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Production and Characterization of Biodiesel from *Citrullus colocynthis* as Alternative Energy Source for Petrol Diesel Engine

By

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Abstract

Biodiesel production from oil-bearing plants have attracted the attention different scholars as a consequence of global issues such as depletion of fossil fuel reserves, global warming, and environmental pollution problems. Hence, this study focused on the production and characterization of biodiesel from Citrullus colocynthis oils using two step transesterification reactions. The raw oils were extracted from Citrullus colocythis seeds, using soxhlet extractor with n-hexane as solvent. The effects of different parameters were studied; including reaction temperature, oil to methanol molar ratio, time of transesterification reaction, and amount of alkaline catalyst in order to achieve the optimum condition to obtain the highest conversion. The results indicate that 60^oC reaction temperature, 6:1 methanol to oil ratio, 90 minutes reaction time and 1.00 wt % NaOH catalysts were taken as optimum conditions for transesterification to achieve the highest conversion of the raw oils to the corresponding biodiesel, which led to biodiesel yield of 98.53%. The physicolcchemcal properties of the raw oil; acid value, saponification value, free fatty acid value, iodine value, PH value, peroxide value, density, specific gravity, calorific value, flash point, fire point, moisture content, and viscosity were characterized and compared with the reported standard values. Based on these finding, the raw oil of the plant was acceptable for prior transesterfication reaction process. Therefore, the produced biodiesels and its blends were further analyzed to determine its fuel properties, such as kinematic viscosity, caloric value, flash and fire point, pour and cloud point, water content, moisture content, ash content, distillation temperature, PH, free fatty acid, acid value and others as per ASTM 6751 standard methods and compared with reported values. For comparison, commercially available petro-diesels fuels were analyzed using the same tests used for the produced biodiesels. The qualities of all the tested parameters of the biodiesel were found to be within international acceptable biodiesel and diesel standards. Hence, the produced biodiesel is used as alternative source of energy for petrodiesel engine without further modifications.

1. Introduction

In Ethiopia, large-scale investments in bio-fuels have a recent history with the first large-scale bio-fuel feedstock production being established in 2006 by the UK-based bio-fuel company, Sun Bio-fuels. Since 2006, Ethiopia has become a major destination for foreign direct investment in bio-fuels in Africa. Although most of the bio-fuel investments have not yet been implemented, in 2011, the amount of capital that the bio-fuel companies committed to invest in bio-fuels accounted up to 50% of foreign direct investment flow at the national level (Bossio et al., 2012).

The bitter apple (*Citrullus colocynthis* L.) a species of flowering plant in the family, Cucurbitaceae is selected for this study. *C. colocynthis*, commonly known as the colocynth, bitter apple, bitter cucumber, egusi is a viny plant native to the Mediterranean Basin and Asia, especially Turkey, but now a days it is widespread throughout Western Africa, Arabia, the Mediterranean, semi-desert areas of northern Africa, tropical, Asia, Australia and Ethiopia (Jeffrey, 1995). *C. colocynthis* is currently less expensive vegetable oil, can be used as feedstock in the production of biodiesel and the resulting fuel is superior for cold winters (Oduvaldo, 2007). Biodiesel produced from *C. colocynthis* has a remarkable advantage regarding lubricity because of its high energy value and positive fuel properties (Berman et al., 2011).

The competition between edible oil and fuel needs may cause worldwide disproportion in the food industry and market demands. Moreover, using all edible oils for biodiesel production will not be enough for worldwide fuel demand. Therefore, there is a need for nonedible and inexpensive feedstock such as *C. colocynthis* oil for biodiesel production. The fatty acid in a *C. colocynthis* made up of triglycerides; 67-73% linoleic acid, 4-5% linoleic acid, 10-16% of oleic acid, 12% palmitic and 5-8% stearic acids. Besides being used as a source of biodiesel, the oil can also be used for manufacturing cosmetics, candles, soaps, and lubricant (Kumar and Sharma, 2011; Taufiq-Yap et al, 2011).

Environmental concerns, production cost, associated hazards and sustainability issues have bedeviled fossils utilization. However, biodiesel usage is less affected by most of these factors as it's renewable, environmentally friendly and produced from varieties of feedstock in respective regions. Biodiesel is defined by American Societies and Test Materials (ASTM) Internationally as a fuel composed of monoalkyl esters of long-chain fatty acids derived from renewable vegetable oils or animal fats meeting the requirements of ASTM D6751 (ASTM, 2008). The word "Bio" relates to its being renewable and of biological origin while "diesel" relates to the application in diesel engines (Zhang et al., 2003).

Among the most promising sources, vegetable oils and animal fats have attracted much attention as a potential resource for the production of biodiesel, which is quite similar to conventional diesel in its main characteristics and can be easily blended with diesel fuel in any proportion with minor or no modifications to the engine as well as fuel system (Agarwal and Das, 2001). The production and use of biodiesel have increased significantly in many countries around the world using numerous feedstock sources (Mulugetta, 2008).

Biodiesel is an alternative and renewable bio-fuel that could help to diminish the dependency of the import from petroleum-producing countries (Van Gerpen, 2005). The most common way to produce biodiesel is the transesterification method, which refers to a catalyzed chemical reaction involving vegetable oil and alcohol to yield fatty acid alkyl esters (i.e. biodiesel) and glycerol as by-product (Zhang et al., 2003).

Biodiesel is biodegradable and readily available but it is not toxic. Moreover, it has a high heat value, and it contains higher amount of oxygen (10-11%) that ensures more complete combustion of hydrocarbons (Global Farmer, 2009) and it is essentially free of sulphur and polycyclic aromatic hydrocarbons (Demirbas, 2003). These properties reduce CO, CO_2, SO_x, NO_x, hydrocarbons and particulate matter emissions when compared with petro-diesel (Fukuda et al., 2001; Du et al., 2004). Biodiesel is being used in both public and private fleet vehicles because it is environmentally friendly and it offers a reduction in some emissions without requiring any modifications to the vehicle of biodiesel (Abdulkareem et al., 2011).

Nowadays, all Ethiopian petroleum products are imported either through the port of Djibouti or Sudan. Besides the cost of fuel, long distance transportation adds to the cost of the fuel getting to Addis Ababa that causes a large burden on Ethiopia's trade balance. One main issue is that around 70% of Ethiopian export earnings is paid for the import of petroleum products. Amongst the identified alternative renewable energy sources, bio-fuels in particular energy crops has received attention as a promising and sustainable energy sources of which biodiesel has arisen as a potential replacement for a petro diesel substitute that minimize the escalating budgetary pressure for diesel oil.

Biodiesel has several advantages over fossil fuels especially in landlocked countries like Ethiopia. For a truly renewable source of fuel, crops or other similar agricultural sources would have to be considered. Every year Ethiopia needs to import fuel from other countries and even now due to the lack of technology in converting vegetable oil into biodiesel and the country needs to import food items from abroad. But, there are some native plants which grow well in the fallow lands that can play a major role for producing the non-edible oil which could be converted biodiesel or directly used as a source of alternative fossil fuel in Ethiopia. This can save the country's foreign currency.

Environmental pollution is one of the factors that have led to the search for alternative sources of energy. Thus, biodiesel is considered as a perfect alternative source of energy that can compete with fossil fuel in terms of performance and efficiency. However, the production of biodiesel from vegetable oils and animal fats also raised a serious concern of food crisis and the critics of the production of biodiesel from edible are making it difficult to justify the use of edible oil for fuel. Hence the need to produce biodiesel from non-edible oils such as *C. colocynthis* oil that are grown in large quantities and waste lands in Ethiopia has been the focus of this study. Results obtained on characterization of the oils and biodiesel produced from the non-edible oils are hereby presented.

Biodiesel obtained from *C. colocynthis* seed oil is non-toxic, biodegradable and an excellent substitute for petroleum based diesel fuel. The energy content, cetane number and viscosity of biodiesel are similar to those of petroleum based diesel fuel. Moreover, engines operating on biodiesel have been reported to have a significantly lower impact upon the environment than those operating on petroleum diesel. Therefore, the potential production of biodiesel from *C. colocynthis* seed oil in Ethiopia is associated to the solution of economic, environmental, and social problems. To the best of the researchers' knowledge, there were no further studies on the production and characterization of biodiesel from *C. colocynthis* in Ethiopia. The overall objective of this work is to produce and characterize biodiesel from *C. colocynthis* seed oil and compare with conventional diesel and report standard values.

2. Literature review

a. Bio-fuel development in Ethiopia

To support the development of the bio-fuel sector in the country, the Ethiopian government has made two main policy amendments. First, in 2009, the government introduced an ethanol blending policy that sets a blending mandate of 5 % ethanol with 95 % gasoline. The blending mandate was increased to 10 % in early 2011 and there is a plan to increase it further to 25 % in 2014. Secondly, the government has made many amendments to its agricultural development and taxation policies to attract investments in large-scale agricultural projects including bio-fuels. According to Rahmato, biofuel related investments are dubbed "open door policy". Moreover; there are many incentives to attract investors to invest in bio-fuels and other agricultural projects (Rahmato, 2011).

In order to ensure the country's continuous development program and the national fuel security, it is important to increase fuel utilization and substituting the demand by locally produced fuels such as biofuel. This document "The Ethiopian Biofuel Development and Utilization Strategy " targets supply of fuels from locally produced biofuel and the objective of the strategy is to ensure the production of biofuel without affecting food self sufficiency, import substitution and improve balance of payment. Although production of bio-diesel is not yet started, various types of plant species which can be used for producing bio-diesel grow in the country. Favorable air condition and suitable soil type for bio-diesel development is available across the coutnry.

b. Biodiesel

Biofuels are becoming big policy and big business as countries around the world are looking to decrease petroleum dependence, reduce greenhouse gas emissions in the transportation sector, and support agricultural interests. The production of biodiesel from bitter oil is technically feasible. The major constraint has been the high price paid for the oil as industrial oil because of high demand by the chemical industries to manufacture very high value products (Drown, 2001; Refaat, 2009; Berman et al., 2011).

