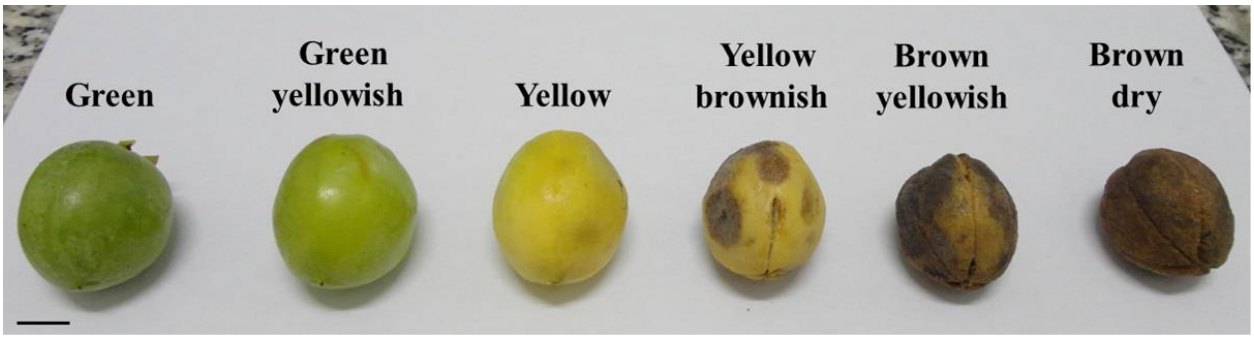


Figure 2-1. Jatropha fruits at different stages of maturity (Brito, et al., 2015).

Silip et al (Silip, et al., 2011) studied the variation of oil content and free fatty acids level in oil extracted from fruits of different maturity levels. Similar to Saragih et al (2007) results, they found out that oil yield was more in ripen fruits than green ones. They also found out that the percentage of free fatty acids decreased as Jatropha fruits matured. Immature (green) fruits contained relatively high levels of FFA whereas dry fruits contained least FFA as indicated in *Figure 2-2*. Free fatty acids content in biodiesel feedstock is important because its content will determine transesterification processes to be used. Therefore it is preferable to use feedstock with less percentage of FFA.

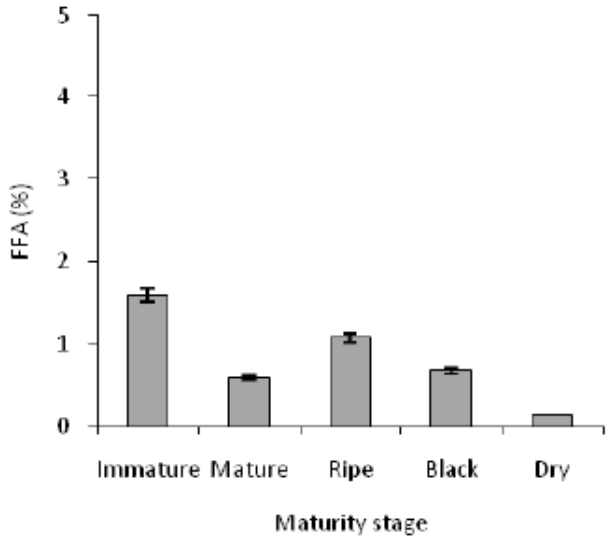


Figure 2-2. Variation in the percentage FFA in Jatropha fruits at different maturity levels (Silip, et al., 2011).

2.2.1. Variation of Oil Content in Jatropha Seeds at Different Fruit Maturity Stages

Oil content in Jatropha seeds have been investigated by a number of researchers, and most of them focusing on the final maturity stage of the fruit (brown dry). However, some researchers in other parts of the world made an effort to investigate variation of oil content in Jatropha seeds at different fruit maturity stages. Negasu (Negasu, 2015) investigated how maturity of Jatropha fruits affect the seed oil yield and germination of the seeds. Jatropha fruits were harvested at different fruit maturity levels namely, green fruit, yellow fruit and brown on mother tree fruit and brown dried fruit dropped on the ground. They recorded high concentration of oil in brown fruit on mother tree and the low concentration of oil was recorded for green fruit. According to their results, oil yield decreased slightly in brown dried fruit dropped on the ground. Sowmya et al (Sowmya, et al., 2012) also investigated the effects of Jatropha fruits maturity stages on seed quality parameters such as oil content, germination and protein content. Their findings on variation of seed oil content are shown in Table 2-1. Saragih et al (Saragih, et al., 2007) studied the variation of oil content in Jatropha seeds at different fruit maturity stages in Indonesia, Malinau District. They harvested Jatropha fruits at different levels of maturity (green, yellow green, yellow and brown). They found out that the maturity of the fruit is linked with the composition of seed and oil from the kernel. According to their results, oil level in the seed kennel is relatively high when the fruits are yellow and low when they are green. However, this findings appear to vary from place to place around the world as shown in Table 2-1, therefore there is need to carry out further research in other geographical areas such as Botswana to find out how seed oil content varies with fruit maturity which is the main focus of the present study.

Table 2-1. Variation of seed oil content in Jatropha seeds at different fruit maturity stages from different geographical origins.

Maturity Stage	**Oil Content (Reference)**					
	Ethiopia (Adami Tulu district) (Negasu, 2015)	**Brazil (State of Bahia)** (Brito, et al., 2015)	**Indonesia (Malinau District)** (Saragih, et al., 2007)	**India (Bangalore)** (Sowmya, et al., 2012)	**India (Sirmour District)** (Ahmad & Sultan, 2015)	**India**
Green	22.88	17.23	6.06	12.74		
Yellow	31.45	23.10	24.4	30.27	36.03	30.82
Yellow brown	34.59	26.11		36.83	46.70	30.00
Brown dry	32.80	23.28	17.72	43.75	48.41	29.06

2.3. Properties of Jatropha Seed Oil and Derived Biodiesel

The chemical and physical properties of oil and derived biodiesel defines its quality. These properties include among others: fatty acid composition, free fatty acid content, iodine value, saponification value, peroxide value, viscosity, density, flash point, cloud point, energy content, pour point and moisture content. Jatropha seed oil quality (physical and chemical properties) is dependent on several factors such as the environment, genetics, processing and storage (Achten, et al., 2008; Bojan & Durairaj, 2012). Properties of Jatropha curcas seed oil vary with their origins hence location as highlighted in Table 2-2. It is pertinent to mention that previous researchers who analysed Jatropha seed oil and derived biodiesel for physiochemical properties only focused on the final fruit maturity stage (brown dry). Little effort has been made in studying the effect of Jatropha fruit maturity on both seed oil and biodiesel quality, which is the main focus of the present study. In the production of biodiesel, the composition of the seed oil (feedstock) affects the quality of the methyl ether (biodiesel). Jatropha seed oil contains triglycerides as the main component. This means that the oil is an ester in which three fatty acid molecules are bound to a trihydric alcohol (glycerol) (Francis, 2014; Buasri, et al., 2009). Previous studies have revealed that Jatropha seeds contain up to 30 wt.% oil of which 21% are saturated fatty acids and 79% are unsaturated fatty acids (Raja, et al., 2011; Achten, et al., 2008; Akbar, et al., 2009). However variation of saturated and unsaturated fatty acids ratio with fruit maturity have not been studied as highlighted in the subsequent paragraph.

Several investigations have been carried out to determine both the physical and chemical properties of biodiesel produced from Jatropha seed oil from brown dry fruits (Lu, et al., 2009; Raja, et al., 2011; Adebayo, et al., 2011; Bobade, et al., 2013; Rao & Rao, 2013; Hiwot, 2018; Turinayo, et al., 2015). There is scares information on production of biodiesel using other stages of maturity, therefore, information on properties of biodiesel produced from Jatropha seed oil from different fruit maturity stages is limited.

Chemical and physical properties of Jatropha curcas seed oil and derived biodiesel from brown dry fruits harvested from various geographical origins are shown in Table 2-2. As previously mentioned, there is scares information on physiochemical properties of Jatropha seed oil harvested at other stages of fruit maturity.

Table 2-2. Chemical and physical properties of Jatropha seed oil and derived biodiesel from brown dry fruits from different geographical origins.

*SO=Jatropha seed oil, BD= derived biodiesel

Parameter	Value (references)															
	Nigeria (Garba, et al., 2013; Belewu, et al., 2010)		**Brazil** (Oliveira, et al., 2009)		**India** (Raja, et al., 2011)		**Malaysia** (Salimon & Abdullah, 2008)		**Rwanda** (Ntaganda, et al., 2014)		**Indonesia** (Silitonga, et al., 2014)		**West Bengal, India** (Tiwari, et al., 2007)	
	SO	BD	SO	BD	SO	BD	SO	BD	SO	BD	SO	BD	SO	BD	SO	BD
Density (g/ml)	0.725	0.868	0.922	0.883					0.900	0.877	0.922	0.882	0.940	0.880		
Kinematic Viscosity (mm^2/s)	20.49	9.6	30.686	4.016	36.92	4.82	36.0		-	7.891	28.35	4.48	24.5	4.8		
Flash point (ºC)	150	140		117	214	128					190.5	160.5	225	135		
Calorific value (MJ/kg)	37.2	47.22	40.31	41.72	39.76	42.80					38.961	40.224	38.65	42.00		
Free fatty acids (%)	0.36		4.2				1.03		1.09	1.143	12.76	0.196	14.07	0.20		
Cloud Point (ºC)	14				11	8						-3				
Pour Point (ºC)	4		-2	-5	6	-2						-2	4	2		
Iodine Value (mg/g)	512.7						135.85		218.28	267.44						
Peroxide value							0.66		1.2	4.4						
Cetane number																
Moisture content (%)	0.101		0.052	0.003			0.02		2.4	-			1.4	0.025		
Oxidation Stability at 110ºC												9.41				
Ash Content (%)													0.8	0.012		

2.3.1. Fatty acid composition of Jatropha curcas seed oil

Fatty acids are the main component of lipids and they exist as triglycerides or triacylglycerols (TAGs) and free fatty acids in oils and fats (Wiesman, 2009; Gopinath, et al., 2010; Abdulkareem, et al., 2012; Blin, et al., 2013). An illustration of a triglyceride molecule is shown in Figure 2-3. Physicochemical properties of a lipid depend primarily on its fatty acid composition (Ichihara & Fukubayashi, 2010). Previous researchers focused on seed oil derived from brown dry fruits. Variation of these fatty acids profile with fruit maturity is still a gap to be filled. The fatty acids in Jatropha oil have been identified as Oleic, Linoleic, Palmitic, Stearic, palmitoleic, Arachidic, Arachidoleic and Behenic acid (Joshi, et al., 2013; Abdullah, et al., 2013; Adebowale & Adedire, 2006). These fatty acids are classified as saturated, unsaturated and free fatty acids. The main saturated fatty acids in Jatropha oil are palmitic and stearic acids while the main unsaturated fatty acids are oleic and linoleic acids (Nzikou, et al., 2009; Akbar, et al., 2009; Turinayo, et al., 2015). Jatropha oil contains more than 75% unsaturated fatty acids (Achten, et al., 2008; Akbar, et al., 2009). The measure of unsaturation of Jatropha oil or fatty acids is referred to as the Iodine value. Increase in iodine value is directly proportional to increase in unsaturation of the oil or fatty acids. The fatty acids present in Jatropha seed oil from brown dry fruits are shown in *Table 2-3*. The fatty acid profile of Jatropha seed oil vary significantly with geographical origin as demonstrated by the same *Table 2-3*. The present study focuses on fatty acid profile of seed oil at different fruit maturity stages of Jatropha collected from selected locations in Botswana.

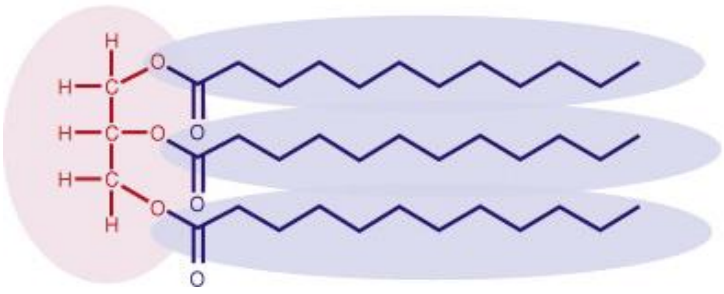


Figure 2-3. Structure of a triglyceride molecule (Wiesman, 2009).

Table 2-3. Fatty acid composition of Jatropha curcas seed oil from brown dry fruits from different geographical origins.

Fatty Acid	**Formula**	**Structure**	**% Fraction (reference)**					
			Nigeria (Adebowale & Adedire, 2006)	**Malaysia** (Abdullah, et al., 2013)	**India (Dehradun)** (joshi, et al., 2011)	**Brazil** (Oliveira, et al., 2009)	(Akbar, et al., 2009)	**Malaysia** (Salimon & Abdullah, 2008)
Oleic acid	$C_{18}H_{34}O_2$	C18:1	12.8	43.32	44.9305	21.8	44.7	46.40
Linoleic acid	$C_{18}H_{32}O_2$	C18:2	47.3	36.70	33.4068	47.4	32.8	31.96
Palmitic acid	$C_{16}H_{32}O_2$	C16:0	11.3	13.19	15.3974	13.5	14.2	13.89
Stearic acid	$C_{18}H_{36}O_2$	C18:0	17.0	6.36	6.2653	6.1	7.0	7.16
Arachidic acid	$C_{20}H_{40}O_2$	C20:0	4.7				0.2	
Arachidoleic acid	$C_{20}H_{38}O_2$	C20:1	1.8					
Behenic acid	$C_{22}H_{44}O_2$	C22:0	0.6					
Palmitoleic acid	$C_{16}H_{30}O_2$	C16:1		0.40			0.7	0.61
Lauric acid	$C_{12}H_{24}O_2$	C12:0				5.9		
Miristic acid	$C_{14}H_{28}O_2$	C14:0				2.7		
Others						2.7		

Fatty acid composition has a significant influence in physicochemical properties of seed oil and biodiesel (Hoekman, et al., 2012; Caldeira, et al., 2017; Ruhul, et al., 2016). Therefore biodiesel properties can vary from one feedstock to the next. Fatty acid composition influence properties such as cetane number, energy content, viscosity, density, iodine value, oxidative stability, cloud and pour point. Biodiesel with relatively high cetane number is preferred for better ignition quality in a diesel engine. Cetane number increases with increase in molecular weight for esters and decreases with increasing unsaturation of the fatty acids (Gopinath, et al., 2010; Klopfenstein, 1985). Previous findings (Gopinath, et al., 2013) have shown that energy content (heating value) decreases with increase in unsaturation (double bonds) due to deficiency in hydrogen atoms.

An increase in oleic and linoleic acids decreases cetane number and energy content. Linoleic acid turns to decrease these parameters even more since it is polyunsaturated. On the other hand, an

increase in palmitic acid and stearic acid in seed oil or biodiesel increase cetane number and energy content since they are saturated.

Increasing degree of saturation and trans-configuration in fatty acids increases overall viscosity of seed oil and biodiesel whereas increase in unsaturation decreases viscosity (Caldeira, et al., 2017). Therefore, increase in linoleic acid and oleic acid reduces overall viscosity.

It is recommended to have an increased amount of short chain and saturated methyl ester contents in fuel to reduce carbon monoxide (CO) and nitrogen oxides (NO_X) emission (Ruhul, et al., 2016). In the case of Jatropha oil, palmitic acid has a relatively short chain and is saturated, therefore an increase in palmitic acid content will significantly reduce both CO and NO_X emissions. Stearic acid has a relatively long chain, however it is saturated. Its presence also contributes to reduction of CO and NO_X emissions.

2.3.2. Free Fatty Acids

Free fatty acids (FFA) are fatty acids liberated from their ester linkage with the parent triglyceride molecule. They are fatty acids which are unattached to any glycerol present in the oil (Ali & Abdurrhman, 2013; Mahesar, et al., 2014; Wiesman, 2009). An illustration of a FFA is shown in Figure 2-4. Free fatty acids are one of the important considerations made when selecting a feedstock for biodiesel production (Azhari, et al., 2008; Buasri, et al., 2009; Math, et al., 2010). There are no international regulations on maximum FFA content in feedstock for biodiesel production. However, previous researchers have successfully converted oil with up to 3% free fatty acid content via homogeneous base transesterification (Kombe, et al., 2013; Knothe, et al., 2005; Bojan & Durairaj, 2012; Canakci & Gerpen, 2001). Therefore, it is appropriate to conclude that 3% is the maximum FFA content that a feedstock can be processed to biodiesel via homogeneous base transesterification without acid pretreatment. Some researchers have reported Jatropha seed oil from brown dry fruits having high (>3%) FFA content (Bojan & Durairaj, 2012; Kombe, et al., 2013; Berchmans & Hirata, 2008; Jayasinghe, et al., 2014). Kombe (Kombe, et al., 2012) reported FFA content in Jatropha seeds from brown dry fruits harvested in Tanzania to be as high as 5.96%. Berchmans and Hirata (2008) analysed the free fatty acids present in crude Jatropha seed oil from

brown dry fruits and found out that oleic acid and linoleic acid were the main constituents of free fatty acids found in Jatropha oil. Some researchers have reported that improper handling and storage conditions lead to deterioration of the oil quality and cause water content to increase, for example, exposing the oil to air and sunlight for a long period of time increases the FFA content significantly due to the hydrolysis of triglycerides in the presence of moisture and oxidations (Bojan & Durairaj, 2012; Berchmans & Hirata, 2008; Mahesar, et al., 2014). The levels of FFA in crude Jatropha seed oil also varies with geographical origin as depicted in Table 2-2. In the production of biodiesel, high levels of FFA (>3% w/w) in crude Jatropha seed oil cause formation of soap therefore separation of products will be difficult, resulting, in low yield of biodiesel product (Akbar, et al., 2009; Knothe, et al., 2005; Canakci & Gerpen, 2001). According to Azhari et al (2008) who studied the effect of FFA content in crude Jatropha seed oil as a feedstock for biodiesel production, reported that the yield of biodiesel produced decreased significantly with increasing FFA content in crude Jatropha seed oil. According to their results, they achieved a maximum biodiesel yield of 90% for feedstock with FFA content of 0.5% and a yield of almost zero for feedstock with FFA content of 25.3%. Information on variation of FFA content in Jatropha seed oil with fruit maturity is scares therefore the need to be investigated.

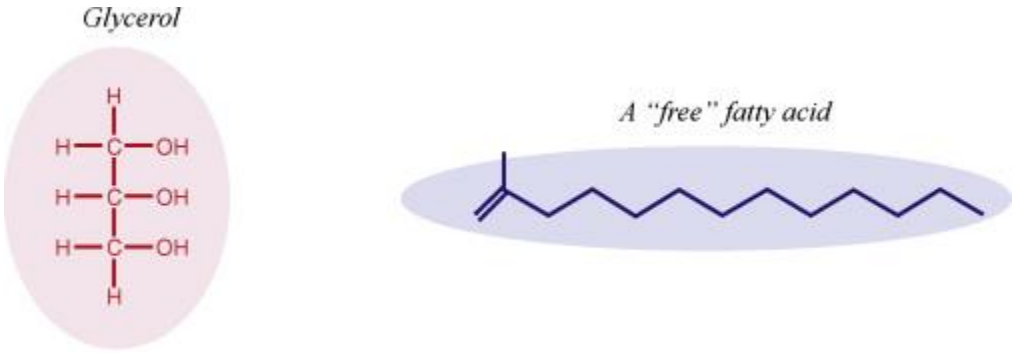


Figure 2-4. Structures of free fatty acid and glycerol molecules (Wiesman, 2009).

2.3.3. Kinematic Viscosity

Viscosity is a measure of a fluid's resistance to flow (Kažys & Rekuvienė, 2011; Blin, et al., 2013; Allah, 2015; Nzikou, et al., 2009; Garba, et al., 2013). Viscosity of a fluid decreases with increase in temperature, therefore viscosity is reported at a specified temperature (Acaroglu & Demirbas, 2007; Wang, et al., 2014). One of the major reasons why vegetable oils or fats are transesterified

to biodiesel is to reduce viscosity. High viscosity of vegetable oils or fats leads to operational problems such as blockage of fuel pipes and injectors, difficulty in pumping and engine deposits when used in diesel engines (Knothe & Steidley, 2005; Yaakob, et al., 2014; Pramanik, 2003; Abdulkareem, et al., 2012). Therefore, it is quite essential to reduce the viscosity of vegetable oils before being used in diesel engines. American Society for Testing and Materials (ASTM D445) specifies viscosity in the range of 1.9 – 6.0 mm^2/s for biodiesel whereas the European standards (EN ISO 3104, ISO 3105) specifies viscosity in the range of 3.5 – 5.0 mm^2/s for biodiesel at 40^oC. Data available in literature about viscosity of Jatropha seed oil and biodiesel from brown dry fruits vary from region to region as indicated in Table 2-2 (Raja, et al., 2011; Oliveira, et al., 2009; Silitonga, et al., 2014; Tiwari, et al., 2007). Previous research focused on viscosity of Jatropha seed oil and biodiesel from brown dry fruits. Viscosity of Jatropha seed oil and derived biodiesel from other stages of fruit maturity is not well documented, hence the need to close the gap.

2.3.4. Moisture content

Moisture content is the amount of water in a substance and is usually expressed as a percentage. Moisture content is one of the important factors determining the quality of seeds, seed oil and derived biodiesel during harvesting, storage and processing (Kraszewski, et al., 1998; Anandalakshmi, et al., 2008; Guzman & Aquino, 2009). Therefore it is important to know the moisture content in order to make a reasonable prediction of the possible storage life of seeds, oil and biodiesel. Orhevba, et al (Orhevba, et al., 2013) carried out an investigation on effect of moisture content on oil yield in Neem seed kernel. They found out that seed kernels with relatively high moisture content yielded less oil as compared to those with relatively low moisture content. Adejumo, et al (Adejumo, et al., 2013) studied the effect of moisture content on yield and characteristics of oil from Moringa Oleifera seeds. Their results were similar to those of Orhevba et al., (2013) since seeds with high moisture content yielded less oil as compared to seeds with low moisture content. Based on their results, seeds with high moisture content are expected to yield less oil. Presence of water in oil triggers side reactions such as saponification which reduces the yield of biodiesel (Moulana, et al., 2013). Therefore, it is advisable to eliminate water present in oil prior to conversion to biodiesel. Silva, et al (2012) determined the moisture content in Jatropha seeds harvested at five different maturity stages from Brazil and their results were as follows, Green: 76%, green yellow: 52%, yellow: 48.3%, yellow brown: 48%, brown dry: 7.57%. Previous

studies have reported moisture content of Jatropha seeds from brown dry fruits to be in the range of 5 to 8% (Bamgboye & Adebayo, 2012; Akowuah, et al., 2012; Joshi, et al., 2011; Silva, et al., 2012). Some previous researchers determined moisture content in Jatropha seed oil and derived biodiesel from brown dry fruits and their findings are shown in Table 2-2. They reported moisture content of Jatropha seed oil from brown dry fruits to be in the range of 0.2 to 2.4%.

2.3.5. Density and Specific Gravity

Density is defined as mass per unit volume and it is expressed as g/cm^3 or Kg/m^3 (Giakoumis & Sarakatsanis, 2018; Barabás & Todoruţ, 2011). In some cases density is expressed as a dimensionless quantity, specific gravity (SG). Specific gravity is simply the ratio of density of a substance to the density of water (Tesfa, et al., 2010). In most cases the density of water is $1g/cm^3$, which only cancels out the units (g/cm^3) and leaves the value unchanged. According to the European Standards (EN ISO 3675, 12185), the acceptable range of biodiesel density is 0.86–0.90 g/cm^3, whereas other international standards like the American Standards (ASTM) are silent on the limits for density and specific gravity of biodiesel fuel. According to Boz et al. and Fazal, (Boz, et al., 2009; Allah, 2015), higher density value of fuel makes injection and atomization of the fuel into the engine difficult resulting in decreased engine performance. Giakoumis et al. and Saxena et al. (Giakoumis & Sarakatsanis, 2018; Saxena, et al., 2013; Bello & Agge, 2010) found out that an increase in the number of double bonds and higher unsaturation of fatty acids in seed oil/lipids and biodiesel result in increase in density. Previous researchers have determined densities and specific gravity of both Jatropha seed oil and derived biodiesel from brown dry fruits as indicated in Table 2-2 (Garba, et al., 2013; Oliveira, et al., 2009; Ntaganda, et al., 2014; Abdulkareem, et al., 2012; Silitonga, et al., 2014; Tiwari, et al., 2007). The effect of fruit maturity on density and specific gravity of both Jatropha seed oil and derived biodiesel has not been covered therefore part of investigation in the present study.

2.3.6. Flash Point

Flash point is an important indicator of the flammability of a chemical. It is defined as the lowest temperature of a liquid at which it forms vapour that when mixed with air ignites resulting in a momentary flash or flame (Garba, et al., 2013; Boz, et al., 2009; Rowley, et al., 2010). Flash point is an important parameter in processing and storage of a fuel (Rowley, et al., 2010). It is used to assess the risk associated to the use or the storage of a flammable liquid. According to ASTM D93, the recommended minimum flash point for biodiesel is 130^{o}C. The flash points of Jatropha seed oil and derived biodiesel from brown dry fruits harvested at different geographical locations, as reported by various researchers are shown in Table 2-2, Section 2.3. Influence of fruit maturity on flash point of Jatropha seed oil and derived biodiesel is not well documented.

2.3.7. Cloud and Pour point

Cloud point (CP) is the temperature at which wax first becomes visible (liquid start to crystalise) when the liquid specimen is cooled while pour point (PP) is the temperature at which the amount of wax out of solution is sufficient to gel (solidify) the liquid specimen (oil/fuel), thus it is the lowest temperature at which the oil/fuel can flow (Bello & Agge, 2010; Saxena, et al., 2013; Boz, et al., 2009). These two properties are particularly important for low temperature operability. The current international standards have not specified limits for cloud and pour point for oils (feedstock) and derived biodiesel. According to (Hoekman, et al., 2012), this is due to large seasonal and geographic temperature variability across the globe. However it is important to determine these two parameters for both seed oil and biodiesel so one can know the lowest temperature at which the fuel can be used. Previous researchers studied CP and PP of Jatropha seed oil and derived biodiesel from brown dry fruits harvested at different geographical locations are shown in Table 2-2. The effect of fruit maturity stage on cloud point and pour point of Jatropha seed oil and derived biodiesel is not well documented thus need to be investigated.

2.3.8. Calorific Value

Calorific value is another important parameter of a fuel and it indicates the amount of energy available in a fuel, the higher the calorific value, the greater the energy contained in the fuel (Oliveira & Silva, 2013; Demirbas, 2005). Fuels with higher calorific value produce more power in the engine whereas fuels with less calorific value tend to burn inefficiently thus causing lot of exhaust and air-pollution (Kumari, et al., 2014). The current international standards have not specified limits for calorific value for oils (feedstock) and derived biodiesel. However, Hoekman et al (Hoekman, et al., 2012) recommends that biodiesel from all sources should have calorific value at least 10% lower than petroleum diesel due to its substantial oxygen content. Biodiesel contains 10 - 12% oxygen by weight (Sivaramakrishnan & Ravikumar, 2012). The effect of fruit maturity on calorific value of Jatropha seed oil and derived biodiesel is not well documented. Calorific values of Jatropha seed oil and derived biodiesel from brown dry fruits are shown in Table 2-2.

2.3.9. Cetane number

Cetane number (CN) is a dimensionless parameter that indicates auto ignition quality of a fuel in compression ignition engines. It determines the delay of the fuel to auto-ignite when injected into the engine, that is the time taken from injection of the fuel into the combustion chamber and self-ignition of the fuel-air mixture (Saxena, et al., 2013; Hoekman, et al., 2012; Aliyu, et al., 2011; Mohammadi & Najafi, 2015; Angelovič, et al., 2014). Relative high cetane number gives lower delay period resulting in smoother engine operation (Bamgboye & Hansen, 2008; Gopinath, et al., 2010; Sivaramakrishnan & Ravikumar, 2012). High cetane number assist in easy engine starting at low temperatures (Sokoto, et al., 2011). On the other hand, low CN results in higher exhaust gas emissions (Ayodeji, et al., 2015). American Society for Testing and Materials (ASTM D613) specifies a minimum cetane number of 47 while the European Standards EN ISO 5165 specifies a minimum cetane number of 51 for biodiesel. Cetane number of biodiesel is influenced by the fatty acid composition of the Methyl esters (Gopinath, et al., 2010; Piloto, et al., 2013). The effect of Jatropha fruit maturity on cetane number of derived biodiesel is yet a gap to be filled. Previous researchers studied cetane number of Jatropha biodiesel from brown dry fruits (Folayan & Ajimotokan, 2018; Shah, et al., 2017; Sivaramakrishnan & Ravikumar, 2012).

2.3.10. Peroxide Value

Peroxide value (PV) is one of the quality characterization parameters for oils and biodiesel. It is a measure of primary oxidation (rancidification) of oils or biodiesel and is measured in milli-equivalents of peroxide units per kg of the oil or biodiesel (mEq/Kg of oil) (Kaleem, et al., 2015; Yaakob, et al., 2014; Marina, et al., 2013; Okparanta, et al., 2018; Cayuela, 2017). During primary oxidation, unsaturated fatty acids react with oxygen and form peroxides. Peroxide value is influenced by factors such as degree of unsaturation of fatty acids in oil, storage conditions, exposure to light and heat. High temperature, exposure to light and oxygen accelerates these reactions (Popa, et al., 2017; Atinafu & Bedemo, 2011). The effect of fruit maturity on peroxide value of Jatropha seed oil and derived biodiesel is also not well documented. Previous research on peroxide value of Jatropha seed oil and derived biodiesel focused on seed oil from brown dry fruits (Salimon & Abdullah, 2008; Ntaganda, et al., 2014; Garba, et al., 2013; Umaru & Aberuagba, 2012; Amabye & Bezabh, 2015). Their findings indicate that peroxide value of Jatropha seed oil and derived biodiesel from brown dry fruits is in the range of 0.6 to 8 and 1 to 5 mEq/Kg respectively. The current international standards have not specified limits for peroxide value for oils (feedstock) and derived biodiesel.

2.3.11. Iodine Value

Iodine value is a quality measurement parameter which determines the amount of unsaturation of fats and oils and is expressed in terms of amount of Iodine absorbed by the sample (Joshi, et al., 2013; Haryati, et al., 1997; Hoekman, et al., 2012). This value can be used to quantify the amount of double bonds (C=C) present in the oil (Sushma, 2014; Rao, et al., 2010). The higher the iodine value, the more double bonds present, hence the higher the unsaturation degree of the oil. Iodine value affect other parameters such as viscosity and cloud point. Saturated oils (low iodine value) tend to have relatively high viscosity and cloud point. Oils which are highly unsaturated (high Iodine value) including biodiesel produced from such oils, are easily oxidized hence increase engine deposits. Iodine values greater than 50 may result in decreased engine life (Rao, et al., 2010). The European biodiesel standards, EN 14111 specifies maximum Iodine value of 120 g I/100g for biodiesel. Other international standards including the American Standards (ASTM) are silent on the limits for Iodine value.

2.4. Biodiesel Production Process

Biofuels are fuels derived from organic matter taken from or produced by plants and animals. Biodiesel is a common example of a biofuel. It is made up of mono Alkyl Esters (fatty acid methyl esters or fatty acid ethyl esters) which have similar characteristics to petro diesel (Kasim, 2012; Moser, 2009; Xue, et al., 2011; Shrestha, et al., 2013). There are several feedstock used in the production of biodiesel. These include vegetable oil, animal fats, waste vegetable oils and microalgae oils. The most common vegetable oils used today for commercial biodiesel production are soybeans and rapeseed oils (Laboratory, 2011; Canakci & Gerpen, 1999; Elbehri, et al., 2013). Feedstock used in biodiesel production vary with location due to climate conditions and availability. For example, rapeseed and sunflower oil are the most used feedstock for biodiesel production in Europe, soybean oil and animal fats are most common in North America (Moser, 2009; Elbehri, et al., 2013). However, these feedstock do not meet the high demand for biodiesel world over. Furthermore, vegetable oils such as soybeans and sunflower oil are used as food for human consumption. Growing crops for fuel competes for land, water, and energy resources vital for the production of food for people, furthermore contribute to increase in food prices (Pimentel, et al., 2009; Ajanovic, 2010). Therefore there is need for additional feedstock for commercial production of biodiesel. The Jatropha curcas oil is of considerable interest as a feedstock for commercial biodiesel production, because it is non edible, therefore has no competition with food demand. Plants or animal oil are transformed into biodiesel through a process called transesterification.

2.4.1. Transesterification Process

Biodiesel is mostly produced via a process called transesterification also known as alcoholysis (Pradhan, et al., 2010; Berchmans & Hirata, 2008; Moser, 2009; Silva, et al., 2013) as mentioned earlier. The vegetable oil including Jatropha seed oil undergoes transesterification reaction with an alcohol such as methanol or ethanol in the presence of an alkali or acid catalyst (Kasim, 2012; Shrestha, et al., 2013; Silva, et al., 2013). A schematic diagram of this process is shown in *Figure 2-5* (Silva, et al., 2013; Kasim, 2012). The stoichiometric molar ratio of alcohol to oil for the transesterification reaction is 3:1, three moles of alcohol and one mole of triglyceride to yield three moles of fatty acid ester and one mole of glycerol. However, the chemical reaction is reversible

therefore excess methanol is required to shift the reaction equilibrium towards completion (product side) (Musa, 2016; Ma & Hanna, 1999). The optimal transesterification reaction of Jatropha oil is carried out using 20 wt% methanol and 1 wt% homogeneous base catalyst (for example, Potassium Hydroxide, Sodium Hydroxide) in relation to oil, and maximum ester yield is achieved after 60 minutes reaction time at 60^0C (Achten, et al., 2008; Rao & Rao, 2013).

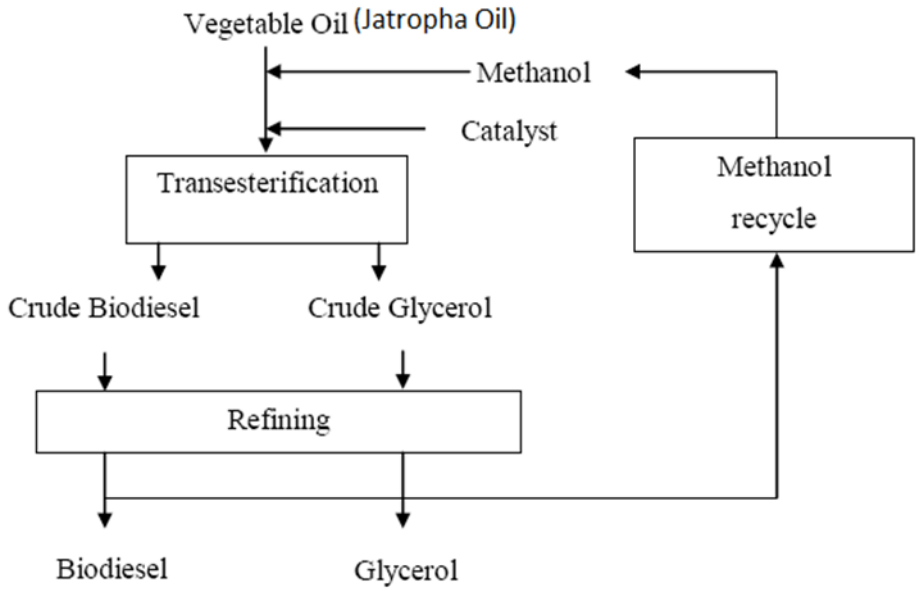
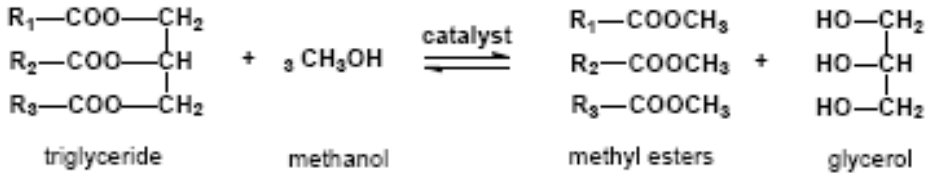


Figure 2-5. Basic schematic diagram for biodiesel production by transesterification process (Kasim, 2012; Silva, et al., 2013).

The reaction involves transesterification of triglycerides present in Jatropha oil to methyl esters with methanol as shown in *Figure 2-6* (Gerpen, et al., 2004; Hillion, et al., 2003).



with R1, R2, R3 = hydrocarbon chain from 15 to 21 carbon atoms

Figure 2-6. Transesterification reaction using an alkali catalyst (Hillion, et al., 2003; Gerpen, et al., 2004).

When triglycerides react with an alcohol (methanol or ethanol), the three fatty acid chains are released from the glycerol skeleton and combine with the alcohol to yield fatty acid alkyl esters (fatty acid methyl esters or fatty acid ethyl esters) (Buasri, et al., 2009). The most commonly used catalyst materials for converting triglycerides to biodiesel are sodium hydroxide, potassium hydroxide and sodium methoxide (Gerpen, et al., 2004; Adebayo, et al., 2011). Alkali or base catalysts are used in feedstock that contain small amounts (< 3%) of free fatty acids. Essentially all of the current commercial biodiesel producers use base catalysed reactions (Gerpen, et al., 2004). If the feedstock contains relatively high levels of free fatty acids (greater than 3%) as indicated in Section 2.3.2, the free fatty acids react with the base catalyst to form soap (Canakci & Gerpen, 2001; Kartika, et al., 2012). Acid catalysts such as sulfuric acid and phosphoric acid can also be used in esterification reaction, however acid catalysed reactions are considered to be too slow (in reaction) for industrial processing. Base catalysed reactions are relatively fast, with residence times from about 5 minutes to about 1 hour (Gerpen, et al., 2004; Abdalla & Oshaik, 2013; Rao & Rao, 2013). When an acid catalyst is used, free fatty acids are converted into biodiesel as shown in *Figure 2-7*.