In biodiesel production, oil is extracted from oil seeds by mechanical crushing or solvent extraction. Byproduct is a protein – rich residue cake that can be used for animal feed. Oil is filtered, washed, decanted dried, and heated. It is reacted with methanol in the presence of base catalyst (typically caustic soda potash) at 60 ^{o}C in a process called transesterification. The biodiesel is purified by washing with warm water. For troubles-free operation in diesel engines, biodiesel must be free of glycerin, catalyst, alcohol and free fatty acids.

Biodiesel which is derived from triglycerides by transesterification and from the fatty acids by esterification has attracted considerable attention during the past decade as a renewable, biodegradable, eco-friendly and non-toxic fuel. Several processes have been developed for biodiesel fuel production. Biodiesel is recently used as a substitute for petroleum based diesel due to environmental considerations and depletion of vital resources like petroleum and coal. The possible use of renewable resources as fuels and as a major feedstock for the chemical industry is currently gaining growth. Further as petroleum is a fast depleting natural resource, an alternative renewable way to petroleum is a necessity (Srivastava and Prasad, 2000).

c. Advantages and benefits of biodiesel

The last few years have witnessed both a dramatic increase in the price of oil and an increase in the production of biofuels. Recent trends also show that interest in biofuels is expanding towards developing countries where production costs are relatively inexpensive and gives possibility for biofuel to contend with fossil fuel prices. Biofuel has several advantages over fossil fuels especially in landlocked countries like Ethiopia.

Biodiesels are renewable source of energy, biodegradable, non-toxic, and emit less greenhouse gases than petroleum-based diesel. It has become an interesting alternative to be used in diesel engine, because it has similar properties to the traditional fossil diesel fuel and may, thus, substitute conventional fuel with none or very minor engine modification (Panwar et al., 2010). In comparison the use of biodiesel produces approximately 80% less carbon dioxide emissions, and almost 100% less sulfur dioxide. Combustion of biodiesel alone provides over 90% reduction in total unburned hydrocarbons, and a 75-90% reduction in aromatic hydrocarbons. Biodiesel has a very high flash point (300 F) making it one of the safest of all alternative fuels, from a combustibility point (Hemant et al., 2011; Berman et al., 2011)

For more than two centuries, the world has relied heavily on non-renewable crude oil, out of which about 90% is estimated for energy generation and transportation. Thus an increased environmental concern, tougher maintenance of clean air standards, necessitates the research for viable alternative fuels, which are environmentally friendly (An-Fei HSU, 2002).

Biodiesel is significantly cleaner than petroleum diesel in almost every pollutant category. Emissions from biodiesel and petroleum diesel combustion are compared below for particulates, unburned hydrocarbons, carbon monoxide, nitrous oxides, sulfur oxides, and polycyclic aromatic hydrocarbons.

d. Energy security of biodiesel

The Government of Ethiopia considers biofuels as an opportunity for enhancing food and energy security. The biofuel sector not only seems promising in addressing the energy security issue but also creates more jobs and income that could support the country's goal of poverty reduction. Increasing fuel prices, concerns with climate change and future energy security have led to tremendous global interest in the use of liquid biofuels in the transport sector (Schut et al., 2010; World Bank, 2010; German et al*.,* 2011).

Increasing pressure of population and increasing use of energy in agriculture, industrial and the domestic and public sectors is an area of concern. As India continues to grow at the rate of 8-9.5 percent, energy security has become a core issue. To alleviate concerns over energy security, the Government of India has taken multiple steps in recent years which include encouraging private sector participation, a more holistic approach towards broad basing its supply base, and improving efficiency in the sector as a whole (Hemant et al., 2011).

With agricultural commodity prices approaching record lows, and petroleum prices approaching record highs, it is clear that more can be done to utilize domestic surpluses of vegetable oils while enhancing our energy security. Because biodiesel can be manufactured using existing industrial production capacity, and used with conventional equipment, it provides substantial opportunity for immediately addressing our energy security issues. Biodiesel emerges as one of the most energy-efficient environmentally friendly options in recent times to fullfill the future energy needs. Biodiesel is a renewable diesel substitute that can be obtained by combining chemically any natural oil or fat with alcohol.

The majority of the world energy needs are supplied through petroleum resources. The increase in energy demand and pollution problems caused by industrialization has urged researchers and economists to find new sources of energy. One of the feasible energy sources is the use of plant oils, which is readily available and environmentally acceptable (Meher et al., 2006). The major sources of the world's energy needs are petroleum, coal and natural gases which are fossil-derived and non renewable. The world, at large, depends on petroleum as the energy source for the transportation sector.

Energy production for industrial and domestic purpose has primarily been based upon the combustion of fossil fuels, such as oil and coal and it has been reported that these resources are finite and pose significant environmental impact from their combustion (Carraretto et al., 2004; Abdulkareem and Odigure, 2002). It has been predicted that coal will be a viable energy resource for 90-200years, while the world oil supply is reaching its peak due to over dependence on oil consumption.

Biodiesel is a source of Bio-energy which derived from biofuels. Biofuels are fuels produced directly or indirectly from organic material biomass including plant materials and animal waste. Overall, bioenergy covers approximately 10% of the total world energy demand. Traditional unprocessed biomass such as fuel wood, charcoal and animal dung accounts for most of this and represents the main source of energy for a large number of people in developing countries who use it mainly for cooking and heating (FAO, 2008).

e. Economic importance of biodiesel

Ethiopia has large underutilized labour force, arable land and suitable climate for biofuels. Exploiting these resources to meet one of the major challenges to the economy, reliable and secure access to energy, was the rationale for Ethiopia's biofuel development strategy. The economic importance of bitter oil include synthesis of cosmetics, plasticizers, lubricants, paints, polymers, resins, textile dying, preservatives, printing inks, lacquers, grease, hydraulic fluids, soaps, oil cloth, laxatives, insecticides, fertilizers, utilization in aircraft, and space rockets (Kang et al., 1990; Ellis and Freeman, 2004).

One of the justifications for encouraging the expansion of biofuels in Ethiopia is the possibility of saving scarce foreign currency that is used to import fossil fuels and shifting from high-cost fossil oil to cost-effective biofuels (MoME, 2007). The value of the country's oil imports has increased substantially over time. For instance, the value of oil imports relative to export earnings of the country has increased from 52.7% percent in 2000/1 to 66.9% in 2010/11. The high cost of oil imports has aggravated the country's balance of payments problem, and has serious repercussions on the macroeconomic stability of the country.

The economical growth of any developing country is based on agriculture and industrial sector. The basic power source of industrial sector as well as agriculture sector is diesel fuel. The expansion of economies always comes with increase in the transport (Ramesh and Sampatraja, 2008).

Ethiopia belongs to the non-oil exporting less developed countries (LED) of Africa. Ethiopia imports all of its petroleum products and the demand for petroleum fuel is rising rapidly due to the growing economy of about 10% GDP growth and infrastructure development. In the second quarter of 2007/08, petroleum imports exceeded export earnings by 30% (NBE, 2007). With the recent trends and volatility of oil prices, the country has been forced to develop a biofuel strategy to mitigate the impacts of imported oil on its economy. The strategy encourages the diversification of energy supplies in the transport sector; therefore, biodiesel offers significant opportunities (MoME, 2007).

Biofuels have advanced from being purely experimental fuels to initial stages of commercialization. They are technically competitive with or offer technical advantages compared to conventional diesel fuel. Besides being a renewable and domestic resource, biodiesel reduces most emissions while engine performance and fuel economy are nearly identical compared to conventional fuels. In addition, biofuels create independence from the imported petroleum, they are not depleting natural resources, and they do not pose environmental concerns.

f. Environmental sustainability of biodiesel

Global warming is one of the greatest environmental threats facing our planet which is caused by increasing atmospheric Green House Gases due to human activities since the start of the industrial era (Emission Standards European Union, 2008). Concern over the economics of accessing fossil fuel reserves, and widespread acceptance of the anthropogenic origin of rising CO_2 emissions and associated climate change from combusting such carbon sources, is driving academic and commercial research into new routes to sustainable fuels to meet the demands of a rapidly rising global population.

The search for alternative fuels started when the pollution created by the burning of fossil fuels showed severe environmental problems as biofuels have a significant role in overall reduction of CO_2 emissions (Lapuerta et al., 2008). The catalytic esterification and transesterification solutions to the clean synthesis of biodiesel, the most readily implemented and low cost, alternative source of transportation fuels to meet future societal demands (Adam et al., 2014). The numerous limitations of the conventional homogenous catalysts have become a great challenge for researchers in the area of biodiesel. Therefore, alternative catalysts for clean and environmental benign process are the solution (Ibrahim et al., 2013).

g. Catalytic transesterfication reaction

Vegetable oil and animal fats were investigated well before the energy crisis of the 1970s. The early 1980s sparked renewed interest in alternative fuels. It is also known that Rudolf Diesel (1858-1913), the inventor of the diesel engine that bears his name, had used peanut oil as fuel in his invention. High fuel viscosity in compression ignition is the major problem associated with the use of vegetable oils; viscosities of vegetable oil are ranging 10 to 20 times higher than diesel fuel. The most common method for the production of biodiesel is transesterification and it leads to mono alkyl esters of vegetable oils and fats, now called Bio-diesel and glycerol when used for fuel purposes. The methyl ester produced by transesterification of vegetable oil has a high cetane number, low viscosity and improved heating value compared to those of pure vegetable oil which results in shorter ignition delay and longer combustion duration and hence low particulate emissions (Naik et al., 2008).

Transesterification is a common and well-established chemical reaction in which linear monohydroxy alcohols react with vegetable oils, which are triglycerides of fatty acids; in the presence of a catalyst among alcohols commonly used for the transesterification process are methanol, ethanol, propanol and butanol. The overall process is a sequence of three consecutive and reversible reactions, in which di and monoglycerides are formed as intermediate compounds. The stoichiometric reaction requires 1 mol of triglycerides and 3 mol of alcohol. However, an alcohol excess is required to drive the reaction close to completion (Sharma, and Singh, 2008). Methanol and ethanol are used most frequently, but methanol is preferred because of its physical and chemical properties (polar and shortest chain alcohol) (Ma and Hanna, 1999). The methods of preparation of biodiesel can be classified into: chemical catalytic (base or acid catalysis), biocatalytic (enzyme catalysis) and noncatalytic supercritical alcohol processes; a very good overview of such methods has recently been published (Luque et al*.,* 2008*).* However, alkali catalysts are the most commonly used in the biodiesel industry, since the process is faster and the reaction conditions are moderated. The process of transesterification brings about extreme change in viscosity of vegetable oil (Rao et al*.,* 2008).

Transesterification is the process of separating the fatty acids from their glycerol backbone to form fatty acid alkyl esters and free glycerol. Alkali-catalysed transesterification process is the most common process for production of biodiesel and need to be optimized for different process variables (Stidham et al., 2007). Biodiesel is generally produced from virgin vegetable oils requiring low temperature and pressure and produces over 98% conversion yield, provided the starting oil is low in moisture and free fatty acids, by transesterification (Rashid et al., 2008). Investigations in biodiesel production have searched the optimization conditions for the transesterification process, such as, molar ratio alcohol/oil, alcohol type, reaction time, temperature, water and free fatty acid (FFA) content in the oil, catalyst type and concentration, mixing intensity and organic co-solvent addition (Meher et al., 2006).

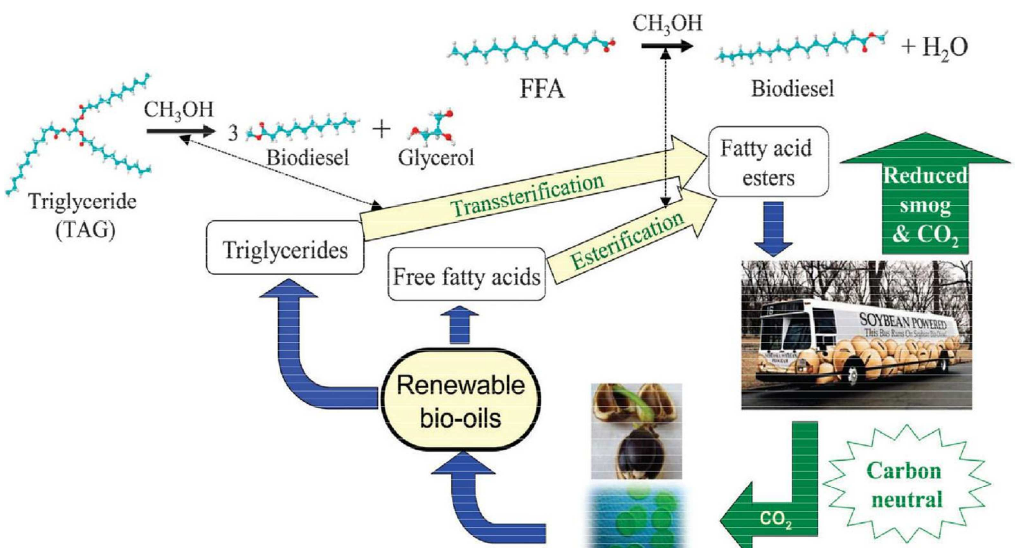

Figure 1 Biodiesel production cycle from renewable bio-oils *via* catalytic transesterification and esterification.

3. Materials and Methods

3.1. Materials and equipment's

3.1.1. Materials

The chemicals used were: solvents (99.5% methanol ACS grade, 99.5 % n-hexane ACS grade and 99.5% chloroform ACS grade Newdlihi, India), anhydrous sodium sulphate (Techno pharmchem Dehi, India), 99% sulphuric acid (Blulux laboratories (p) ltd., India), distilled water, sodium hydroxide pellets AR 99% (Breckland Scientific Supplies, Thetfolrd Norflok, UK), acetic acid (British drug house ltd., UK), ferric chloride (British drug house ltd., England), 37% hydrochloric acid (Blulux laboratories (p) ltd., India), alpha-naphthol (Baker chemical co., USA).

3.1.2. Equipment's

The equipments used were pH meter, distillation apparatus, viscometer, thermostatic hot plate, magnetic stirrer, digital weighing balance, thermometer, petri dish, pippete, separating funnel, burete, oil test centrifuge, pycometer bottles, Abbe refractometer, flash point tester, oven, thimble, soxhlet apparatus, filter paper, blander, water bath, conical flask (different size), measuring cylinder (different sizes), rotary evaporator, Rotary evaporator flasks (different sizes), spatula, condenser, polyethylene bags, and heating mantle.

3.2. Experimental methods

3.2.1. Collection of the plant materials

The Dry seeds of *C. colocynthis* were collected from Awash, Oromia Regional State from October-November, 2015. The shade dried plant materials were chopped into small pieces and finally pulverized into fine powder using a sterile electric grinder and packed in polyethylene bags to avoid entrance of air and any other mixing of surrounding material. The grinned powders were kept in refrigerator at $4^{o}C$ for further analysis.

(a) **(b)** **(c)**

Figure 2: Fruit (a), seed (b) and powder (c) of *C. colocynthis*

3.2.2. Extraction of seed oil

Air dried seeds of *C. colocynthis (*10 Kg) powder extraction was carried out using Soxhlet apparatus with n-hexane as solvent for 8 hrs. 60 g of the ground sample was poured into the thimble and two third volume of the round bottom flask was filled with the solvent. The heating mantle temperature was adjusted to about (60-65) ºC below the boiling point of the solvent. As the solvent was heated continuously, it starts to evaporate and condenses back into the sample in the thimble. The oil extracted, containing some portion of the solvent was then recycled back to the round bottom flask as it refluxes and the total process of reflux continues until total oil extraction was observed. An anhydrous sodium sulphate was added to the extracted oil to remove drops of water and filtered using whatman number one filter paper. A rotary evaporator was used to separate the oil from the solvent, at the temperature of 40ºC under reduced pressure. This extraction procedure process was repeated for many runs to obtain a reasonable oil quantity. The yield of the crude oil was weighted and recorded 52. 06 wt% (weight of oil/weight of seed powder) and it was kept in refrigerator at 4^{o}C for further analysis.

3.2.3. Physicochemical characterization of the crude oil

Prior to the production of biodiesel from *C. colocynthis* seed oil, physicochemical analysis of the crude oil such as density, specific gravity, acid value, free fatty acid, saponification value, iodine value, peroxide value, viscosity, and moisture content were conducted according the standard procedure AOCS method and ASTM D-6751 standards.

Determination of density

The weight of a small empty bottle was determined using an electronic weighing balance. The bottle was then filled to the brim with the oil and the weight of the bottle and oil determined. The procedure was repeated with the labeled of W1 and W2 for weight of an empty bottle and weight of oil plus bottle, respectively.

Determination of specific gravity

Density bottle was used in determining the specific gravity of the oil. A clean and dry stoppered bottle of 25 mL capacity was weighed (W0) and then filled with the oil stoppered and reweighed to give (W1). The oil was substituted with distilled water after washing and drying the bottle and weighed to give (W2).

Determination of moisture content

About 10 g of a clean sample was weighed and then dried in the oven at 105˚C for 5 hrs and the weight was recorded after every 2 hrs. The same procedure was repeated until a constant weight was obtained. After 2 hours, the sample was removed from the oven and placed in the dessicator for 30 minutes to cool then removed and re-weighed.

Determination of ash content

An empty porcelain crucible was weighed empty (W1), 3.0 g of the oil was weighed and poured into the crucible and the crucible reweighed (W2). The crucible was later burnt over a bursen burner to remove all the solvent present. It was taken to the muffle furnace for ashing at 55 ^{o}C for two hours. The crucible was removed and placed in a desicator and allowed to cool. It was reweighed (W3).

Determination of refractive index

Abbe's refractometer was used in the determination of refractive index. This instrument measures the index of refraction by measuring the critical angle of total reflection. In this case, a few drops of the sample were transferred into the glass slide of the refractometer. Water at 30˚C was circulated round the glass slide to keep its temperature uniform.

Through the eyepiece of the refractometer, the dark portion viewed was adjusted to be in line with the intersection of the cross. At no parallax error, the pointer on the scale pointed to the refractive index. The refractometer was calibrated using distilled water where the refractive index of water at that temperature was obtained (Warra *et al*., 2011) The procedure was repeated by using *C. colocynthis* oil from the six regions and their refractive indices were obtained at 30˚C. The mean value for each region was noted and recorded as the refractive index.

PH value

In a dry clean 25 mL beaker, 2 g of the sample was placed followed by 13 mL of hot distilled water and the mixture was stirred slowly. The mixture was then cooled in a cold-water bath to 25˚C. The pH electrode was standardized with buffer solutions (pH 4 and 7) and the electrode immersed into the sample where an average pH of three recordings per sample were recorded (Isah, 2006).