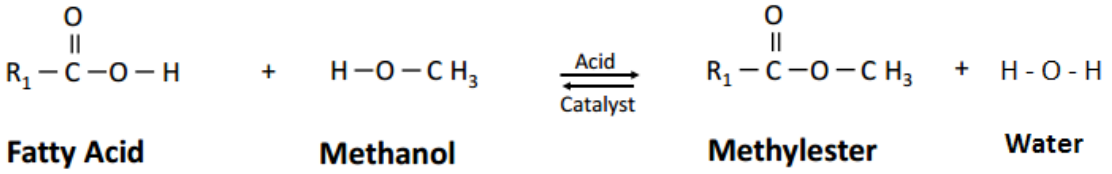


Figure 2-7. Esterification reaction using an acid catalyst.

In cases of high free fatty acids content in the feedstock (greater than 3%), Berchmans and Hirata (Berchmans & Hirata, 2008) suggest using a two-step process acid–base catalysed transesterification process. The first step involves acid esterification for removing or reducing free fatty acids content in the oil, which is mainly a pre-treatment process. The second step is an alkali or base catalysed transesterification process. Achten (Achten, et al., 2008) recommends that the pre-treatment reaction be carried out using methanol at molar ratio of 5:1 (methanol to oil) and 1.4 wt% H_2SO_4 as a catalyst at 60^0C for about 90 minutes.

2.4.2. Quality of Feedstock for Biodiesel Production

The quality of a feedstock is of paramount importance in production of biodiesel since it determines both quality and yield of biodiesel. Generally, feedstock used for biodiesel production are fats and oils, which are triglycerides. The main differences between these feedstock are water content, free fatty acid (FFA) level and saturation level or fatty acid profile (Atadashi, et al., 2012; Canakci & Gerpen, 2001; Canakci & Gerpen, 1999). Thus, this are the main determinants of feedstock quality in biodiesel production. The conversion method used for converting a feedstock to biodiesel is dependent on the level of FFA in the feedstock as mentioned earlier in Section 2.3.2.

2.5. Biodiesel Standardization and Test Methods

The standards for regulating biodiesel quality are based on a variety of factors which vary from region to region. These factors include the types of diesel engines most common in the region, the emissions regulations governing those engines, the development stage and the climatic conditions of the region/country where it is produced and or used, and not least, the use of biodiesel (Barabás & Todoruț, 2011). In the present investigation, the American Standards for Testing and Material (ASTM) and the European standards were used since they cover almost all of the international biodiesel standards. Biodiesel properties and methods of measurement specified by ASTM and European standards are shown in Table 2-4. The European standards have narrower and stringent limits as compared to the American standards.

Table 2-4. Biodiesel properties and methods specified by European and American (ASTM) standards (Barabás & Todoruţ, 2011; Gerpen, et al., 2004; Knothe, 2006).

Property	ASTM Biodiesel standard			European biodiesel standard			Units
	Method	Limits		Method	Limits		
		Min	Max		Min	Max	
Flash point	D93	130		EN ISO 3679	120		^{o}C
Kinematic viscosity at 40 ^{o}C	D445	1.9	6.0	EN ISO 3104, ISO 3105	3.5	5	mm^2/s
Acid value	D664		0.5	EN 14104		0.5	mg KOH/g
Iodine value				EN 14111		120	g I/100 g
Density at 15 ^{o}C				EN ISO 3675, EN ISO 12185	0.86	0.9	g/cm^3
Cloud point	D 2500	report					^{o}C
Pour point							^{o}C
Cetane number	D 613	47		EN ISO 5165	51		
Calorific value	D5865						
Moisture content	D 2709		0.05				%
Sulfated Ash	D874		0.02				Wt.%

Section 2.6 discuss the toxicity of Jatropha seeds and seed oil as reported by various researchers.

2.6. Toxicity of Jatropha Seeds, Seed Oil and Biodiesel

There is considerable work of research study carried out on the toxicity of the Jatropha plant. However, most literature have focused on mature brown dry fruits and little or no attention given to various maturity stages of Jatropha fruits. Most parts of the Jatropha plant are reported to be toxic and these include the seeds, leaves, stem, flower, buds, roots, bark, and wood (Devappa, et al., 2010; Xiao, et al., 2015; Pradhan, et al., 2010; Roach, et al., 2012; Makkar & Becker, 1999; Baldini, et al., 2014). The crude oil extracted from the seeds of the plant also contains toxic chemical compounds. Most researchers focused on the toxicity of the Jatropha seeds. Makkar and Becker (Makkar & Becker, 1999) studied the toxins present in the Jatropha curcas seeds. They identified the toxins as phorbol esters, curcin, tannins, phytates, flavonoids, saponins, vitexine, cyanide and trypsin inhibitor. According to their study the toxicity of the Jatropha curcas seeds vary with location (Makkar & Becker, 1997). They reported that some stains of Jatropha curcas had toxic seeds whereas some had nontoxic seeds. Non-toxic Jatropha curcas have been reported in Veracruz in Mexico (Sanghamitra, et al., 2014; Valdes-Rodriguez, et al., 2013). However, this non-toxic genotype of Jatropha is not as abundant as the toxic genotype. According to (Kumar, et al., 2018), non-toxic Jatropha are those that contain PEs less than 0.05mg/g whereas toxic Jatropha contains PEs of quantity 0.05mg/g or more. *Table 2-5* shows some of the toxins by chemical concentration found in Jatropha seed cake from Ilorin, Nigeria.

Table 2-5. Analyzed phytotoxins content in Jatropha seed cake (brown dry fruits) from Jatropha curcas cultivated in Ilorin, Nigeria (Annongu, et al., 2010).

Phytotoxin	***Concentration (mg/g)***
Phorbol esters	2.79
Total phenols	3.60
Tannins	0.40
Phytates	94.0
Saponins	26.0
Trypsin inhibitors	21.3
Lectins (1/mg meal/ml assay)	102

A number of researchers from various geographical locations characterized Jatropha curcas seed cake, oil and derived biodiesel from brown dry fruits for toxicity (Pradhan, et al., 2010; Punsuvon & Nokkaew, 2013; Xiao, et al., 2015; Devappa, et al., 2013). They determined Phorbol ester

content of Jatropha curcas seed oil, cake and biodiesel using high performance liquid chromatography. According to their findings, the quantity of phorbol esters were recorded as 3.17 and 1.59 mg/g in oil and seed cake, respectively. Their results as shown in *Table 2-6* depict that Phorbol esters were detected in oil and seed cake but not detected in biodiesel. Therefore, based on their results it can be deduced that the biodiesel production process (including transesterification) eliminates phorbol esters from the oil. The toxicity of Jatropha curcas seeds emanates mainly from phorbol esters (Wakandigara, et al., 2013; Wink, et al., 2004; Makkar, et al., 2009; Diwani, et al., 2011). Phorbol esters are distributed in different parts of the Jatropha curcas plant, however they are mainly concentrated in the seed kernel. Analyses have shown that about 70% of the total phorbol esters in Jatropha seeds are dissolved in the oil and the remainder is found in the deoiled seed cake (Makkar & Becker, 2009; Pradhan, et al., 2010; Devappa, et al., 2013). The phorbol ester content has been found to vary from 0.87 - 3.32 mg/g in different varieties of Jatropha seeds (Makkar & Becker, 1997). According to Wink et al (Wink, et al., 2004), the phorbol esters in Jatropha seed cake and oil make it toxic to humans, rodents and livestock if ingested. Musa et al (Musa, et al., 2011) carried out an experimental study where by Jatropha curcas oil was used as an insecticide. They treated maize grains with Jatropha oil. Their findings showed that at 24 hours after treatment, all insects died at an application rate of 1.0 ml/100g grains indicating that Jatropha oil had fatal toxicity to insects. Jatropha intoxication in human showed symptoms such as abdominal pain, vomiting, diarrhoea, miosis, and dehydration (Devappa, et al., 2010).

Table 2-6. Content of phorbol esters in Jatropha oil, seed cake and biodiesel from brown dry fruits cultivated in various geographical locations.

Sample	**Phorbol ester content (mg/g) (References)**			
	Gujarat, India (Pradhan, et al., 2010)	**Thailand** (Punsuvon & Nokkaew, 2013)	**China** (Xiao, et al., 2015)	**Maui, HI, USA)** (Devappa, et al., 2013)
Jatropha oil	3.17	2.93	3.09	3.38
Seed cake	1.59	0.55	-	1.84
Biodiesel	Not detected	-	-	-

Wakandigara et al (Wakandigara, et al., 2013) studied the toxicity of phorbol esters since they are the main toxic compounds found in the Jatropha curcas plant. They found that six different phorbol esters were present in Jatropha seed oil and they classified them as Jatropha factors C_1, C_2, C_3, C_4, C_5 and C_6. The chemical structures of these phorbol esters are shown in Figure 2-8 and Figure 2-9.

There are several methods that can be used to analyse the toxic compounds present in Jatropha plant. However, the most accurate and highly sensitive methods are the high-performance liquid chromatography with diode array detection (HPLC-DAD) and high-performance liquid chromatography with mass spectrometry (HPLC-MS) (European-Food-Safety-Authority, 2015; Devappa, et al., 2013; Baldini, et al., 2014).

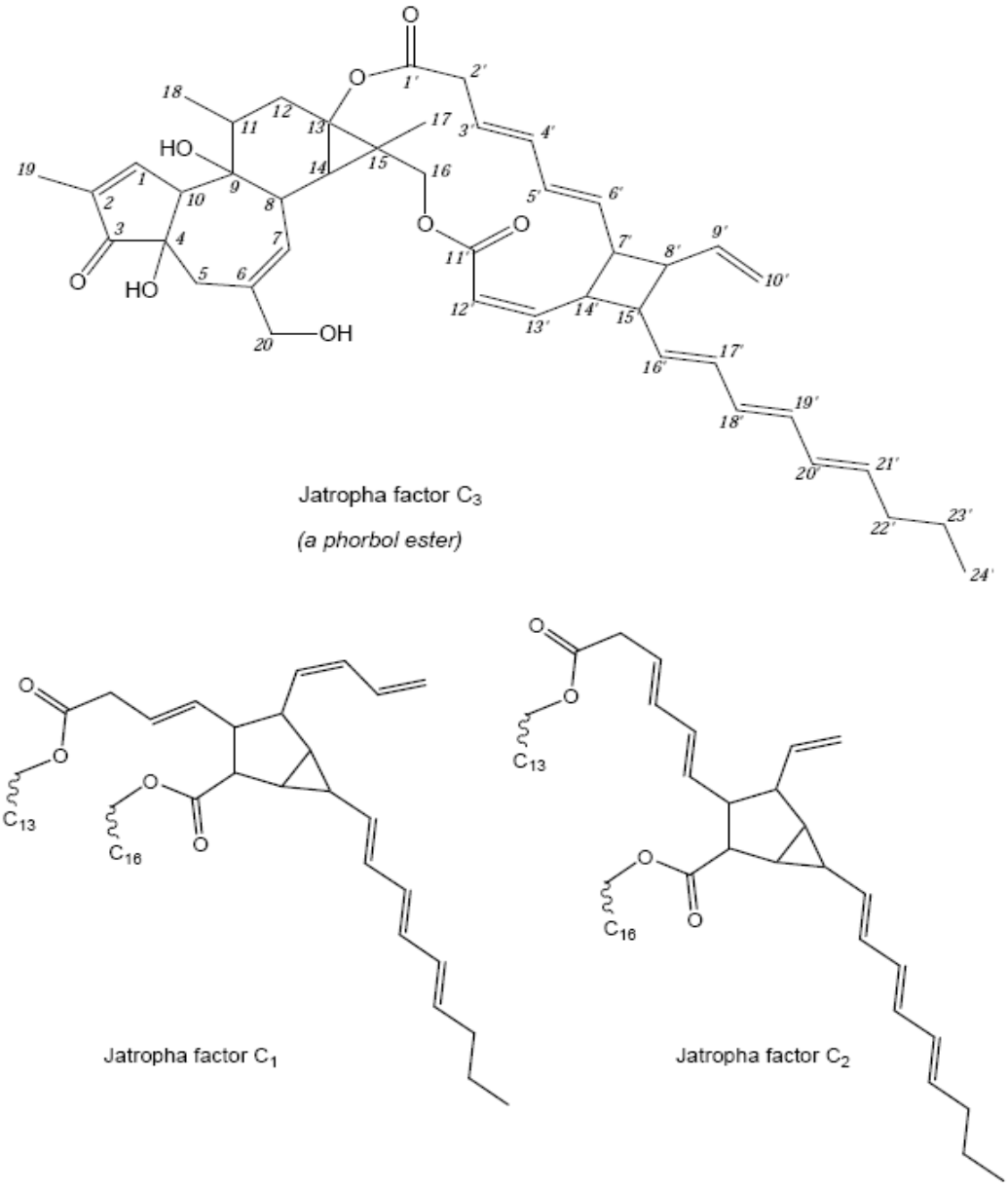


Figure 2-8. Phorbol esters C_1, C_2 and C_3 (Wakandigara, et al., 2013).

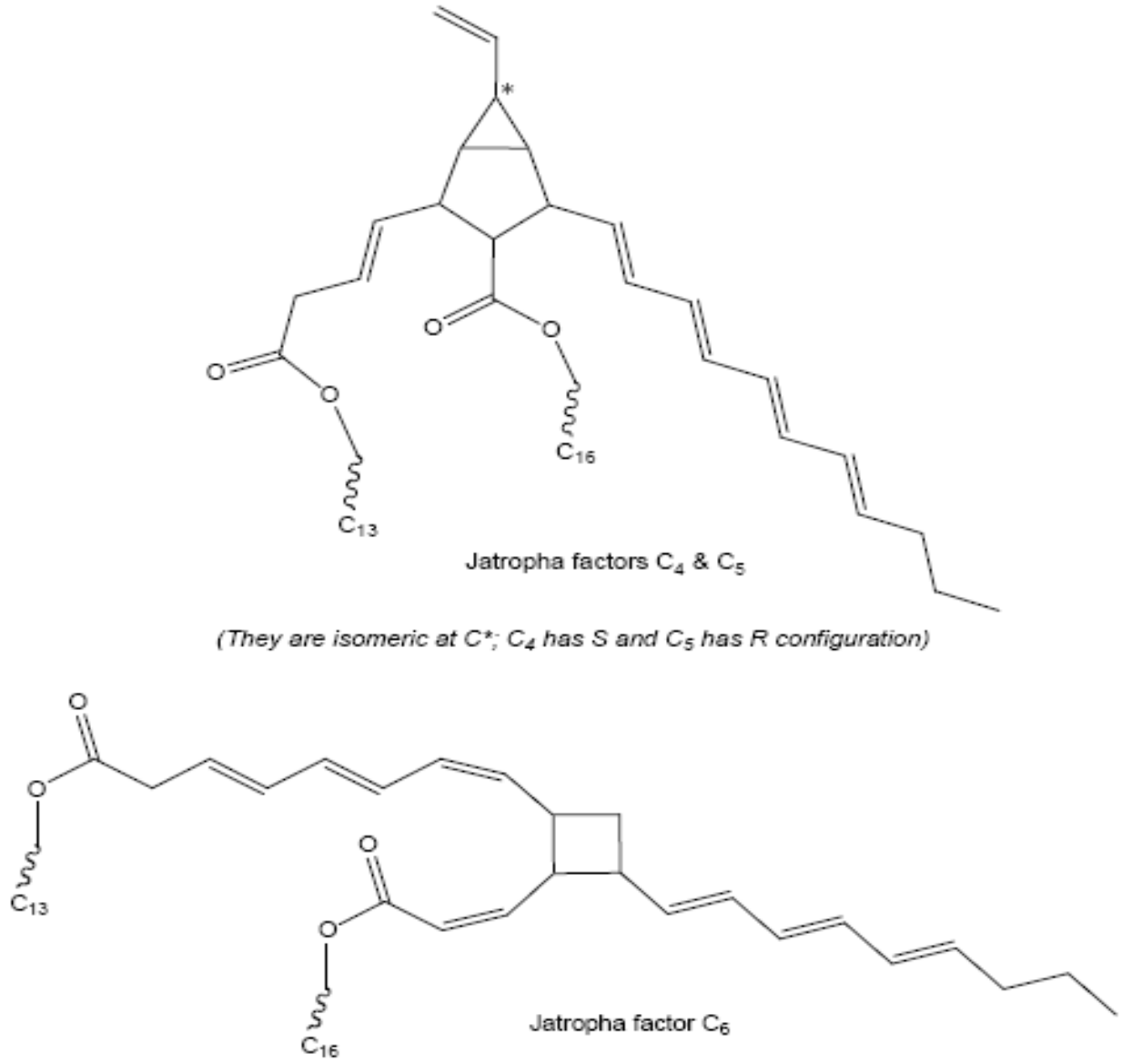


Figure 2-9. Phorbol esters C_3, C_4 and C_5 (Wakandigara, et al., 2013).

Section 2.6.1 discusses various detoxication methods that can be used to detoxify Jatropha oil and seed cake as reported by literature.

2.6.1. Detoxification of Jatropha Seed Oil and Seed Cake

There are several methods (physical, chemical and biological) that can be used to detoxify Jatropha seeds, seed oil and seed cake. Trypsin inhibitors and lectins present in Jatropha can be destroyed by moist heating (Gogoi, et al., 2015). Phorbol esters and phytates are insensitive to heat hence the heat treatment method is not effective in removing Phorbol esters and phytates. High polarity solvents such as methanol and ethanol can be used to remove Phorbol esters and phytates (Gogoi, et al., 2015). Makkar and Becker (Makkar & Becker, 1999) combined the heating and chemical extraction method to detoxify a Jatropha seed meal. They heated the Jatropha seed meal at 120^0C in the presence of 67% moisture for 30 minutes followed by treatment with aqueous methanol. The solvent (methanol) and the toxic phorbol esters were recovered by distillation process. Makkar and Becker suggest that the recovered solvent can be reused and the phorbol esters can be used as insecticides and molluscicides. Punsuvon and Nokkaew (Punsuvon & Nokkaew, 2013) reported 100% elimination of phorbol esters from deoiled Jatropha curcas meal (Jatropha seed cake). Their method of detoxification involved extraction of the seed cake with 0.1 M NaOH in 90% methanol and 85% ethanol. However, their study did not cover the detoxification of Jatropha seed oil. Xiao et al (Xiao, et al., 2015) suggested using ultraviolet irradiation to detoxify Jatropha oil. In their investigations, they exposed Jatropha oil to ultraviolet radiation inside quartz vessels at room temperature for 40 minutes. Ultraviolet (UV) radiation causes phorbol esters to undergo biodegradation. After treatment by UV, the Jatropha oil was washed with 65% ethanol. The Jatropha oil was then separated from ethanol by evaporation (distillation) at 50^oC. According to their results, phorbol esters were completely removed from the oil. Their results also indicated that the detoxification method had no detrimental effects on the quality of Jatropha oil. Detoxification of Jatropha seed oil has received less attention from researchers as compared to the detoxification of Jatropha seed cake. This is so because there has been growing interest in using Jatropha seed cake as a feed to livestock due to its high nutritional value (Gogoi, et al., 2015; Annongu, et al., 2010; Makkar & Becker, 1997). The present study is not intended to cover detoxification of Jatropha seed cake or biodiesel produced from Jatropha oil plant.

2.7. Storage of Jatropha Seeds, Seed Oil and Derived Biodiesel

The shelf life of seeds, seed oil and derived biodiesel may vary from days to years depending on storage conditions. Degradation of seed oil is mainly due to chemical reactions namely, hydrolysis and oxidation (Rodrigues, et al., 2015; Sharma & Mann, 2015; Andersson & Lingnert, 1998; Yaakob, et al., 2014). During hydrolysis of seed oil, water attacks the ester linkage of triacylglycerol and produces di- and mono-acylglycerols, glycerol, and free fatty acids as shown in Figure 2-10. During oxidation of seed oil, oxygen react with triacylglycerols forming hydroperoxides as primary oxidation products which then decompose into secondary oxidation products such as aldehydes, ketones and alcohols (Sharma & Mann, 2015). Oxidation generally occurs with unsaturated fatty acids.

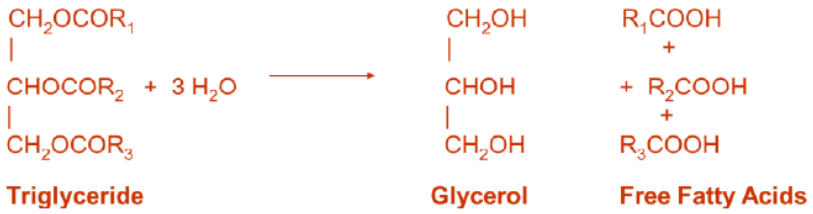


Figure 2-10. Hydrolysis of oil (triglycerides) producing free fatty acids.

After the final fruit maturity stage (brown dry), the quality of Jatropha seeds and seed oil continue to change during storage. During storage, seed oil quality and quantity can remain at the initial level or decline significantly depending on storage conditions (Šimić, et al., 2007). According to Simic et al, who investigated influence of storage conditions on maize, soybean and sunflower seeds, unfavourable storage conditions, particularly temperature and moisture, contribute significantly to acceleration of seed deterioration during storage. Their findings indicate that unfavourable storage conditions result in decline of seed oil quality and quantity. Sisman and Delibas (Sisman & Delibas, 2005) investigated how storage conditions such as moisture and temperature affected sunflower seeds and seed oil quality (free fatty acids) and oil content. According to their findings, oil content in sunflower seeds declined by 2.8% over a period of 3 months of storage while free fatty acids (FFA) content increased by 55% over the same storage period. The quality and quantity of seed oil during storage is mostly affected by temperature, moisture and the storage duration (Andersson & Lingnert, 1998; Akowuah, et al., 2012; Chen &

Ahn, 1998). Akowuah et al., (2012) investigated the influence of storage of Jatropha seeds on seed oil yield and FFA content. Their findings revealed that oil content in Jatropha seeds decreased from 35.5% to 31% whereas FFA content increased from 7.8% to 32% after four months of storage. Rodrigues et al., (2015) investigated the storage stability of Jatropha seed oil over a period of 42 days in the dark, at 35^{o}C and 75% and 92% relative humidity. After the 42 days of storage, FFA content increased from 0.8% to 7.4% and 25%, respectively. This clearly indicates that moisture has a high effect in hydrolysis of seed oil.

Chapter 3 gives details on research methodology, which covers harvesting of fruits, oil extraction from seeds, analyses of the seed oil and biodiesel produced from it for physical and chemical properties (quality).

Chapter 3

3. Methodology

3.1. Introduction

This chapter addresses methods and equipment used in collecting data, experimental setup and procedures used in the present investigation. It covers field work and laboratory work from harvesting of Jatropha fruits at different maturity stages in selected geographical locations in Botswana, to analysis of the seed oil and biodiesel in the laboratory. The crude seed oil was analyzed for yield and quality (physical and chemical properties) before being converted to biodiesel using transesterification process as described later in this Chapter. The converted biodiesel was also analyzed for physical and chemical properties. These properties were determined according to international test methods and standards including the American Society for Testing and Materials (ASTM) standard methods and European standards. The level of free fatty acids present in the oil determined the transesterification method (one-step or two-step processes) and type of catalyst (alkali or acid) to be used (Berchmans & Hirata, 2008; Bouaid, et al., 2016; Chai, et al., 2014).

3.2. Harvesting of Jatropha Fruits

Seeds harvested from Jatropha found and adapted to Botswana climatic conditions were used. In Botswana, Jatropha plants start to flower in November and bear fruits from mid-December till May. It takes about three weeks from flowering for the fruit to turn yellow and further two weeks for it to become brown dry. Therefore, Jatropha fruits can be harvested from January until May in Botswana. Jatropha fruits were harvested at various maturity stages (mature green, green yellow, yellow, yellow brown and brown dry) from selected 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^{o}S latitude, 27.7514^{o}E longitude) and Shashe (21.433^{o}S latitude, 27.450^{o}E longitude). These geographical locations are indicated in Figure 3-1. These are areas with relatively high concentration of Jatropha plants in Botswana. Harvesting or collection of Jatropha fruits was carried out between the months of March to May 2017/2018. 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. The maturity of the fruits/seeds were categorised in terms of colour

change as demonstrated by *Figure 3-2*. Seeds extracted from mature green fruits have a pale seed coat and have immature to no kernel as shown in Figure 3-3. Therefore, very little oil can be extracted from green fruits. This being the reason, seed oil was extracted from four maturity stages namely, green yellow, yellow, yellow brown and brown dry.

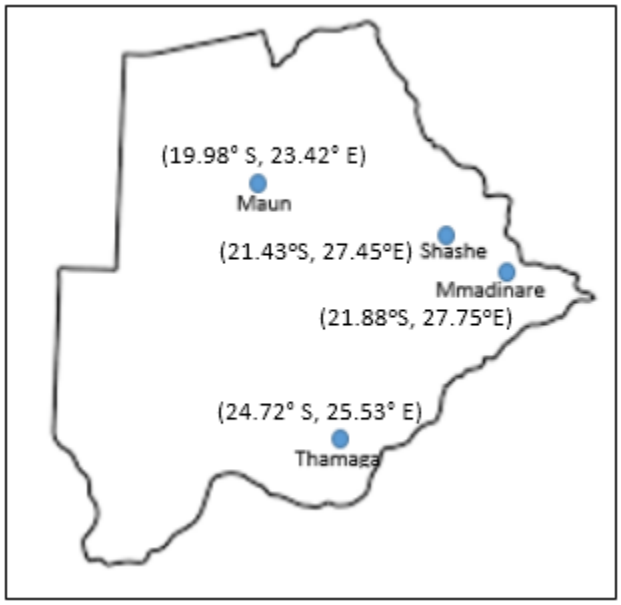


Figure 3-1. Geographical locations where Jatropha fruits were harvested in Botswana.

Figure 3-2. Jatropha fruits at five different maturity stages namely, mature green, green yellow, yellow, yellow brown and brown dry.

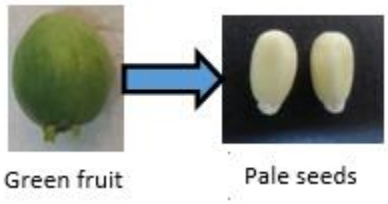


Figure 3-3. Seeds extracted from mature green Jatropha fruits have a pale seed coat and the seed kernel is inmature.

The influence of climatic conditions on Jatropha plant is outside the scope of this study. However, a brief overview of the climatic conditions of the areas where Jatropha fruits were harvested in Botswana is provided in Table 3-1. The average rainfall and temperature was recorded over a period of 10 years (from 2009 to 2019) by the Department of Meteorological Services. Detailed information on rainfall and temperature recorded in those areas is provided in Appendix F. Generally, the climate of Botswana is classified as semi-arid.

Table 3-1. A brief description of the climatic conditions of collection sites where Jatropha fruits were harvested in Botswana (En.climate-data.org, 2020; Wit & Bekker, 1990).

Location	**Climatic Elements**			
	Average annual rainfall (mm)	Annual Temperature (°C)		Soil type
		Av. Min	Av. Max	
Maun	489	16	31	Alluvial and Sandveld
Mmadinare	343	13	29	Hardveld
Thamaga	416	12	28	Hardveld
Shashe	476	13	29	Hardveld

After harvesting, seeds were removed from the fruits then dried as explained in Section 3.3.

3.3. Drying of Jatropha Seeds

Drying Jatropha seeds is essential to reduce the moisture content of fresh harvested seeds to a safe storage level. Moreover, processing techniques such as mechanical oil extraction requires dry seeds to maximize de-oiling of the seeds hence reduce residual oil left in the seed cake. The seeds were removed from the fruits within 24 hours of harvesting and dried naturally (air dying) for 10 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. The seeds were considered dry when the reduction in weight, which was recorded on daily basis remained constant. A sample of 100g from each batch was monitored for change in weight. 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. The initial and final weight of the seeds were used to determine the moisture content in seeds harvested at different maturity stages.

Dried Jatropha seeds were then analysed for oil yield as described in the following Section 3.4.

3.4. Determination of Seed Oil Yield

Oil yield/content is the total amount of lipid/oil contained in seeds expressed in terms of percentage. The most common of all the solvent extraction methods for oil yield determination is the conventional soxhlet method and it has been widely used by previous researchers including (Achten, et al., 2008; Ogunleye & Eletta, 2012; Ntaganda, et al., 2014; Bello & Agge, 2010; Bhuiya, et al., 2015; Liauw, et al., 2008; Sayyar, et al., 2009). However, more precise and advanced methods such as Filter Bag Technology (FBT) developed by ANKOM Technology have been developed recently as an alternative to the conventional Soxhlet method. This alternative method was approved by American Oil Chemists' Society as an Official Procedure, Am 5-04 (AOCS, 2005). It accelerates the process by performing the oil extraction under relatively high pressure and elevated temperatures. Advantages of the FBT method such as high precision, low purchase and using costs, and shorter analyses time have been echoed by some previous researchers who have adopted the method including (Seenger, et al., 2008; Liu, 2010; Srigley & Mossoba, 2017).

The oil yield/content in Jatropha seeds harvested at four different maturity stages was determined using Filter Bag Technology according to American Oil Chemists' Society (AOCS) Standard Procedure Am 5-04. Dried Jatropha seeds were de-shelled to obtain the kernels. The seed kernels were then ground into powder using Mellerware Aromatic Grinder (29105), thus increasing the surface area for extraction. The process is as demonstrated by Figure 3-4. 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^{o}C for 3 hours according to manufactures specifications. The samples were then allowed to cool to room temperature for 15 minutes 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 shown in Figure 3-5 which follows the AOCS Standard Procedure Am 5-04. Petroleum ether was used as a solvent for this 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. Extraction temperature was then set at 90^{o}C and extraction time at 60 minutes. Extraction process was started with the instrument in automatic operation. 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 105ºC for 30 minutes to get rid of the solvent. The samples were placed in a desiccator for 15 minutes to cool to room temperature. Each filter bag was re-weighed.

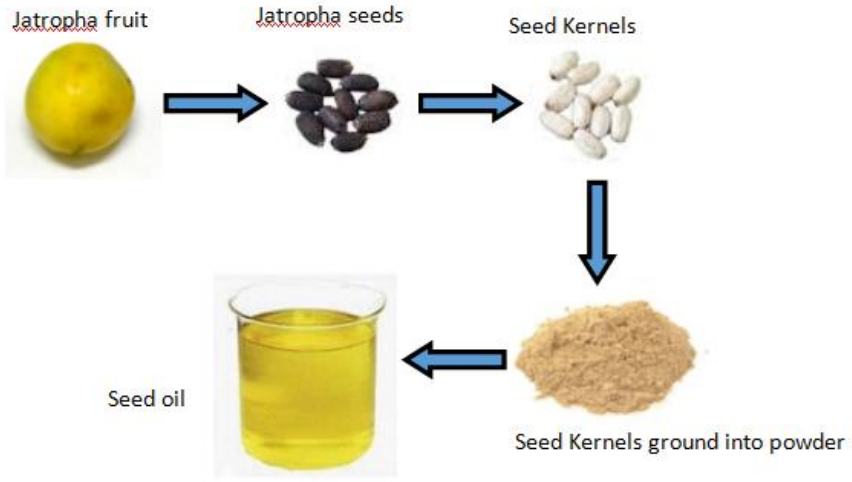


Figure 3-4. An illustration of processing of Jatropha fruits to obtain seed kernels for oil yield determination via solvent extraction method.

The percentage oil content in Jatropha seeds was determined by recording the weight of dried seeds before extraction of the oil then recording the weight of the seeds (seed cake) after oil extraction. This procedure was repeated five times for each sample then calculated the average. Oil yield was then calculated using *Equation 3-1*.

$$\%\ Oil\ yield = \frac{W_2 - W_3}{W_1} \times 100\%$$

Equation 3-1

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

Figure 3-5. Ankom XT15 fat extractor.

Source: Sebele Agriculture research lab

Table 3-2. Technical specifications for Ankom XT15 extractor used for extracting fats and oils.

Technical Specifications	
Sample Size	1.0 – 3.0 grams
Fat/Oil Range	0% - 100%
Samples per batch	Up to 15
Operating Temperature	90°C
Dimensions	13" wide x 20" deep x 31" high
Power Requirements	110 – 240V, 50/60 Hz
Weight	44Kg

After determination of oil content in Jatropha seeds from four different maturity stages, oil was then extracted from the seeds as described in Section 3.5.

3.5. Oil Extraction from Jatropha Seeds

Oil was extracted from dried Jatropha seeds harvested at four different maturity stages using an electric powered Kern Kraft KK40 mechanical screw press. This equipment is shown in Figure 3-6. Mechanical screw press is relatively cheaper and less complicated as compared to solvent extraction method. Therefore, it is the best choice when extracting larger quantities of seed oil. It is worth noting that mechanical extraction method was not used to determine oil content in seeds, but rather to extract larger quantities of oil to be used in other experimental activities such as production of biodiesel and characterization of physicochemical properties, as described in the following subsequent Sections 3.6 to 3.18. The screw was first heated to about 85^{o}C before oil extraction commenced using a band heater, in order to enhance oil extraction process thus increasing the amount of oil produced. Seeds were then fed into the feeding hopper while the screw was rotating at a specified speed (20rpm). Unlike with solvent extraction method, seeds were fed into the screw press as whole seeds (without de-shelling). The equipment was operated at a speed of 20 revolutions per minute (r.p.m). Faster speeds reduce oil extraction efficiency thus reduction in amount of oil extracted from the seeds. Extracted oil was discharged through the oil outlet holes while the seed cake was ejected through the nozzle of 15mm opening. The oil was left in a container for about 24 hours for residue to settle to the bottom. The oil was then separated from the residue.

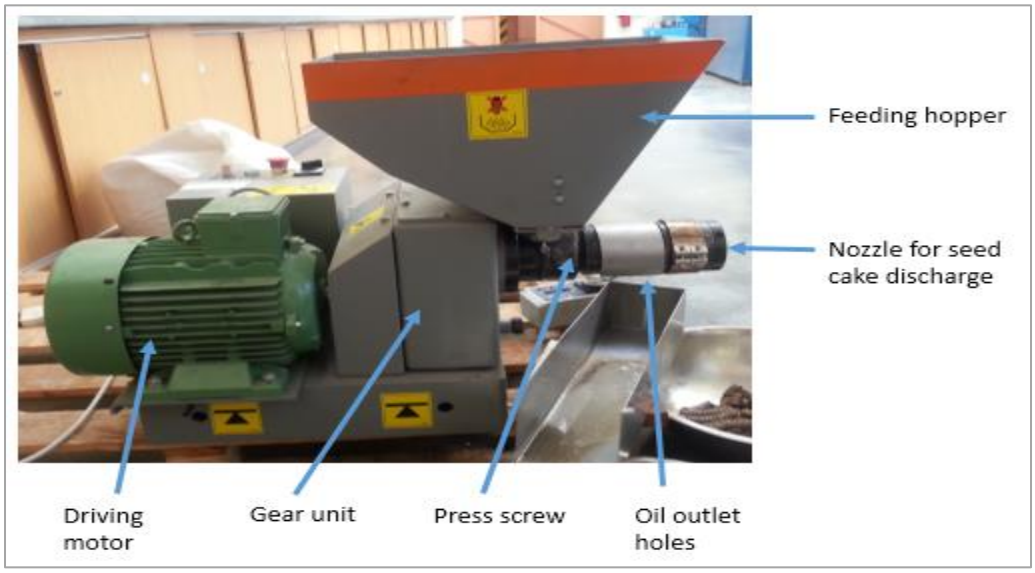


Figure 3-6. An electric powered Kern Kraft KK40 mechanical screw press.

Source: University of Botswana, Department of Mechanical Engineering

3.6. Determination of Acid value/Free Fatty Acids Content

Free fatty acids content, discussed in more detail in Section 2.3.2, can also be expressed in terms of acid value. The acid value is defined as number of milligram of KOH necessary to neutralize 1.0 g of the sample (Ali & Abdurrhman, 2013; Kardash & Tur'yan, 2005; Vitz, et al., 2017; Ramos, et al., 2009). Free fatty acids (%) and acid value are related by Equation 3-4. Free fatty acids are one of the most important quality measures of a feedstock used in the production of biodiesel as mentioned earlier in Section 2.3.2. Therefore, it is essential to determine the level of free fatty acids present in Jatropha seed oil or any other feedstock used for biodiesel production prior to the processing stage. There are several methods that can be used in determination of free fatty acids. These include titration method, HPLC, capillary gas chromatography and capillary electrophoresis (Ali & Abdurrhman, 2013; Silitonga, et al., 2013). The most common free fatty acids content determination method is the titration method (Azhari, et al., 2008; Bojan & Durairaj, 2012; Berchmans & Hirata, 2008; Mu'azu, et al., 2013). This method is cheaper and easy to use. The content of free fatty acid and acid value of Jatropha oil were determined using the titration method in accordance to ASTM D664 test method.