Determination of viscosity

A viscometer was used to determine the viscosity of the oil. Chloroform was poured first in the viscometer and the time at which the chloroform reached the bottom of the equipment was taken. The oil was then poured into the viscometer and the time taken far the oil to reach the bottom of the equipment was taken and recorded in mm^2/s. The procedure was repeated a number of times and the average value was taken which was then multiplied with the viscometer calibration to give the kinematic viscosity.

Determination of Acid Value and Free Fatty Acid

2 g of the oil was measured and poured in a beaker. A neutral solvent (a mixture of petroleum ether and ethanol) was prepared and 50 mL of it was taken and poured into the beaker containing the oil sample. The mixture was stirred vigorously for 30 minutes. 0.50 g of potassium hydroxide (KOH) pellet was measured and placed in a separate beaker and 0.1M KOH was prepared, 3 drops of phenolphthalein indicator was added to the sample and was titrated against 0.1M KOH till the color change observed turned pink and persisted for 15 minutes.

Determination of saponification value

The alcoholic KOH was freshly prepared by dissolving KOH pellet in ethanol. 4 g of oil was measured and poured into a conical flask. 25 mL of the alcoholic KOH was added to it, a blank was used. The sample was well covered and placed in a steam water bath for 30 minutes shaking it periodically, 1 mL of phenolphthalein was added to the mixture and titrated against 0.5M HCl to get the end point.

Determination of iodine value

The oil was poured into a small beaker; a small rod was added to it. 2 g of the oil was weighed and poured into a glass-stopper bottle of about 250 mL capacity. 10 mL of carbon tetrachloride was added to the oil to dissolve. 20 mL of Wij's solution was added and a stopper was inserted and allowed to stay in the dark for 30 minutes. 15 mL of potassium iodide solution (10%) and 100 mL of water was introduced and the mixture was thoroughly mixed and titrated with0.1M sodium thiosulphate solution using starch as indicator.

Determination of peroxide value

2 g of oil was weighed into a clean drying boiling tube, 1g of powdered potassium iodide and 20 mL of solvent mixture (2 volume of glacial acetic acid + 1 volume of chloroform) was added, the tube was placed in boiling water so that the liquid boils within 30 seconds and was also allowedto boil vigorously for not more than 30 seconds. The content was quickly poured into a flask containing 20 mL of potassium iodide solution; the tube was washed out with 25 mL of distilled water and was titrated with 0.002 M sodium thiosulphate solution using starch as indicator. A blank was also carried out at the same time.

Determination of flash point and fire point

The flash point was determined using a small strong heat resistance glass cup and a heating mantle. The sample was poured into the cup and heated gradually while being stirred to distribute heat uniformly in the cup and the temperature was monitored using a thermometer. At regular interval temperatures, the cup was exposed to naked flame, the temperature at which the oil increases the flame like a flash but does not supports combustion was recorded, which was the flash point of the sample. The temperature at which the oil catches fire (supports combustion) was noted and recorded which gives the fire point of the sample.

Determination of cloud point and pour point

The sample was poured in a test tube to a certain level and a thermometer was inserted and sealed alongside with the test tube. It was then placed in a freezer and monitored at intervals. The temperature at which some traces of cloudy suspension appears in the test tube was noted which is the cloud point of the sample and the temperature at which the oil solidifies (freezes) was noted which is the pour point of the sample.

3.2.4. Two-step transesterification of the crude oils

In order to avoid the problem of saponification, a two-step transesterfication was used for synthesis of biodiesel from *C. colocynthis* seed oil.

Acid catalyzed etherification

This was considered as a pre-treatment for the crude oils in order to reduce their water content which was the main cause of soap formation and subsequently, reduce its free fatty acid. The oil was heated in the reaction glass tube to 60 ^{o}C and a solution of concentrated H_2SO_4 acid (1.0% based on the oil weight) in methanol (30% v/v) was heated to 45 ^{o}C and added to the reaction glass tubes. The resulting mixture was stirred on a magnetic stirrer for 1 hr and the content was poured into a separating funnel and allowed to settle for 2 hrs. The methanol-water fraction at the top layer was removed and the oil was decanted to be used for transesterification reaction

Base catalyzed transesterification reaction

50 mL of oil was measured and poured into a 150 mL conical flask and heated to a temperature of 45 ^{o}C using a water bath. A solution of sodium methoxide was prepared in

a 500 mL beaker using 1.00 g of NaOH pellet and 150 mL of anhydrous methanol. The solution was properly stirred until the NaOH pellet was completely dissolved in it. The sodium methoxide solution was then poured into the warm oil and stirred vigorously for 90 minutes using a magnetic stirrer and the mixture was left to settle for 24 hr in a separating funnel. After settling, the upper layer which was biodiesel decanted into a separate beaker while the lower layer which comprises of glycerol and soap was collected from the bottom of the funnel. The quantity of biodiesel collected was measured and recorded.

3.2.5. Purification of the produced biodiesel

Washing

Biodiesel must be washed to remove any remaining methanol, glycerin, catalyst, soaps and other impurities. Water used was warmed to about 45 ^{o}C and passed through the esters to allow soluble material, excess catalyst and other impurities to stick to the water and be settled to the bottom of the vessel. The water was removed from the vessel periodically until the wash water drained out was clear or the pH of the biodiesel becomes relatively neutral.

Drying

The biodiesel washing sometimes leaves the biodiesel looking a bit cloudy. This means there's still a little water in it. It was heated slowly to 90-100 ^{o}C and held there until all moisture present was evaporated (i.e. dry).

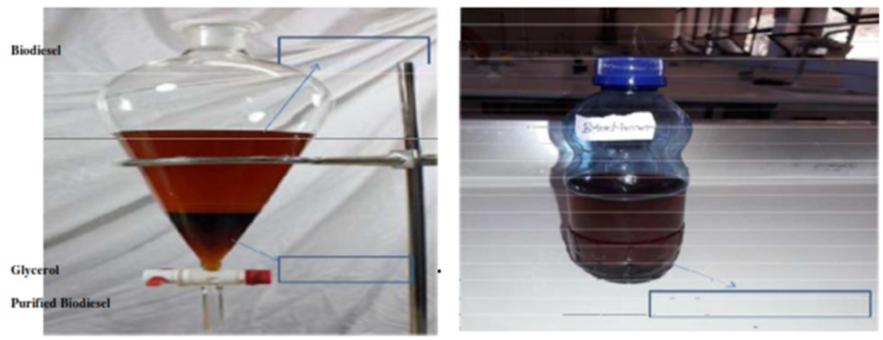

Figure 3: Separation and purification of biodiesel

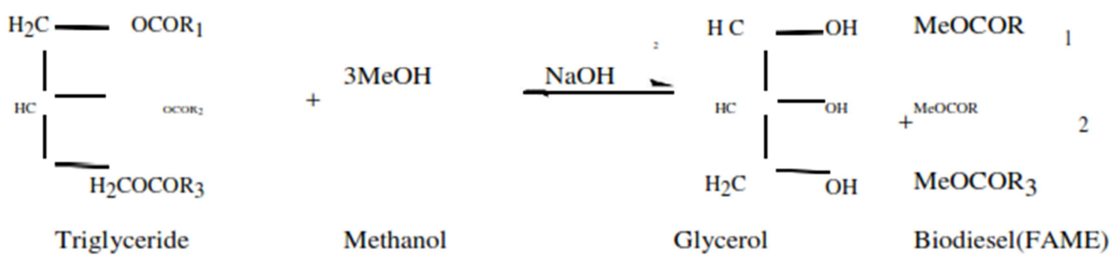

Figure 4: Schematic representation for the alkali catalyzed transesterification of triglyceride

3.2.6. Characterization of the Produced biodiesel

The physicochemical characterization analysis and comparative test were carried out according to the ASTM-D6751 specification standards. Specific gravity, density, acid value, free fatty acid, refractive index, iodine value, peroxide, moisture content, ash content, kinematic viscosity saponification values, flash and fire point, cloud and pour point were determined using the same procedure as the characterization of the extracted seed oil.

3.2.7. Preparation and Characterization of Blends of Biodiesel

At present the amount of biodiesel available is less than that of diesel. The biodiesel blended with diesel by volume as B5 (5% biodiesel and 95% diesel fuel) was prepared as first 95% (95 mL) of diesel fuel was taken in reactor vessel then 5 %(5mL) biodiesel was introduced in the same vessel. The mixture was then stirred (300 rpm) at 40 ^{o}C for 15 minutes. Other blends were prepared as same method B10 (10% biodiesel and 90% diesel fuel), B20 (20% biodiesel and 80% diesel fuel), and B100 (100% biodiesel and 00%

diesel fuel). The physicochemical properties of the blends of biodiesel were characterized according to the ASTM D6751 standard method and compared with the conventional diesel fuels.

3.2.8. Fatty Acid analysis using GC

The fatty acid composition of *C. colocynthis* oil was determined by Gas Chromatography (Shimadzu GC-17A) equipped with capillary column BPX 70 (30 m × 0.25 m × 0.25 µm) and FID detector. The column temperature was programmed at 120°C with an increment of 3°C per minute for 57 minutes whereas the injector and detector temperatures were set at 260°C and 280°C, respectively. Fatty acid methyl ester (FAME) was prepared according to PORIM Official Test Method (1995) with some modifications. The identification of the peaks was performed by comparing the retention times with standard methyl linoleic acid and other individual fatty acid methyl ester.