Potassium Hydroxide (KOH) solution with normality of 0.1M was prepared by dissolving 5.61g of white deliquescent KOH pellets in 10ml of deionized water then adding 1L of 99% ethanol. The solution was left in a tightly stoppered bottle for 24 hours to ensure complete dissolution. The solution (KOH) was standardized by pipetting 50ml of 0.1M hydrochloric acid (HCL) into a conical flask then adding 5 drops of phenolphthalein indicator. The HCL was then titrated with Potassium Hydroxide solution until permanent pink colour appeared. (1ml of 0.5M HCL acid is equivalent to 0.02806g of KOH)

Oil sample of about 2g was charged into a conical flask. 50ml of a mixture of 95% ethanol/ toluene, 1/1, v/v and 5 drops of phenolphthalein indicator were added to the flask. The mixture was then titrated with KOH while stirring vigorously until a permanent pink colour appeared. The acid value and free fatty acids were then calculated using *Equation 3-2* and *Equation 3-3*, respectively. The experiment was repeated five times for each sample then calculated the average value.

$$Acid\ Value = \left(\frac{V \text{ x N x } MW_{KOH}}{W}\right) \quad \text{(Silitonga, et al., 2013)}$$

Equation 3-2

$$\%FFA = \left(\frac{V \text{ x N x } MW_{C18:1}}{W \text{ x } 1000}\right) \quad \text{(Silitonga, et al., 2013)}$$

Equation 3-3

Where:

V = potassium hydroxide solution consumed in the titration (mL)

N = normality of the potassium hydroxide solution

W = weight of oil sample (g)

MW = molecular weight (g/mol)

MW_{KOH} = 56.1g/mol

$MW_{C18:1}$ = 282.5 g/mol (expressed as oleic acid)

$$Acid\ Value \sim 2 \times \%FFA$$

Equation 3-4

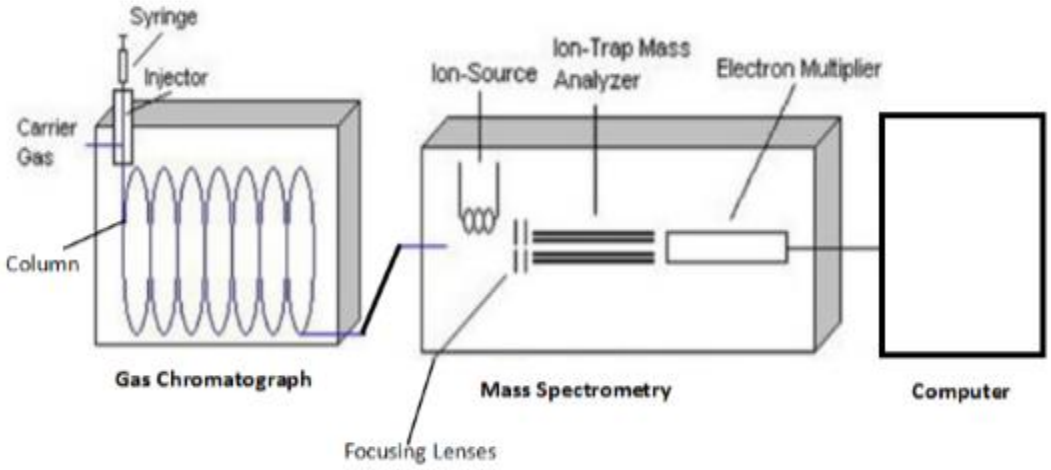
3.7. Determination of Fatty Acid Composition

The fatty acid profile of Jatropha seed oil is discussed in more detail in Section 2.3.1. As mentioned earlier previous researchers focused mainly on seed oil derived from brown dry fruits. The present study focuses on chemical composition (fatty acid profile) of seed oil derived from Jatropha fruits at different maturity stages.

The chemical composition (fatty acid profile) of the seed oil together with the biodiesel derived from it was analyzed using Gas Chromatography – Mass Spectrometry (GC-MS) as described by (Botineștean, et al., 2012; Ruhul, et al., 2016; Abdullah & Hassan, 2014; Ramos, et al., 2009). The fatty acids in the seed oil were converted to methyl esters (biodiesel) by transesterification process before they were injected into the gas chromatograph since GC analyzes fatty acids either as free fatty acids or as fatty acid methyl esters (FAME). Seed oil (triglycerides) is not volatile hence conversion to more volatile FAME.

The composition and quantity of fatty acid methyl ester (FAME) was determined according to test method ASTM D6584, using Agilent Technologies GC System 7890A gas chromatograph (GC) equipped with a HP-5MS capillary column (30m x 250µm x 0.25µm) and an automated injector. The equipment setup is shown in Figure 3-7. The GC was connected to Agilent Technologies 5975C mass spectrometer with Triple-Axis detector. Helium was used as carrier gas at a pressure of 72kPa and flow rate of 64mL/min according to manufacturers' specification. 1µL of sample was injected using an automated injector. The initial oven temperature was set to 100^{o}C for 4 minutes, which was then increased at a rate of 7^{o}C/min to 235^{o}C, then 10^{o}C/min to 300^{o}C for 7 minutes. The injector and detector temperatures were set to 325^{o}C. Total run time was about 36 minutes.

(a)



(b)

Figure 3-7. (a)Agilent Technologies GC System 7890A gas chromatograph (GC) equipped with a HP-5MS capillary column (30m x 250µm x 0.25µm) connected to Agilent Technologies 5975C mass spectrometer, (b) a schematic diagram of a Gas Chromatography – Mass Spectrometry setup.

Source: (a) University of Botswana, Department of Health Sciences

3.8. Determination of Calorific Value and Sulfated Ash

3.8.1. Calorific Value

The calorific value of biodiesel was determined using Oxygen Bomb Calorimeter, IKA C200, in accordance to test method ASTM D5865. About 1g of sample was prepared and charged into a crucible. A piece of cotton thread (with gross calorific value of 50J) was attached to the fuse wire with the other end of the thread immersed in the sample. The bomb head (with sample) was then inserted into the bomb cylinder then the screw cap was screwed firmly to a solid stop. The bomb was charged with oxygen to a pressure of 30 bar to ensure complete combustion of the sample. When the bomb was ready it was put into the calorimeter. The bomb was handled carefully to ensure that the sample was not disturbed. Distilled water was filled to the mark into the calorimeter. The calorimeter was then started and run for about 26 minutes. Data was displayed and recorded through the computer. Each experiment was repeated three times then calculated the average, though the variation was not significant.

3.8.2. Sulfated Ash

Sulfated ash is the amount of unburnt matter after complete combustion. Weight of crucible was recorded before and after combustion of the fuel in Bomb Calorimeter. The difference in weight were recorded as unburnt matter (sulfated ash).

3.9. Determination of Kinematic Viscosity

Viscosity of seed oil and biodiesel was determined using a digital electronic viscometer (Fungilab Premium Series) shown in Figure 3-8 in accordance to ASTM D445. Specifications for Fungilab Premium digital electronic viscometer are shown in Table 3-3. The viscometer was connected to a Thermo-scientific (Hake AC 150) water heater which heats and circulate 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 15ml 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 the tests to

ensure accurate results. Low viscosity adaptor (LCP spindle) was used for both seed oil and biodiesel since it can be used for fluids with viscosities as low as 1cp. 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 bath was circulated around the test cup to control the temperature of the sample. The sample was heated gradually from room temperature (about 20^{o}C) to 50^{o}C while recording the corresponding viscosity at each unit temperature increment. A maximum temperature of 50^{o}C was set to avoid thermal decomposition of the oil due to excessive heat. Viscosity at 40^{o}C was noted, as specified by the American Society for Testing and Materials (ASTM D445). Viscosity recording of each sample was repeated five times then calculated the average value.

Figure 3-8. Fungilab Premium digital electronic viscometer used for measuring viscosity and Thermo-scientific (Hake AC 150) water heater.

Source: University of Botswana, Department of Mechanical Engineering

Table 3-3. Specifications for the Fungilab Premium digital electronic viscometer used for measuring viscosity.

Specifications
Precision: ± 1% of full scale
Resolution: With low viscosity adapter: 0.01 For lower than 10.000 viscosity cP: 0.1 For viscosity equal to or above 10.000 cP: 1
Repeatability: 0.2%
Thermometer features: Temperature margins: 0ºC to +100ºC 32ºF to 212.0 ºF Resolution: 0.1ºC / 0.1722 ºF Precision: +/- 0.1 ºC Type of probe: PT100
Supplied at 100-240 VAC, 50/60 Hz
Measuring Range (cP) : 1 - 106.000.000
Speed (r.p.m) : 0.01 - 250

3.10. Determination of Density and Specific Gravity

The density and specific gravity of seed oil and biodiesel were determined using KEM Kyoto Electronics density meter, Figure 3-9, in accordance to the test method ASTM-D1298. Specifications for the test instrument are presented in Table 3-4. Before performing sample density measurement, the density of pure water was determined to ensure accuracy of the instrument. The precision of the instrument is $1 \pm 0.001 g/cm^3$. Factor calibration was carried out whenever the difference was greater than $\pm 0.001 g/cm^3$. Density measurement was carried out by filling the cell with sample then recording the reading from the display screen. Five recordings were carried out for each sample then calculating the average value. The cell was cleaned using ethanol before measuring a different sample then allowed to dry for 30 minutes according to manufacturer's specifications.

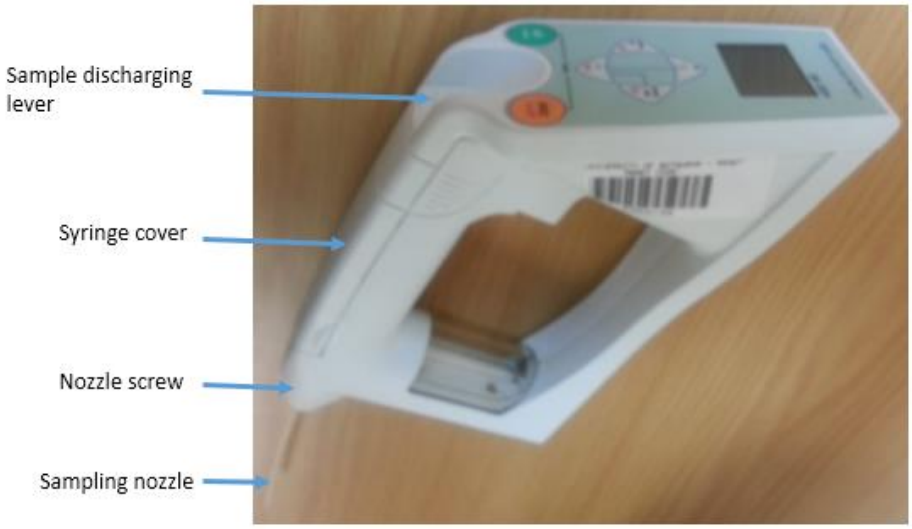


Figure 3-9. Portable KEM Kyoto Electronics density meter.

Source: University of Botswana, Department of Mechanical Engineering

Table 3-4. Specifications for KEM Kyoto Electronics density meter used for measuring density.

Specifications	
Range	0.0000 to 2.0000 g/cm^3
Precision	±0.001 g/cm^3
Resolution	0.0001 g/cm^3
Temperature range	0 to 40°C

3.11. Determination of Cetane Number

Cetane number is a function of molecular weight and percentage composition of individual fatty acid methyl esters (FAME). Moreover, it also depends on percentage composition of unsaturated fatty acids, that is number of double bonds (Azam, et al., 2005; Sokoto, et al., 2011). Saponification number, Iodine value and Cetane number can be calculated by relating molecular weight and fractional composition of individual FAMEs. This has enabled previous researchers to develop a

mathematical model to predict the cetane number of biodiesel based on fractional composition of fatty acid methyl esters. Klopfenstein developed a mathematical model for estimation of cetane number of pure fatty acid esters which was later further modified by Krisnangkura (Krisnangkura, 1986) to calculate a mixture of fatty acid esters and published in the Journal of the American Oil Chemists' Society. This method (mathematical equation) have since been adopted and proved by a number of researchers to yield results very close if not the same to those determined experimentally (Azam, et al., 2005; Eevera, et al., 2009; Sokoto, et al., 2011). Therefore, cetane number was calculated using Equation 3-5, Equation 3-6 and Equation 3-7 developed by Klopfenstein (Krisnangkura, 1986). The molecular masses of fatty acid methyl esters found in Jatropha seed oil and derived biodiesel are provided in the Appendix A.

$$SN = \sum \left(\frac{560 \text{ x } A_i}{MW_i}\right)$$

Equation 3-5

$$IV = \sum \left(\frac{254 \text{ x D x } A_i}{MW_i}\right)$$

Equation 3-6

$$CN = 46.3 + \left(\frac{5458}{SN}\right) - (0.225 \text{ x IV})$$

Equation 3-7

Where:

CN = Cetane Number

SN = Saponification Number

IV = Iodine Value

MW_i = Molecular mass of a particular ester (FAME)

A_i = Percentage composition of the ester (fatty acid)

D = Number of double bonds

3.12. Determination of Cloud and Pour Point

Cloud point and pour point were determined using Normalab NTE 450 automatic cloud and pour point analyzer connected to Tamson low temperature circulator TLC80 in accordance to ASTM D2500 and ASTM D97, respectively. The equipment setup for determining cloud and pour point is shown in Figure 3-10. Specifications for the Normalab NTE 450 automatic cloud and pour point analyzer are shown in Table 3-5. The Tamson low temperature circulator which uses methanol as a cooling liquid was started and left for about 2 hours 30 minutes to cool down the methanol to about - 60^{o}C. Sample test tube with reflective bottom was filled with 50 mL of sample (seed oil or biodiesel) to the mark then attached to the measurement head. Cloud and pour point test was set to start at 10^{o}C, which is well above the expected cloud and pour point values for both seed oil and biodiesel. Testing was then started and lasted for about 1 to 2 hours for each sample. The test was automatically stopped once the cloud and pour points were reached and recorded on the display screen.

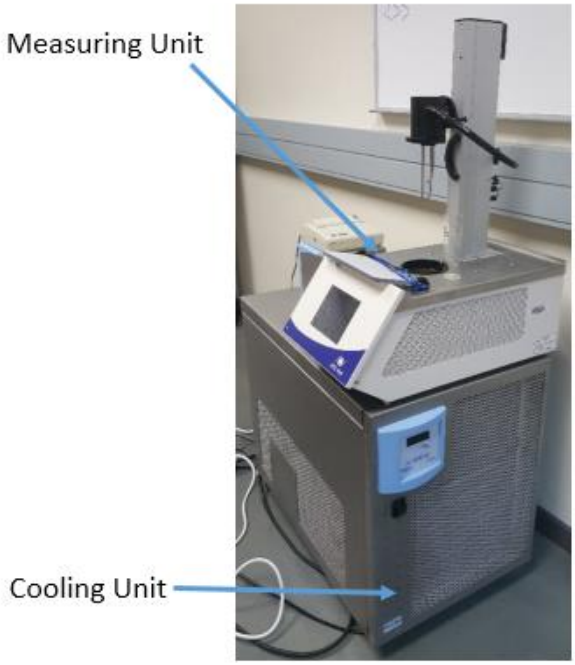


Figure 3-10. Normalab NTE 450 automatic cloud and pour point analyzer.

Source: University of Botswana, Mechanical Engineering Lab (ME 251)

Table 3-5. Specifications for the Normalab NTE 450 automatic cloud and pour point analyzer.

Specifications
Pour point • Detection by ultrasonic sensor • Temperature range from -75°C to 51°C (Lowest temperature depends on the cryostat type) • Temperature measurement resolution : 1°C • Tilting intervals: every 3°C or 1°C (parametrable)
Cloud point • Detection by optical fibre • Intervals of 1°C • Temperature measurement resolution: 0.1°C • Temperature range from -75°C to 49°C (Lowest temperature depends on the cryostat type)
Size: (W) 270x (D) 500x (H) 600 mm
Net weight: (+/- 25kg)
Voltage: AC 230V – 50/60Hz

3.13. Determination of Moisture Content

Jatropha seeds from all maturity stages were measured for moisture content immediately after they were removed from the fruits as well as after drying. The recording was done daily until seeds were completely dry as mentioned earlier in Section 3.3. Therefore variation in moisture content per maturity stage was analyzed. Moisture content of seed oil extracted was also determined. The processes of determining moisture content was performed using an electronic moisture analyser (KERN DBS VERSION 1.3) shown in Figure 3-11 in accordance to ASTM D6304. Specifications for electronic moisture analyser (KERN DBS VERSION 1.3) are shown in Table 3-6. 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 10g of sample was weighed. The sample was evenly distributed on the sample dish then placed on the weighing chamber. The lid was closed then sample drying was automatically started. A drying temperature of 120^{o}C was set to ensure that all the water content in the sample was evaporated. Rapid Drying Mode (RDM) was used since the samples did not form skin during the

dying process. The dying process ended when preset weight loss (ΔM) of 0.01% remained constant for 30 seconds following manufactures operation procedure. Drying time varied between 2 minutes to 1 hour depending on the moisture content of each sample. The weight loss was then recorded as percentage of moisture content.

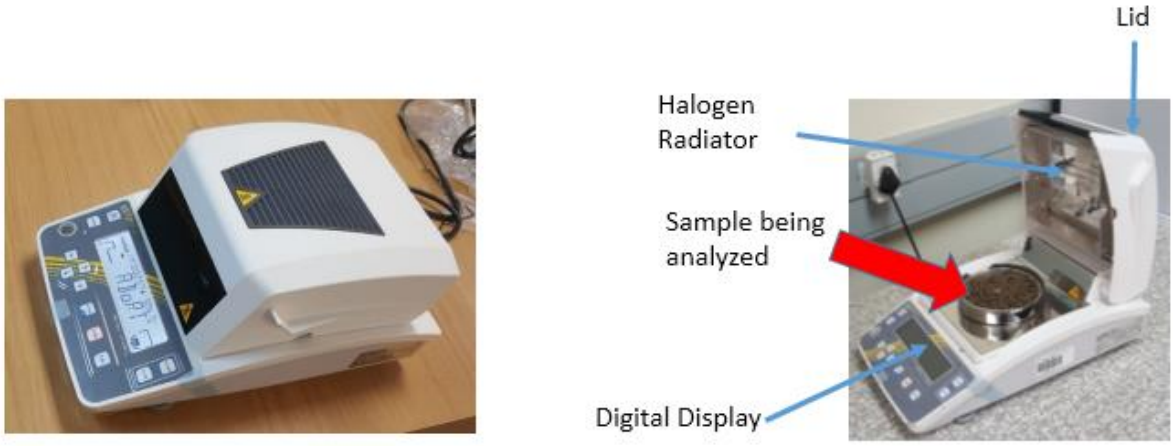


Figure 3-11. Electronic moisture analyser (KERN DBS VERSION 1.3) used for determining moisture content.

Source: University of Botswana, Mechanical Engineering Lab (ME 251)

Table 3-6. Specifications for Electronic moisture analyser (KERN DBS VERSION 1.3) used for determining moisture content.

Specifications	
Radiator	Halogen (1×400W)
Temperature range	50ºC – 200ºC
Maximum load	60g
Minimum load	0.02g
Linearity	± 0.003 g
Readability	Weight 0.001g
	Moisture 0.01%
Weighing units	(%) moisture
Dimensions (mm)	202(B) ×336(D) ×157(H)
Net weight	4.2 kg
Electric supply	AC 220 – 240 V 50/60 Hz
Power consumption	Rating 430 VA

3.14. Determination of Flash point

The flash point of Jatropha seed oil and derived biodiesel was performed using Tanaka apm - 8 flash point tester (Pensky Martens Closed Cup) according to the procedure described by (Dave, et al., 2014) in accordance to ASTM D92. The closed cup flash point tester is shown in Figure 3-12 and its specifications are presented in Table 3-7. The test cup was filled with oil sample to the specified mark and the cup closed with a test cover then placed in the assembly unit. Dave et al (2014) suggest that the temperature of the test cup and test specimen should be at least 18°C below the expected flash point. The oil sample was at room temperature (about 25^{o}C) at beginning of each test run. The oil was then heated at a rate of 5^{o}C per minute. The temperature at which the first flash occurs was recorded and displayed on the digital display screen as demonstrated by Figure 3-12. The same temperature was then recorded as the flash point.

Figure 3-12. Automated Abel closed cup tester used for measuring flash point.

Source: University of Botswana, Department of Mechanical Engineering Lab (250)

Table 3-7. Specifications of the Automated Abel closed cup tester used for measuring flash point.

Specifications	
Test Methods	ASTM D92, ISO 13736, ISO 1516/1523, IP 170
Measuring Range	+10°C to +110°C for abl-8a -30°C to +110°C for abl-8l (with an optional chiller)
Ignition source	Gas flame or Electric Coil
Temperature Sensor	PT-100 in stainless steel sheath
Flash Detector	CRC Thermocouple
Power consumption	250W
Size	230(W) × 470(D) × 385(H) mm
Net Weight	16kg

3.15. Determination of Iodine Value

Iodine value was determined according to Wijs method reported by Rao et al (2010) (Rao, et al., 2010), in accordance with ASTM D1959-97 test method. 0.7g of oil sample was weighed into a dry iodine flask. 15 mL of carbon tetrachloride (CCl_4) and 20 mL of Wijs solution (iodine monochloride in glacial acetic acid) was added to the flask to dissolve the sample. The mixture was then stored in a dark room for 30 minutes at room temperature (about 25^{o}C). Thereafter, 20 ml of potassium iodide solution was added to the flask. The mixture was then titrated with sodium thiosulphate solution using starch as an indicator. The experiment was repeated without the oil sample to obtain a blank value (control value). The iodine value of the solution was then calculated using Equation 3-8. An iodine meter was then used to compare and verify the results obtained by titration.

$$IV = \frac{12.69\, M_{ST}\,(V_B - V_A)}{W_S} \qquad \text{(Rao, et al., 2010)}$$

Equation 3-8

Where:

IV = Iodine value

M_{ST} = Concentration of sodium thiosulphate solution (moles/L),

W_S = Weight of the sample (g),

V_A = Volume of sodium thiosulphate solution consumed for the sample (mL),

V_B = Volume of sodium thiosulphate solution consumed for the blank value (mL).

3.16. Determination of Peroxide Value

Peroxide value of Jatropha seed oil and derived biodiesel from four different fruit maturity stages was determined according to AOCS method Cd 8-53 test standard for peroxide value. 1.5mL of oil sample was weighed into a 250 mL conical flask. 10mL of chloroform was added into the conical flask to dissolve the sample then 10mL of glacial acetic acid was also added to the solution. 1mL of saturated Potassium Iodide solution was added to the solution in 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 minutes at room temperature. Thereafter, 75mL of deionized water was added to the solution and 1mL of starch was added as an indicator. The mixture was then titrated with 0.01N sodium thiosulphate solution until the blue colour disappeared (turns colourless). 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 Equation 3-9. The experiment was repeated five times for each sample then calculated average.

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

Equation 3-9

Where:

PV = Peroxide value

m = Weight of the sample (g),

V_A = Volume of sodium thiosulphate solution consumed for the sample (mL),

V_B = Volume of sodium thiosulphate solution consumed for the blank value (mL).

M_{ST} = Concentration of sodium thiosulphate solution (moles/L)

3.17. Conversion of Jatropha Seed Oil to Biodiesel

Jatropha seed oils derived from four different fruit maturity stages were converted to biodiesel. The level of free fatty acids present in the oil determined the conversion method (one-step or two-step) (Berchmans & Hirata, 2008) and type of catalyst (alkali or acid) to be used. Acid esterification is used as a pretreatment method to reduce the level of FFA in oil to below 3% as mentioned earlier in Section 2.3.2.

3.17.1. Homogeneous Base Transesterification

This method was used for seed oils with free fatty acid content less than 3%. Prior to conversion processes, seed oil was heated at 105^{o}C for approximately 1 hour to remove water which may interfere with transesterification reaction. The seed oil was transferred to a three neck round bottom flask (reactor) after cooling to temperatures around 60^{o}C. Potassium hydroxide pellets (0.4% w/w of KOH to oil ratio) were dissolved in methanol (80% v/v of methanol to oil ratio) then the solution was added to the reactor. The reactor was then placed on a heating mantle and the mixture was heated at 60^{o}C (optimum temperature for transesterification reaction) throughout the reaction process. The reaction was carried out at atmospheric pressure. A condenser was fitted to the reactor to reduce loss of methanol during the reaction process. The mixture was stirred vigorously throughout the reaction process which lasted for 1 hour. The experimental set up for the transesterification reaction is shown in Figure 3-13. The reaction products were then poured into a separating funnel, as shown in Figure 3-14, and left for at least 24 hours to allow glycerol and methyl esters to separate under gravity. After separation, excess methanol was recovered from the methyl esters using a rotary evaporator (BUIKICHI Rotavapor R-114).

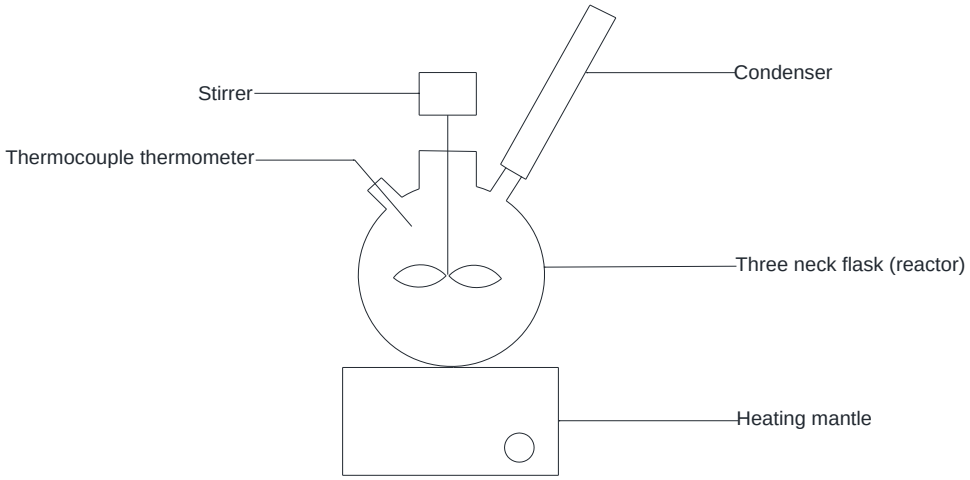


Figure 3-13. Experimental set up for the transesterification reaction.

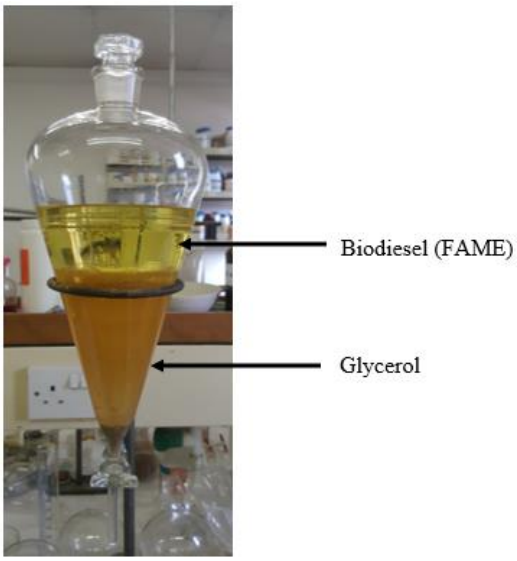


Figure 3-14. Separating funnel used for separation of FAME and glycerol.

3.17.2. Acid Esterification

Jatropha seed oil was poured into a reactor. Concentrated sulphuric acid 98%, (1% v/v of H_2SO_4 to oil ratio) was mixed with methanol (50% v/v of methanol to oil ratio) and the reactor was charged with the mixture. The mixture in the reactor was reacted at a temperature of 60^{o}C for 1 hour while stirring continuously for the entire process. The mixture was transferred into a funnel

separator then allowed to settle for two hours. The oil and FAME mixture at the bottom was separated from the methanol-water at the top. The pre-treated oil was then reacted further using the Homogeneous Base Transesterification method.

3.17.3. Excess Methanol Removal

After separation, biodiesel contains some methanol. Methanol is completely soluble in both FAME and glycerol (Zhou, et al., 2003). The excess methanol was removed from biodiesel using a rota vapour under vacuum.

3.17.4. Washing of the Methyl Ester (Biodiesel)

This is the final step in producing biodiesel. The methyl esters (biodiesel) obtained contains traces of catalyst such as KOH and other impurities including soaps, methanol and glycerol. These contaminants were removed by washing the biodiesel with hot deionized water at a temperature of 80^{o}C since they are more soluble in water than in biodiesel. Using hot water speeds up the process of transferring the impurities from the biodiesel to the water, while using cold water results in emulsions (Tech, 2018). The biodiesel was mixed with water at a ratio of 1:2 (water: biodiesel), then the mixture was stirred gently for 10 minutes. Stirring the mixture vigorously or violently results in an emulsion which is difficult to separate (Tech, 2018; Gerpen, 2017; Saifuddin & Chua, 2004; Atadashi, et al., 2011). The mixture was then allowed to settle in a separating funnel for 2 hours as demonstrated by Figure 3-15. Thereafter, water was drained off from the bottom. Washing process was repeated 3 times to remove as much impurities as possible. The biodiesel was then heated for 1 hour at 105^{o}C to remove traces of water as described by (Kartika, et al., 2013).

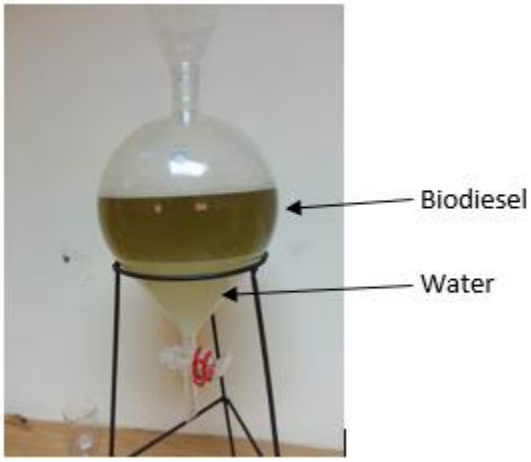


Figure 3-15. Separation of biodiesel and water after washing. Water settles at the bottom while biodiesel remains at the top.

During washing there is a possibility of formation of emulsions, especially when the level of impurities in biodiesel are relatively high. Emulsions are a mixture of water and biodiesel and other stuff like soap and glycerol (Tech, 2018). There are many methods of treating emulsification, and using table salt (NaCl) is the fastest (Anon., 2008).

3.18. Toxicity Analysis

Toxicity of Jatropha, particularly of the seeds including seed oil, have been discussed in Section 2.6. Knowledge in toxicity is important when handling the plant and its by-products. It helps in coming up with proper safety precautions. The seed cake, crude seed oil and biodiesel from different maturity stages (green yellow, yellow, yellow brown and brown dry) of Jatropha fruits were tested for toxicity. This involved testing the presence and quantity of phorbol esters.

3.18.1. Extraction of phorbol esters

Seed cake: Extraction of phorbol esters (PEs) from Jatropha seed cake was carried out using a method described by Wang et al (Wang, et al., 2013) with some modifications. Phorbol esters were extracted from seed cake samples from screw press using methanol. 5g samples were extracted with 50mL of methanol. The seed cake/methanol mixture was placed in an ultrasound bath for 15 minutes. The methanol extract was then filtered from the seed cake residue using a paper filter

(Grade 1: 11 µm). The seed cake residue was extracted two more times, then the methanol extracts were combined then concentrated using a rotary evaporator (BUIKICHI Rotavapor R-114).

Seed oil: Phorbol esters were extracted from Jatropha seed oil using a modified method described by Nishshanka et al and Roach et al (Nishshanka, et al., 2016; Roach, et al., 2012). 25 mL of Jatropha seed oil (obtained from screw press extraction) was extracted with 25 mL of methanol. The seed oil/ methanol mixture was stirred vigorously using a magnetic stirrer at 60^{o}C for 5 minutes. The layers were allowed to separate through gravity then the methanol layer was collected. The oil sample was extracted three times then the methanol layers were combined then concentrated using a rotary evaporator (BUIKICHI Rotavapor R-114).

3.18.2. Phorbol esters analysis

The presence and quantity of phorbol esters (PEs) was determined using High-Performance Liquid Chromatography (HPLC) according to a method described by (Makkar & Becker, 1997; Baldini, et al., 2014; Wink, et al., 2004) with some modifications. A schematic diagram showing setup of high-performance liquid chromatography is shown in Figure 3-16. The HPLC equipment used was Agilent Technologies 1260 infinity with manual injection shown in Figure 3-17. Formic acid 0.14% and acetonitrile (15:85) were used as mobile phase. These solvents were filtered then degased by ultra-sonication before used. The elution was in isocratic mode at a flow rate of 1mL/min. Separation was performed using a reverse phase column (Supelco analytical 10cm x 4mm, 5µm) with temperature set at 25^{o}C. The detector wavelength was set at 280 nm. Sample injection volume was 20 µL.

Phorbol-12-myristate 13-acetate (PMA) purchased from Sigma-Aldrich was used as a standard. The standard come in quantities of 5mg per bottle. Phorbol esters in the samples were analysed using the standard addition method. The standard addition method is more precise and accurate than the external calibration method. A stock solution of the standard was prepared by dissolving 5mg of PMA standard in 5ml of HPLC grade methanol thus having a concentration of 1000ppm. First, the standard solution with known concentration was injected and run to get the chromatogram of the pure standard. The same was repeated on the sample to get the chromatogram of the sample. The sample was then spiked with PMA standard solution in order to increase the ion signal intensities of PE in the sample. 6 different spiked aliquots were prepared for each sample such that each aliquot had a different concentration and evenly spaced amounts of spike. These

spiked aliquots of the sample were then used to generate a calibration line which was used to calculate the concentration of PE in the sample. The calibration line was plotted as concentration of the spiked sample (x-axis) versus their peak area (y-axis).

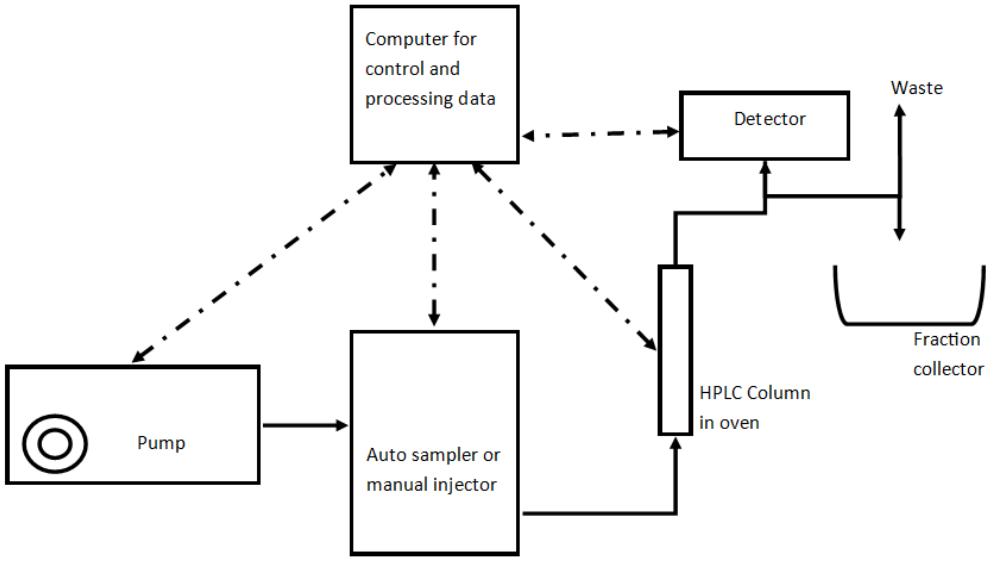


Figure 3-16. Schematic diagram showing setup of high-performance liquid chromatography (HPLC).

Figure 3-17. Agilent Technologies 1260 infinity High-Performance Liquid Chromatograph.

3.19. Standard Errors

Experimental measurements were repeated then calculated an average value in order to improve accuracy of the results. Therefore it was necessary to calculate the standard error. The standard error was calculated according to Equation 3-10 and Equation 3-11.

$$s = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}}$$

Equation 3-10

$$SE = \frac{s}{\sqrt{n}}$$

Equation 3-11

Where

S = Standard deviation

SE = Standard error

n = number of measurements

x_i = i^{th} measurement from the sample

$\bar{x}$ = Average of the measurements

3.20. Statistical Analysis

The influence of fruit maturity on seed oil content and some quality parameters of seed oil and derived biodiesel 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% ($\alpha = 0.05$). If the p-value is less than or equal to the significance level, the differences between the means are statistically significant. If the p-value is greater than the significance level, the differences between the means are not statistically significant. Similarly, if F-values greater than the critical F-value shows that the variation is statistically significant whereas F-values less than the critical F-value shows that the variation is not statistically significant. For statistically significant differences, post hoc tests were conducted using Tukey's method.

Chapter 4 presents and discusses results from the present study following the methodology used in the present chapter.

Chapter 4

4. Presentation and Discussion of Results

4.1. Introduction

This chapter presents and discusses results on how fruit maturity of Jatropha curcas found in Botswana affect seed oil yield and quality (physicochemical properties) of seed oil and derived biodiesel. The analysis were performed using different biodiesel standard methods (ASTM and European standards). The influence of fruit maturity on phorbol ester content (toxicity) in Jatropha seed oil and seed cake is also presented and discussed in this chapter. For simplicity, only a sample of the results obtained from the investigation have been presented and discussed in this Chapter. This enables the main findings of the research to be identified and explained. The remainder of the results from the investigation, which comprises mostly of the experimental data are presented in Appendix A with a brief commentary to explain the significance of the obtained in each case.

4.2. Drying Trend of Jatropha seeds

Jatropha seeds from four different fruit maturity stages were dried as described in Section 3.3. During drying, Jatropha seeds lost moisture (water content) hence experienced reduction in weight. Total weight reduction varied with fruit maturity indicating variation in moisture content with fruit maturity. Seeds extracted from green yellow fruits experienced relatively high weight reduction indicating highest moisture content. Seeds extracted from brown dry fruits experienced least weight reduction indicating relatively least moisture content. Moisture content in Jatropha seeds reduces continuously as the fruits matures from green to brown dry as shown in Figure 4-1. After approximately four days of drying, weight of the seeds from the three maturity stages, namely green yellow, yellow, yellow brown and brown dry remained constant. Drying seeds naturally does not eliminate all the moisture from the seeds. According to (Keneni & Marchetti, 2019; Rao, et al., 2006) there is always residual moisture in seeds after drying. After drying, moisture content of seeds equilibrate with the relative humidity of the air surrounding it, and this residual moisture in seeds is termed Equilibrium Moisture Content (EMC) of the dried seeds. After performing oven drying, 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 (Anandalakshmi, et al., 2008; Akowuah, et al., 2012; Guzman & Aquino, 2009). Therefore, it is appropriate to conclude that natural drying method is recommendable for drying Jatropha seeds from any maturity stage. Some previous researchers (Aquino, et al., 2009; Silva, et al., 2012; Silva, et al., 2017) who used natural drying method on Jatropha seeds reported relatively higher equilibrium moisture content (8 to 10%) after dying. This may have been due to higher relative humidity since the final moisture content after natural drying is influenced mostly by humidity of the environment. 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.

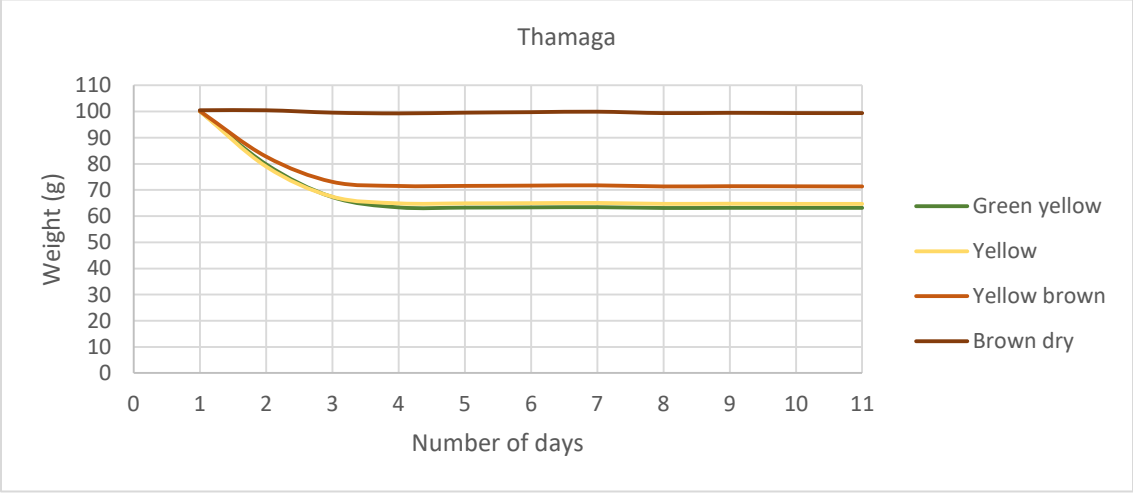


Figure 4-1. Drying trend of Jatropha Seeds harvested at four different maturity stages from Thamaga area.

The percentage weight loss of the seeds during drying is equivalent to the moisture content of the seeds. Moisture content of the seeds calculated from percentage weight loss of the seeds during drying, that is immediately after extraction from the fruits and after natural drying is shown in Figure 4-2. Before drying, seeds extracted from green yellow fruits have highest moisture content of 40.6%. The data in Figure 4-2 depict that 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%. These results show a similar trend to those reported by Silva et al (Silva, et al., 2012) who also determined moisture content in Jatropha

seeds at various maturity stages. They reported that moisture content in Jatropha seeds decreased from 52% to 17.7% as the fruits matured from green yellow to brown. Aquino et al (Aquino, et al., 2009) also reported a similar trend of decreasing moisture content (40% to 19.4%) in seeds as Jatropha fruits matures from green yellow to brown.

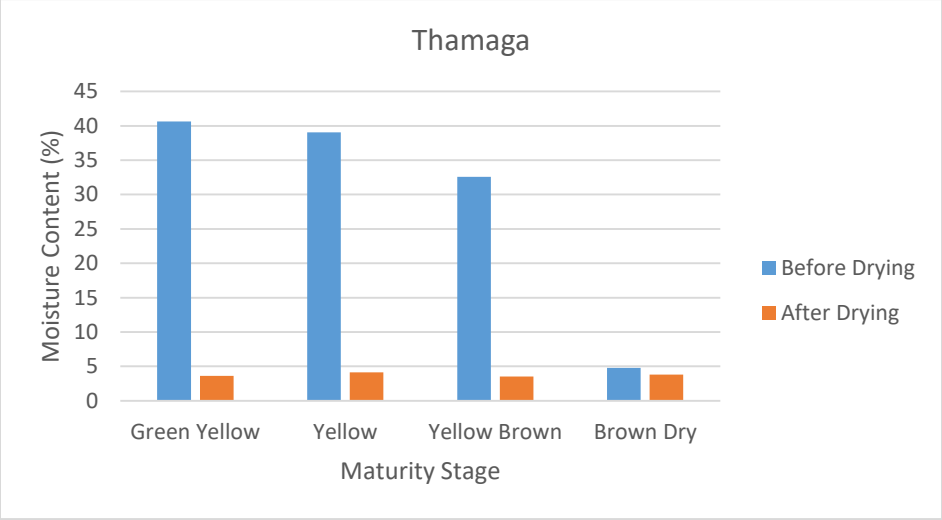


Figure 4-2. Moisture content in Jatropha seeds immediately after harvest (before drying) and after the natural drying process at four different maturity stages.

4.3. Effect of Fruit Maturity on Jatropha Seed Oil Content/Yield

Oil yield in Jatropha seed kernel ranges from 38.7% to 45.8%, thus a variation of 7% for the four maturity stages and four selected geographical locations investigated as shown in Figure 4-3. Jatropha seeds harvested in Mmadinare area recorded relatively highest oil yield of 45.8% from yellow fruits followed by Thamaga (44.9%) then Maun (41.7%). Seeds from Shashe area recorded least oil yield of 40.4% from the same maturity stage. Overall, Jatropha seeds from Mmadinare area yields relatively highest oil content in all maturity stages whereas those from Shashe area yield relatively lowest oil content. Effect of fruit maturity on Jatropha seed oil yield is statistically significant, as indicated in Table A-24, Appendix A 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. Influence of geographical location on Jatropha seed oil content is statistically significant. Post Hoc Tukey test results in Appendix E indicate that variation of seed oil content with geographical location is significant

between all the four investigated geographical locations. 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 by Figure 4-3. This results agrees with those reported by Saragih et al (Saragih, et al., 2007) who studied variation of oil content in Jatropha seeds at different fruit maturity stages in Malinau District, Indonesia as discussed in Section 2.2.1. However, the latter researcher did not show the oil accumulation trend over the various fruit maturation stages as demonstrated in the present study.

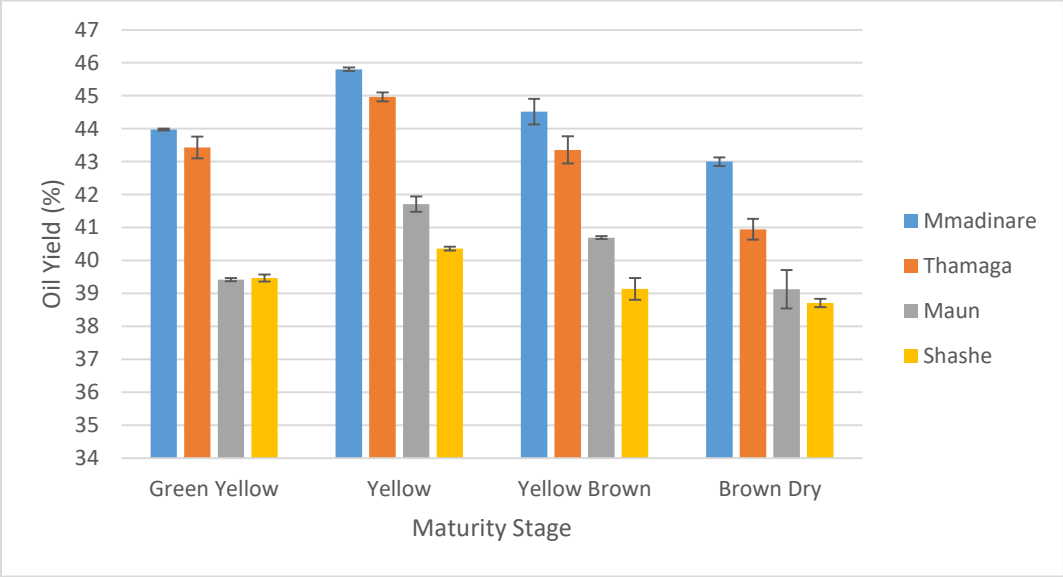


Figure 4-3. Seed kernel oil yield/content of Jatropha seeds harvested at four different fruit maturity stages from four different geographical locations.

It is important to note that the seed coat is also part of the seed, therefore need to be taken into consideration when expressing the overall seed oil yield/content in the seed instead of kernel oil content. However, the seed coat does not contain oil. Both the seed coat and kernel were weighed during de-shelling in order to get the mass ratio of seed coat to kernel. Seed oil yield was then re-calculated accounting for the seed coat weight in order to make a good comparison with mechanical screw press seed oil yield because during mechanical screw press, the seed coat is not removed. The mass ratios of seed coat to kernel is shown in Figure 4-4. These mass ratios applies for all the area studied. Therefore oil content in whole seeds (taking seed coat into account) was

determined according to relationship in *Equation 4-1* for green yellow seeds and *Equation 4-2* for yellow, yellow brown and brown dry seeds.

$$\%\ Oil\ yield = \frac{\%\ Oil\ yield\ (solvent\ extraction)}{100} \times 65$$

Equation 4-1

$$\%\ Oil\ yield = \frac{\%\ Oil\ yield\ (solvent\ extraction)}{100} \times 67$$

Equation 4-2

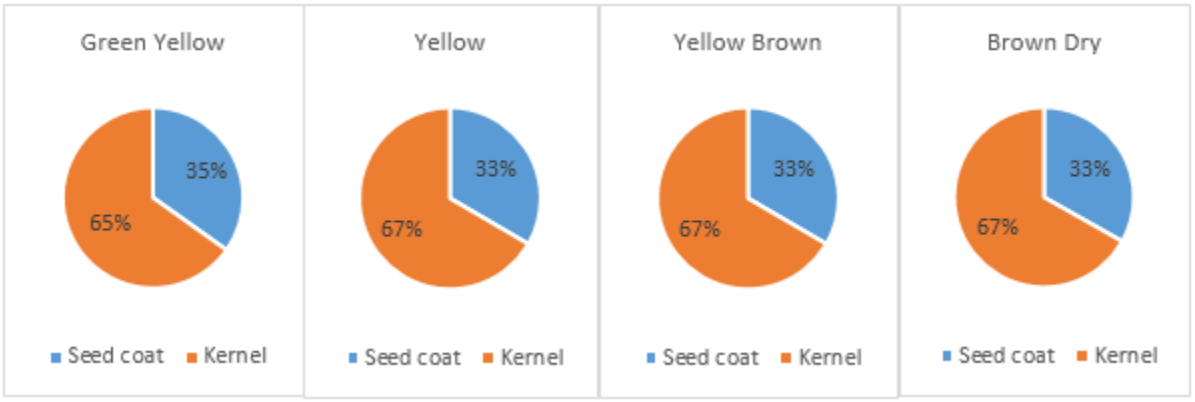


Figure 4-4. Mass ratio of seed coat to seed kernel of Jatropha seeds harvested at four different maturity stages.

It is quite clear that the yield changes due to the additional weight of the seed coat, however, the overall yield trend remains unchanged as indicated by the recalculated seed oil yield for whole seeds (including the seed coat mass) shown in Figure 4-5. The seed oil yield of whole seeds reduces slightly as compared to the yield of seed kernels alone (without including the seed coat). Based on the above observations, it is appropriate to conclude that the seed oil content of whole Jatropha seeds ranges from 25 to 31%, thus 17% less than what is reported in the literature by several authors including (Negasu, 2015; Ahmad & Sultan, 2015; Sowmya, et al., 2012; Gandure & Ketlogetswe, 2011). However, the results in Figure 4-5 indicate that seeds from the yellow stage have the highest oil content.

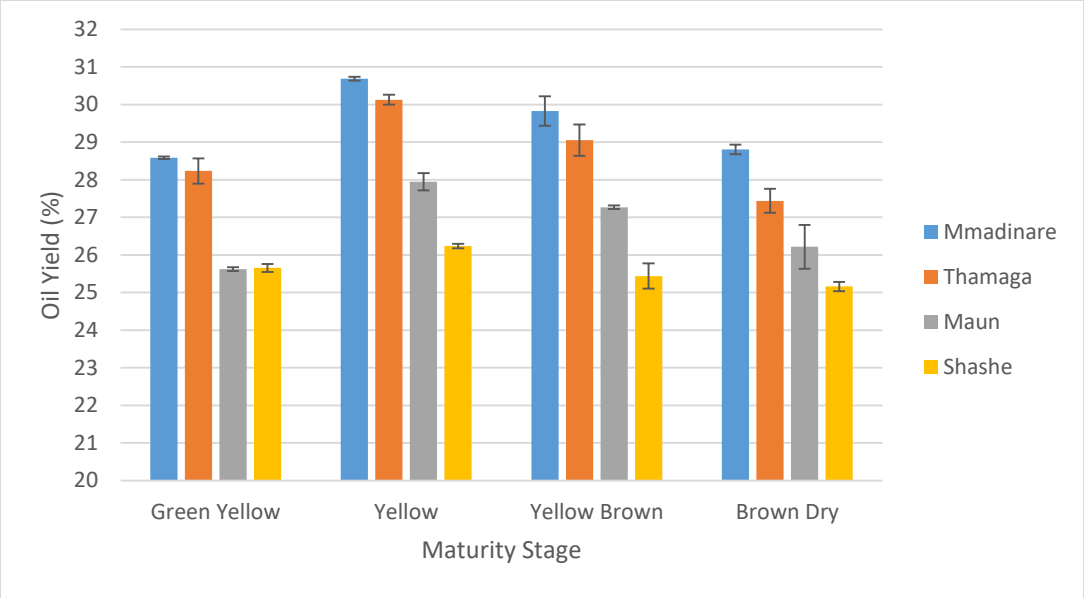


Figure 4-5. Re-calculated seed oil yield of Jatropha fruits harvested at four different maturity stages from three different locations, taking into consideration the seed coat mass.

4.3.1. Accumulation of Oil in Jatropha Seeds

Oil content in Jatropha seeds varies as the fruit matures. Seed oil content increases as the fruit 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 as depicted in Figure 4-6. This decline in seed oil content start as the fruit turns yellow brown and declines further as the fruit turns brown dry. For all the areas studied, variation of seed oil yield with fruit maturity follows the same cubic trend. Oil yield in Jatropha seeds reaches its peak when the fruit turns yellow, thereafter, as the fruit turns brown dry oil content in seeds reduces by about 6 to 9% as depicted in Figure 4-6 and Figure 4-8 which is further explained in the subsequent paragraph.

Accumulation of seed oil in Jatropha seeds during maturation follows a similar cubic trend, irrespective of geographical location as shown in Figure 4-6. Baud and Lepiniec (Baud & Lepiniec, 2009) 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 also reported by Eastmond and Rawsthorne (Eastmond & Rawsthorne, 2000) 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 (Chia, et al., 2005) 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 stated that during the final maturation stage of the seeds, some of the triglycerides undergo β-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'** (Angelovici, et al., 2010). In the case of Jatropha, it is appropriate to conclude that seed desiccation occurs when the fruit turns yellow brown to brown dry as depicted in Figure 4-6.

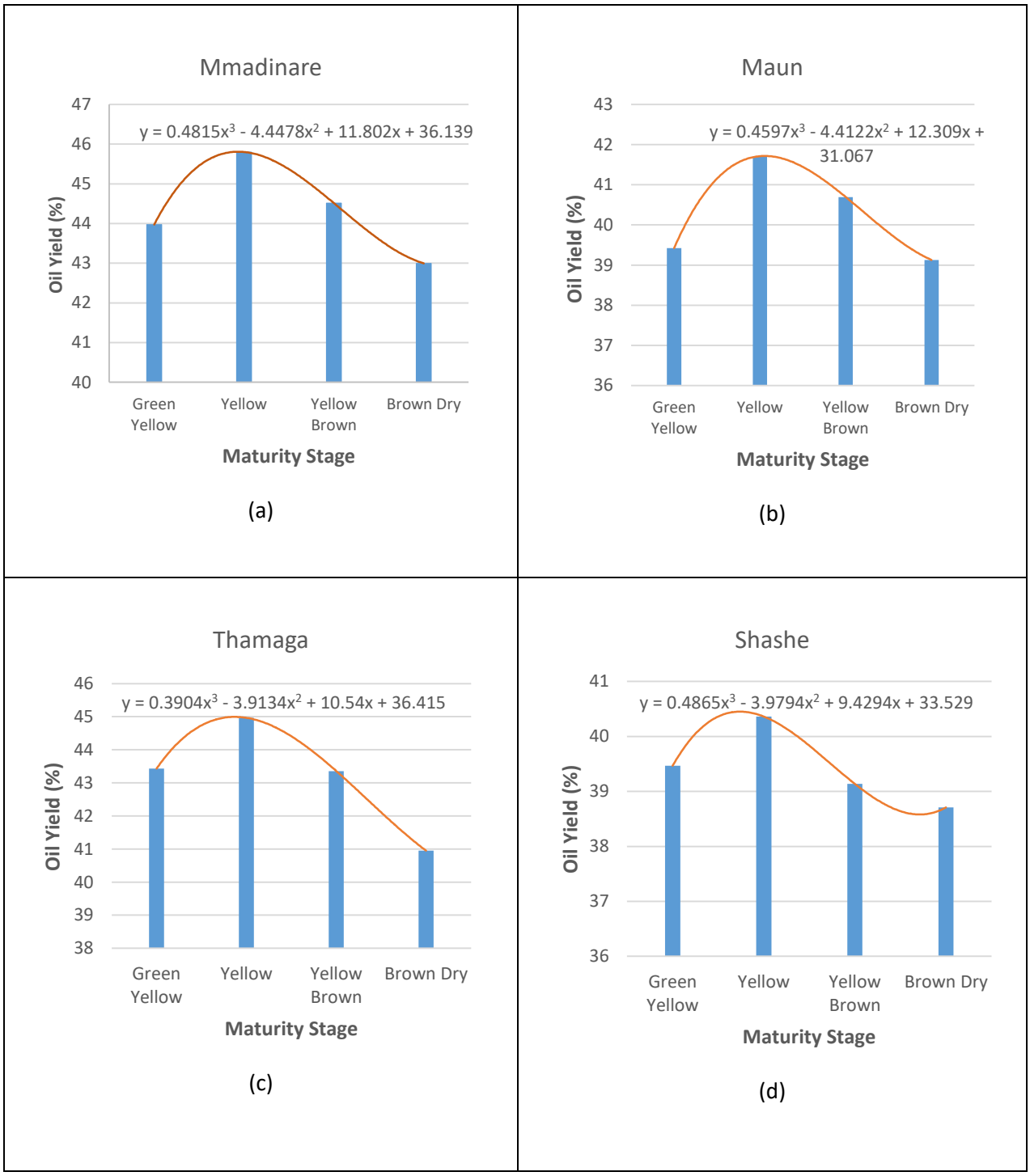


Figure 4-6. Cubic trend of seed oil content variation with fruit maturity of Jatropha fruits harvested in four different geographical locations: (a) Mmadinare, (b) Maun, (c) Thamaga and (d) Shashe.

Generally, accumulation of oil in Jatropha seeds 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, y is the oil yield in seeds and x is the maturity stage. Solving these equations results in x = 1, 2, 3 and 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 as shown in Figure 4-7 and Figure 4-8. 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 4-8. The trend demonstrated in Figure 4-8 suggest that synthesis of triglycerides (oil) in Jatropha seeds occurs from when the fruit is mature green up to the point when the fruit turns yellow. After the yellow stage, oil content in Jatropha seeds start to decline continuously until the final maturation stage (brown dry).

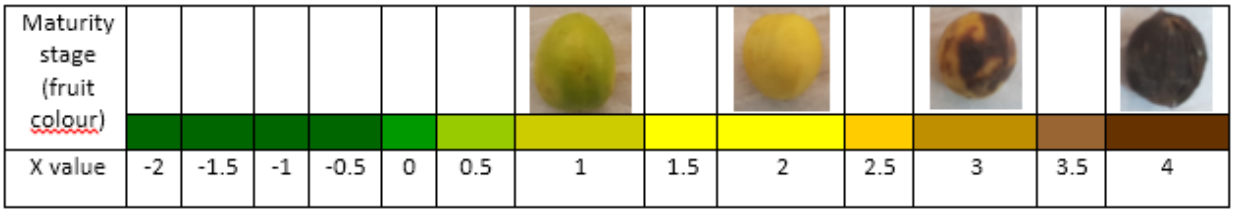


Figure 4-7. Jatropha fruits/ seeds maturity scale showing correspondence of fruit colour to the x-value.

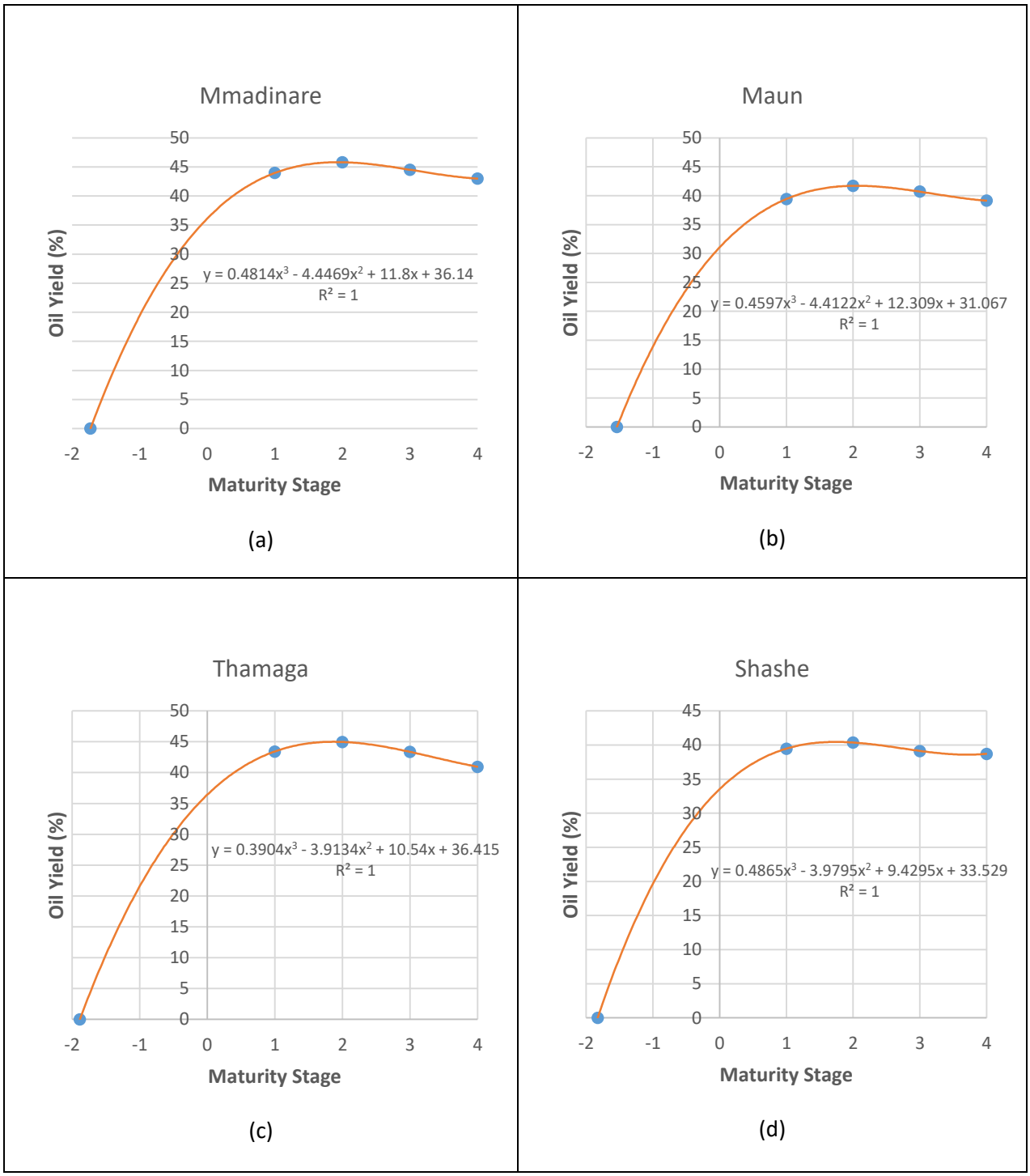


Figure 4-8. Accumulation of oil in Jatropha seeds from various geographical locations, (a)Mmadinare, (b)Maun, (c)Thamaga and (d)Shashe, from zero up to the final maturity stage (brown dry).

4.4. Effect of Fruit Maturity on Free Fatty Acid Content in Jatropha Seed Oil

Free fatty acid content in Jatropha seed oil increase continuously as fruits matures from green yellow to brown dry as shown in Figure 4-9. Seed oil derived from green yellow and yellow fruits contains relatively low Free Fatty Acids (FFA) while seed oil derived from brown dry fruits contains relatively highest FFA content as depicted in Figure 4-9. Analysis of variance reveal that effect of fruit maturity on FFA content in all the four geographical locations is statistically significant since the significance values are less than 0.05 as demonstrated by Table A-24 in Appendix A. Free fatty acid content in Jatropha seed oil from the four different fruit maturity stages and four different geographical locations investigated is in the range of 0.2 to 0.75%. Highest FFA content of 0.75% was recorded in Jatropha seed oil from brown dry fruits from Mmadinare area. However, the content of FFA across all the four maturity stages and all the four geographical locations in Botswana is well within the 3% limit recommended by previous researchers for efficient production of biodiesel with homogeneous base transesterification as described in Section 2.3.2. In other parts of the world, some researchers reported FFA content in Jatropha seed oil from brown dry fruits to be as high as 14% as indicated in Section 2.3.2 (Silitonga, et al., 2014; Tiwari, et al., 2007; Folaranmi, 2013; Shambhu, et al., 2012). However, some previous researchers who analysed Jatropha seed oil from brown dry fruits such as (Garba, et al., 2013; Belewu, et al., 2010) reported similar values as those presented in Figure 4-9. Free fatty acids in Jatropha seed oil turns to increase as the fruit matures from green yellow to brown dry follow a third order polynomial trend as shown in Figure 4-9. The trend appear to suggest 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. This trend reveals that the lipid/oil in Jatropha seeds is synthesized as triglycerides, then later some of the triglycerides breaks down into free fatty acids. This liberation of fatty acids from the glycerol molecule occurs continuously throughout the maturation process resulting in relatively higher FFA content in successive maturity stages. As reported by Berchmans and Hirata (Berchmans & Hirata, 2008) high levels of free fatty acids reduce methyl ester yield during transesterification process. Therefore, Jatropha seed oil from green yellow and yellow fruits will be the most favorable for transesterrification process in biodiesel production. Results in Figure 4-9 have a logical trend as compared to those reported by Silip et al (Silip, et al., 2011) who made an effort to investigate variation of FFA in Jatropha seed oil at different fruit

maturity stages and their results did not show any conclusive trend as indicated in Section 2.2. According to the results of the present investigation, seed oil from green yellow and yellow fruits is of relatively better quality than the other fruit maturity stages.

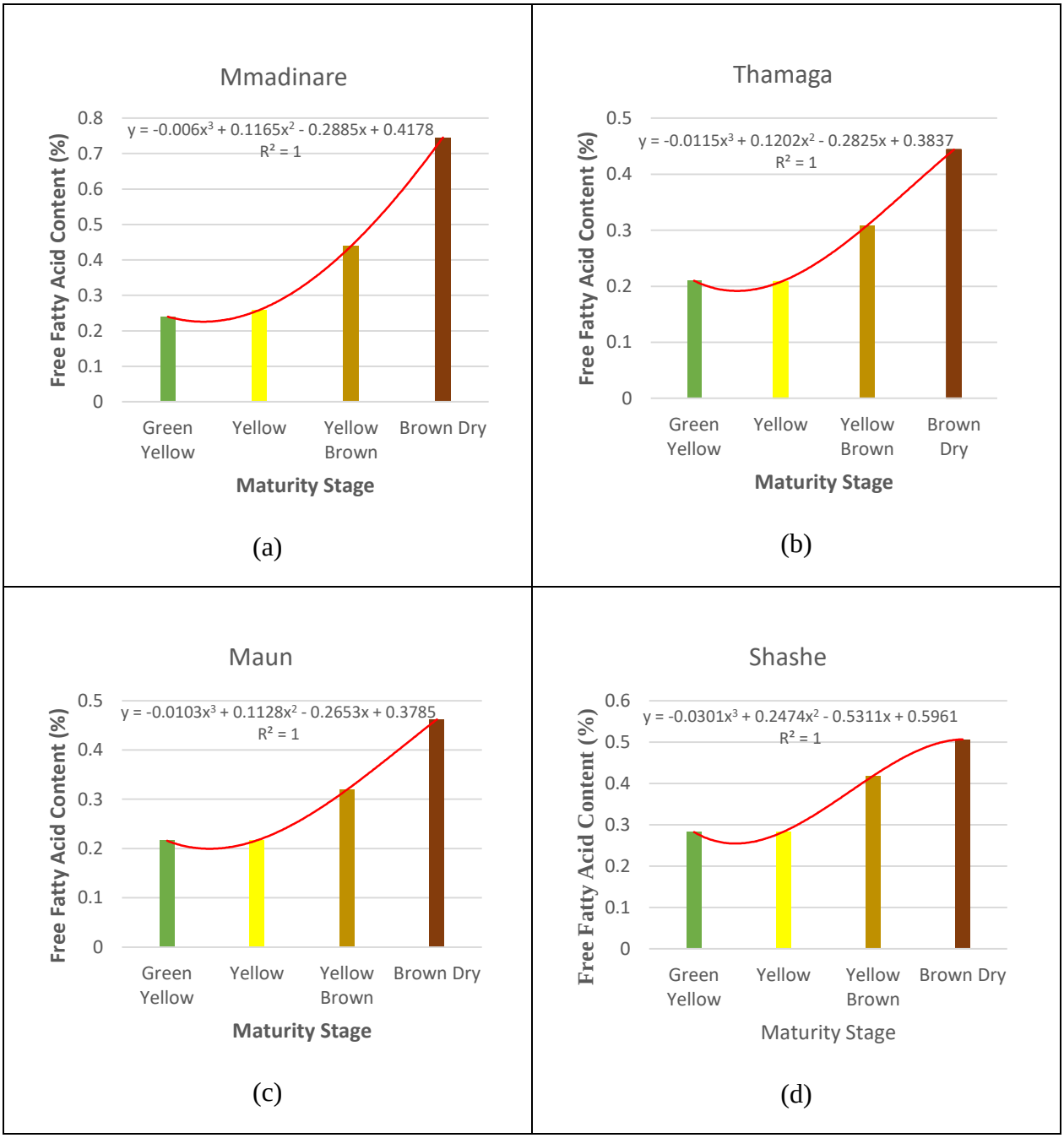
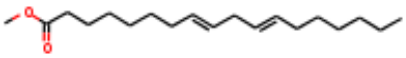
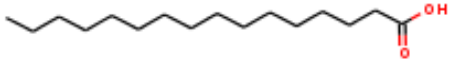
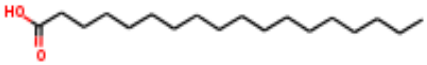
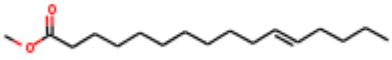
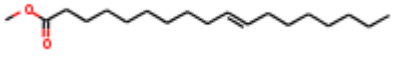


Figure 4-9. Variation of free fatty acid content in seed oil with fruit maturity of Jatropha harvested in (a)Mmadinare, (b)Thamaga, (c)Maun and (d)Shashe areas.

4.5. Effect of Fruit Maturity on Fatty Acid Composition of Jatropha Seed Oil and Derived Biodiesel

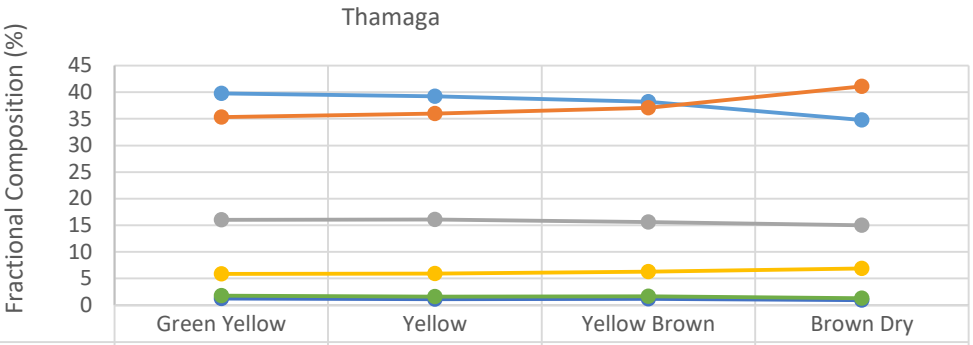
The main fatty acids in Jatropha seed oil derived from different fruit maturity stages and selected geographical locations investigated in Botswana were found out to be Linoleic acid, Oleic acid, Palmitic acid, Stearic acid, 11-Hexadecenoic acid and 10-Octadecenoic acid as demonstrated by the results in Figure 4-10, Figure 4-11, Figure 4-12 and Figure 4-13. These findings are consistent with the findings reported by previous researchers including (Adebowale & Adedire, 2006; Abdullah, et al., 2013; Joshi, et al., 2013; Akbar, et al., 2009; Gopinath, et al., 2010) who characterized Jatropha seed oil from brown dry fruits in various geographical origins. Structures of these fatty acids methyl esters (FAME) are shown in Table 4-1. Linoleic acid is a polyunsaturated fatty acid, having two double bonds. Oleic acid, 11-Hexadecenoic acid and 10-Octadecenoic acid are mono unsaturated fatty acids. Palmitic acid and Stearic acid are both saturated fatty acids.

Table 4-1. Chemical description of fatty acids found in Jatropha seed oil in the form of fatty acid methyl esters (FAME).

Fatty Acid	Formula	Structure	Molecular Structure
Linoleic acid (14,17-Octadecadienoic acid)	$C_{19}H_{34}O_2$	C19:2	
Oleic acid (9-Octadecenoic acid)	$C_{19}H_{36}O_2$	C19:1	
Palmitic acid (Hexadecanoic acid)	$C_{17}H_{34}O_2$	C17:0	
Stearic acid (Octadecanoic acid)	$C_{19}H_{38}O_2$	C19:0	
11-Hexadecenoic acid	$C_{17}H_{32}O_2$	C17:1	
10-Octadecenoic acid	$C_{19}H_{36}O_2$	C19:1	

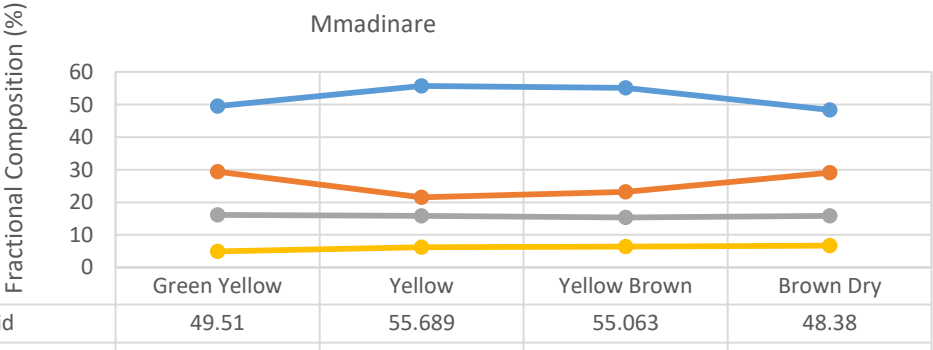
Fractional compositions of fatty acids in Jatropha seed oil at different fruit maturity stages are shown in Figure 4-10, Figure 4-11, Figure 4-12 and Figure 4-13 of Jatropha harvested in Thamaga, Mmadinare, Maun and Shashe areas respectively. Linoleic and oleic acids make a significant portion of Jatropha seed oil across all maturity stages of the fruit. For all maturity stages, unsaturated fatty acids make up 77 - 80% of total fatty acids in Jatropha seed oil. Therefore Jatropha seed oil is more unsaturated. Similar findings were reported by (Abdullah, et al., 2013; Nzikou, et al., 2009; Joshi, et al., 2013) who found out that fractional composition of linoleic and oleic acids in Jatropha seed oil from brown dry fruits contributes over 70% of total fatty acids. Results reveal that these unsaturated fatty acids, particularly linoleic and oleic acids are the most affected by fruit maturity than the other fatty acids found in Jatropha seed oil. Their fractional composition appear to decrease as fruit maturation advances. The data in Figure 4-10, Figure 4-11, Figure 4-12 and Figure 4-13 show that as Jatropha fruits matures from yellow to brown dry (during seed desiccation stage), fractional composition of linoleic acid reduces by 8 to 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) is a result of breakdown of some of the unsaturated fatty acids. Therefore, there is a logical relationship between the trend in fatty acid composition in Jatropha seed oil and oil content. According to Chia, Pike and Rawsthorne (Chia, et al., 2005) 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 is the most unstable fatty acid in Jatropha seed oil. However unsaturated fatty acids have advantages too as mentioned earlier in Section 2.3.1. One of the advantages of increase in unsaturated fatty acids is that they lower both cloud and pour points of biodiesel hence improved cold weather usability. 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 as demonstrated by the same figures. Palmitic and Stearic acids which makes 20 - 23% of the total composition 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. Based on all these, it is appropriate to conclude that

maturity stage have an effect on fatty acid composition of Jatropha seed oil. Generally, the effect is similar in all the investigated geographical locations in Botswana.



	Green Yellow	Yellow	Yellow Brown	Brown Dry
Linoleic acid	39.766	39.231	38.198	34.789
Oleic acid	35.323	35.992	37.09	41.079
Palmitic acid	16.003	16.098	15.617	15.029
Stearic acid	5.892	5.938	6.284	6.897
11-Hexadecenoic acid	1.251	1.131	1.164	0.925
10-Octadecenoic acid	1.765	1.61	1.65	1.282

Figure 4-10. Fractional composition of fatty acids in Jatropha seed oil at different fruit maturity stages of Jatropha harvested in Thamaga area.



	Green Yellow	Yellow	Yellow Brown	Brown Dry
Linoleic acid	49.51	55.689	55.063	48.38
Oleic Acid	29.35	21.503	23.191	29.054
Palmitic acid	16.178	15.851	15.37	15.887
Stearic acid	4.962	6.176	6.376	6.68
11-Hexadecenoic acid				
10-Octadecenoic acid				

Figure 4-11. Fractional composition of fatty acids in Jatropha seed oil at different fruit maturity stages of Jatropha harvested in Mmadinare area.