4. Result and Discussion

4.1. Physiochemical properties of *C. colocynthis* oil

The extracted crude oil was yellowish brown in color and it gets darkened during the storage, sweet odor and bitter taste. The percentage yield of the extracted oils was 52.06 %. This yield was higher than the values reported for Jatropha oil (52%) and lower than for Castor oil (56%) (Van Gerpen *et al*., 2004). In order to utilise the crude oil as a feedstock for the production of biodiesel; the physiochemical properties characterization such as density, specific gravity, ash content, pH, viscosity, acid value, saponifcation value, free fatty acid, moisture content, peroxide value and iodine value were very important. The results obtained on the properties of oils were compared with that of AOCS and ASTM D6751standard values. The physicochemical properties of the crude oil were reported and summarized in Table 1.

The moisture content of the oil was seen to be 3.5%; these agree with ASTM D6751 standard specification range (1.25 – 5.88%). The ash content of the crude oil was 5.83 %, it is within the specified standard value by ASTM. Ash content is important parameter for the determination of heating value; high heating value of oils is expected with lower ash content. Refractive index of the crude oil at 30ºC was investigated to be 1.45 which is in agreement with the ASTM specified standard (1.245 and 1.675). Refractive index of an oil is the ratio of speed of light at a defined wavelength to its speed in the oil itself. This value varies with wavelength and temperature, the degree and type of unsaturation, the type of substitutions of component fatty acids. Refractive index is widely used in quality control to check for the purity of materials and to follow hydrogenation and isomerization reactions.

Due to presence of free fatty acids in the *C. colocynthis* oil, it was necessary to determine the pH of the oil since it tells the state of oil as to whether the dissociation has taken place or not. The pH value (6.35) of the crude oils was summarized in Table 1. As seen in the table, the oil had a moderate pH value as compared with other AOCS specification standard oils. This is linked to the lower free fatty acids which are justified by its acid value and % FFA.

The iodine value shows the level of unsaturation of the oil and also influences the oxidation and deposition formed in diesel engines. The iodine value could be used to quantify the amount of double bond present in the oil which reflects the susceptibility of the oil to oxidation. Iodine value obtained for *C. colocynthis* was 102 gI_2/100 g values fall within the acceptable AOCS limit. As the iodine value is below 115 gI_2/100g the crude oil classified as non-drying oils. The saponification value obtained from the oil was 195 and it is within the range specified by AOCS standards. The saponification value of the oil is a measure of the tendency of the oil to form soap during the transesterification reaction. The presented result (195 mg KOH/g) revealed a similar saponification value with *Prunus armeniaca, Prunus persica, Datura metel*, and *Hyptis suaveolens* seed oil (Indra et al., 2013). Peroxide value is the most common indicator of oil oxidation. The unrefined vegetable oils are characterized by greater peroxide value (16 meq/kg), compared to refined oils. Low values of peroxide are indicative of low levels of oxidative rancidity of the oils and also suggest presence or high levels of antioxidant (Kyari, 2008). The peroxide values are low and are pointers to the fact that the oils may not be easily susceptible to deterioration.

Acid value of the crude oil was 1.85 mg KOH/g. This value showed an agreement with ASTM D664 specification standard (0.5-0.8 mg KOH/g). The high acid value indicates that more quantity of the base will be required to neutralize the acidity of the oil to be transestrified and it also indicates high free fatty acid content in the crude oil. The percentage free fatty acid content of the oils was also determined as 2.75 %. As the free fatty acid content is greater than 2.50%, it needs further pretreatment in order to remove the formation of soap which is a serious problem for the base catalyzed biodiesel production. Furthermore, oils with high acid value tend to deactivate base catalyst used during transesterification. This signifies that the ester yield of the oil will be higher due to the increase catalyst activation and decrease soap formation during the process (Leung and Guo, 2006).

The viscosity of the crude oil at 40ºC was 28.70 mm^2/s, which is higher than the ASTM specification standard; however, it is lower than to that reported for Jatropha oil 31.5 mm^2/s and moringa oil 33.50 mm^2/s (Van Gerpen et al., 2004). The high viscosity of the oils indicates that the oil cannot be used directly in diesel engines due to its low flow capability, thus there is need for further transesterification process. Those oils with low viscosity value indicate that they are light and so probably highly unsaturated; the high value might be as a result of suspended particles still present in the crude oil (Nangbes et al., 2013). The density (0.874 g/cm^3) of the oil was determined at 30ºC and the result was within the specified range of ASTM D1298 specification standard. Density of seed/vegetable oils is dependent on their fatty acid composition, minor components and temperature (Fakhri and Qadir, 2011). The specific gravity of the crude oil at 30ºC was 0.924; this value was also in agreement with the value in the ASTM D287 standard of (0.89-0.99). The result showed that most of the reviewed oils are less dense than water and therefore would be useful in cream production as it will make the oils flow and spread easily on the skin (Oyeleke et al., 2012b). According to Yahaya et al. (2012), specific gravity is commonly used in conjunction with other figures in assessing the purity of oil.

The flash point of the crude oil was determined as 157ºC, which was greater than that specified standard value of ASTM D93. This makes the oil well suited for biodiesel production with respect to safety in handling, storage and transportation. Safety in handling the fuel is also exhibited in their high fire point values of 183ºC. The cloud point and pour point of the extracted oil was 8 ºC and 10ºC, respectively. The cloud pointas well as pour point of the oil were similar to that specified in the ASTM standard specification, making it suitable for making biodiesel production and to be used in cold places without the use of additives to improve their cold filter plugging point (Raheman and Phadatare, 2004).

Table 1. Physiochemical properties of *C. colocynthis* oils with ASTM D6751 standard values.

Parameter	Unit	Experimental Value	ASTM D6751	Remark
Yield	%	52.06	-	
Odour	-	Sweet odour	-	
colour	-	Yellowish brown	-	
Moisture content	%	3.5	1.25-5.88	
Density	g/cm^3	0.874	0.86-0.90	
Specific gravity	-	0.924	0.86-0.99	
Ash content	%	5.83	4.5-10.00	
Acid value	mgKOH/g	1.85	0.80-1.00	
Free acid value	%	2.75	3.00-5.00	
viscosity	mm^2/s	28.70	6.0 max	
saponification	mgKOH/g	195	-	
Value				
Iodine Value	$gI_2/100g$	102	-	
Flash point	^oC	157	90-130.00	
Cloud point	^oC	8	-15-5.00	
Fire point	^oC	183	-	
Pour point	^oC	10	-15-10.00	
Peroxide value	meq/kg	16.08	-	
Refractive index	-	1.45	1.245-1.675	
Ph value	-	6.35	5.5-6.8	

4.2. Characterization of produced biodiesel and its blends

Biodiesel (FAME) yield obtained after the transesterfication process was found to be 98.53 % using optimal conditions of 6:1 methanol to oil molar ratio, 1.00 wt% NaOH reaction catalyst, 60 ^{o}C reaction temperature and 90 minute reaction time. According the data summarized in the table 2 the fuel properties of the biodiesel and its blends were discussed.

The kinematic viscosity value determined for the biodiesel at 40 ºC was (5.75) mm^2/s falls within the ASTM D445 specification standard. This value is in agreement with the international standard of China (1.9- 6.0); USA 1.9-6.0 and EU 3.5-5.0. The higher the viscosity the poorer the atomization of the fuel and operation of the injection would be less accurate. Furthermore, at decreased temperature viscosity of the biodiesel increases (Antony et al., 2011). The kinematic viscosity of the crude oil was 28.70mm^2/s and reduced to within the ASTM limits for biodiesel after transesterification and blending with diesel fuels. The transesterification process principally works to reduce viscosity of raw oil and the result was compared with raw oil which was much reduced but higher than the conventional diesel fuel. The viscosity of biodiesel is higher than that of diesel fuel because biodiesel have lubricating effect which reduces wears and tears of the engine. One of the reasons why biodiesel is used as an alternative fuel instead of pure vegetable oils or animal fats is as a result of its reduced viscosity which enhances fuel flow characteristics. In addition, kinematic viscosity is an important parameter regarding fuel atomization and combustion as well as fuel distribution. High viscosity affects the atomization of a fuel upon injection into the combustion chamber and thus leads to the formation of engine deposits. Kinematic viscosity is influenced by factors such us increase of fatty acid chain length, increasing degree of saturation or oxygenated moiety, position, number, and nature of double bonds in the fatty acid methyl ester (Knothe *et al*., 2003). Generally, the hydrocarbons in petrodiesel exhibit lower viscosity in a narrower range than the fatty esters comprising biodiesel and related fatty compounds with dibenzothiophene affecting viscosity of a low-viscosity solvent less than a long chain hydrocarbon and fatty acid esters.

The Specific gravity and densities at 30 ºC of the produced biodiesel under study were 0.880 and 0.890 g/cm^3, respectively. The results were in agreement with the ASTM D1298 and ASTM D287 standard for biodiesel and diesel fuels, respectively. The higher the mass of the biodiesel fuel, the higher the energy for work output per unit volume (Nurugesan and Neluzchezhain, 2009). Biodiesel is slightly heavier than conventional diesel fuel but it is lighter in comparison to density of raw oils.

Refractive index of the produced biodiesels at 30ºC was 1.42 and is also in agreement with the ASTM D1500 specification standard. The refractive index was decreased tremendously after transesterification reaction as compared to that of their raw oils, which indicates that heavier molecules have been converted to lighter ones, enabling interface detection, enhancing lubricity and increasing functionality of the fuel oil (Antony *et al*., 2011). Refractive index and refractive dispersion can be used in conjunction with other properties to characterize fuels.