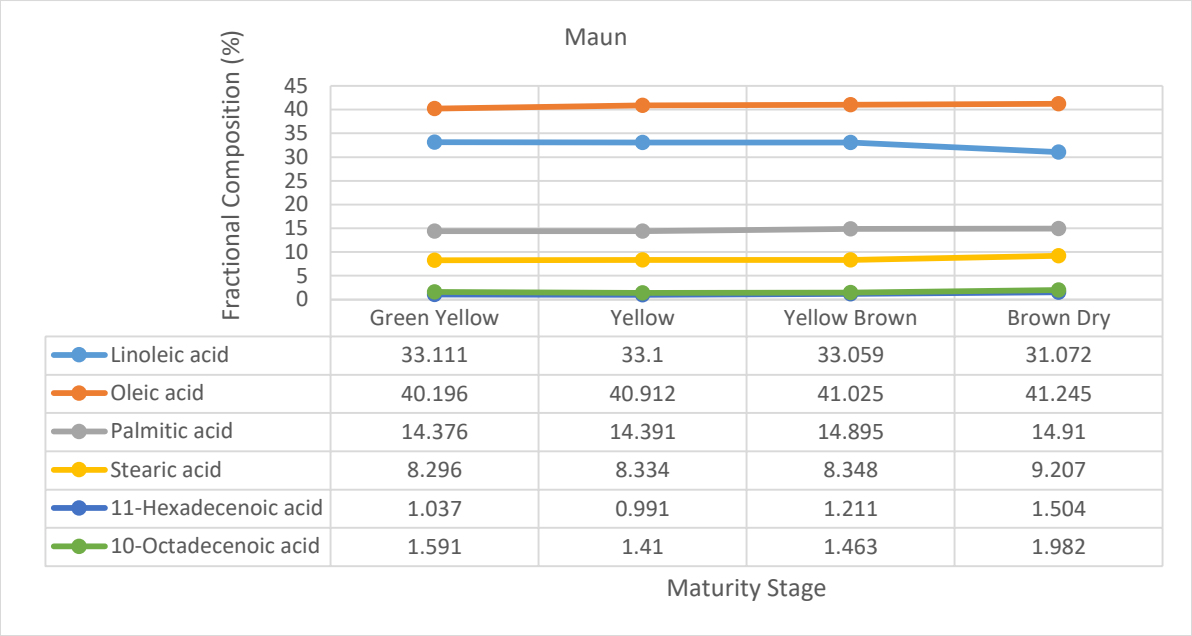


Figure 4-12. Fractional composition of fatty acids in Jatropha seed oil at different fruit maturity stages of Jatropha harvested in Maun area.

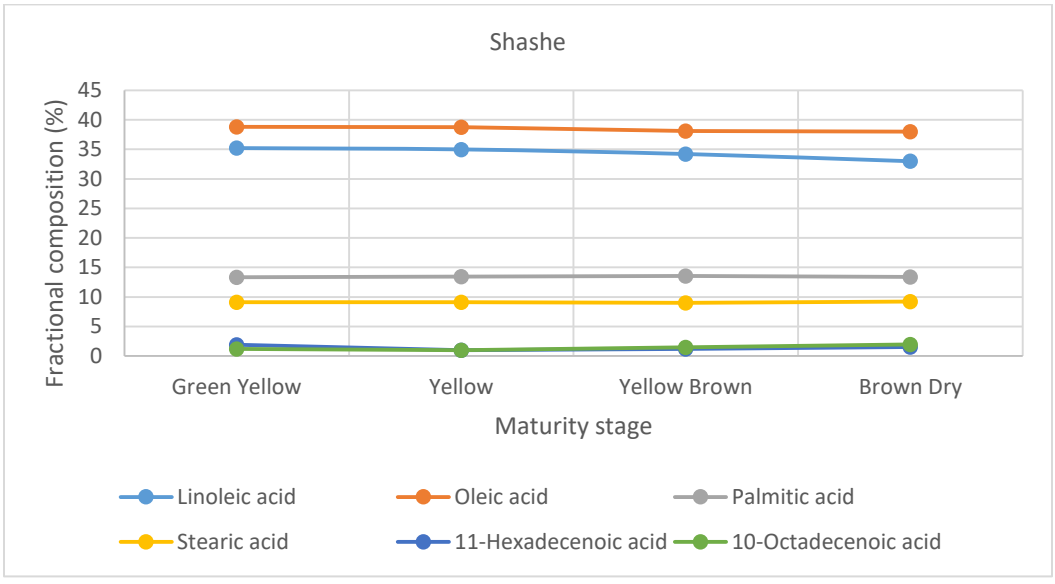


Figure 4-13. . Fractional composition of fatty acids in Jatropha seed oil at different fruit maturity stages of Jatropha harvested in Shashe area.

4.6. Effect of Fruit Maturity on Peroxide Value of Jatropha Seed Oil and Derived Biodiesel

The data in Figure 4-14 show that the Peroxide Value (PV) of Jatropha seed oil increases gradually and lineally as fruits matures from green yellow to brown dry and ranges from 1.2 to 3.7 mEq/Kg. As a result, the peroxide value of biodiesel derived from the oil follows the same trend and ranges from 2.1 to 4.4 mEq/Kg oil. However, the peroxide value of derived biodiesel is slightly greater than that of seed oil. Analysis of variance test results presented in Table A-24 and Table A-25, Appendix A reveal that effect of fruit maturity on Jatropha seed oil and derived biodiesel is statistically significant since the significance values are less than 0.05. The PV of oils from all the investigated geographical locations in Botswana (Mmadinare, Thamaga, Shashe and Maun) follow the same linear trend and in the same range. Previous researchers have reported that Jatropha seed oil experiences oxidation during storage (Mohammed, et al., 2014; Kondratowicz-Pietruszka, 2007; Moussa, et al., 2015). However, results from the present investigation reveal 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 (Desouky, et al., 2009) who investigated the influence of fruit maturity stage on peroxide values of Arbequina, Bouteillan and Koroneiki cultivars oil. They found out that peroxide value of extracted oils increased as fruits matured from green to purple and increased further in black fruits. Sohaimy et al (Sohaimy, et al., 2016) 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 to 11.9 mEq/Kg oil) as fruits matured from green to black. There is currently no official limit on maximum degree of oxidation allowable for the fuel to be used in diesel engines nor for feedstock used for biodiesel production. Although the PV of biodiesel is not regulated by both the ASTM and European standards, increase in PV of both seed oil and biodiesel results in deterioration of feedstock and fuel quality. Increase in PV of seed oil and biodiesel results in increase in viscosity, increase in cetane number and increase in corrosiveness of the fuel (Zuleta, et al., 2012; Canakci, et al., 1999; Ismail & Ali, 2015). The results in Figure 4-14 appears to suggest that seed oil and biodiesel from early stages of fruit maturity (green yellow and yellow) have relatively low PV, therefore of better quality.

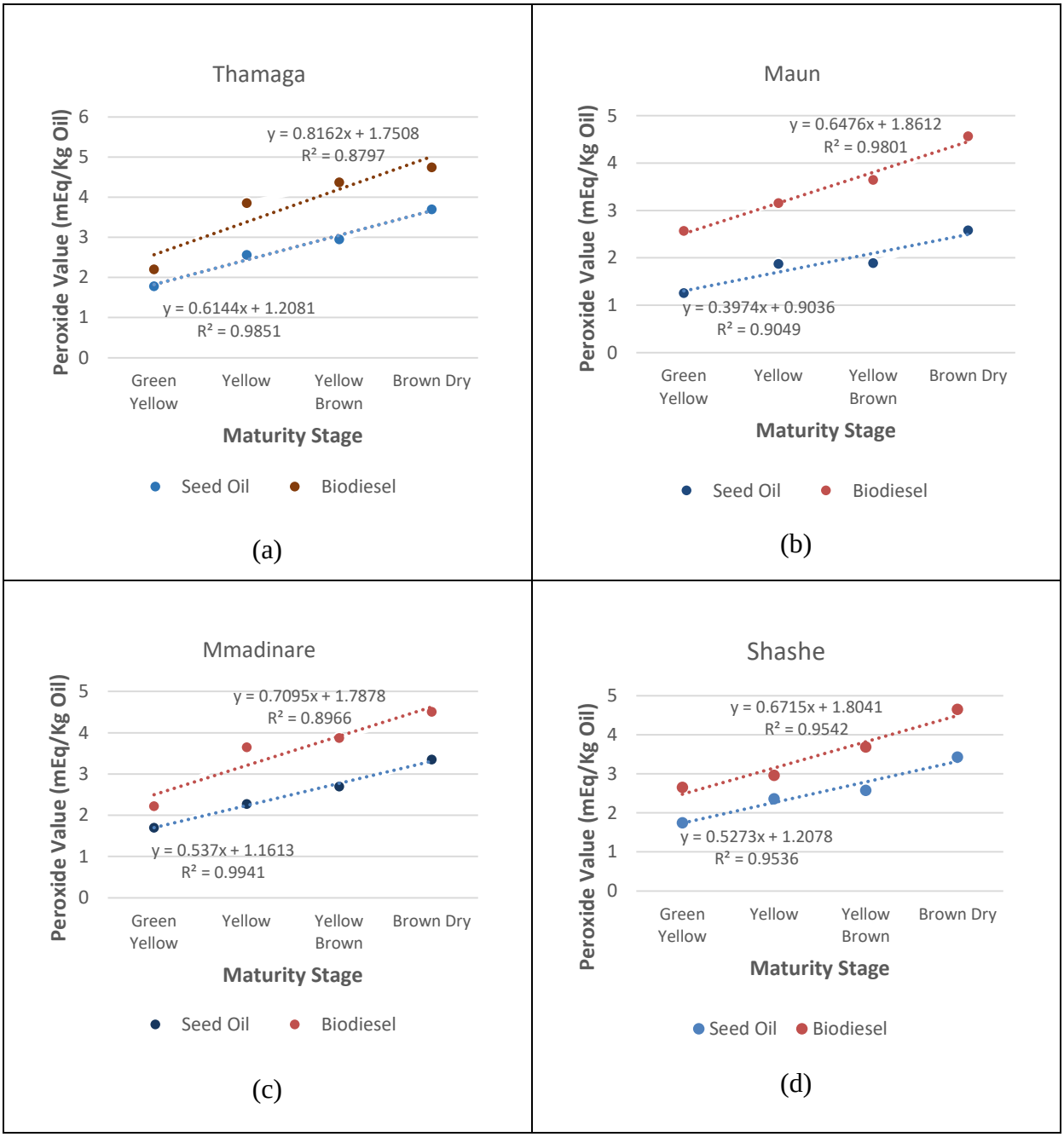


Figure 4-14. Variation of Peroxide Value in Seed oil and derived biodiesel with fruit maturity of Jatropha harvested in (a)Thamaga, (b)Maun, (c)Mmadinare and (d)Shashe areas.

4.7. Effect of Fruit Maturity Stage and Temperature on Kinematic Viscosity of Jatropha Seed Oil and Derived Biodiesel

The kinematic viscosity of both seed oil and biodiesel of Jatropha at four different fruit maturity stages was recorded at temperature range from 22^{o}C (room temperature) to 48^{o}C. The data in Figure 4-15 indicate that viscosity of seed oil does not change much with fruit maturity. As Jatropha fruits matures from green to brown dry, viscosity of seed oil remains almost the same. Viscosity of Jatropha seed oil ranges from 8.8 to 9 mm^2/s at 40^{o}C for all fruit maturity stages and for the three geographical locations investigated. The results show that there is a rapid drop in seed oil viscosity with increase in temperature at relatively lower temperature (up to 30^{o}C). Thereafter, seed oil viscosity reduces gradually with increase in temperature until it becomes almost constant after 40^{o}C as shown in Figure 4-15. There is 67% change in Jatropha seed oil viscosity as temperature increases from 22 to 48^{o}C. Biodiesel produced from the seed oil also changes in viscosity as temperature increase from 22 to 48^{o}C as demonstrated by Figure 4-16. However, biodiesel viscosity only changes slightly with change in temperature as compared to seed oil. There is 35% change in biodiesel viscosity as temperature increases from 22 to 48^{o}C. Percentage change in viscosity of Jatropha biodiesel is almost half that of Jatropha seed oil over the same temperature range. Therefore, Jatropha biodiesel can be used over a wider temperature range with relatively less change in viscosity as compared to seed oil. Viscosity of Jatropha biodiesel is in the range of 2.3 to 2.4 mm^2/s at 40^{o}C in all fruit maturity stages and all the geographical locations investigated. Previous researchers including (Silitonga, et al., 2014; Garba, et al., 2013; Raja, et al., 2011; Oliveira, et al., 2009; Tiwari, et al., 2007) reported a wide range of Jatropha seed oil and biodiesel viscosity as indicated in Table 2-2 Section 2.3. Testing equipment may have contributed to this variation in results from previous studies. Both the ASTM and European biodiesel standards requires that kinematic viscosity of biodiesel be specified at 40^{o}C. American Society for Testing and Materials standards requires that biodiesel kinematic viscosity be within the range of 1.9 to 6 mm^2/s while European standards specifies a narrower range of 3.5 to 5 mm^2/s. Jatropha biodiesel kinematic 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 high influence on the viscosity of both Jatropha seed oil and derived biodiesel.

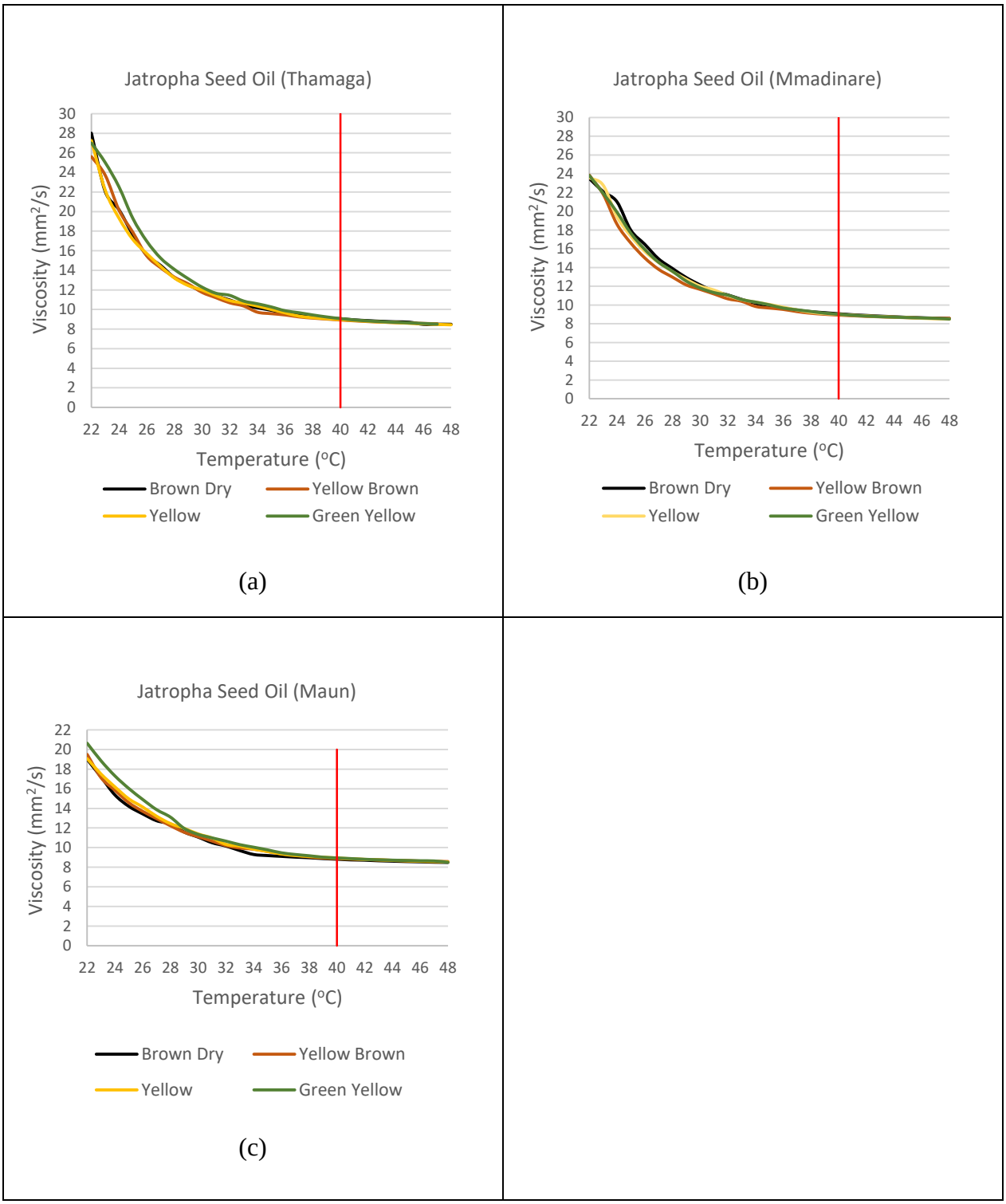


Figure 4-15. Variation of Jatropha seed oil viscosity from four different fruit maturity stages with temperature, harvested from different geographical locations; (a) Thamaga area, (b) Mmadinare and (c)Maun. (NB: 1 mm^2/s = 1 centiStokes).

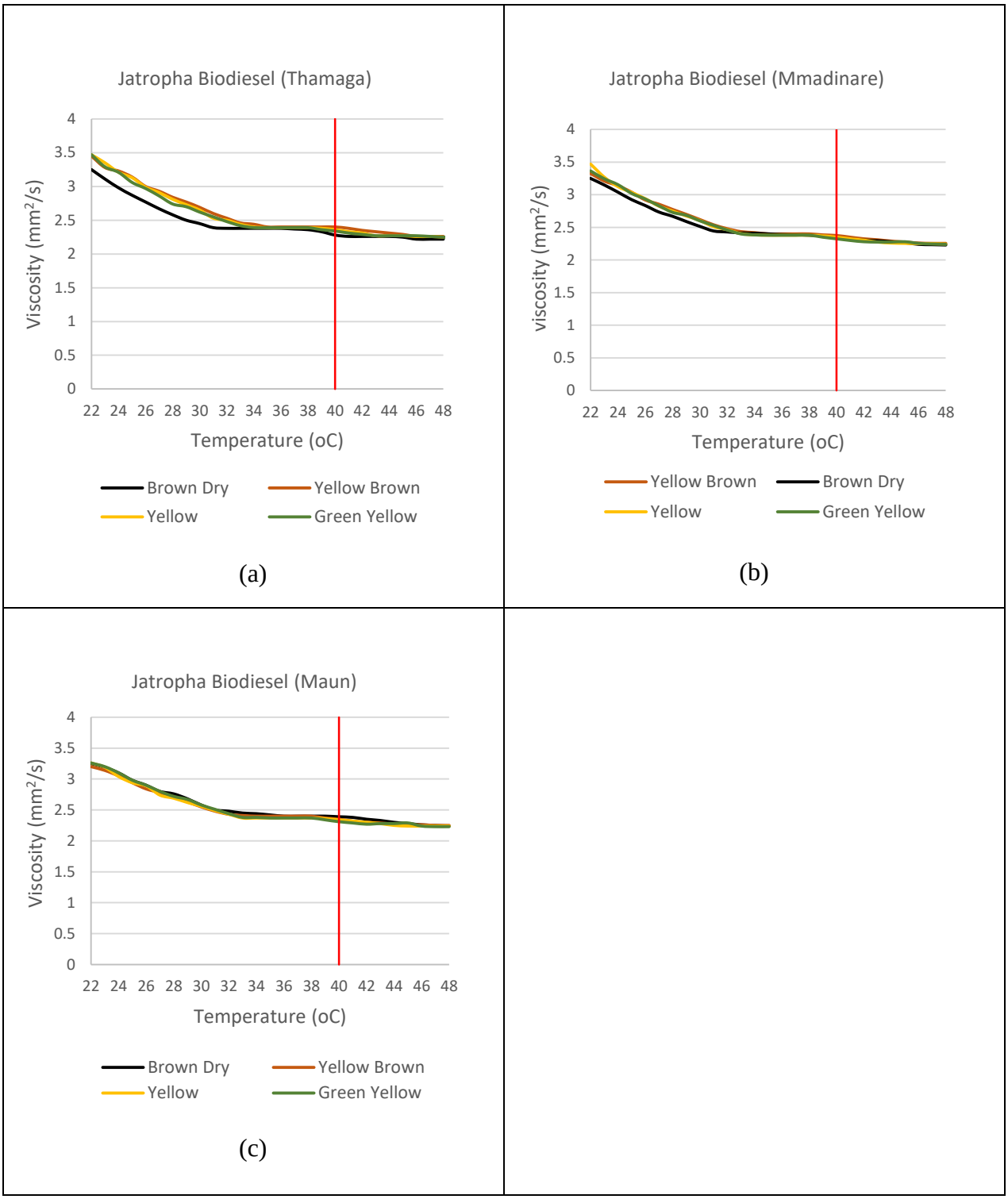


Figure 4-16. Variation of Jatropha biodiesel viscosity from four different fruit maturity stages with temperature, harvested from different geographical locations; (a) Thamaga area, (b) Mmadinare and (c) Maun. (NB: 1 mm^2/s = 1 centiStokes).

Conversion of Jatropha seed oil to biodiesel greatly reduces its viscosity as depicted in Figure 4-17. At 40°C, viscosity of Jatropha seed oil reduces by 6 mm^2/s from 9 mm^2/s after conversion to biodiesel. This indicates that using Jatropha seed oil in diesel engines may result in difficulty in fuel flow in pipes and atomization in fuel injectors as highlighted by several authors including (Yaakob, et al., 2014; Knothe & Steidley, 2005; Abdulkareem, et al., 2012). Relatively high viscosity of Jatropha seed oil is one of the main reasons it is converted to biodiesel before use in diesel engines. A similar trend of reduction in viscosity of Jatropha seed oil and derived biodiesel with increase in temperature was reported by Dubey et al (Dubey, et al., 2011). They reported that viscosity of Jatropha seed oil reduced by 74.8% after transesterification to biodiesel. Rao et al (Rao, et al., 2009) also reported that transesterification of Jatropha oil to biodiesel reduced its viscosity from 36 to 4.8mm^2/s at 40°C. They also reported that viscosity of Jatropha oil and derived biodiesel decreased remarkably with increasing temperature. Chalatlon et al (Chalatlon, et al., 2011) also echoed that the viscosity of Jatropha oil decreased remarkably with increasing temperature. Furthermore, they reported that instead of conversion to biodiesel, viscosity of raw Jatropha oil may be reduced by blending with petro-diesel since up to 10% blends were within the ASTM limits.

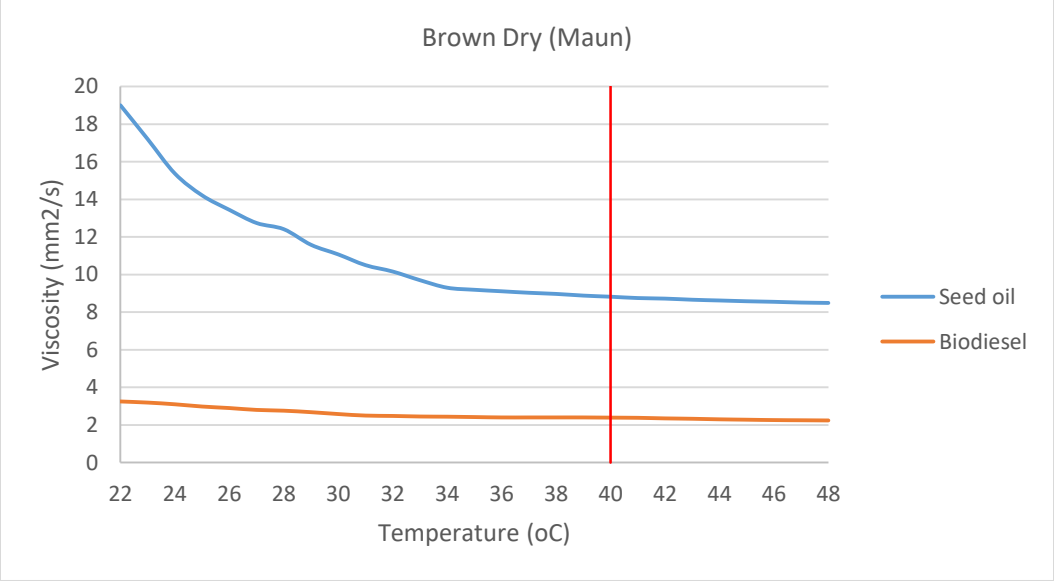


Figure 4-17. Comparison of both seed oil and biodiesel viscosity variation with temperature.

4.8. Effect of Fruit Maturity on Moisture Content of Jatropha Seeds, Seed Oil and Derived Biodiesel

Moisture content in extracted Jatropha seed oil ranges from 0.23 to 0.30% in all the maturity stages and four different geographical locations investigated as shown in Figure 4-18 (a). Moisture content in biodiesel derived from Jatropha seed oil ranges from 0.03 to 0.05% in all the fruit maturity stages investigated, as indicated in Figure 4-18 (b). Since seed oil and biodiesel are derived from dried seeds, moisture content in seed oil and biodiesel is not influenced much by fruit maturity as is the case in seeds as demonstrated in section 4.2. 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 (He, et al., 2007; Fregolente, et al., 2015; Fregolente & Maciel, 2012). This being the case, moisture content in biodiesel is likely to vary from time to time. Therefore, it is advisable to store biodiesel in an air-tight container to avoid absorption of moisture. Available information indicate that presence of water in oil triggers side reactions such as saponification during transesterification process, resulting in reduction in biodiesel yield as discussed previously in Section 2.3.4. Therefore, it is necessary to eliminate water present in oil prior to conversion to biodiesel. A common method for removing moisture from oil prior to conversion to biodiesel is by heating the oil at 100 -105^{o}C for about an hour. For large scale production, vacuum is applied in addition to heating to accelerate the moisture removal process (Refaat, 2010; Sudhir, et al., 2007). Joshua Folaranmi and Kywe (Folaranmi, 2013; Kywe & Oo, 2009) reported moisture content in Jatropha seed oil similar to those in the present study. However, their study focused only on brown dry maturity stage. Some researchers (Ntaganda, et al., 2014; Tiwari, et al., 2007) reported relatively higher moisture content (1.4 and 2.4%) in Jatropha seed oil from brown dry stage. The oil may have absorbed moisture due to its hygroscopic nature as highlighted earlier.

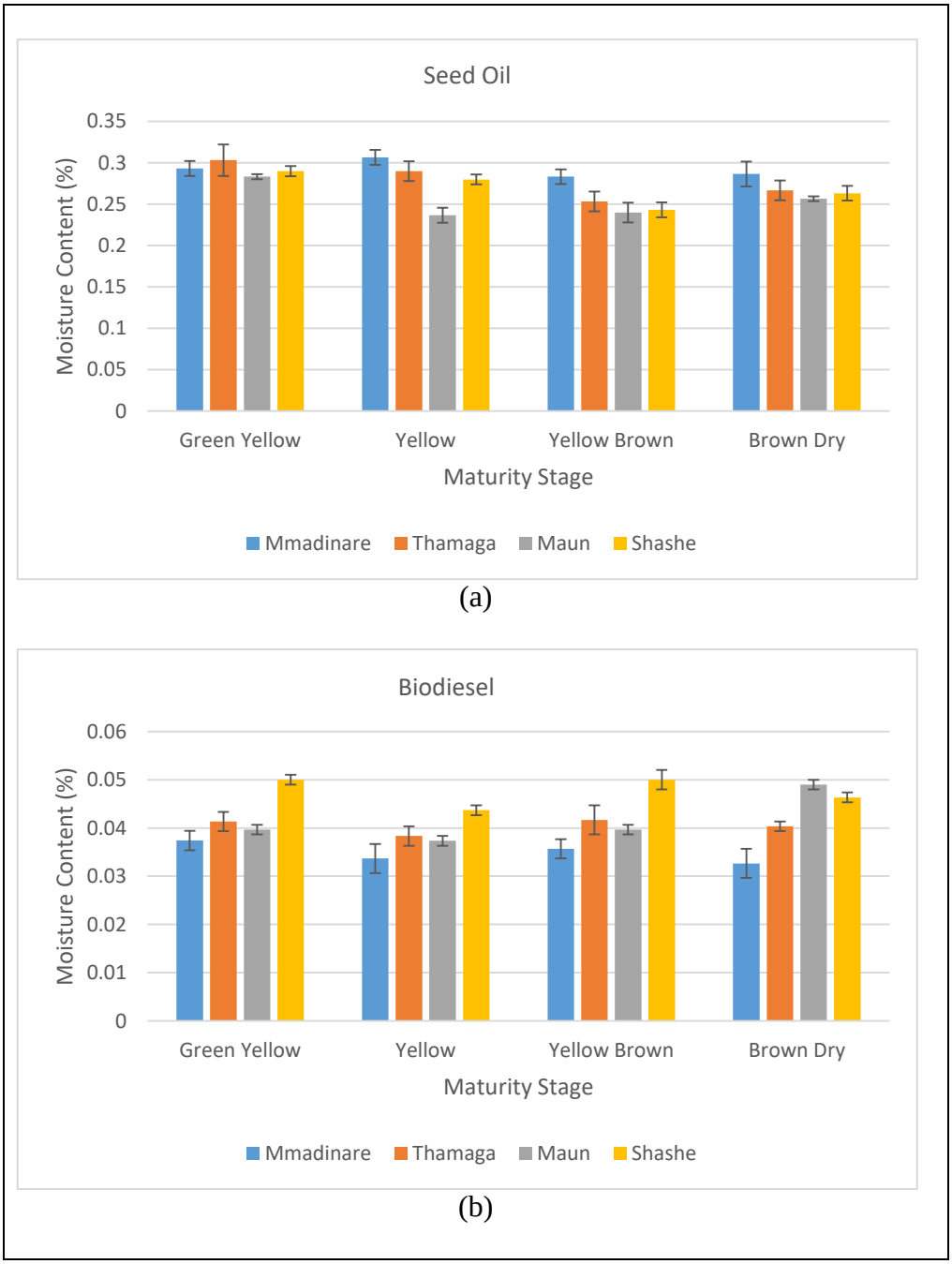


Figure 4-18. Moisture content in (a) Jatropha seed oil and (b) biodiesel derived from seeds harvested at four different fruit maturity stages from four different geographical locations.

4.9. Effect of Fruit Maturity on Density of Jatropha Seed Oil and Derived Biodiesel

Variation in density of both Jatropha seed oil and derived biodiesel with fruit maturity is insignificant as it appears in Figure 4-19. The densities seem not to be affected much by fruit maturity. Density of Jatropha seed oil is 0.91g/cm^3 and that of biodiesel is 0.87g/cm^3. After converting seed oil to biodiesel, the density drops by 0.04g/cm^3, which is equivalent to 4.4%. Previous researchers (Ntaganda, et al., 2014; Silitonga, et al., 2014; Oliveira, et al., 2009) who characterized Jatropha seed oil from brown dry fruits reported similar results as indicated in Section 2.3. The European standards (EN ISO 3675, EN ISO 12185) specifies density of 0.86 to 0.9g/cm^3 for biodiesel. The ASTM standard is silent on biodiesel density. Jatropha biodiesel density at all fruit maturity stages is well within the standards specifications as depicted in Figure 4-19. 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 negative impact in atomization of the fuel as echoed by (Allah, 2015; Boz, et al., 2009). Results have revealed that harvesting Jatropha fruits at different fruit maturity stages have no significant impact on the density of the seed oil.

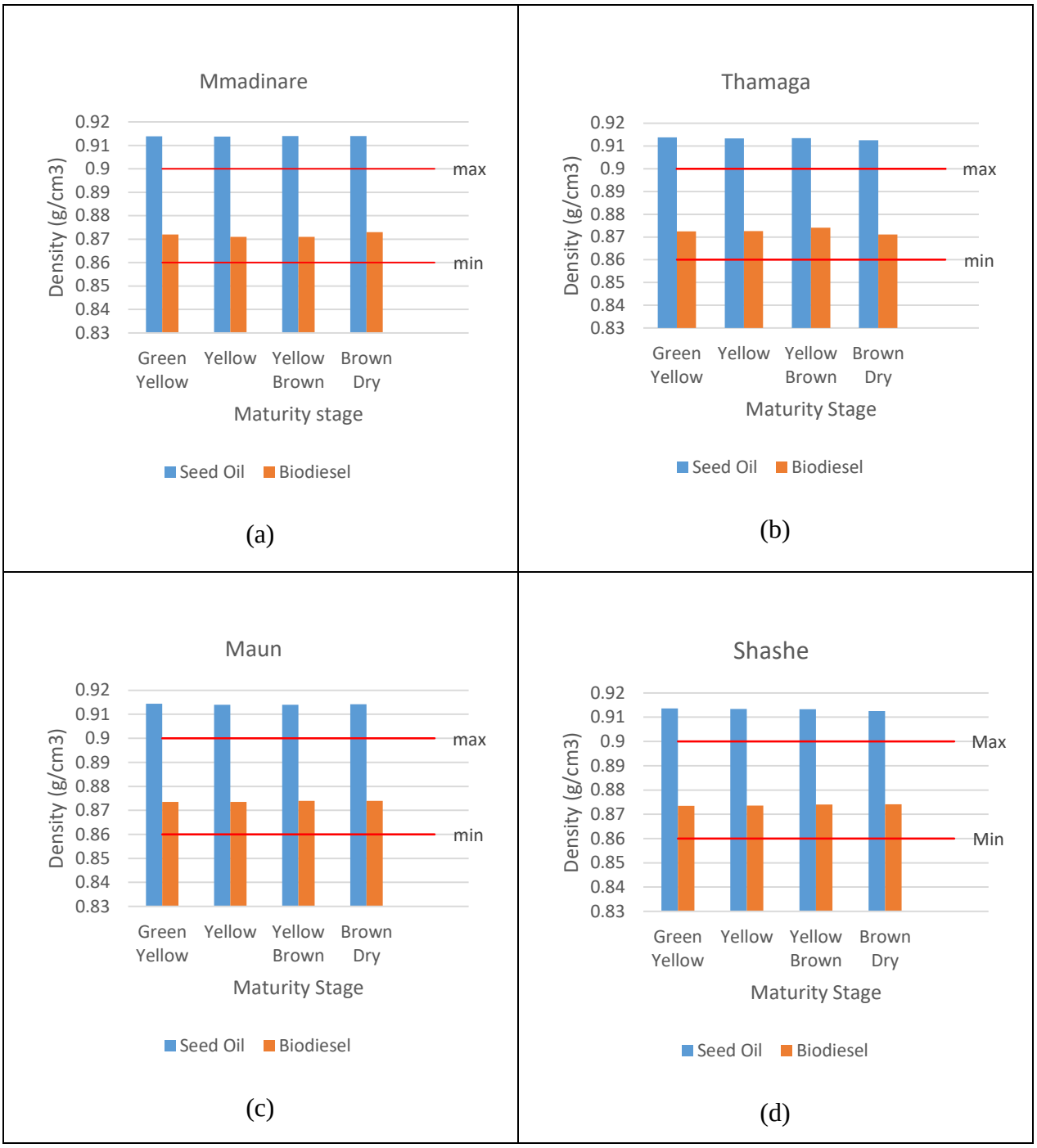


Figure 4-19. Densities of both Jatropha seed oil and derived biodiesel at four different fruit maturity stages harvested in (a)Mmadinare, (b)Thamaga, (c)Maun and (d)Shashe areas. The limits for biodiesel densities specified by European biodiesel standards (EN ISO 3675) are indicated by horizontal red lines.

4.10. Effect of Fruit Maturity on Calorific Value of Jatropha Seed Oil and Derived Biodiesel

Calorific value (CV) indicates the amount of energy available in a fuel, the higher the calorific value, the greater the energy contained in the fuel. The significance of calorific value of a fuel is discussed in Section 2.3.8. The calorific value of Jatropha seed oil ranges from 38.238 to 38.721MJ/Kg whereas that of derived biodiesel ranges from 39.282 to 39.924MJ/Kg as shown in Figure 4-20. This indicates that energy content of biodiesel is 3% greater than in seed oil. Results in Figure 4-20 show that there is no positive correlation between Jatropha fruit maturity and calorific values of both seed oil and derived biodiesel. Moreover, analysis of variance test results in Table A-24 in Appendix A highlight that effect of fruit maturity on calorific value is not statistically significant since the significance values are more than 0.05 and the F-values are less than the critical F-value (4.07). Therefore, harvesting Jatropha fruits/seeds at different fruit maturity stages may not improve the calorific value of seed oil and derived biodiesel. However, this variation of calorific value with fruit maturity is small, which is 1.3 and 1.6% for seed oil and biodiesel, respectively. The calorific value of petro-diesel is roughly 45MJ/Kg, therefore the calorific value of Jatropha biodiesel is 11% less than that of petro-diesel. There are currently no regulations on calorific value of both seed oil and biodiesel by international standards. However, previous researchers (Garba, et al., 2013; Oliveira, et al., 2009; Raja, et al., 2011; Silitonga, et al., 2013) who determined the calorific value of Jatropha seed oil and derived biodiesel from brown dry fruits reported a similar range. According to Kumari et al (Kumari, et al., 2014), fuels with higher calorific value produce more power in the engine whereas fuels with less calorific value tend to burn inefficiently producing a lot of exhaust emissions thus air-pollution. Therefore, Jatropha biodiesel will produce relatively less power in a combustion engine as compared to petro-diesel. Results from this investigation have revealed that different maturity stages may not improve the CV of both seed oil and derived biodiesel. Reports from previous studies (Abu-Hamdeh & Alnefaie, 2015; Barad, et al., 2017) have revealed that the CV of biodiesel may be improved by blending biodiesel with petro-diesel.

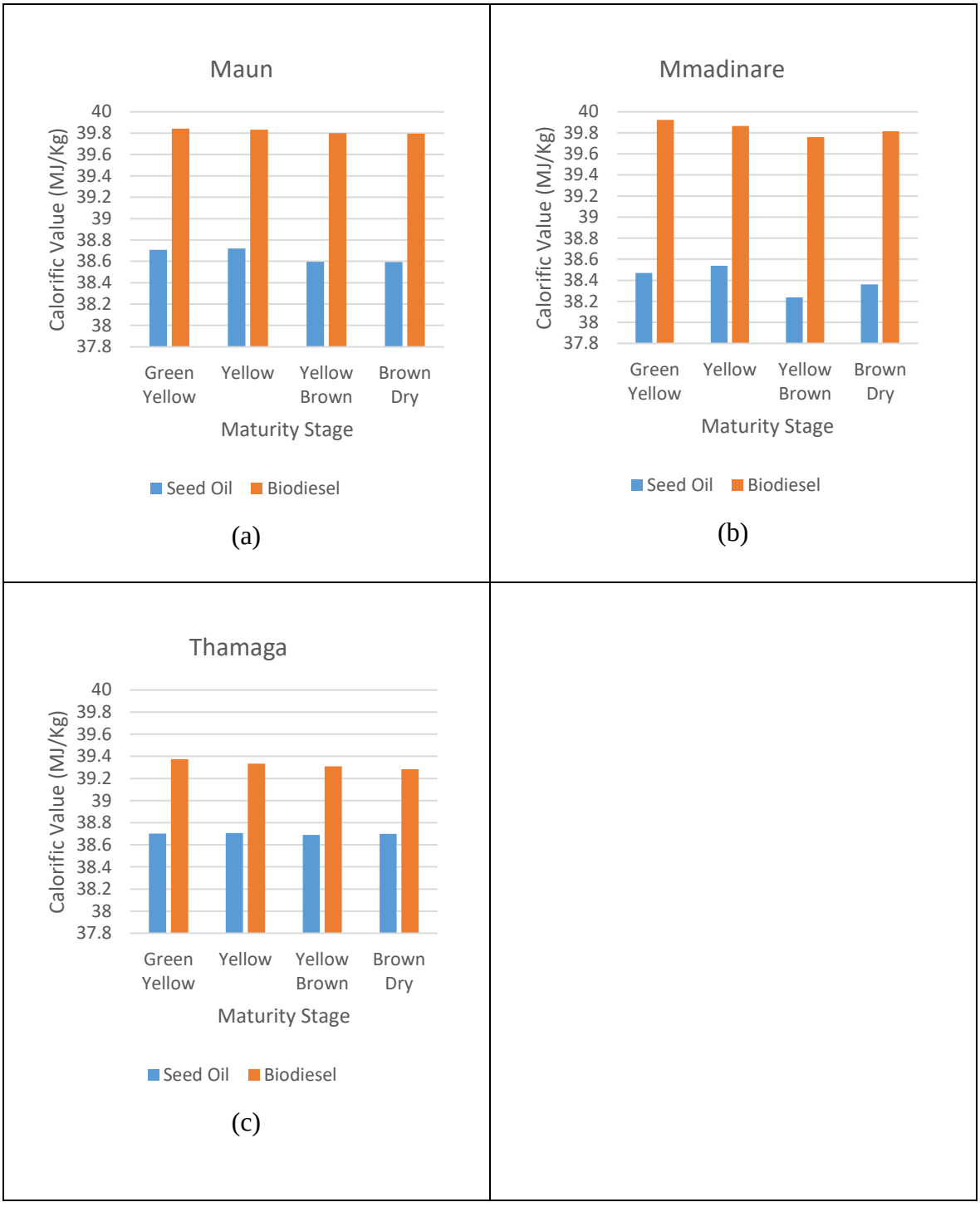


Figure 4-20. Calorific values of both Jatropha seed oil and derived biodiesel at four different fruit maturity stages harvested in (a) Maun, (b) Mmadinare and (c) Thamaga.

4.11. Effect of Fruit Maturity on Cloud Point and Pour Point of Jatropha Seed Oil and Derived Biodiesel

The effect of fruit maturity on cloud and pour points of Jatropha seed oil and derived biodiesel is quite minimal based on the results presented in Figure 4-21 and Figure 4-22. Cloud and pour point of both Jatropha seed oil and derived biodiesel remain unchanged during various fruit maturity stages. Therefore, harvesting Jatropha fruits at different maturity stages may not improve both the cloud and pour point of Jatropha seed oil and derived biodiesel. The cloud and pour point of both Jatropha seed oil and derived biodiesel is the same for all the selected geographical locations investigated in Botswana. Results reveal that the cloud and pour point of Jatropha seed oil is -4^{o}C and -5^{o}C, respectively for all the four maturity stages while the cloud and pour point of derived biodiesel is 2^{o}C and 1^{o}C, respectively. The cloud and pour point of Jatropha seed oil are lower than those of derived biodiesel. Kywe and Oo (Kywe & Oo, 2009) reported pour point of -1°C for biodiesel from Jatropha seed oil from brown dry fruits which is 2^{o}C lower than results presented in Figure 4-22. Shambhu, et al (Shambhu, et al., 2012) reported cloud and pour point of 9^{o}C and 3^{o}C respectively for Jatropha seed oil from brown dry stage and cloud and pour point of 4^{o}C and 1^{o}C respectively for derived biodiesel. Their results for Jatropha biodiesel are similar to those of the present study whereas results for Jatropha seed oil differ. Findings across the literature on cloud and pour point of Jatropha seed oil and derived biodiesel vary as indicated in Table 2-2 in Section 2.3. Results from the present study indicate that usage of Jatropha biodiesel at temperatures below 2^{o}C will cause it to solidify. Therefore this findings reveal that Jatropha biodiesel may be used at temperatures above 2^{o}C to avoid blockage of pipes. At temperatures below 2^{o}C, Jatropha biodiesel may be blended with petro-diesel to improve its low temperature operability.

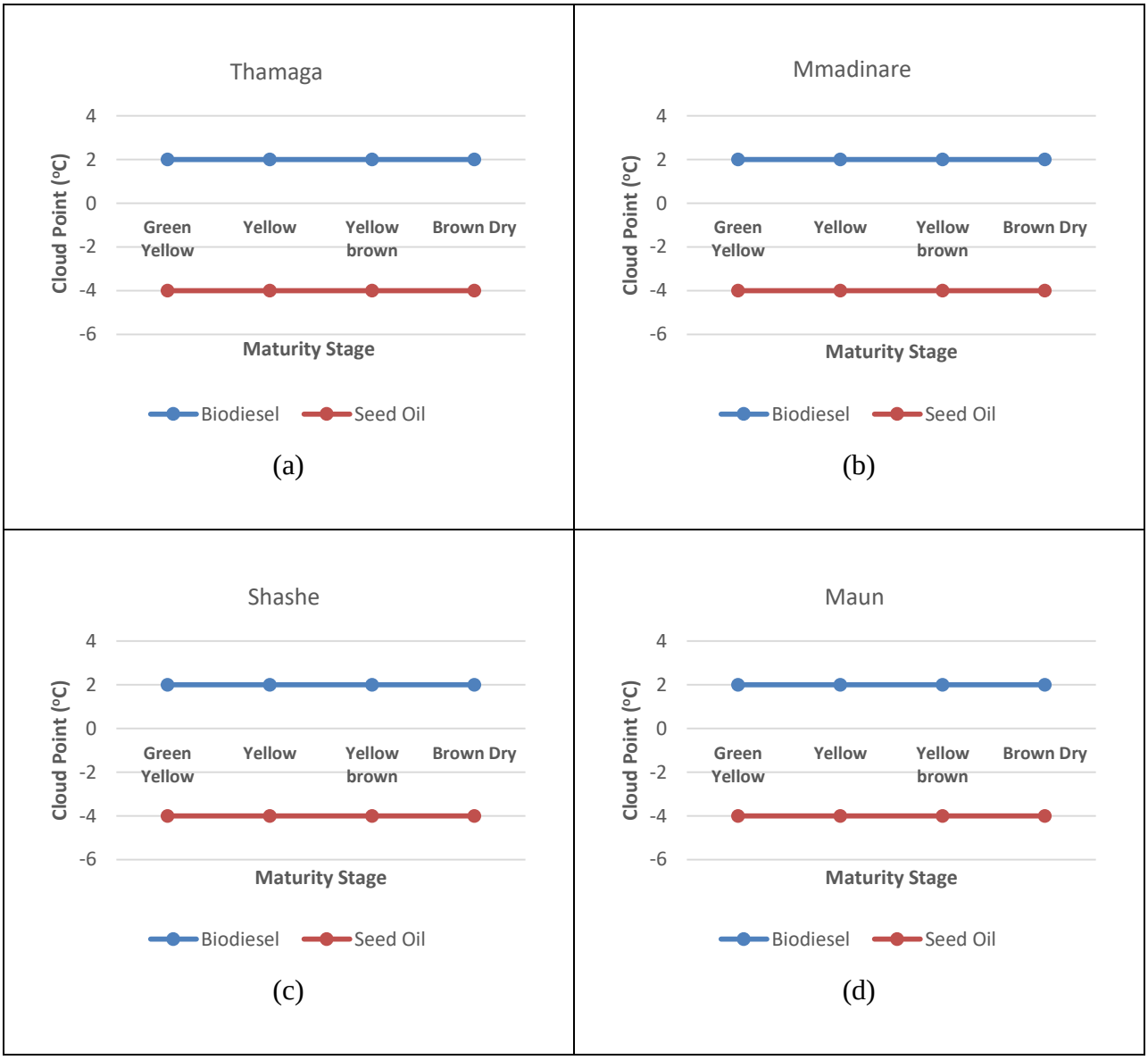


Figure 4-21. Cloud points of both Jatropha seed oil and derived biodiesel at four different fruit maturity stages harvested in (a) Thamaga, (b) Mmadinare, (c) Shashe and (d) Maun.

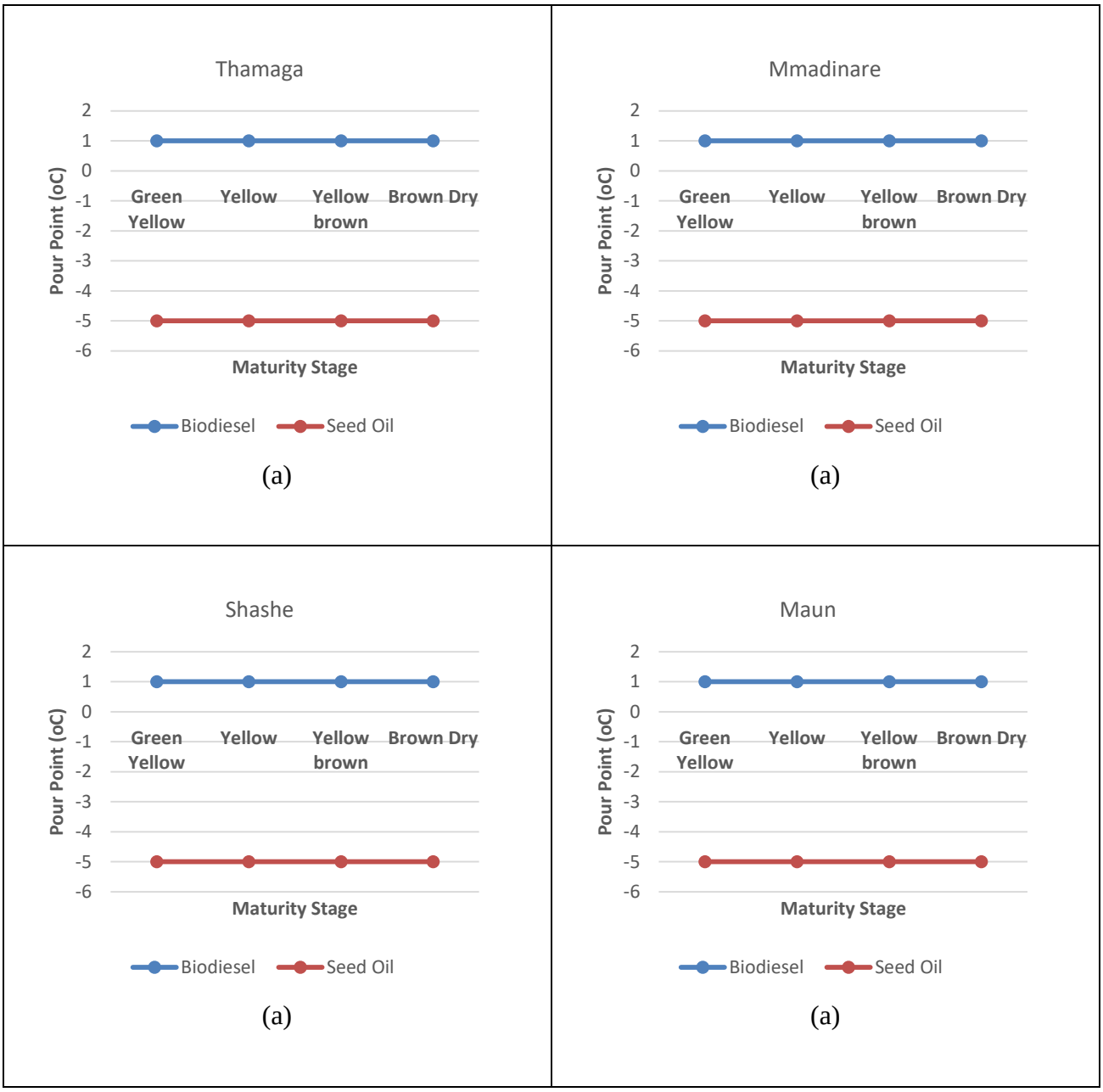


Figure 4-22. Pour points of both Jatropha seed oil and derived biodiesel at four different fruit maturity stages harvested in (a) Thamaga, (b) Mmadinare, (c) Shashe and (d) Maun.

4.12. Effect of Fruit Maturity on Flash point of Jatropha Seed Oil and Derived Biodiesel

The flash point of Jatropha seed oil ranges from 212 to 220^{o}C and that of derived biodiesel ranges from 137 to 142^{o}C during the different fruit maturity stages investigated as depicted by Figure 4-23. This results are in agreement with the findings by a number of researchers including (Raja, et al., 2011; Tiwari, et al., 2007) who characterised Jatropha seed oil and derived biodiesel from brown dry fruits in India. Furthermore, the flash point of derived biodiesel from all the different fruit maturity stages and geographical locations in Botswana complies with some international biodiesel standards such as ASTM D93, which recommends minimum flash point for biodiesel of 130^{o}C. Generally, the flash point of seed oil is 54 to 55% greater than that of biodiesel. This implies that converting Jatropha seed oil to biodiesel reduces its flash point by 54 to 55%. There is no clear trend in variation of flash point of seed oil and derived biodiesel with fruit maturity from all the geographical locations investigated. Therefore, it is appropriate to conclude that fruit maturity does not have any influence on the flash point of seed oil and derived biodiesel. As highlighted earlier in Section 2.3 the influence of fruit maturity on flash point of Jatropha seed oil and derived biodiesel is not well documented by previous researchers.

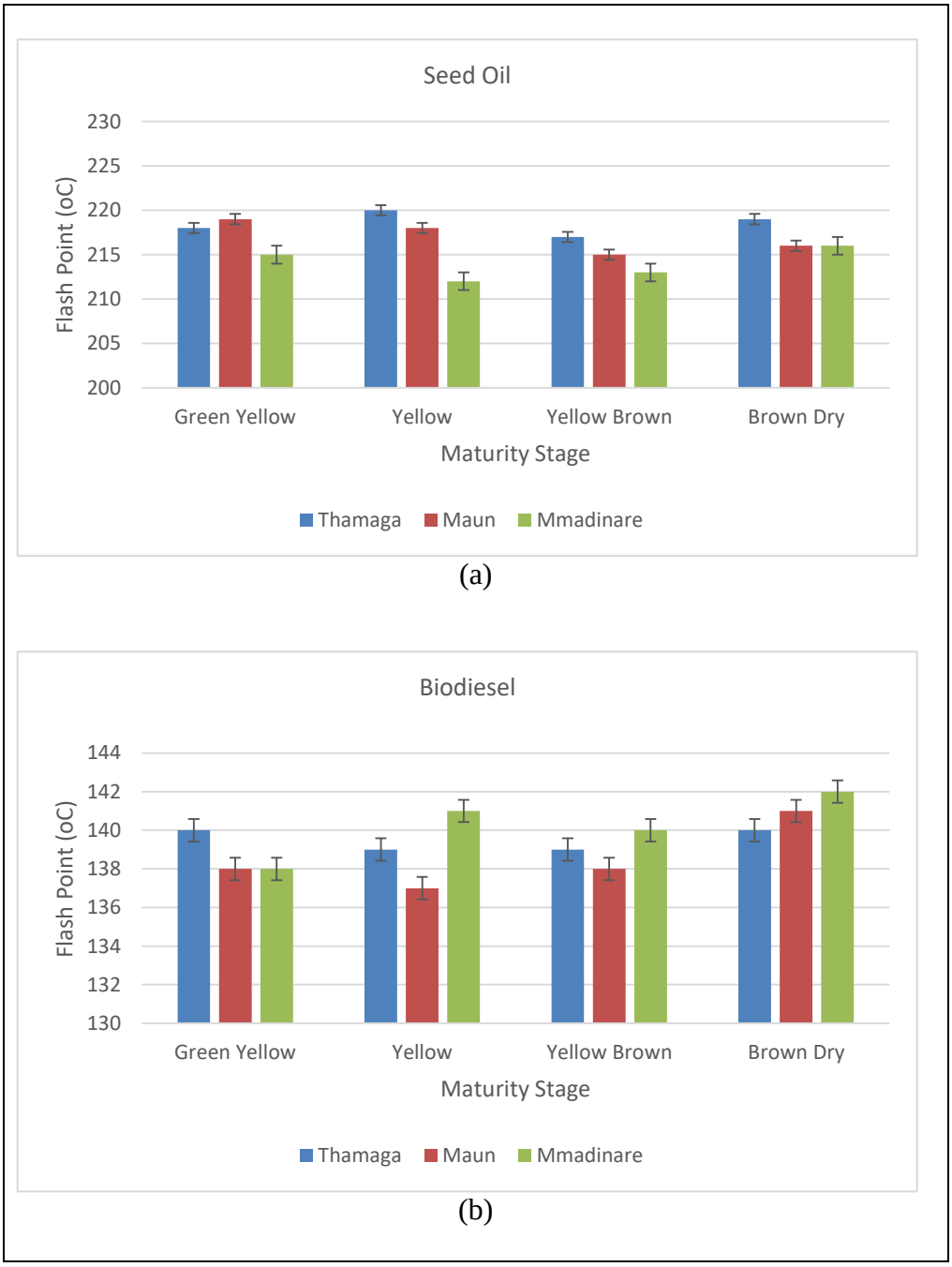


Figure 4-23. Flash point of (a) Jatropha seed oil and (b) derived biodiesel from selected geographical locations in Botswana at different fruit maturity stages.

4.13. Effect of Fruit Maturity on Cetane Number of Jatropha Biodiesel

Biodiesel derived from seeds harvested in Shashe area have relatively highest cetane number of 54.7 from brown dry fruits whereas those harvested from Mmadinare recorded relatively least cetane number of 50.3 from the same maturity stage. Analysis of variance results indicate that influence of fruit maturity on cetane number of Jatropha derived biodiesel is statistically significant. Generally, the cetane number of biodiesel increases slightly as Jatropha fruits matures from green yellow to brown dry as shown in Figure 4-24. This is due to decrease in fractional composition of unsaturated fatty acids (linoleic and oleic acid) as demonstrated by results in Figure 4-10, Figure 4-11, Figure 4-12 and Figure 4-13 in Section 4.5. It should be noted that cetane number of biodiesel is influenced by fatty acid composition of the seed oil (feedstock). As mentioned earlier in Section 4.5, unsaturated fatty acids in seed oil undergo degradation due to autoxidation during maturation of fruits/seeds as supported by Chia et al (Chia, et al., 2005). Decrease in unsaturated fatty acids in seed oil results in increase in cetane number. Therefore, the results in Figure 4-24 indicate that biodiesel derived from the final stage of fruit maturity (brown dry) have relatively high cetane number than biodiesel derived from early stages of fruit maturity (green yellow and yellow). American Society for Testing and Materials (ASTM D613) specifies a minimum Cetane number of 47 while the European standards EN ISO 5165 specifies a minimum Cetane number of 51 for biodiesel. Biodiesel from all the maturity stages and all the geographical locations investigated comply with the ASTM regulations for cetane number. However, biodiesel derived from seeds harvested in Mmadinare (48.8 to 50.3) is slightly below the minimum requirement set by the European standards for biodiesel. The significance of cetane number in a diesel engine is discussed in Section 2.3.9. Variation of biodiesel cetane number with geographical location is a result of variation in fatty acid composition in Jatropha seed oil in various geographical locations. Previous researchers including (Sivaramakrishnan & Ravikumar, 2012; Maina, 2014; Dangoggo, et al., 2018; Reddy, et al., 2018) who studied Jatropha seed oil from brown dry stage reported similar results on cetane number of Jatropha biodiesel in the range of 50 to 62. Generally, cetane number of biodiesel is greater than that of petro-diesel which is about 48, resulting in higher combustion efficiency as well as smoother combustion for biodiesel as highlighted by Bhatia (Bhatia, 2014).

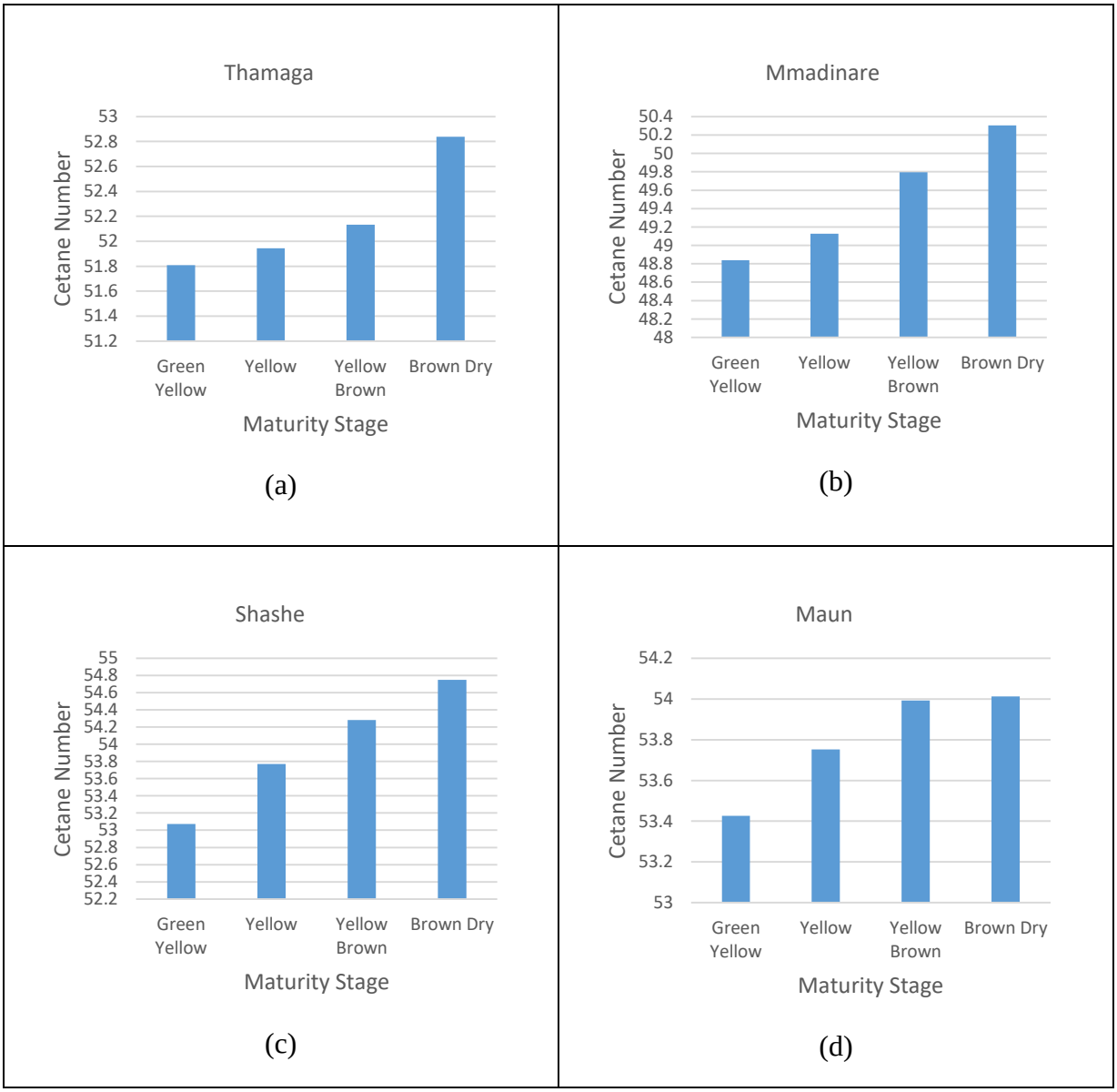


Figure 4-24. Cetane number of Jatropha biodiesel derived from four different fruit maturity stages, harvested in (a)Thamaga, (b)Mmadinare, (c)Shashe and (d)Maun.

4.14. Effect of Maturity on Iodine Value and Saponification Value of Jatropha Seed Oil

Iodine values of Jatropha seed oil decrease gradually with successive maturity stage as indicated Figure 4-25. This implies that the degree of unsaturation of Jatropha seed oil decreases continuously as fruits mature from green yellow to brown dry. This decrease in double bonds makes seed oil and derived biodiesel from final maturity stages (brown dry) relatively more stable as compared to earlier maturity stages (green yellow and yellow). Therefore, seed oil and derived biodiesel from earlier maturity stages (green yellow and yellow) are relatively more susceptible to oxidation than those from final maturity stages (brown dry). The decrease in seed oil iodine value with successive maturity stage may be due to degradation of some of the unsaturated fatty acids during final maturation stages which also contributes significantly to decline in lipid content in Jatropha seeds. Iodine values of Jatropha seed oil from all the four maturity stages are within the maximum limit specified by the European biodiesel standards, EN 14111 of 120 g I/100g.

Saponification values of Jatropha seed oil are in the range of 186 to 196 mg/g as indicated in Table 3. Variation of saponification value with maturity stage does not follow any particular trend, therefore there is no correlation between maturity with change in saponification value.

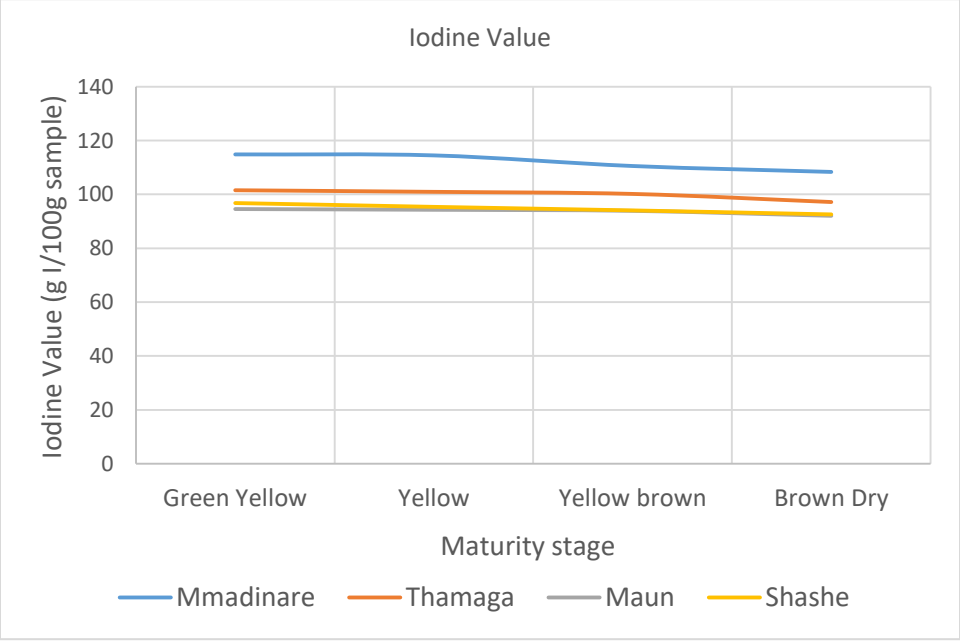
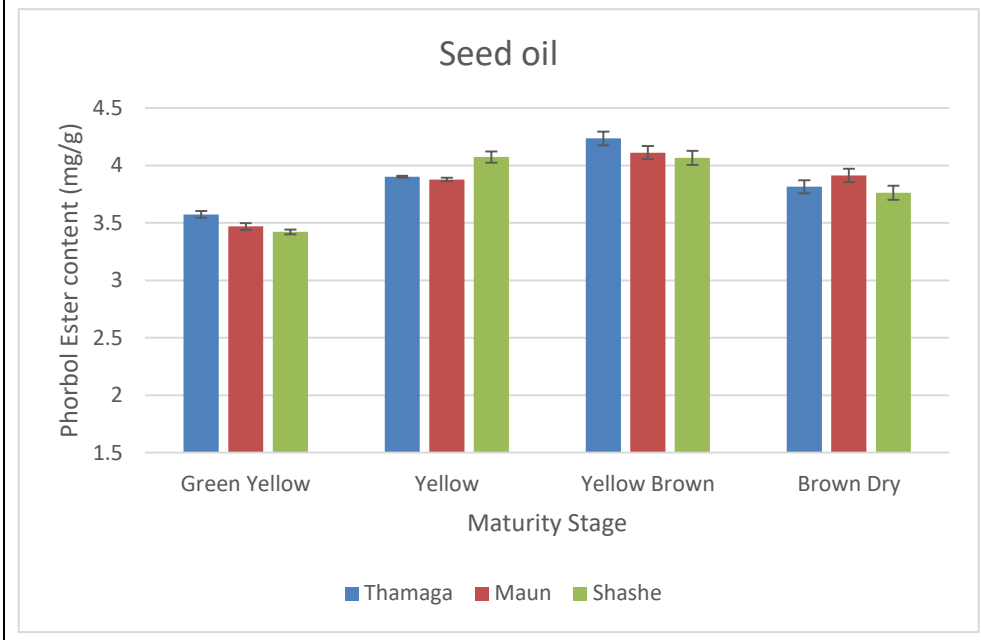


Figure 4-25. Variation of Iodine value with maturity stage of Jatropha biodiesel from four different geographical locations in Botswana.

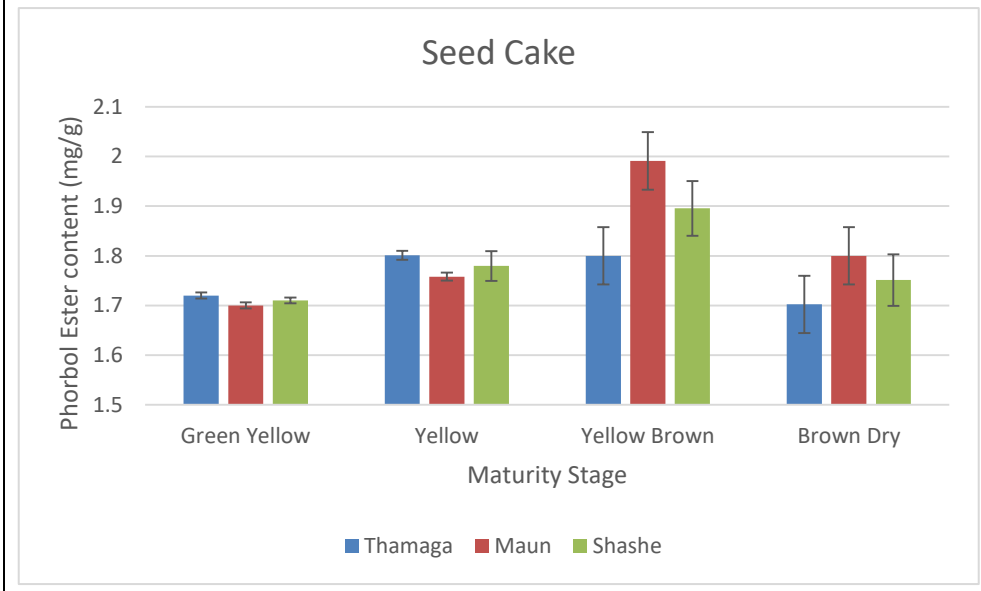
4.15. Effect of Fruit Maturity on Phorbol Ester Content (Toxicity) in Jatropha Seed Oil and Seed Cake

Phorbol ester content in Jatropha seed oil ranges from 3.4 to 4.2 mg/g whereas in seed cake ranges from 1.7 to 2.0 mg/g during the four different fruit maturity stages as illustrated by Figure 4-26. The highest PE content in seed oil and seed cake was recorded from yellow brown stage in all the investigated geographical locations. At this maturity stage, seed oil from Thamaga has relatively the highest concentration of PEs (4.24 mg/g), followed by Maun (4.11mg/g) and seed oil from Shashe recorded relatively the least concentration (4.0 mg/g). These results are in line with those reported by (Devappa, et al., 2013; Pradhan, et al., 2010) in United States of America and India, respectively, from brown dry fruits as shown in Section 2.6. The influence of fruit maturity stage on phorbol ester content in both seed oil and seed cake is statistically significant as demonstrated in Table A-26 in Appendix A. The influence of location in PE content is insignificant as demonstrated in Table A-27 in Appendix A. The p-values are all greater than 0.05 indicating that variation is statistically insignificant. Phorbol ester content in both Jatropha seed oil and seed cake increase as the fruit matures from green yellow to yellow brown as depicted by the data in Figure 4-26. Thereafter, the PE content decrease by 5 to 10% as the fruits turns brown dry. Generally, this particular trend applies for all the selected geographical locations investigated. This indicate that Jatropha seed oil and seed cake from yellow brown fruits is the most toxic since PE content in this particular maturity stage is at the peak. Phorbol ester accumulation trend in Jatropha seeds, as depicted in Figure 4-26 suggest that PEs in Jatropha seeds are synthesised continuously during maturation of seeds until they reach peak level when the fruit turns yellow brown. It is likely that the PEs start to degrade as Jatropha fruits turns brown dry from yellow brown since their concentration start to decrease. The seed cake have relatively lower concentration of PEs than the seed oil. Partly, some of the phorbol esters in seed cake may be contributed by residual oil which is left in the cake after oil extraction. The data in Figure 4-26 appear consistent with reports by previous researchers who reported that about 70% of the total phorbol esters in Jatropha seeds are dissolved in the oil and the remainder is found in the deoiled seed cake (Makkar & Becker, 2009; Pradhan, et al., 2010; Devappa, et al., 2013). In conclusion, Jatropha seed oil and seed cake from all the three investigated geographical locations in Botswana (Thamaga, Maun, Shashe) are toxic since their PE content is way above the threshold reported by (Kumar, et al., 2018) of 0.05 mg/g which distinguishes between the toxic and non-toxic Jatropha genotypes. However, the toxicity

level varies with fruit maturity stage. Those from yellow brown fruits are relatively the most toxic, as confirmed by the results of this study. Variation of PE content in both seed oil and seed cake from all the three geographical locations under review have a uniform trend. Jatropha seed oil and seed cake from all the four maturity stages and three geographical locations investigated in Botswana are toxic. Therefore, precaution must be taken when handling both the oil and seed cake from all the maturity stages to avoid ingestion by any means due to high concentration of phorbol esters. In other parts of the world, non-toxic Jatropha have been reported as mentioned earlier in Section 2.6.



(a)



(b)

Figure 4-26. Phorbol ester content in (a) Jatropha seed oil and (b) seed cake from selected geographical locations in Botswana at different fruit maturity stages.

4.16. Analysis of Variance (ANOVA) on Influence of Fruit Maturity on Jatropha Seed Oil Content and Quality Parameters of Both Seed Oil and Derived Biodiesel

Analysis of variance (ANOVA) assess the amount of variability between and within group means in order to determine whether the mean differences are statistically significant (Frost, 2019). Therefore, the larger the differences between the means, the more variation present. In the present study, ANOVA was used to assess the influence of fruit maturity on Jatropha seed oil content and quality parameters of seed oil and derived biodiesel. According to Table E-1 in Appendix E, the critical F-value is 4.07. A higher influence of fruit maturity on any parameter corresponds to having a higher variance between group means (variance in the numerator). Therefore, F-values greater than 4.07 shows that the influence of fruit maturity is statistically significant. The significance values for oil content in Jatropha seeds harvested in Thamaga, Maun and Shashe are 0.032, 0.001 and 0.002, respectively. Therefore, effect of fruit maturity on Jatropha seed oil yield is statistically significant because the significance values are less than 0.05. However, variance in oil content in Jatropha seeds harvested from Mmadinare is not statistically significant. Results reveal that effect of fruit maturity on most of the quality parameters is statistically significant since the significance values are less than 0.05. Moreover, the F-values for those parameters are greater than 4.07 which shows that the effect is statistically significant. These parameters include FFA, peroxide value, density, viscosity, cetane number and moisture content. The effect of fruit maturity on calorific value is not statistically significant since the significance values are more than 0.05 and the F-values are less than the critical F-value.

Conclusions derived from the results and findings of the present study are presented in Chapter 5.

Chapter 5

5. Conclusions and Recommendations for Further Research

5.1. Introduction

This chapter presents conclusions from the present work and recommendations on future investigations.

5.2. Conclusions

The effect of fruit maturity on seed oil and biodiesel yield and quality (physical and chemical properties) of Jatropha Curcas adapted to Botswana climatic conditions have been investigated. According to the results of this investigation, yellow is the optimum maturity stage that Jatropha fruits can be harvested to get the highest seed oil yield possible. Accumulation of oil in Jatropha seeds reaches its peak when fruits are yellow, thereafter oil content in seeds starts to decline continuously until the final maturity stage. Harvesting Jatropha fruits when they are yellow increases seed oil output by 6 to 9% as compared to harvesting the fruits on their final maturity stage (brown dry). This can significantly increase feedstock availability in large scale biodiesel production. Degradation of unsaturated fatty acids (particularly linoleic acid) during seed desiccation stage contributes significantly to decline in lipid content in Jatropha seeds. Fatty acid composition of Jatropha seed oil varies with fruit maturity. Fractional composition of saturated fatty acids remain almost constant throughout the maturation stages for all the areas under review. On the other hand, fractional composition of unsaturated fatty acids, which makes up 77 - 80% of the total lipid, declines continuously with each successive maturity stage. Moreover, seed oil from early stages of fruit maturity (green yellow and yellow fruits) have relatively low free fatty acids content (0.20-0.27%) and peroxide value (1.2-2.5mEq/Kg Oil) hence of better quality. As a result, derived biodiesel also have relatively low peroxide value (2.2-3.6mEq/Kg Oil). This indicates that the quality of both seed oil and derived biodiesel can be improved by using seed oil from early stages of fruit maturity (green yellow and yellow). Cetane number of biodiesel derived from Jatropha seed oil increases (from 49 to 54) as fruits matures from green to brown dry due to decrease in fractional composition of unsaturated fatty acids. Biodiesel derived from the final maturity stage has relatively highest cetane number. Parameters affected the most by fruit maturity

are seed oil yield/ content, free fatty acid content, fatty acid profile, peroxide value, water content and cetane number. Other quality parameters such as viscosity, energy content, density, flash point, pour point and cloud point of the seed oil and derived biodiesel remain almost constant throughout the maturation process of Jatropha fruits. Overall, the yellow stage has more advantages in terms of seed oil content and quality of both oil and biodiesel. Therefore, it is recommended that Jatropha fruits should be harvested when yellow instead of brown dry in order to optimize both quantity and quality of seed oil hence derived biodiesel. Results from this study have confirmed that seeds of Jatropha found in Botswana (Thamaga, Maun, Shashe) are toxic, and those from yellow brown fruits are relatively the most toxic, due to the presence of phorbol esters. Phorbol ester content in Jatropha seed oil and seed cake do vary with fruit maturity (3.4 to 4.2mg/g in seed oil and from 1.7 to 2.0mg/g in seed cake), and peak PE content is attained when fruits are yellow brown irrespective of geographical location.