The acid value (mg KOH/g) of the methyl esters was 0.68 mg KOH/g. This was in agreement to the ASTM D664 specification standard (0.5-0.8 max) mg KOH/g. This lower acid value of the biodiesel may be due to the absence of air and water contact with the biodiesel fuel. A lower acid value will also decrease % FFA which helps to protect the corrosion of engine parts as the amount of water is decreases in the fuel. Biodiesel with high acid value can cause corrosion of oil tank and other relevant component.

The cloud point and pour point of the produced biodiesel was -6.5ºC and -13 ºC, respectively and this was in agreement with the ASTM D2500 specification standards. This is an indication that the fuel will perform satisfactorily in both tropical and cold climatic conditions (Raheman and Phadatare, 2004). Biodiesel has a higher cloud points and pour points compared to the existing diesel fuels. These problems can be minimized by blending with diesel fuels. Cloud point and pour point have implications on the use of biodiesel in cold weather applications. The cloud point is the temperature at which wax first becomes visible when the fuel is cooled. The cloud point is the most common measure of the tendency of a fuel to crystallize. This signifies that *C. colocynthis* has a high tendency of forming cloudy crystals easily in cold temperature. Pour point measures the lowest temperature at which the biodiesel is observed to flow easily. It is an important property of biodiesel as it determines the lowest temperature at which the fuel can still be moved before it forms gels.

The flash point is the lowest temperature at which an applied ignition source will cause the vapours of the fuel to ignite. It is therefore a measure of tendency of a sample to form a flammable mixture with air. The flash point and fire points of the produced methyl esters were 141ºC and 175^{o}C, respectively, but the flash point result was out of the ASTM D93 standard (90 -130ºC). The flash point of biodiesel is generally higher than those of petrol diesel fuels. Biodiesel flash points higher than 90^{o}C are safer than diesel in terms of storage and transportation from the standpoint of fire hazard. Flash point reduces after transesterification but decreases with increasing amount of diesel in the blend due to the diluting effects of diesel that has lower flash point (Ertan and Canakci, 2009. The flash point of raw oil decreases after transesterification processes that show improvement in its volatile characteristics. The fire points of the produced biodiesel value were lower than that reported for jatropha biodiesel having a fire point of up to 296ºC (Ullman *et al*., 1990). The fire point is the lowest temperature at which a fuel will sustain burning for 5 seconds.

The distillation temperature of the raw oil and the produced biodiesel (B100) at 60^{o}C were determined to be 296 ^{o}C and 293^{o}C, respectively, which differs by 3°C. However; the temperature was reduced to 275 °C, 270 °C and 267^{o}C for B20, B10 and B5, respectively, because diesel fuels contain much heavier components. The calorific value of biodiesel was 70 MJ/L which indicates the energy available in it was very lower in comparisons with conventional diesel fuels. This lower energy content of biodiesel leads to higher consumption of biodiesel in order to achieve yield of diesel in the engine (Da-Silva, 2013).

The saponification value was increased from195.00 mg KOH/g to 202.30 mg KOH/g after transesterification because of the effect of alkaline catalyst used, but reduced with blending as diesel does not contain saponifying catalyst. Peroxide value increased after transesterification and decreased with increasing amount of diesel in the blending. Iodine value of the raw oil was 102 g I_2/100g of which triglyceride was the major component and the value was decreased after transesterification with increasing proportion of diesel.

Biodiesel is also potentially subject to hydrolytic degradation caused by the presence of water. Fuel contaminated with water can cause engine corrosion and breakdown. From the results obtained, bitter apple has traces of bottom sediment and water with 0.04%vol values conforms to the ASTM D 6751 set of standard whose maximum allowable limit is 0.05%vol. Since *C. colocynthis* fatty acid methyl ester has a legible amount of bottom water and sediment, it has been taken as better quality of fuel and acknowledged as great advantage over fossil diesel.

Table 2: Comparison of physiochemical properties of *C. colocynthis* biodiesel, blends and diesel with ASTM D6751 standard values.

Property	**Unit**	**ASTM** Test Method	**Expt.** Value of Biodiesel	**Expt. Value of blends**			Expt. Value of petro diesel	**ASTM D 6751 Standard for Biodiesel**	**Remark**
			B100	B20	B10	B5			
Yield	%	-	98.53	-	-	-		-	
FAME									
Density	g/cm^3	ASTM D1298	0.890	0.850	0.842	0.830	0.832	0.860 – 0.890	
Specific	-	ASTM D287	0.880	0.863	0.845	0.834	0.825	0.860 – 0.890	
Gravity									
Kinematic	mm^2/s	ASTM D445	5.75	3.420	2.840	1.760	1.320	1.900 -6.00	
Viscosity									
at 40oC									
Acid value	mgKOH/	ASTM D664	0.68	0.57	0.39	0.26	0.189	0.50 -0.80 max	
	g								
Water	%	ASTM	0.04					0.05 max	
content									
Flash Point	^oC	ASTM D93	141	96	89	83	67	130 -170.00max	
Cloud	^oC	ASTM D2500	-6.5	-	-	-	-		
Point								-3.00 to 12.00	
Pour point	^oC	ASTM D97	-13	-	-	-	-	-15 00 to 10.00	
Fire point	^oC	ASTM D 445	175	-	-	-	78		
Caloric	MJ/L	ASTM D6751	70	216	254	295	310	-	
value									
Refractive		ASTM D1500	1.42	1.46	1.48	1.51	1.53	1.33-1.55	
index									
Iodine value	$gI_2/100g$	ASTM D2075	96.02	91.07	88.26	80.40	-	-	
Peroxide	meq/kg		17.24	15.80	14.35	13.42	-	-	
value									
Saponificati	mgKOH/g		202.30	165.49	162.60	154.20	-	-	
on value									
Distillation	^oC	ASTM D86	293	275	270	267	260	-	
temperature		ASTM D1160							

4.3. Optimization of Parameters

Transesterification is the reaction between triglycerides and methanol to produce fatty acid methyl ester (FAME) and free glycerol as byproducts. It involves reaction between the oil which is the feedstock and an alcohol, usually methanol in the presence of a basic catalyst sodium hydroxide to give corresponding methyl esters. The main factors influencing the transesterification reactions are the methanol to oil molar ratio, catalyst concentration, reaction temperature and reaction time, more amount of methanol to oil ratio which were used to drive the equilibrium to a maximum fatty acid methyl ester yields.

Results obtained on the effect of temperature on the yield of the produced biodiesel from *C. colocynthis* oil as the feed stock were presented in Table 3. The maximum FAME yields were obtained at the temperature of 60^oC with the percentage of 98.53%. This is because the reaction temperature at 60^oC, the molecules of the triglycerides of *C. colocynthis* had high kinetic energy and this thus increased the collision rate and therefore improved the overall process by favouring the formation of biodiesel while at the lower temperatures of 50^oC and 55^oC with corresponding percentage yielding 92.08% and 96.85%, respectively at 6:1 methanol to oil molar ratio, there was lesser collision of reacting molecules and thus, reduced biodiesel yield as reported in Table 3. The higher reaction temperature would favour endothermic reaction, thus increasing the rate of reaction as well as the methyl ester concentration. However; at 65^oC, it was noticed that there was a drop in percentage of methyl ester yield which is because at the reaction temperature (65^oC), there was increased vaporization of the methanol used in the transesterification process due to the proximity of the reaction temperature (65^oC) to the boiling point of methanol (64.7^oC). This is probably due to the fact that methanol boils at this temperature which probably led to evaporation and subsequent loss of the solvent, thus lowering the conversion of oil to biodiesel.

Based on the investigated results on the effect of reaction temperature on the yield of biodiesel production, it can be realized that the optimum conditions for the production of fatty acid methyl ester from crude oil of *C. colocynthis* through two step transesterification process are methanol to oil molar ratio of 6:1, catalyst concentration of 1.00wt%, reaction time of 90 minutes and reaction temperature at 60^oC.

According the data reported in table 3, biodiesel yield with respect to time for 3:1, 4.5:1 and 6:1 methanol to oil molar ratio at different percentage of catalyst NaOH and reaction temperature were increased. It has been observed that there is higher yield in case of 6:1 molar ratio as compared to 3:1 and 4.5:1 molar ratio. It may be because of the amount of methanol using for 6:1 molar ratio was more than that of 3:1 and 4.5:1 molar ratio. In all these reactions fatty acid methyl esters were produced. The stoichiometric relation between methanol and the oil was 3:1. However, an excess of methanol is usually more appropriate to improve the reaction towards the desired product side.

The yield of biodiesel for 0.5% NaOH is less as compared to 0.75% and 1% NaOH. It may be because of 0.5% NaOH is not sufficient to enhance the reaction rate. The maximum yield of biodiesel produced at the highest catalytic reaction conditions was 98.53 for 6:1 molar ratio, at 60^{o}C reaction temperature and 90 minutes reaction time. It can be seen that the conversion of *C. colocynthis* oil to biodiesel increased steadily in the first 90 minutes. The reaction temperature, catalyst, and molar ratio of methanol to oil at fixed reaction time showed a linear relationship.

4.3.1. Effect of methanol to oil molar ratio

In transesterification process, excess amount of catalyst produces excess soap over time and gives lower yield. For this reason, a fixed amount of catalyst and reaction temperature was used, while the methanol to oil molar ratio was varied from 3:1 to 6:1 and reaction time was also varied from 30 to 90 minutes. The yield of fatty acid methyl acid was increased linearly as molar ratio and reaction time was increased dramatically. The maximum fatty acid methyl ester yield (98.53%) was obtained at 90 minutes and 60^{o}C reaction time and temperature, respectively.

4.3.2. Effect of reaction temperature

The effect of reaction temperature on the conversion of *C. colocynthis* crude oil to biodiesel was studied by varying from 50 to 60^{o}C with 5^{o}C difference in each reactions process. The yield of the produced biodiesel was increased linearly as reaction temperature was increased with constant difference. Maximum yield of biodiesel (98.53%) was produced at the reaction temperature of 60^{o}C. At a fixed time the reaction temperature had a linear relationship with methanol to oil molar ratio and reaction catalyst.

4.3.3. Effect of catalyst concentration

The effect of catalyst on yield of fatty acid methyl ester was studied with catalyst varying from 0.5% to 1.00% NaOH with 0.25% difference in each batch of reactions by considering the weight of the crude oils. The yield of fatty acid methyl ester was increased on the increase of catalyst concentration and maximum results were achieved 98.53% at 1.00% catalyst concentration. The reaction catalyst had showed a linear relationship with reaction temperature and methanol to oil molar ratio at fixed reaction time.

4.3.4. Effect of reaction time

The effect of the reaction time on *C. colocynthis* oil conversion to fatty acid methyl ester was conducted. The reaction time was varied from 30 to 90 minutes with 15 minutes difference for each transesterfication reaction process. The yield of fatty acid methyl ester was increased as reaction time was increased and maximum yield of 98.53% were obtained at 90 minuet reaction times. The reaction time had showed a linear relationship with methanol to oil molar ratio at fixed reaction temperature and catalyst.

Table 3 Yield (%) of biodiesel and time for different molar ratio, temperature and catalyst concentration

Temperature (^oC)	%catalyst (NaOH)	Molar ratio 3:1		Molar ratio 4.5:1		Molar ratio 6:1	
		Tim(min)	Yield (%)	Tim(min)	Yield (%)	Tim(min)	Yield (%)
50	0.5	30	79.64	30	81.13	30	84.35
		45	80.38	45	82.18	45	85.46
		60	82.73	60	85.23	60	88.70
		75	84.14	75	87.44	75	90.05
		90	86.21	90	88.98	90	92.08
55	0.75	30	82.08	30	85.34	30	88.37
		45	84.26	45	87.46	45	91.25
		60	86.79	60	89.18	60	93.27
		75	88.42	75	91.29	75	95.68
		90	90.13	90	93.74	90	96.85
60	1	30	85.25	30	87.82	30	91.09
		45	87.15	45	89.53	45	94.16
		60	89.27	60	91.37	60	96.85
		75	91.06	75	93.41	75	97.42
		90	93.48	90	95.65	90	98.53

4.4. Fatty acid methyl ester composition

Fatty acid methyl ester composition of *C. colocynthis* oil was determined using gas chromatography (GC) analyzer equipped with a flame ionization detector (FID). The sample of *C. colocynthis* seeds oil was esterified to bring them into a vaporous phase, transforming the fatty acid into fatty acid methyl esters using alkaline catalyzed methanolysis process. Accordingly, the GC spectrum thirteen fatty acid methyl esters were determined in the esterified oil and its components were presented in Table 4. As per the reported data in Table 4, the major component of oil was linoleate (C18:2; 60.34) followed by oleate (C18:1; 12.46), palmitate (C16:0; 10.59), stearate (C18:0; 8.73), linolenate (C18:3; 1.76) and myristate (C14:0; 1.42) methyl esters and other trace amount of fatty acid methyl esters were also determined.

The fatty acid profile of biodiesel has strong influence on its physicochemical properties such as iodine value, saponification value, viscosity and cloud point (Ramadhas et al*.,* 2009). As linoleic acid is the dominant component of the fatty acid profiles, the property of the biodiesel is mainly dictated by this chemical. Blending biodiesel with the conventional diesel fuels has very little effect on the proportion of the fatty acids presented in the oil. The fatty acid composition of the raw materials employed in transesterification process dictates, to a large extent, the fuel properties of biodiesel produced. Different feedstocks of seed oils utilized in biodiesel production certainly have different chemical compositions and undeniably different fuel properties.

Table 4 fatty acid methyl ester profile of *C. colocynthis* seed oil

S.NO	Methyl esters	Equivalent Chain length	Retention Time	Composition (%)	remark
1	Linoleate	C18:2	21.340	60.34	
2	Oleate	C18:1	21.123	12.46	
3	Palmitate	C16:0	17.135	10.59	
4	Stearate	C18:0	25.642	8.73	
5	Linolenate	C18:3	29.237	1.76	
7	Myristate	C14:0	11.976	1.42	
7	Arachidate	C20:1	25.735	0.98	
8	Behenate	C22:0	26.163	0.94	
9	Palmitoleate	C16:1	30.673	0.87	
10	Docosatrienoate	C22:3	21.839	0.54	
11	Eicosadienoate	C20:2	25.524	0.09	
12	Lignocerate	C24:0	34.161	0.06	
13	Nervnoate	C24:1	34.328	0.07	
14	Total saturated FAME composition (%)		21.74		
15	Total unsaturated FAME composition (%)		77.11		
16	Total FAME composition (%)		98.85		

5. Conclusion and Recommendation

World biodiesel production is on the increase due to rising crude oil prices, decreasing fossil fuel reserves and environmental concerns. Ethiopia wants to join global biodiesel producers as it would decrease its dependence on fossil fuels and imported oil thereby promote renewable energy which would eventually decrease pollution and help to halve unemployment rate and poverty of the nation. Biodiesel produced form vegetable oil is seen as the ideal replacement for fossil diesel in Ethiopia.

The current research focused on the production of biodiesel from non-edible oil as alternative energy that will replace the existing conventional diesel fuels. The production of biodiesel from edible vegetable oil is not economically feasible due to the fact that it will lead to food crisis while trying to solve energy problems. Hence, it is more desirable to use non-edible oils as a feedstock in the production of biodiesel, which is the focus of this study. Based on the results of physicochemical analysis of the crude seed oil, it can be concluded that it is suitable feed stock for the production of biodiesel. The raw oil was transesterified using methanol in the presence of sodium methoxide to produce 98.53% biodiesel (FAME) at 6:1 methanol to oil molar ratio, 60^oC temperature, 90 minute time and 1.00% NaOH catalyst reaction conditions. The properties of the biodiesel produced were tested and compared with conventional diesel fuel and found to be in confinement with the ASTM-D6751 specification standard for biodiesel fuels. *C. colocynthis* raw oil and its biodiesel confirm that the biodiesel promotes clean and environmental friendly, nontoxic, biodegradable and renewable bio-energy alternative fuels.

The physico-chemical properties of the biodiesel are similar to those of diesel fuels thus confirming that it can be used in diesel engine with little modification or blending with conventional diesel fuels. So, blends are have the potential to reduce the over burden of the imports of diesel fuel and the properties of blending tend toward those of diesel as the proportion of diesel increases. Bio-diesel is found to be better substitute for petroleum diesel and also most advantageous over petro-diesel for its being environmentally friendly. According the GC chromatogram fatty acid methyl ester composition bitter apple was found to be synonymous in chemical constituents with those of sunflower, groundnut, and soybean biodiesel which have been well established and widely published in international journals.

According to the findings of the current study, the following recommendations are made:

- The Ethiopia government must realize its role in the development of a biodiesel industry in the country and not be too ignorant to learn from countries with an already developed biodiesel industries.
- When it comes to biodiesel, commercial production does not necessarily mean a few hugely centralized plants; one must not forget the small producers which could at the end of the day also contribute quite a bit to the production of renewable energy.
- The by-product of transesterification reaction, glycerol, when purified can be used in its traditional application (pharmaceutical, cosmetics and food industries) and can also be used in recently developed applications in the fields, polymers, surfactants and lubricants. It is recommended producing glycerol with the purity of up to 99.5–99.7% using vacuum distillation or ion exchange refining processes is viable.

References

A.O.A.C., (1990). Official Methods of Analysis of the Association of Official Analytical Chemists, 14^{th} edn. Washington, D.C, USA

Abdulkareem, A.S., Uthman, H., Afolabi, A.S., Awonebe, O.L., (2011). Extraction and Optimization of Oil from Moringa Oleifera Seed as an Alternative Feedstock for the production of Biodeisel. Majid N, Mostafa K, editors. Sustainable Growth and Application in Renewable Energy Sources. InTech, 243-268.

Abdulkareem, A.S., Odigure J.O., Kuranga, M.B., (2010). Production and Characterization of Bio-Fuel from Coconut oil. Journal of Energy Source Part A, **32**: 106-114.

Adam, F.L, James, A.B., Jinesh, C.M and Karen, W., (2014). Heterogeneous catalysis for sustainable biodiesel production via esterification and transesterification. Chem. Soc. Rev**., 43**:7887.

Agarwal, A.K., Das L.M. (2001). Biodiesel development and characterization for use as a fuel in compression ignition engines. Journal of Engineering for Gas, Turbines and Power, **123**: 440-447.

Alam, I., Sharing, S.A., Mondal S.C., Alam, J., Khalekuzza-man, M., Anisuzzaman M., Alam, M.F. (2010). *In vitro* micro propagation through cotyledonary node culture culture of castor bean (*Ricinus Communis* L.). Aust. J. Crop Sci, **4**:81-84.

Amoo, I.A., Eleyinmi, A.F., Ilelaboye, N.O.A., Akoja, S.S. (2004). Characterization of oil extracted from Gourd *(Cucurbita maxima)* seed. Journal of Food, Agriculture and Environment, **2**:38-39.

Hsu, A.F. (2002). Immobilization lipase catalyzed production of alkyl esters of restaurant grease as biodiesel. **77(4)**:181-186.