5.3. Recommendations for Future Research

This study is limited to investigating the influence of fruit maturity stage on yield and quality of seed oil and derived biodiesel. Further investigations should be carried out on how oil content in Jatropha seeds changes during storage. Furthermore, investigations may be carried out to assess how various storage conditions impact different quality parameters of Jatropha seeds, seed oil as well as derived biodiesel. This will inform the biofuel industry on the timeliness of harvesting and processing Jatropha seeds for biodiesel production. Information on storage of both feedstock and processed biodiesel is vital to the biodiesel production industry.

A thorough and more detailed economic evaluation assessment on using Jatropha seed oil as a feedstock in biodiesel production in Botswana is also recommended as a future research. Feedstock cost plays an important role in overall production costs of biodiesel. Therefore, information on using Jatropha seed oil as a feedstock in biodiesel production in Botswana will help understand the economic viability of biodiesel production from Jatropha.

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Appendix A

A. Experimental Data

A.1. Moisture Content in Jatropha Seeds

Jatropha seeds from four different maturity stages harvested from selected geographical locations as demonstrated by Figure 3-1 and *Figure 3-2* in Section 3.2 were analyzed for moisture content as described in Section 3.3 and Section 3.13. The results are presented in Table A-1. The seeds were analysed for moisture content immediately after harvesting (before drying) and after the natural drying processes.

Table A-1. Moisture content in Jatropha seeds from four different maturity stages before and after drying.

Location	**Moisture Content in Jatropha Seeds (%)**							
	Green Yellow		Yellow		Yellow Brown		Brown Dry	
	Before Drying	After Drying	Before Drying	After Drying	Before Drying	After Drying	Before Drying	After Drying
Mmadinare	40.564	3.626	39.490	4.109	32.280	3.509	4.869	3.787
Thamaga	40.655	3.717	39.040	3.658	32.576	3.805	4.779	3.696
Maun	40.753	3.815	39.088	3.706	32.670	3.899	5.471	4.388
Shashe	40.564	3.626	39.424	4.042	32.180	3.409	4.903	3.820
	Standard Error (±)							
Mmadinare	0.050	0.050	0.370	0.370	0.452	0.452	0.074	0.074
Thamaga	0.129	0.129	0.103	0.103	0.165	0.165	0.062	0.062
Maun	0.300	0.300	0.027	0.027	0.124	0.124	0.163	0.163
Shashe	0.052	0.052	0.398	0.398	0.552	0.552	0.042	0.042

A.2. Seed Oil Yield Variation with Fruit Maturity

Oil in Jatropha fruits is stored in seeds (specifically in the seed kernel). Depending on method of oil extraction, oil can be extracted from Jatropha seeds either as whole seeds (kernels with seed coat) or isolated kernels. For the present study, seed oil yield is divided into two, actual yield and screw press yield. The actual seed oil yield is the total lipid content in seeds, and it was determined using solvent extraction as described in Section 3.4. Screw press yield is the amount of oil that is extractable using mechanical screw press and is dependent on the efficiency of the pressing equipment. Therefore, screw press yield will always be lower than the actual seed oil yield.

A.2.1. Actual Seed Oil Yield

The actual seed oil content in Jatropha seed kernel harvested at four different fruit maturity stages from four different geographical locations in Botswana is shown in Table A-2. Since the experimental measurements were repeated five times, Standard Errors (SE) were determined for each average value.

Table A-2. Oil yield/content in Jatropha seed kernel harvested at four different fruit maturity stages.

Location	**Jatropha Seed Oil Yield (%)**			
	Green Yellow	Yellow	Yellow Brown	Brown Dry
Mmadinare	43.974	45.803	44.515	42.998
Thamaga	43.433	44.966	43.357	40.949
Maun	39.423	41.713	40.695	39.128
Shashe	39.466	40.362	39.137	38.709
	Standard Error (±)			
Mmadinare	0.032	0.052	1.390	0.127
Thamaga	0.333	0.132	1.416	0.319
Maun	0.049	0.232	0.046	0.581
Shashe	0.108	0.060	0.333	0.123

A.2.2. Screw Press Seed Oil Yield

Pressing Jatropha seeds using a mechanical screw press results in seed cake, slurry and seed oil. Slurry is a mixture of seed oil and very fine seed cake debris. The slurry and seed oil are separated by sedimentation. Figure A-1 shows Jatropha seed oil after mechanical screw press of Jatropha seeds derived from Jatropha fruits at four different maturity stages. The percentage weight fraction of seed oil, slurry and seed cake after mechanical screw press are shown in Table A-3. Screw press seed oil yield will not form part of the discussion of results since they do not indicate the actual oil content in seeds. During mechanical screw press, some of the oil remains in seed cake as residual oil while some of the oil forms part of the slurry. Therefore, screw press yield will always be lower than the actual seed oil yield.

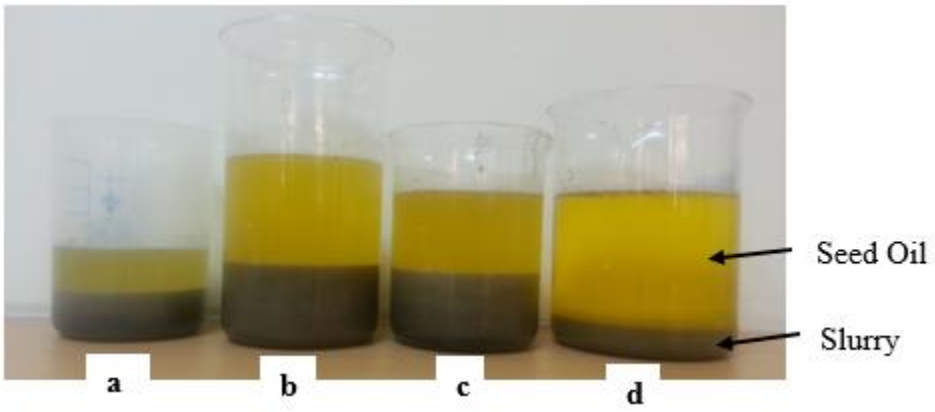

Figure A-1. Jatropha seed oil after mechanical screw press, the slurry settles at the bottom while the oil remains at the top. (a) Green yellow, (b) Yellow, (c) Yellow brown, (d) Brown Dry.

Table A-3. Percentage weight fraction of seed oil, slurry and seed cake after mechanical screw press.

	Green Yellow	Yellow	Yellow Brown	Brown Dry
Oil Yield (%)	18.39	26.81	22.60	18.64
Slurry (%)	15.53	6.26	4.22	6.60
Seed Cake (%)	66.80	66.93	73.18	74.76

A.3. Free Fatty Acids Content and Acid Value of Jatropha Seed Oil at Different Fruit Maturity Stages

The amount of free fatty acids and acid value in Jatropha seed oil at different fruit maturity stages was analysed as described in Section 3.6. Results obtained from four different geographical locations namely, Mmadinare, Thamaga, Maun and Shashe are presented in Table A-4 and Table A-5, respectively. The relationship between free fatty acid content (%) and acid value is indicated in Section 3.6.

Table A-4. Free fatty acids content in Jatropha Seed oil at different fruit maturity stages of Jatropha harvested in Mmadinare, Thamaga, Maun and Shashe areas.

Location	**Free Fatty Acids Content (%)**			
	Green Yellow	Yellow	Yellow Brown	Brown Dry
Mmadinare	0.240	0.259	0.440	0.746
Thamaga	0.210	0.208	0.309	0.444
Maun	0.216	0.217	0.319	0.462
Shashe	0.274	0.279	0.413	0.502
	Standard Error (±)			
Mmadinare	0.001	0.000	0.009	0.015
Thamaga	0.002	0.002	0.003	0.039
Maun	0.001	0.002	0.000	0.039
Shashe	0.005	0.003	0.002	0.004

Table A-5. Acid Values of Jatropha Seed oil at different fruit maturity stages of Jatropha harvested in Mmadinare, Thamaga, Maun and Shashe areas.

Location	**Acid Value (mg KOH/g oil)**			
	Green Yellow	Yellow	Yellow Brown	Brown Dry
Mmadinare	0.476	0.514	0.873	1.481
Thamaga	0.417	0.413	0.613	0.881
Maun	0.428	0.430	0.634	0.918
Shashe	0.544	0.554	0.820	0.997
	Standard Error (±)			
Mmadinare	0.003	0.000	0.019	0.029
Thamaga	0.003	0.004	0.006	0.077
Maun	0.003	0.005	0.001	0.077
Shashe	0.009	0.007	0.005	0.008

A.4. Fatty Acid Composition of Jatropha Seed Oil at Different Fruit Maturity Stages

The fatty acid composition of Jatropha seed oil at different fruit maturity stages was analysed as described in Section 3.7. Results are presented in *Table A-6*, Table A-7, Table A-8 and Table A-9 for Mmadinare, Thamaga, Maun and Shashe areas, respectively. Results for other geographical locations are presented in Table A-10 and A-11.

Table A-6. Fatty acid profile of Jatropha seed oil at different maturity stages harvested in Mmadinare area.

Fatty Acid	**Formula**	**Structure**	**Composition (%)**			
			Green Yellow	**Yellow**	**Yellow Brown**	**Brown Dry**
14,17-Octadecadienoic acid **Linoleic acid**	$C_{19}H_{34}O_2$	C19:2	49.510	55.689	55.063	48.380
9-Octadecenoic acid **Oleic acid**	$C_{19}H_{36}O_2$	C19:1	29.350	21.503	23.191	29.054
Hexadecanoic acid **Palmitic acid**	$C_{17}H_{34}O_2$	C17:0	16.178	15.851	15.370	15.887
Octadecanoic acid **Stearic acid**	$C_{19}H_{38}O_2$	C19:0	4.962	6.176	6.376	6.680
11-Hexadecenoic acid	$C_{17}H_{32}O_2$	C17:1	-	-	-	-
10-Octadecenoic acid	$C_{19}H_{36}O_2$	C19:1	-	-	-	-
Total Composition (%)						

Table A-7. Fatty acid profile of Jatropha seed oil at different maturity stages harvested in Thamaga area.

Fatty Acid	**Formula**	**Structure**	**Composition (%)**			
			Green Yellow	**Yellow**	**Yellow Brown**	**Brown Dry**
14,17-Octadecadienoic acid **Linoleic acid**	$C_{19}H_{34}O_2$	C19:2	39.766	39.231	38.198	34.789
9-Octadecenoic acid **Oleic acid**	$C_{19}H_{36}O_2$	C19:1	35.323	35.992	37.090	41.079
Hexadecanoic acid **Palmitic acid**	$C_{17}H_{34}O_2$	C17:0	16.003	16.098	15.617	15.029
Octadecanoic acid **Stearic acid**	$C_{19}H_{38}O_2$	C19:0	5.892	5.938	6.284	6.897
11-Hexadecenoic acid	$C_{17}H_{32}O_2$	C17:1	1.251	1.131	1.164	0.925
10-Octadecenoic acid	$C_{19}H_{36}O_2$	C19:1	1.765	1.610	1.650	1.282
Total Composition (%)						

Table A-8. Fatty acid profile of Jatropha seed oil at different maturity stages harvested in Maun area.

Fatty Acid	**Formula**	**Structure**	**Composition (%)**			
			Green Yellow	**Yellow**	**Yellow Brown**	**Brown Dry**
14,17-Octadecadienoic acid **Linoleic acid**	$C_{19}H_{34}O_2$	C19:2	33.111	33.100	33.059	33.072
9-Octadecenoic acid **Oleic acid**	$C_{19}H_{36}O_2$	C19:1	40.196	40.912	41.025	38.945
Hexadecanoic acid **Palmitic acid**	$C_{17}H_{34}O_2$	C17:0	14.376	14.391	14.895	15.289
Octadecanoic acid **Stearic acid**	$C_{19}H_{38}O_2$	C19:0	8.296	8.334	8.348	9.207
11-Hexadecenoic acid	$C_{17}H_{32}O_2$	C17:1	1.037	0.991	1.211	1.504
10-Octadecenoic acid	$C_{19}H_{36}O_2$	C19:1	1.591	1.410	1.463	1.982
Total Composition (%)						

Table A-9. Fatty acid profile of Jatropha seed oil at different maturity stages harvested in Shashe area.

Fatty Acid	**Formula**	**Structure**	**Composition (%)**			
			Green Yellow	**Yellow**	**Yellow Brown**	**Brown Dry**
14,17-Octadecadienoic acid **Linoleic acid**	$C_{19}H_{34}O_2$	C19:2	35.212	34.991	34.211	32.987
9-Octadecenoic acid **Oleic acid**	$C_{19}H_{36}O_2$	C19:1	38.786	38.771	38.111	37.999
Hexadecanoic acid **Palmitic acid**	$C_{17}H_{34}O_2$	C17:0	13.322	13.445	13.55	13.41
Octadecanoic acid **Stearic acid**	$C_{19}H_{38}O_2$	C19:0	9.113	9.100	9.001	9.207
11-Hexadecenoic acid	$C_{17}H_{32}O_2$	C17:1	1.907	1.002	1.221	1.500
10-Octadecenoic acid	$C_{19}H_{36}O_2$	C19:1	1.201	0.998	1.473	1.980
Total Composition (%)						

Table A-10. Fatty acid profile of Jatropha seed oil from different fruit maturity stages (yellow and brown dry) harvested in Tutume area, Botswana.

Fatty Acid	**Formula**	**Structure**	**Composition (%)**			
			Green Yellow	**Yellow**	**Yellow Brown**	**Brown Dry**
14,17-Octadecadienoic acid **Linoleic acid**	$C_{19}H_{34}O_2$	C19:2		41.185		65.487
9-Octadecenoic acid **Oleic acid**	$C_{19}H_{36}O_2$	C19:1		37.745		*
Hexadecanoic acid **Palmitic acid**	$C_{17}H_{34}O_2$	C17:0		14.447		21.269
Octadecanoic acid **Stearic acid**	$C_{19}H_{38}O_2$	C19:0		6.623		10.022
11-Hexadecenoic acid	$C_{17}H_{32}O_2$	C17:1	-	-	-	-
10-Octadecenoic acid	$C_{19}H_{36}O_2$	C19:1	-	-	-	-
Total Composition (%)						

Table A-11. Fatty acid profile of Jatropha seed oil from brown dry fruits harvested in Sebele area, Botswana.

Fatty Acid	**Formula**	**Structure**	**Composition (%)**			
			Green Yellow	**Yellow**	**Yellow Brown**	**Brown Dry**
14,17-Octadecadienoic acid **Linoleic acid**	$C_{19}H_{34}O_2$	C19:2				34.815
9-Octadecenoic acid **Oleic acid**	$C_{19}H_{36}O_2$	C19:1				40.818
Hexadecanoic acid **Palmitic acid**	$C_{17}H_{34}O_2$	C17:0				14.603
Octadecanoic acid **Stearic acid**	$C_{19}H_{38}O_2$	C19:0				7.447
11-Hexadecenoic acid	$C_{17}H_{32}O_2$	C17:1				0.925
10-Octadecenoic acid	$C_{19}H_{36}O_2$	C19:1				1.393
Total Composition (%)						

A.5. Peroxide Value of Jatropha Seed Oil and Derived Biodiesel

Jatropha seed oil and derived biodiesel from four different fruit maturity stages were analysed for peroxide value as described in Section 3.16. Results are presented in Table A-12.

Table A-12. Peroxide Values of Jatropha seed oil and derived biodiesel at different fruit maturity stages from various geographical locations in Botswana.

Location	**Peroxide Value (mEq/Kg Oil)**							
	Green Yellow		Yellow		Yellow Brown		Brown Dry	
	Seed Oil	Biodiesel	Seed Oil	Biodiesel	Seed Oil	Biodiesel	Seed Oil	Biodiesel
Mmadinare	1.698	2.217	2.274	3.650	2.697	3.872	3.347	4.508
Thamaga	1.776	2.199	2.559	3.850	2.948	4.372	3.694	4.746
Maun	1.255	2.565	1.872	3.155	1.886	3.639	2.575	4.562
Shashe	1.741	2.648	2.360	2.957	2.575	3.683	3.427	4.644
	Standard Error (±)							
Mmadinare	0.098	0.124	0.115	0.023	0.022	0.113	0.073	0.117
Thamaga	0.082	0.218	0.126	0.047	0.218	0.347	0.126	0.089
Maun	0.188	0.118	0.154	0.248	0.258	0.084	0.066	0.118
Shashe	0.078	0.124	0.055	0.230	0.101	0.044	0.067	0.174

A.6. Kinematic Viscosity of Jatropha Seed Oil and Derived Biodiesel at Different Fruit Maturity Stages

The determination of kinematic viscosities of Jatropha seed oil and derived biodiesel from four different fruit maturity stages were performed as described in Section 3.9. The kinematic viscosities recorded at 40^{o}C are shown in Table A-13. The results for Shashe do not form part of the results presented in Table A-13 because there was not enough seed oil from the area to perform kinematic viscosity tests.

Table A-13. Kinematic viscosities of Jatropha seed oil and derived biodiesel at different fruit maturity stages from various geographical locations in Botswana, measured at 40°C.

Location	**Kinematic Viscosity at 40°C (mm^2/s)**							
	Green Yellow		Yellow		Yellow Brown		Brown Dry	
	Seed Oil	Biodiesel	Seed Oil	Biodiesel	Seed Oil	Biodiesel	Seed Oil	Biodiesel
Mmadinare	9.01	2.33	9.00	2.35	8.93	2.38	9.07	2.34
Thamaga	9.07	2.34	9.06	2.36	8.925	2.4	9.06	2.28
Maun	8.95	2.31	8.94	2.34	8.88	2.35	8.82	2.39
	Standard Error (±)							
Mmadinare	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006
Thamaga	0.006	0.006	0.006	0.006	0.012	0.006	0.012	0.006
Maun	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.006

A.7. Moisture Content in Jatropha Seed Oil and Derived Biodiesel at Different Fruit Maturity Stages

Jatropha seed oil and biodiesel from four different maturity stages were analysed for moisture content as described in Section 3.3 and Section 3.13. The results are presented in Table A-14.

Table A-14. Moisture content in Jatropha seed oil and derived biodiesel from four different maturity stages.

Location	**Moisture Content (%)**							
	Green Yellow		Yellow		Yellow Brown		Brown Dry	
	Seed Oil	Biodiesel	Seed Oil	Biodiesel	Seed Oil	Biodiesel	Seed Oil	Biodiesel
Mmadinare	0.293	0.037	0.307	0.034	0.283	0.036	0.287	0.033
Thamaga	0.303	0.041	0.290	0.038	0.253	0.042	0.267	0.040
Maun	0.283	0.040	0.237	0.037	0.240	0.040	0.257	0.049
Shashe	0.290	0.05	0.280	0.044	0.243	0.050	0.263	0.046
	Standard Error (±)							
Mmadinare	0.009	0.002	0.009	0.003	0.009	0.002	0.015	0.003
Thamaga	0.019	0.002	0.012	0.002	0.012	0.003	0.012	0.001
Maun	0.003	0.001	0.009	0.001	0.012	0.001	0.003	0.001
Shashe	0.006	0.001	0.006	0.001	0.009	0.002	0.009	0.001

A.8. Density of Jatropha Seed Oil and Derived Biodiesel at Different Fruit Maturity Stages

The densities of both Jatropha seed oil and derived biodiesel at four different fruit maturity stages harvested from selected geographical locations in Botswana are presented in Table A-15.

Table A-15. Density of seed oil and biodiesel from Jatropha seeds harvested at different maturity stages from various locations in Botswana.

Location	**Density (g/cm^3)**							
	Green Yellow		Yellow		Yellow Brown		Brown Dry	
	Seed Oil	Biodiesel	Seed Oil	Biodiesel	Seed Oil	Biodiesel	Seed Oil	Biodiesel
Mmadinare	0.9139	0.8720	0.9138	0.8719	0.9140	0.8719	0.9140	0.8730
Thamaga	0.9138	0.8725	0.9133	0.8726	0.9135	0.8741	0.9126	0.8711
Maun	0.9144	0.8735	0.9140	0.8735	0.9141	0.8740	0.9142	0.8739
Shashe	0.9136	0.8735	0.9134	0.8736	0.9133	0.874	0.9125	0.8741
	Standard Error (±)							
Mmadinare	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Thamaga	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Maun	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Shashe	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

A.9. Calorific Values of Jatropha Seed Oil and Derived Biodiesel at Different Fruit Maturity Stages

Jatropha seed oil and derived biodiesel from four different fruit maturity stages were analysed for energy content/calorific value as described in Section 3.8. Results are presented in Table A-16.

Table A-16. Calorific value of seed oil and biodiesel derived from four different fruit maturity stages.

Location	Calorific Value (MJ/Kg)							
	Green Yellow		Yellow		Yellow Brown		Brown Dry	
	Seed Oil	Biodiesel	Seed Oil	Biodiesel	Seed Oil	Biodiesel	Seed Oil	Biodiesel
Mmadinare	38.471	39.924	38.537	39.865	38.238	39.760	38.362	39.814
Thamaga	38.702	39.374	38.707	39.333	38.690	39.310	38.699	39.282
Maun	38.707	39.842	38.721	39.832	38.597	39.800	38.592	39.796
Shashe								
	Standard Error (±)							
Mmadinare	0.077	0.093	0.052	0.047	0.158	0.126	0.105	0.033
Thamaga	0.062	0.044	0.084	0.015	0.046	0.010	0.019	0.022
Maun	0.081	0.075	0.096	0.108	0.011	0.107	0.039	0.139
Shashe								

A.10. Cloud Point and Pour Point of Jatropha Seed Oil and Derived Biodiesel at Different Fruit Maturity Stages

Jatropha seed oil and derived biodiesel from four different fruit maturity stages were analysed for both cloud and pour points as described in Section 3.12. Results from this analysis are presented in Table A-17 and Table A-18.

Table A-17. Cloud point of Jatropha seed oil and derived biodiesel at different fruit maturity stages from various geographical locations in Botswana.

Location	Cloud Point (ºC)							
	Green Yellow		Yellow		Yellow Brown		Brown Dry	
	Seed Oil	Biodiesel	Seed Oil	Biodiesel	Seed Oil	Biodiesel	Seed Oil	Biodiesel
Mmadinare	-4	2	-4	2	-4	2	-4	2
Thamaga	-4	2	-4	2	-4	2	-4	2
Maun	-4	2	-4	2	-4	2	-4	2
Shashe								

Table A-18. Pour Point of Jatropha seed oil and derived biodiesel at different fruit maturity stages from various geographical locations in Botswana.

Location	Pour Point (°C)							
	Green Yellow		Yellow		Yellow Brown		Brown Dry	
	Seed Oil	Biodiesel	Seed Oil	Biodiesel	Seed Oil	Biodiesel	Seed Oil	Biodiesel
Mmadinare	-5	1	-5	1	-5	1	-5	1
Thamaga	-5	1	-5	1	-5	1	-5	1
Maun	-5	1	-5	1	-5	1	-5	1
Shashe								

A.11. Flash Point of Jatropha Seed Oil and Derived Biodiesel at Different Fruit Maturity Stages

Flash point of both Jatropha seed oil and derived biodiesel from four different fruit maturity stages, harvested from selected geographical locations in Botswana was determined as described in Section 3.14. Results are presented in Table A-19.

Table A-19. Flash Point of Jatropha seed oil and derived biodiesel at different fruit maturity stages from various geographical locations in Botswana.

Location	Flash Point (°C)							
	Green Yellow		Yellow		Yellow Brown		Brown Dry	
	Seed Oil	Biodiesel	Seed Oil	Biodiesel	Seed Oil	Biodiesel	Seed Oil	Biodiesel
Mmadinare	215.00	138.00	212.00	141.00	213.00	140.00	216.00	142.00
Thamaga	218.00	140.00	220.00	139.00	217.00	139.00	219.00	140.00
Maun	219.00	138.00	218.00	137.00	215.00	138.00	216.00	141.00
Shashe							218.00	139.00
	Standard Error (±)							
Mmadinare	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58
Thamaga	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58
Maun	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58
Shashe							0.58	0.58

A.12. Cetane Number of Jatropha Biodiesel Derived from Different Fruit Maturity Stages

The cetane number of biodiesel derived from Jatropha seed oil from four different fruit maturity stages, harvested from selected geographical locations in Botswana was determined as described in Section 3.11. Results of the biodiesel cetane number are presented in Table A-20.

Table A-20.Cetane number of Jatropha biodiesel derived from different fruit maturity stages from various geographical locations in Botswana.

Location	**Cetane Number**			
	Green Yellow	Yellow	Yellow Brown	Brown Dry
Mmadinare	48.840	49.128	49.795	50.304
Thamaga	51.808	51.944	52.132	52.839
Maun	53.427	53.753	53.993	54.012
Shashe	53.073	53.771	54.280	54.748
	Standard Error (±)			
Mmadinare	0.006	0.006	0.006	0.006
Thamaga	0.006	0.006	0.006	0.007
Maun	0.006	0.006	0.006	0.032
Shashe	0.006	0.234	0.006	0.006

A.13. Iodine Value and Saponification Number of Jatropha Seed Oil and Derived Biodiesel at Different Fruit Maturity Stages

Table A-21. Iodine values of Jatropha biodiesel derived from four different maturity stages.

Location	**Iodine Value (g I/100g sample)**			
	Green Yellow	Yellow	Yellow Brown	Brown Dry
Mmadinare	114.855	114.489	110.552	108.349
Thamaga	101.556	100.960	100.184	97.179
Maun	94.574	94.294	93.898	92.056
Shashe	96.804	95.380	94.065	92.579

Table A-22. Saponification values of Jatropha seed oil derived from four different maturity stages.

Location	**Saponification Number (mg/g)**			
	Green Yellow	Yellow	Yellow Brown	Brown Dry
Mmadinare	192.299	190.922	192.392	192.304
Thamaga	192.467	192.456	192.363	192.153
Maun	192.144	190.380	189.384	192.015
Shashe	191.145	188.655	187.271	186.420

A.14. Phorbol Ester Content in Jatropha Seed Oil and Seed Cake at Different Fruit Maturity Stages

Phorbol ester (PE) content in Jatropha seed oil and seed cake from four different fruit maturity stages, harvested from selected geographical locations in Botswana was determined as described in Section 3.18. Results of phorbol ester content in Jatropha seed oil and seed cake are presented in Table A-23. The chromatograms of pure Phorbol-12-myristate 13-acetate (PMA), Jatropha seed oil and seed cake are shown in Figure A-2. The retention time of the PE is 5.7 minutes.

Table A-23. Phorbol ester content in Jatropha seed oil and seed cake from four different fruit maturity stages, harvested from selected geographical locations in Botswana.

Location	**Phorbol ester content (mg/g)**							
	Green Yellow		Yellow		Yellow Brown		Brown Dry	
	Seed Oil	Seed Cake	Seed Oil	Seed Cake	Seed Oil	Seed Cake	Seed Oil	Seed Cake
Thamaga	3.574	1.720	3.902	1.801	4.235	1.800	3.814	1.702
Maun	3.469	1.700	3.877	1.758	4.112	1.991	3.913	1.800
Shashe	3.422	1.710	4.074	1.780	4.066	1.896	3.764	1.751
	Standard Error (±)							
Thamaga	0.030	0.006	0.009	0.009	0.059	0.058	0.058	0.058
Maun	0.030	0.006	0.015	0.008	0.058	0.058	0.058	0.058
Shashe	0.020	0.006	0.048	0.030	0.062	0.055	0.061	0.052

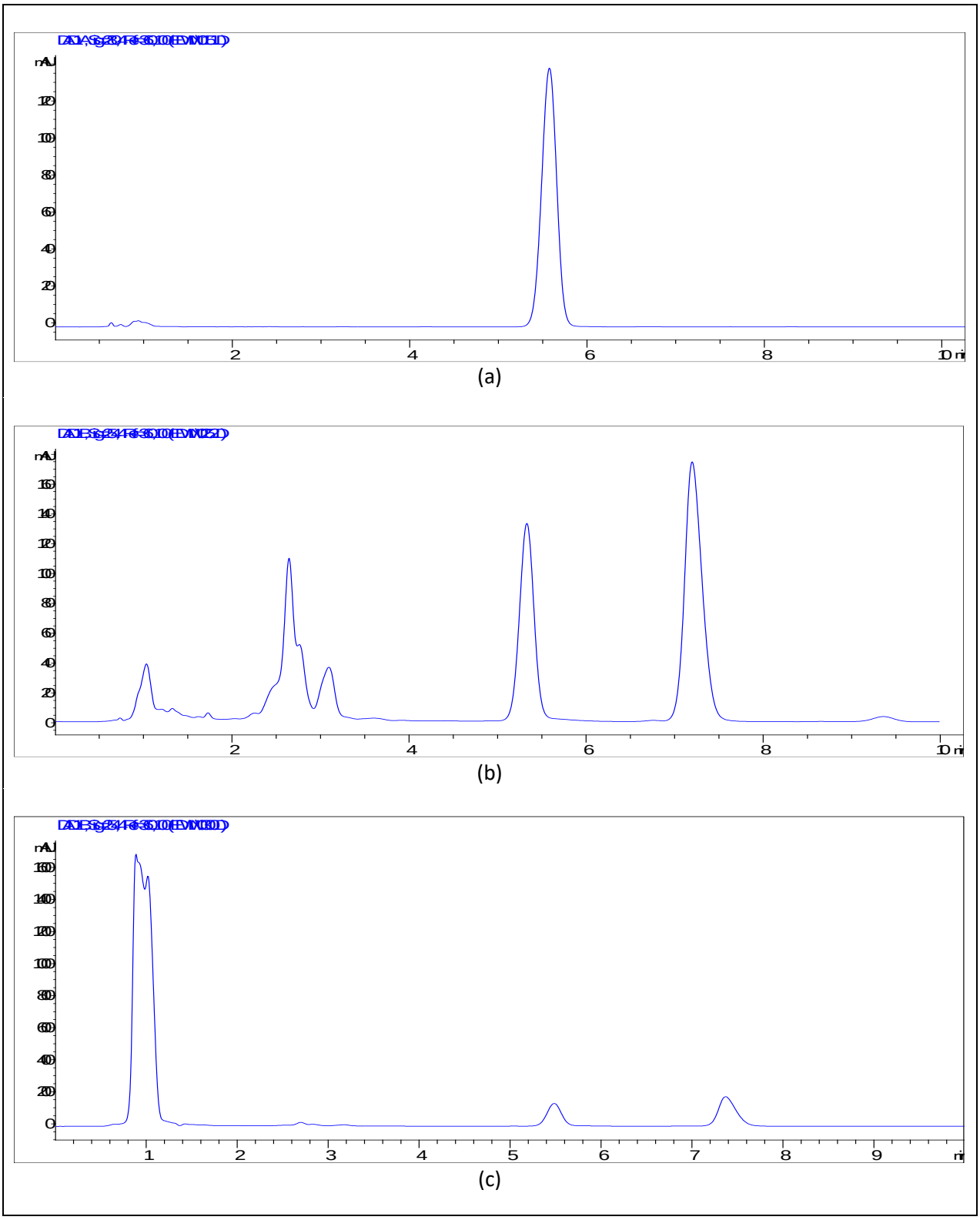

Figure A-2. Chromatograms of (a) pure Phorbol-12-myristate 13-acetate (PMA), (b) Jatropha seed oil and (c) Jatropha seed cake.

A.15. Statistical Analysis (ANOVA)

Analysis of variance (ANOVA) on influence of fruit maturity on Jatropha seed oil content and quality parameters of both seed oil and derived biodiesel was performed as described in Section 3.20. The obtained results of F-value and significance level (p-value) are presented in Table A-24 and Table A-25 for seed oil and derived biodiesel, respectively. The degrees of freedom within samples is 8, and the degrees of freedom between samples is 3. According to the data in Table E-1, Appendix E, the critical F-value is 4.07. F-values greater than 4.07 shows that the variation is statistically significant. On the other hand, significance values equal to or less than 0.05 shows that variation is statistically significant. Significance values greater than 0.05 shows that variation is not statistically significant.

Table A-24. Analysis of variance (ANOVA) on influence of fruit maturity on Jatropha seed oil content and quality parameters.

Parameter	Location	Mean Square		F Test	Significance
		Between groups	Within groups		
Oil Content	Mmadinare	4.148	1.462	2.837	0.006
	Thamaga	8.262	1.677	4.926	0.032
	Maun	4.280	0.297	14.389	0.001
	Shashe	1.475	0.106	13.847	0.002
Density	Mmadinare	0.000	0.000	2.750	0.112
	Thamaga	0.000	0.000	78.000	0.000
	Maun	0.000	0.000	11.000	0.003
	Shashe	0.000	0.000	70.000	0.000
Free fatty acids	Mmadinare	0.170	0.000	731.150	0.000
	Thamaga	0.032	0.001	28.596	0.000
	Maun	0.047	0.001	41.187	0.000
	Shashe	0.036	0.000	921.401	0.000
Peroxide value	Mmadinare	1.450	0.022	67.139	0.000
	Thamaga	1.916	0.065	29.692	0.000
	Maun	0.873	0.098	8.944	0.006
	Shashe	1.458	0.018	81.605	0.000
Viscosity	Mmadinare	0.010	0.000	98.750	0.000
	Thamaga	0.013	0.000	53.600	0.000
	Maun	0.011	0.000	108.750	0.000
	Shashe				
Calorific value	Mmadinare	70508.306	33440.167	2.108	0.177
	Thamaga	146.444	10164.417	0.014	0.997
	Maun	14419.889	13.133	1.098	0.405
	Shashe				
Moisture content	Mmadinare	0.000	0.000	0.958	0.458
	Thamaga	0.002	0.001	2.647	0.120
	Maun	0.001	0.000	7.794	0.009
	Shashe	0.001	0.000	7.517	0.010
Flash Point	Mmadinare	10.000	1.000	10.000	0.004
	Thamaga	5.000	1.000	5.000	0.031
	Maun	10.000	1.000	10.000	0.004
	Shashe				

Table A-25. Analysis of variance (ANOVA) on influence of fruit maturity on Jatropha derived biodiesel quality parameters.

Parameter	Location	Mean Square		F Test	Significance
		Between groups	Within groups		
Cetane Number	Mmadinare	1.304	0.000	11994.578	0.000
	Thamaga	0.628	0.000	5525.098	0.000
	Maun	0.238	0.001	287.972	0.000
	Shashe	1.887	0.041	45.995	0.000
Density	Mmadinare	0.000	0.000	2.750	0.112
	Thamaga	0.000	0.000	450.750	0.000
	Maun	0.000	0.000	20.750	0.000
	Shashe	0.000	0.000	26.000	0.000
Peroxide value	Mmadinare	2.807	0.032	88.788	0.000
	Thamaga	3.787	0.133	28.373	0.000
	Maun	2.140	0.072	29.594	0.000
	Shashe	2.363	0.075	31.366	0.000
Viscosity	Mmadinare	0.001	0.000	14.000	0.002
	Thamaga	0.008	0.000	75.000	0.000
	Maun	0.003	0.000	32.750	0.000
	Shashe				
Calorific value	Mmadinare	14842.889	21088.750	0.704	0.576
	Thamaga	4566.306	2024.917	2.225	0.159
	Maun	1581.111	35977.667	0.44	0.987
	Shashe				
Moisture content	Mmadinare	0.000	0.000	0.600	0.633
	Thamaga	0.000	0.000	0.583	0.643
	Maun	0.000	0.000	26.769	0.000
	Shashe	0.000	0.000	9.018	.006
Flash Point	Mmadinare	8.750	1.000	8.750	0.007
	Thamaga	1.000	1.000	1.000	0.441
	Maun	9.000	1.000	9.000	0.006
	Shashe				

Table A-26. Analysis of variance (ANOVA) on influence of fruit maturity on phorbol ester content in Jatropha seed oil and seed cake from three different geographical locations.

Parameter	Location	Mean Square		F Test	Significance
		Between groups	Within groups		
Phorbol ester content (Seed Oil)	Thamaga	0.247	0.006	42.317	0.000
	Maun	0.225	0.006	38.395	0.000
	Shashe	0.208	0.008	26.994	0.000
Phorbol ester content (Seed cake)	Thamaga	0.008	0.005	15.660	0.027
	Maun	0.052	0.005	10.189	0.004
	Shashe	0.019	0.005	38.120	0.006

Table A-27. Analysis of variance (ANOVA) on influence of geographical location (Thamaga, Maun and Shashe) on phorbol ester content in Jatropha seed oil and seed cake at four different fruit maturity stages.

Parameter	Maturity Stage	Mean Square		F Test	Significance
		Between groups	Within groups		
Phorbol ester content (Seed Oil)	Green Yellow	0.007	0.013	1.648	0.269
	Yellow	0.014	0.016	2.591	0.155
	Yellow Brown	0.054	0.064	2.552	0.158
	Brown dry	0.050	0.063	2.405	0.171
Phorbol ester content (Seed cake)	Green Yellow	0.000	0.000	3.000	0.125
	Yellow	0.002	0.001	2.150	0.198
	Yellow Brown	0.029	0.010	3.007	0.125
	Brown dry	0.007	0.009	0.697	0.534

Appendix B

B. Molecular masses of fatty acid methyl esters found in Jatropha seed oil

Table B-1. Molecular masses of fatty acid methyl esters found in Jatropha seed oil and derived biodiesel

Fatty Acid	Formula	Structure	Molecular Mass
Linoleic acid (14,17-Octadecadienoic acid)	$C_{19}H_{34}O_2$	C19:2	294.479
Oleic acid (9-Octadecenoic acid)	$C_{19}H_{36}O_2$	C19:1	296.495
Palmitic acid (Hexadecanoic acid)	$C_{17}H_{34}O_2$	C17:0	270.457
Stearic acid (Octadecanoic acid)	$C_{19}H_{38}O_2$	C19:0	298.511
11-Hexadecenoic acid	$C_{17}H_{32}O_2$	C17:1	268.411
10-Octadecenoic acid	$C_{19}H_{36}O_2$	C19:1	296.495

Appendix C

Gas Chromatography Data

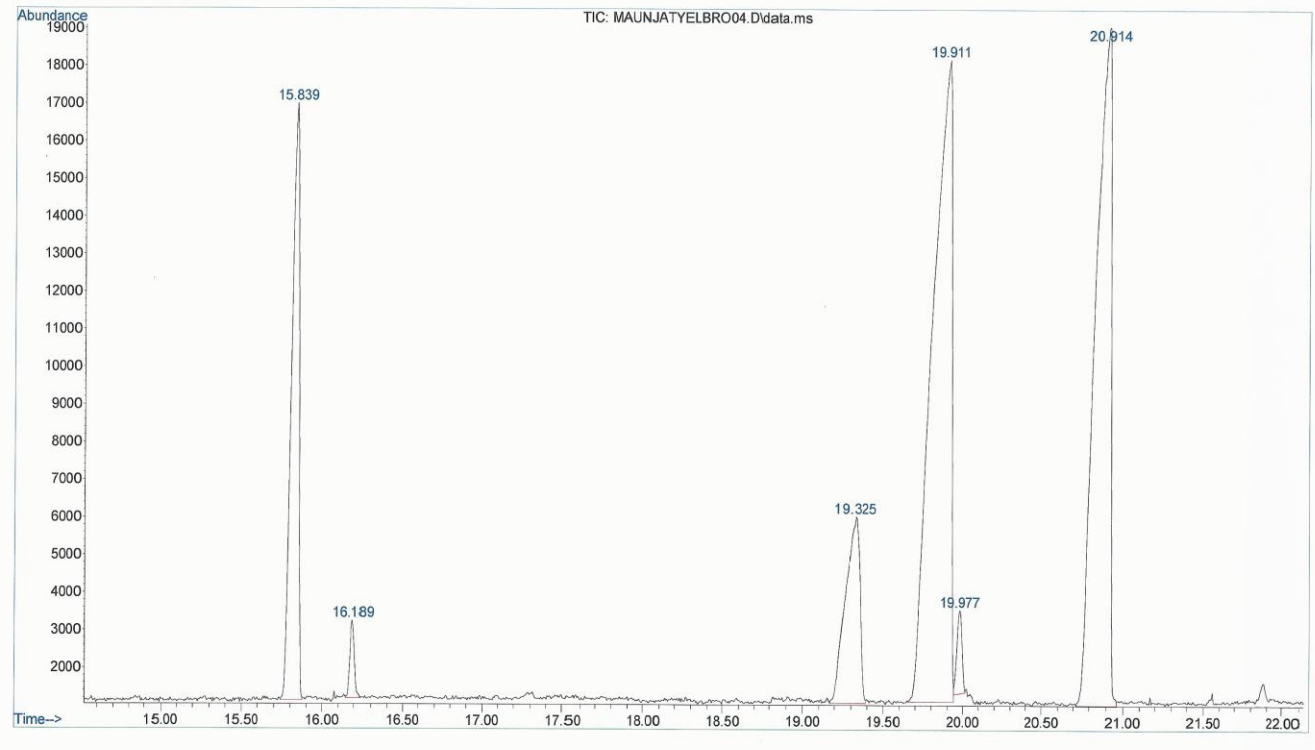

Figure C-1. Chromatogram of Jatropha biodiesel (FAMEs) analysed using Agilent Technologies GC System 7890A gas chromatograph.

Integration Parameters: rteint.p
Integrator: RTE
Smoothing : OFF Filtering: 5
Sampling : 1 Min Area: 2.5 % of largest Peak
Start Thrs: 0.2 Max Peaks: 15
Stop Thrs : 0 Peak Location: TOP

If leading or trailing edge < 100 prefer < Baseline drop else tangent >
Peak separation: 5

Method : C:\ENVDEMO\GCMETHOD\PESTPCB.M
Title : 91CLPPEST / OLM01.8

Signal : TIC: MAUNJATYELBRO04.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total	
1	15.839	1928	1945	1951	rBV	15856	51899	36.31%	14.895%	Palmitic
2	16.189	1996	2003	2011	rVB3	2059	4218	2.95%	1.211%	11-Hexadecenoic
3	19.325	2496	2522	2533	rBV	4951	29087	20.35%	8.348%	Stearic
4	19.911	2577	2619	2624	rBV2	17059	142950	100.00%	41.025%	Oleic
5	19.977	2624	2630	2636	rVB3	2187	5099	3.57%	1.463%	10-octadecenoic
6	20.914	2753	2785	2793	rBV2	18035	115191	80.58%	33.059%	Linoleic

Sum of corrected areas: 348444

Figure C-2. Results from GC-MS showing fractional composition of fatty acids found in Jatropha seed oil.

Appendix D

Statistical Data and Results

Table D-1. F-Critical values at 0.05 significance level (Anon., 2019).

		Degrees of freedom between samples								
		1	2	3	4	5	6	7	8	9
Degrees of freedom within samples	1	161.4476	199.5000	215.7073	224.5832	230.1619	233.9860	236.7684	238.8827	240.5433
	2	18.5128	19.0000	19.1643	19.2468	19.2964	19.3295	19.3532	19.3710	19.3848
	3	10.1280	9.5521	9.2766	9.1172	9.0135	8.9406	8.8867	8.8452	8.8123
	4	7.7086	6.9443	6.5914	6.3882	6.2561	6.1631	6.0942	6.0410	5.9988
	5	6.6079	5.7861	5.4095	5.1922	5.0503	4.9503	4.8759	4.8183	4.7725
	6	5.9874	5.1433	4.7571	4.5337	4.3874	4.2839	4.2067	4.1468	4.0990
	7	5.5914	4.7374	4.3468	4.1203	3.9715	3.8660	3.7870	3.7257	3.6767
	8	5.3177	4.4590	4.0662	3.8379	3.6875	3.5806	3.5005	3.4381	3.3881
	9	5.1174	4.2565	3.8625	3.6331	3.4817	3.3738	3.2927	3.2296	3.1789
	10	4.9646	4.1028	3.7083	3.4780	3.3258	3.2172	3.1355	3.0717	3.0204
	11	4.8443	3.9823	3.5874	3.3567	3.2039	3.0946	3.0123	2.9480	2.8962
	12	4.7472	3.8853	3.4903	3.2592	3.1059	2.9961	2.9134	2.8486	2.7964
	13	4.6672	3.8056	3.4105	3.1791	3.0254	2.9153	2.8321	2.7669	2.7144
	14	4.6001	3.7389	3.3439	3.1122	2.9582	2.8477	2.7642	2.6987	2.6458
	15	4.5431	3.6823	3.2874	3.0556	2.9013	2.7905	2.7066	2.6408	2.5876
	16	4.4940	3.6337	3.2389	3.0069	2.8524	2.7413	2.6572	2.5911	2.5377
	17	4.4513	3.5915	3.1968	2.9647	2.8100	2.6987	2.6143	2.5480	2.4943
	18	4.4139	3.5546	3.1599	2.9277	2.7729	2.6613	2.5767	2.5102	2.4563
	19	4.3807	3.5219	3.1274	2.8951	2.7401	2.6283	2.5435	2.4768	2.4227
	20	4.3512	3.4928	3.0984	2.8661	2.7109	2.5990	2.5140	2.4471	2.3928
	21	4.3248	3.4668	3.0725	2.8401	2.6848	2.5727	2.4876	2.4205	2.3660
	22	4.3009	3.4434	3.0491	2.8167	2.6613	2.5491	2.4638	2.3965	2.3419
	23	4.2793	3.4221	3.0280	2.7955	2.6400	2.5277	2.4422	2.3748	2.3201
	24	4.2597	3.4028	3.0088	2.7763	2.6207	2.5082	2.4226	2.3551	2.3002
	25	4.2417	3.3852	2.9912	2.7587	2.6030	2.4904	2.4047	2.3371	2.2821
	26	4.2252	3.3690	2.9752	2.7426	2.5868	2.4741	2.3883	2.3205	2.2655
	27	4.2100	3.3541	2.9604	2.7278	2.5719	2.4591	2.3732	2.3053	1.7306
	28	4.1960	3.3404	2.9467	2.7141	2.5581	2.4453	2.3593	2.2913	2.2360
	29	4.1830	3.3277	2.9340	2.7014	2.5454	2.4324	2.3463	2.2783	2.2229
	30	4.1709	3.3158	2.9223	2.6896	2.5336	2.4205	2.3343	2.2662	2.2107
	40	4.0847	3.2317	2.8387	2.6060	2.4495	2.3359	2.2490	2.1802	2.1240
	60	4.0012	3.1504	2.7581	2.5252	2.3683	2.2541	2.1665	2.0970	2.0401
	120	3.9201	3.0718	2.6802	2.4472	2.2899	2.1750	2.0868	2.0164	1.9588
	∞	3.8415	2.9957	2.6049	2.3719	2.2141	2.0986	2.0096	1.9384	1.8799

Table D-2. Post Hoc Tukey test on influence of geographical location (Mmadinare, Thamaga, Maun and Shashe) on Jatropha seed oil content.

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) Location	(J) Location	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Green Yellow	1	2	.45848998	.25278841	.334	-.3510275	1.2680075
		3	4.46825012*	.25278841	.000	3.6587326	5.2777676
		4	4.42541324*	.25278841	.000	3.6158958	5.2349307
	2	1	-.45848998	.25278841	.334	-1.2680075	.3510275
		3	4.00976014*	.25278841	.000	3.2002427	4.8192776
		4	3.96692327*	.25278841	.000	3.1574058	4.7764407
	3	1	-4.46825012*	.25278841	.000	-5.2777676	-3.6587326
		2	-4.00976014*	.25278841	.000	-4.8192776	-3.2002427
		4	-.04283688	.25278841	.998	-.8523544	.7666806
	4	1	-4.42541324*	.25278841	.000	-5.2349307	-3.6158958
		2	-3.96692327*	.25278841	.000	-4.7764407	-3.1574058
		3	.04283688	.25278841	.998	-.7666806	.8523544
Yellow	1	2	.83137037*	.19359446	.011	.2114128	1.4513280
		3	4.08401294*	.19359446	.000	3.4640553	4.7039705
		4	5.43495767*	.19359446	.000	4.8150001	6.0549153
	2	1	-.83137037*	.19359446	.011	-1.4513280	-.2114128
		3	3.25264257*	.19359446	.000	2.6326850	3.8726002
		4	4.60358730*	.19359446	.000	3.9836297	5.2235449
	3	1	-4.08401294*	.19359446	.000	-4.7039705	-3.4640553
		2	-3.25264257*	.19359446	.000	-3.8726002	-2.6326850
		4	1.35094473*	.19359446	.001	.7309871	1.9709023
	4	1	-5.43495767*	.19359446	.000	-6.0549153	-4.8150001
		2	-4.60358730*	.19359446	.000	-5.2235449	-3.9836297
		3	-1.35094473*	.19359446	.001	-1.9709023	-.7309871
Yellow Brown	1	2	1.15826573	1.42310102	.846	-3.3990047	5.7155361
		3	3.81969227	1.42310102	.104	-.7375781	8.3769627
		4	5.37807638*	1.42310102	.022	.8208060	9.9353468
	2	1	-1.15826573	1.42310102	.846	-5.7155361	3.3990047
		3	2.66142654	1.42310102	.311	-1.8958439	7.2186969
		4	4.21981065	1.42310102	.070	-.3374598	8.7770811
	3	1	-3.81969227	1.42310102	.104	-8.3769627	.7375781
		2	-2.66142654	1.42310102	.311	-7.2186969	1.8958439
		4	1.55838412	1.42310102	.702	-2.9988863	6.1156545
	4	1	-5.37807638*	1.42310102	.022	-9.9353468	-.8208060
		2	-4.21981065	1.42310102	.070	-8.7770811	.3374598
		3	-1.55838412	1.42310102	.702	-6.1156545	2.9988863
Brown Dry	1	2	2.04969131*	.48552496	.012	.4948695	3.6045132
		3	3.87001018*	.48552496	.000	2.3151883	5.4248320
		4	4.28907065*	.48552496	.000	2.7342488	5.8438925
	2	1	-2.04969131*	.48552496	.012	-3.6045132	-.4948695
		3	1.82031886*	.48552496	.023	.2654970	3.3751407
		4	2.23937934*	.48552496	.007	.6845575	3.7942012
	3	1	-3.87001018*	.48552496	.000	-5.4248320	-2.3151883
		2	-1.82031886*	.48552496	.023	-3.3751407	-.2654970
		4	.41906048	.48552496	.823	-1.1357614	1.9738823
	4	1	-4.28907065*	.48552496	.000	-5.8438925	-2.7342488
		2	-2.23937934*	.48552496	.007	-3.7942012	-.6845575
		3	-.41906048	.48552496	.823	-1.9738823	1.1357614

*. The mean difference is significant at the 0.05 level.

1 = Mmadinare, 2 = Thamaga, 3 = Maun and 4 = Shashe

Table D-3. One way analysis of variance on influence of geographical location (Mmadinare, Thamaga, Maun and Shashe) on Jatropha seed oil content.

Parameter	Maturity Stage	Mean Square	F Test	Significance
Oil Content	Green Yellow	Between groups: 17.894	186.682	0.000
		Within groups: 0.096		
	Yellow	Between groups: 20.127	358.010	0.000
		Within groups: 0.056		
	Yellow Brown	Between groups: 18.043	5.940	0.020
		Within groups: 3.038		
	Brown Dry	Between groups: 11.520	32.578	0.000
		Within groups: 0.354		

Appendix E

Maximum and Minimum Temperatures Recorded in ^{o}C (2009 to 2019)

(Source: Department of Meteorological Services, Botswana)

1. Maun

Mean Monthly Maximum temperatures

Year	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	**Annual Aver.**
2009/10	23.4	28.3	33.0	35.1	33.6	33.8	32.5	32.1	30.4	29.3	28.8	25.5	**30.5**
2010/11	25.3	29.2	34.2	37.0	33.4	31.9	30.3	32.4	31.4	30.1	28.3	26.1	**30.8**
2011/12	24.5	28.8	34.2	36.0	35.9	31.7	33.1	31.4	32.7	31.4	30.0	26.2	**31.3**
2012/13	26.3	29.6	33.5	34.4	33.7	32.5	32.2	33.8	34.3	32.3	29.1	27.6	**31.6**
2013/14	26.4	29.0	33.4	35.2	34.1	29.8	31.3	30.4	29.5	28.0	30.1	26.1	**30.3**
2014/15	26.0	29.6	32.7	35.5	32.2	32.1	33.1	34.5	33.1	31.6	29.4	25.7	**31.3**
2015/16	27.0	30.6	33.8	37.2	35.3	35.0	34.3	33.8	33.1	31.0	27.6	26.4	**32.1**
2016/17	25.8	29.0	32.9	34.7	34.1	33.0	28.5	29.2	29.6	28.2	27.1	26.2	**29.9**
2017/18	26.3	28.3	32.9	34.8	33.9	32.4	32.4	28.6	29.4	30.0	28.5	26.4	**30.3**
2018/19	24.4	30.7	33.8	34.3	35.4	35.0	33.3	32.4	35.7	32.0	29.8	26.4	**31.9**
Monthly Average	**25.5**	**29.3**	**33.4**	**35.4**	**34.2**	**32.7**	**32.1**	**31.9**	**31.9**	**30.4**	**28.9**	**26.3**	

Mean Monthly Minimum temperatures

Year	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	**Annual Aver.**
2009/10	7.8	10.6	16.7	20.6	19.7	21.2	21.2	20.7	19.4	17.9	14.2	8.4	**16.5**
2010/11	10.8	11.1	16.5	20.5	20.9	20.6	20.2	20.0	19.8	17.5	13.0	7.2	**16.5**
2011/12	7.4	10.8	15.8	19.8	21.9	20.5	20.7	20.3	18.9	14.9	12.1	9.0	**16.0**
2012/13	7.6	11.2	15.9	20.2	20.9	19.9	20.1	20.0	19.1	14.6	11.6	9.3	**15.9**
2013/14	9.9	12.1	17.3	19.9	21.5	20.0	20.8	20.3	19.7	16.4	13.6	9.6	**16.8**
2014/15	9.1	12.5	16.9	20.4	21.1	21.3	20.8	21.1	20.5	16.8	12.4	9.3	**16.9**
2015/16	12.9	11.8	17.4	21.7	21.5	22.6	22.6	21.4	20.5	16.9	11.9	9.3	**17.5**
2016/17	8.2	12.0	16.9	22.0	22.3	12.0	20.5	20.5	18.3	15.6	11.7	10.1	**15.8**
2017/18	15.4	11.5	15.5	19.9	19.4	20.7	20.2	19.9	19.4	16.1	11.5	8.4	**16.5**
2018/19	9.2	13.2	14.9	18.7	20.6	21.5	19.9	20.1	20.4	17.9	13.2	9.3	**16.6**
Monthly Average	**9.8**	**11.7**	**16.4**	**20.4**	**21.0**	**20.0**	**20.7**	**20.4**	**19.6**	**16.5**	**12.5**	**9.0**	

2. Mmadinare

Mean Monthly Maximum temperatures

Year	July	Aug	Sept	Oct	Nov	Dec	Jan	feb	March	April	May	June	**Annual Av.**
2009/10	20.9	25.2	29.6	31.1	30.5	32.6	32.2	31.3	31.7	26.4	26.0	22.0	**28.3**
2010/11	22.1	25.0	30.2	33.5	31.2	31.8	28.4	29.8	32.6	26.8	26.4	23.8	**28.4**
2011/12	22.0	25.0	30.7	32.0	32.6	29.4	32.3	34.0	32.7	28.4	27.2	24.0	**29.2**
2012/13	24.1	27.3	29.6	30.1	31.6	31.7	29.6	31.4	30.8	27.8	26.2	25.2	**28.8**
2013/14	23.3	26.5	31.0	30.7	34.0	29.8	30.4	28.5	28.5	28.5	26.5	24.6	**28.5**
2014/15	23.8	26.3	29.7	31.3	31.2	30.3	31.6	34.1	31.2	28.0	29.3	23.8	**29.2**
2015/16	25.2	28.4	28.7	33.4	32.8	35.3	33.3	33.5	29.9	29.7	25.6	24.1	**30.0**
2016/17	23.2	26.7	30.0	31.4	31.9	31.6	29.1	28.0	28.2	27.0	26.1	24.9	**28.2**
2017/18	24.5	26.3	30.0	30.5	31.4	32.2	31.7	28.8	29.9	28.8	27.1	25.0	**28.8**
2018/19	21.9	29.6	33.2	31.0	31.7	31.6	32.9	32.0	33.5	29.5	27.3	24.3	**29.9**
Monthly Average	**23.1**	**26.6**	**30.3**	**31.5**	**31.9**	**31.6**	**31.1**	**31.1**	**30.9**	**28.1**	**26.8**	**24.2**	

Mean Monthly Minimum temperatures

Year	July	Aug	Sept	Oct	Nov	Dec	Jan	feb	March	April	May	June	**Annual Av.**
2009/10	5.75	7.47	13.90	18.27	18.53	20.95	21.71	20.98	19.68	17.42	13.53	7.08	**15.44**
2010/11	7.65	8.26	13.75	18.37	20.01	20.01	19.90	18.31	18.83	16.39	17.31	5.87	**15.39**
2011/12	5.50	7.60	12.30	16.90	20.30	19.80	20.40	19.10	19.60	14.40	10.40	7.30	**14.47**
2012/13	6.90	9.60	15.00	18.00	18.70	20.80	20.50	19.40	18.20	14.30	9.80	7.80	**14.92**
2013/14	8.10	10.20	14.80	16.70	20.60	20.40	20.60	18.40	14.50	14.50	9.20	6.20	**14.52**
2014/15	5.70	8.00	12.50	16.00	19.20	19.60	19.10	22.30	18.40	15.30	10.50	7.10	**14.48**
2015/16	7.60	9.30	14.20	17.30	17.40	21.30	20.50	22.30	19.00	15.00	10.10	7.10	**15.09**
2016/17	6.30	8.10	14.20	17.40	19.90	8.10	19.70	19.50	16.30	14.00	9.00	6.80	**13.28**
2017/18	6.90	8.40	13.42	16.40	16.20	18.80	19.93	19.20	18.00	14.50	10.90	6.80	**14.12**
2018/19	7.40	11.30	12.80	13.30	17.70	19.03	20.00	19.70	19.40	16.40	10.00	6.90	**14.49**
Monthly Average	**6.78**	**8.82**	**13.69**	**16.86**	**18.85**	**18.88**	**20.23**	**19.92**	**18.19**	**15.22**	**11.07**	**6.90**	

3. Thamaga

Mean Monthly Maximum temperatures

Year	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	**Annual Av.**
2009/10	19.6	23.8	28.9	29.7	29.6	32.4	30.3	31.3	28.6	24.9	24.3	21.5	**27.1**
2010/11	21.3	24.8	30.4	33.1	31.7	31.6	28.7	30.4	30.7	25.2	23.8	22.3	**27.8**
2011/12	20.9	24.9	30.6	32.0	32.5	30.3	32.2	33.0	31.6	27.5	26.7	22.4	**28.7**
2012/13	23.0	26.1	28.7	30.7	32.9	29.3	31.2	33.2	31.3	27.6	25.5	23.7	**28.6**
2013/14	22.6	25.0	30.6	30.9	34.3	28.9	32.6	30.2	27.1	25.7	26.7	23.3	**28.2**
2014/15	22.2	25.7	30.4	31.6	30.3	32.2	32.7	35.1	31.6	27.9	27.9	21.9	**29.1**
2015/16	22.9	28.0	28.9	35.4	33.4	35.5	33.8	34.7	30.3	28.9	24.2	22.2	**29.9**
2016/17	22.4	25.7	29.5	31.6	31.7	31.8	30.2	28.1	29.5	28.9	25.3	23.0	**28.2**
2017/18	24.5	25.6	29.5	39.1	31.5	31.7	32.0	29.4	28.8	27.4	25.7	23.7	**29.1**
2018/19	21.4	28.2	31.8	30.5	33.4	35.2	34.0	32.0	34.3	27.4	26.4	23.6	**29.9**
Monthly Average	**22.1**	**25.8**	**29.9**	**32.5**	**32.1**	**31.9**	**31.8**	**31.7**	**30.4**	**27.1**	**25.7**	**22.8**	

Mean Monthly Minimum temperatures

Year	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	**Annual Av.**
2009/10	2.9	6.2	12.2	16.7	16.3	18.8	12.8	19.9	18.3	15.4	10.4	4.0	**12.8**
2010/11	5.7	7.0	12.4	17.2	18.7	19.3	19.4	18.6	18.1	14.4	8.4	2.7	**13.5**
2011/12	1.6	4.6	10.5	15.1	17.9	18.3	18.3	20.1	17.1	11.9	7.4	3.8	**12.2**
2012/13	3.9	8.1	11.2	16.5	19.2	18.8	20.1	19.6	18.0	13.4	7.3	5.0	**13.4**
2013/14	6.3	7.7	14.0	16.4	19.7	19.6	20.2	19.7	17.9	11.9	7.5	3.7	**13.7**
2014/15	3.4	5.8	10.0	15.0	17.3	18.0	16.9	17.2	16.2	13.5	6.3	3.7	**11.9**
2015/16	5.7	8.4	14.1	18.6	18.1	22.6	21.6	22.0	18.1	15.6	9.7	6.2	**15.1**
2016/17	4.1	6.8	14.0	18.2	19.8	6.8	19.7	19.6	16.0	15.6	7.3	5.1	**12.8**
2017/18	5.0	7.6	12.1	15.6	15.6	18.8	19.3	19.4	17.1	14.0	7.8	3.5	**13.0**
2018/19	4.4	8.8	11.6	15.0	18.8	21.3	20.7	20.0	19.7	15.2	8.8	4.3	**14.1**
Monthly Average	**4.3**	**7.1**	**12.2**	**16.4**	**18.1**	**18.2**	**18.9**	**19.6**	**17.7**	**14.1**	**8.1**	**4.2**	

4. Shashe

Mean Monthly Maximum temperatures

Year	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	**Annual Av.**
2009/10	21.5	26.2	30.5	32.3	31.1	33.0	32.1	31.3	30.6	27.1	26.9	23.9	**28.9**
2010/11	23.3	25.8	31.8	35.0	32.2	30.8	28.1	29.9	32.3	28.1	27.0	25.2	**29.1**
2011/12	23.0	26.4	31.9	33.5	33.1	29.3	32.7	33.7	32.6	28.2	28.0	24.6	**29.8**
2012/13	24.7	27.9	31.3	31.9	32.6	31.4	29.8	32.4	31.3	29.1	27.0	25.6	**29.6**
2013/14	24.0	27.8	32.3	32.4	33.1	29.5	30.3	29.3	28.2	27.2	28.4	25.5	**29.0**
2014/15	24.6	26.4	31.0	32.8	32.3	30.1	31.2	33.8	31.6	27.8	29.2	25.0	**29.7**
2015/16	26.3	28.7	30.0	35.0	34.5	35.1	33.4	33.6	29.3	28.9	25.7	24.5	**30.4**
2016/17	23.6	26.8	30.3	31.9	31.7	31.0	31.1	30.5	30.0	27.7	26.3	25.1	**28.8**
2017/18	24.2	27.1	30.3	32.4	31.2	32.2	31.1	28.2	29.1	28.2	27.1	24.8	**28.8**
2018/19	21.8	30.4	33.4	31.4	32.9	33.3	32.5	31.3	33.3	30.4	27.8	24.5	**30.3**
Monthly Average	**23.7**	**27.3**	**31.3**	**32.9**	**32.5**	**31.6**	**31.2**	**31.4**	**30.8**	**28.3**	**27.3**	**24.9**	

Mean Monthly Minimum temperatures

Year	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	**Annual Av.**
2009/10	5.4	6.3	12.4	17.4	17.2	19.2	20.0	19.9	18.4	16.2	11.8	5.4	**14.1**
2010/11	8.1	7.8	12.5	17.4	18.7	18.7	18.8	17.6	18.0	15.2	9.1	3.4	**13.8**
2011/12	3.9	7.1	12.2	16.9	19.7	18.8	18.8	19.2	16.7	10.6	7.1	4.3	**12.9**
2012/13	3.7	6.7	13.7	18.2	18.5	19.4	19.0	18.2	17.4	13.0	7.8	6.2	**13.5**
2013/14	6.4	9.2	13.9	16.3	19.4	19.9	19.4	18.6	17.9	25.0	6.9	3.4	**14.7**
2014/15	3.4	5.8	10.0	15.0	17.3	18.0	16.9	17.2	16.2	17.7	6.3	3.7	**12.3**
2015/16	5.6	4.8	13.3	16.0	17.2	19.9	18.4	21.0	17.7	13.2	8.1	4.0	**13.3**
2016/17	4.3	4.9	13.1	17.1	19.3	4.9	18.8	18.5	14.8	19.8	5.2	4.1	**12.1**
2017/18	3.2	6.3	12.6	15.4	14.5	19.3	18.8	19.0	16.6	12.6	8.0	2.6	**12.4**
2018/19	5.1	8.2	11.0	13.0	17.5	18.0	18.3	17.7	16.7	14.4	6.3	3.9	**12.5**
Monthly Average	**4.9**	**6.7**	**12.5**	**16.3**	**17.9**	**17.6**	**18.7**	**18.7**	**17.0**	**15.8**	**7.7**	**4.1**	

Rainfall Recorded in millilitres (2009 to 2019)

(Source: Department of Meteorological Services, Botswana)

1. Maun

Year	jul	aug	sept	oct	nov	dec	jan	feb	march	april	may	jun	Total
2007/08	0	0	0	22.4	71.8	64.8	205.5	81.7	42.7	0	17.5	0	506.4
2008/09	0	0	0	0	28.4	41.2	83	39.1	118.3	0	20.7	57.7	388.4
2009/10	0	0	0	40.3	31.3	109.5	222.7	182	65.4	96.6	1	0	748.8
2010/11	0	0	0	0.2	45.3	84.3	186.2	46.3	130.5	25.1	0	0	517.9
2011/12	0	0	0	0	48.4	140.5	54.6	62.9	15.4	0	0	0	321.8
2012/13	0	0	0	11	28.6	99	207.9	3.1	5.2	0	0	0	354.8
2013/14	0	0	0	7	20.8	142.9	143.4	86.8	156	27.2	0	0	584.1
2014/15	0	0	1.4	1.3	23	78.5	75.9	37.3	53.7	98.5	0	0	369.6
2015/16	0	0	0	4.1	8.8	32.9	77.2	60.6	131.7	26.1	0	0	341.4
2016/17	0	0	0	0	64	150.6	219.7	276.7	33.4	44.4	0	0	788.8
2017/18	0	0	0	5.9	24.4	75.8	56.6	223.3	171.2	4.2	0	0	561.4
2018/19	0	0	0	43.8	12.2	55	181.1	93.5	0	1	0	0	386.6
Average													**489.2**

2. Shashe

Year	jul	aug	sept	oct	nov	dec	jan	feb	march	april	may	jun	Total
2008/09	0	0	0	4.4	34.1	59.7	149	173.2	63.8	0	15.5	65.8	565.5
2009/10	1	0	18.6	16.6	60.5	85.2	66.5	72.4	37.4	98.9	20.4	0.9	478.4
2010/11	0	0	0	32.3	92.3	117.4	198.5	25.8	14.3	60	3.1	0	543.7
2011/12	0	0	0	5.2	115.9	125.3	0	15.8	31.1	0	0	0	293.3
2012/13	0	0	0	29.5	83.3	54.4	208	4.2	5.4	6.6	0	0	391.4
2013/14	0	0	0	24.7	31.8	133.3	193.6	94.4	221.5	17.4	0	0	716.7
2014/15	0.6	0	0	1.5	105.3	176.1	3.4	65.3	38.3	37.6	0	2.7	430.8
2015/16	0	0	40.5	0	48.7	44.8	95	154.2	113.2	0	0	0	496.4
2016/17	0	0	0.2	6.6	2.4	113.1	203.6	227.1	37.1	12.1	0	0	602.2
2017/18	0	0	0	22.3	n/a	n/a	4.3	287	95.3	22	0	0	430.9
2018/19	1.5	0	0.9	0	85	55.5	41.7	92.8	9.2	8.2	0	0	294.8
Average													**476.7**

3. Thamaga

Year	jul	aug	sept	oct	nov	dec	jan	feb	march	april	may	jun	Total
2005/06	0	0	0	0	48.8	86.2	134.1	191.1	160	13.4	5	2.5	641.1
2006/07	0	3.5	0	11.7	51.6	21.5	19.7	1.1	30.6	33.3	0	6.5	179.5
2007/08	0	0	15.5	94.4	108	94.7	195.5	65.5	180.6	12.5	0	0	766.7
2008/09	0	0	0	30	111.9	5.5	269.6	78	32.5	0	0	103	630.5
2009/10	14	0	27.6	48.3	123.9	43	36.3	15.7	35.3	41.2	9.8	0	395.1
2010/11	0	0	0	0	47.3	136	150.5	37	46.5	161.7	1.5	24.1	604.6
2011/12	0	0	0	0	85.9	13	61.5	35.5	15.8	0	0	0	211.7
2012/13	0	0	0	55.6	6	83.7	27	0	39.4	0	0	0	211.7
2013/14	9	0	0	32.5	12.1	89.1	94.1	83.3	90	0	0	0	410.1
2014/15	0	0	0	0	44	0	n/a	n/a	n/a	112.9	0	0	156.9
2015/16	0	0	0	0	75.9	37	56.4	9.2	83	63	13	15.1	352.6
2016/17	0	0	0	27.7	58.4	8	143	215.5	14.7	58.1	0	0	525.4
2017/18	0	0	0	81.6	13.3	0	125.2	50.5	160.3	56	0	0	486.9
2018/19	0	0	0	23.3	6.1	53.1	54.4	15.9	28.9	74.4	0	0	256.1
Average													**416.4**

4. Mmadinare

Year	jul	aug	sept	oct	nov	dec	jan	feb	march	april	may	jun	Total
1995/96	0	0	0	n/a	n/a	n/a	227.4	101.7	0	0	0	0	329.1
1996/97	47	0	0	0	n/a	368.3	148.5	16	50.1	17	3	0	649.9
1997/98	0	0	24.5	44	62	75.5	106.4	18.4	9.3	0	0	0	340.1
1998/99	0	0	0	39	103.9	24	26	29	20.6	0	0	0	242.5
1999/00	0	0	0	9.5	95.8	66.1	109.4	117.1	0	0	0	0	397.9
2000/01	0	0	0	0	41.8	48.6	150	252.7	52.3	31	0	0	576.4
2001/02	0	0	0	16	108	63.4	18	0	0	0	0	0	205.4
2003/04	0	0	0	25.5	13	56.5	64.5	52.3	119.1	0	0	0	330.9
2004/05	0	0	0	0	0	98.2	11.1	0	0	0	0	0	109.3
2005/06	0	0	0	0	52	62.6	66	175	0	0	0	0	355.6
2008/09	0	0	0	5	73.5	n/a	124	82	30	0	0	0	314.5
2009/10	0	0	7	30	76.3	0	41.5	69	17	35	0	0	275.8
2010/11	0	0	0	20	92.6	0	159.7	0	2	16.8	0	0	291.1
2011/12	0	0	0	12.1	71.1	0	78	20	n/a	0	0	0	181.2
2012/13	0	0	0	44.3	53	44.5	275	0	0	0	0	0	416.8
2013/14	0	0	0	n/a	n/a	n/a	173	139	146	0	0	0	458
Average													**343.0**

Appendix F

Publications

1. Published Articles

Jonas, M., Ketlogetswe, C., Gandure, J., 2020. Effect of Fruit Maturity on Some Physicochemical Properties of Jatropha Seed Oil and Derived Biodiesel, ACS Omega, 5(23), pp. 13473-13481.

Jonas, M., Ketlogetswe, C., Gandure, J., 2020. Variation of Jatropha Curcas Seed Oil Content and Fatty Acid Profile with Fruit Maturity Stage, *Heliyon, Elsevier Ltd*., 6(1)

Jonas, M., Ketlogetswe, C., Gandure, J., 2018. Influence of Jatropha Fruit Maturity on Seed Oil Yield, Composition and Heat of Combustion of Derived Biodiesel, Energy and Power Engineering, pp. 77-86

2. Articles submitted for Publication

Jonas, M., Ketlogetswe, C., Gandure, J., Quantification of Phorbol Esters Content in Jatropha Seed Oil and Seed Cake at Different Fruit Maturity Stages

3. Conference Papers

Jonas, M., Ketlogetswe, C., Gandure, J., Effect of Jatropha Curcas Fruit Maturity on Seed Oil and Biodiesel Quality: A Review, International Renewable Energy Conference (IREC), Gaborone, Botswana; 26-28th October, 2016.

Jonas, M., Ketlogetswe, C., Gandure, J., Production of Biodiesel using Jatropha and Beef tallow as Feedstock in Botswana (Poster), Energy Pitso Conference, Gaborone, Botswana; 15th November, 2016.

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Article

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

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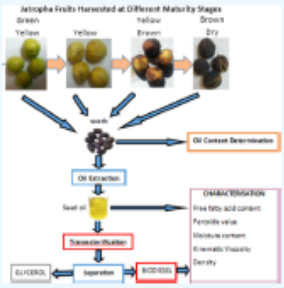
ABSTRACT: The quality of a feedstock in biodiesel production is of paramount importance, and Jatropha seed oil is no exception. This study investigates the influence of the fruit maturity stage on the physicochemical properties of Jatropha seed oil and the derived biodiesel. Free fatty acid content, 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, biogradable, and nontoxic and having reduced emissions by 53−70% when compared with petro-diesel.[1−3] 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.[4,5] Quality of feedstock in terms of physical and chemical properties is an important factor in biodiesel production. Atadashi et al.,[6] 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.[7−9] These free fatty acids react with the alkaline catalyst during biodiesel production to produce soaps that makes separation difficult.[10,11] 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,[12] 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,[13,14] higher density values of

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Research article

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

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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 petro-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].

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Influence of Jatropha Fruit Maturity on Seed Oil Yield, Composition and Heat of Combustion of Derived Biodiesel

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Abstract

Maturity of Jatropha fruits has influence on oil yield, chemical composition and physicochemical properties of derived biodiesel. Oil yield was determined using soxhlet extraction while biodiesel was prepared through the process of transesterification. Fatty acid profile was determined according to test method EN 14103 using Agilent Technologies GC System 7890. The calorific value of biodiesel was determined using Oxygen Bomb Calorimeter, IKA C200 according to test method ASTM D5865. Results showed that Yellow Jatropha fruit seeds have the highest oil yield and energy content, coupled with the best mix of fatty acid methyl esters.

Keywords

Fruit Maturity, Biodiesel, Oil Yield, Energy Content, Fatty Acid Profile

1. Introduction

Jatropha plant has received much attention as a potential feedstock for biodiesel production. The plant bares seeds are rich in oil, which can be converted into biodiesel. It has a good adaptation to a large variety of soil and climatic conditions including dry and semiarid conditions which are unsuitable for food production [1] [2]. During the maturation process of fruits, seeds undergo physical and chemical changes that determine the quality of the oil [3]. Maturity of fruits is usually determined with colour change once they have reached their maximum growth size. During maturation, Jatropha fruits change colour from green to green yellow, yellow, yellow brown and finally brown dry. Feedstock of low quality in terms of physical and chemical properties requires pretreatment prior to conversion to biodiesel which increases costs of production. For example,