Raja, S.A., Smart, D.S.R., Lindon, C., Lee, R. (2011). Biodiesel production from jatropha oil and its characterization. Research journal of chemical societies, **1(1)**:81-87.

Armstrong, W.P., (1982). Not Beavers, Stars or Sons of Jupiter. Environment Southwest, 496:47.

ASTM, (2008). Standard specification for biodiesel fuel (B-100) blend stock for distillate fuels. In: Annual Book of ASTM Standards, ASTM International, West Conshohocken, and Method D6751-08.

Berman, P., Nisri S., Wiesman, Z. (2011). Castor oil biodiesel and its blends as alternative fuel. Biomass Bioenergy, **35**:2861-2866.

Bossio, D., Erkossa T., Dile, Y., McCartney, M., Killiches, F., Hoff, H. (2012). Water Implications of Foreign Direct Investment in Ethiopia's Agricultural Sector. Journal of Water Alternatives, **5(2)**:223-242.

Canoira, L., Galean, J.G., Alcantara, R., Lapuerta, M., Contreras, R.Y. (2010). Fatty acid methyl esters (FAMEs) from castor oil: Production process assessment and synergistic effects in its properties. Renewable Energy, **35**:208-217.

Carraretto, C., Macor A., Mirandola, A. (2004). Biodiesel as alternative fuel: Experimental analysis and energetic evaluation. Journal of Energy, **29**:2195-2211.

Comar, V., Tilley, D., Felix, E., Turdera, M., Neto, M.C. (2004). Comparative emergy evaluation of castorbean (*Ricinus Communis*) production systems in Brazil and the U.S. Proceedings of IV Biennial International Workshop. Advances in Energy Studies, 227-237.

Conceicao, M.M., Candeia, R.A., Silver, F.C., Bezerra, A.F., Fernandes, V.J., Souza, A.G. (2007). Thermochemical Characterization of Castor Oil Biodiesel Renewable and Sustainable Energy Reviews. **11(5)**: 964-975.

Da-Silva, MLCP. (2013). Relationship between cetane no and calorific value for biodiesel from viscera oil blends with mineral diesel. International conference on renewable energies and power quality (ICREPQ'13).

Demirbas, A. (2003). Biodiesel fuels from vegetable oils via catalytic and non-catalytic supercritical alcohol transesterifications and other methods: a survey. Energy Conversion and Management, **44**: 2093-2109.

Deore A.J. and Johnson, T.S. (2008). High frequency plant regeneration from leaf-disc cultures of *Jatropha curcas* L.: an important bio-diesel crop. Plant Biotechnol Rep, **2**:7-11.

Drown, D.C., Harper, K., Frame, E. (2001). Screening vegetable oil alcohol esters as fuel lubricity enhancers. J. Am. Oil Chem. Soc. 78:579-584.

Ellis, F. and Freeman, H.A. (2004). Rural Livelihood and Poverty Reduction Strategies in Four African Countries. The Journal of Development Studies, **40(4)**:1-30

Ertan, A., Canakci, M. (2009). Characterization of key fuel properties of methyl ester Diesel Blends. Fue.l, **88(1)**:75-80.

Fakhri, N. A. and Qadir, H. K. (2011). Studies on Various Physico-Chemical Characteristics of Some Vegetable Oils. Journal of Environmental Science and Engineering, **5**:844-849.

German, L., Schoneveld, G.C., Gumbo, D. (2011). The local social and environmental impacts of smallholder-based biofuel investments in Zambia. Ecology and Society, **16(4)**:12.

Hemant, Y.S., Panwar, N.L., Bamniya, B.R. (2011). Bio Diesel from Castor Oil - A Green Energy Option. Low Carbon Economy. **2**:1-6

Ibrahim, H., Ahmed, A.S., Bugaje, I.M., Mohammed-Dab, Ugwumma, C.D. (2013). Synthesis of Bulk Calcium Oxide (Cao) Catalyst and its Efficacy for Biodiesel Production.

Journal of Energy Technologies and Policy, **3**:12.

Indra, R., Bachheti R.K., Archana, J. (2013). Chemical composition, mineral and nutritional value of wild *Bischofia javanica* seed. International Food Research Journal, **20(4)**:1747-1751.

Isah, A.G. (2006). Production of Detergent from Castor Oil. Leonardo Electronic Journal of Practices and Technologies, **9**:153-160.

Janaun, J., and Ellis, N. (2010). Perspectives on Biodiesel as a Sustainable Fuel. Renewable and Sustainable Energy Reviews, **14(4)**:1312-1320.

Kang, H.Y., Matsushima, N., Sameshima, K., Takamura, N. (1990). Termite resistance tests of hardwoods of Kochi growth: The strong termiticidal activity of Kagonoki (*Litsea coreana* Léveillé). Mokuzai Gakkaishi, **36**:78-84.

Knothe, G., Matheaus, A.C., Ryan, T.W. (2003). Cetane numbers of branched and straight-chain fatty esters determined in an ignition quality tester. Fuel, **82**:971–975.

Kyari, M. Z. (2008). Extraction and characterization of seed oils. International Agrophysics, **22**:139-142.

Kumar, A. and Sharma, S. (2011). Potential non-edible oil resources as biodiesel feedstock: an Indian perspective. Renewable and Sustainable Energy Reviews, **15:(4)**:1791-1800.

Lapuerta, M., Armas, O., Rodriguez-Fernandez, J. (2008). Effect of biodiesel fuels on diesel emissions. Progress in Energy and Combustion Science, 34: 198-223.

Leung, D.Y.C and Guo, Y. (2006). Transesterification of neat and used frying oil: Optimization for biodiesel production. Fuel Process Technology, **87(10)**:883–890.

Luque, R., Herrero-Davila, L., Campelo, J. M., Clark, J. H., Hidalgo, J. M., Luna, D., Marinasa, J. M., Romero, A. A. (2008): Energy and Environmental Science, **1**:542-564.

Ma. F., Hanna M.A., (1999). Biodiesel production: A review. Bioresource Technology. **70**:1-15.

Meher, L.C., Dharmagadda V.S.S., Naik S.N., (2006). Optimization of alkali-catalyzed transesterification of Pongamia pinnata oil for production of biodiesel. Bioresource Technology. **97(12)**:1392-1397.

Ministry of Mines and Energy of Ethiopia report, (2007), Addis Ababa, **2**:1-54.

MoME, (2007). Biofuel development and utilization strategy. Addis Ababa: The Federal Democratic Republic of Ethiopia.

Mulugetta, Y., (2008). Evaluating the economics of biodiesel in Africa. Renew Sust Energy Rev. **13**:1592-15989.

Mutlu, H., Meier M.A.R., (2010). Castor oil as a renewable resource for the chemical industry. Eur. J. Lipid Sci. Technol., **112**:10-30.

Naik, M., Meher L.C., Naik S.N., Das L.M., (2008). Production of biodiesel from high free fatty acid Karanja (*Pongamia pinnata*) oil. Biomass and Bio energy, **32**:354-357.

Nangbes, J.G., Nvau J.B., Buba W.M., Zukdimma A.N., (2013). Extraction and characterization of castor (*Ricinus Communis*) seed oil. The International Journal of Engineering and Science. **2(9)**:105-109.

Nurugesan, A., Neluzchezhain N., (2009). Production and analysis of biodiesel from non edible oils. A review and sustainable energy review. **13**:825-834.

Oduvaldo, C.M.P.J., (2007). Comparison between Polyurethanes Containing Castor Oil (Soft Segment and Cancellous Bone Autograft in the Treatment of Segmental Bone Defect Induced in Rabbits". J. Biomater. Appl. 21(3): 283-297.

Ogunniyi, D.S., (2006). Castor oil: A vital industrial raw material. Bioresour. Technol. 97(9): 1086-1091.

Oyeleke, G.O., Afolabi O., Olayiwola O.A., Adetoro R.O., (2012a). Oil quality characteristics and effects of temperature variations on some functional properties of horse eye *(Dioclea reflexa)* seed flour. Journal of Environmental Science, Toxicology and Food Technology. **2(2):**38-42.

Panwar, N.L., Shrirame H.Y., Bamniya B.R., (2010). CO_2 Mitigation Potential from Biodiesel of Castor Seed Oil in Indian Context. Clean Technologies and Environmental Policy. **12(5)**: 579-582.

Panwar, N.L., Shrirame Y.H., Rathore N.S., Jindal S., Kurchania A.K., (2010). Performance Evaluation of a Die-sel Engine Fueled with Methyl Ester of Castor Seed Oil. Applied Thermal Engineering. **30(2-3)**:245-249.

Phillips, R.M., 1999. Annuals and Biennials. London: Macmillan. ISBN 0-333-74889-1 p. 106. pp.1097-1107.

Raheman, H.A.G., 2004. Phadatare Diesel engine emissions and performance from blends of Karanja methyl ester and diesel. Biomass and Bio energy. **27**: 393-397.

Rahmato, D., (2011). Land to Investors: Large-scale land transfer in Ethiopia. Forum of Social Studies (FSS) Addis Ababa, FSS Policy Debate Series No.1.

Ramadhas, A.S, Jayaraj S., Muraleedharan C., (2009). Biodiesel Production from High FFA Rubber Seed Oil. Fuel. 84(4):335-340.

Ramesh., D., Sampatraja, A., (2008). Investigation on Performance and emission characteristics of diesel engine with jatropha biodiesel and its blends. Agric Engg Int: CIGR e-J Sci Res Develop, 10, EE 07 01.

Rao, G., Lakshmi N., Sampath S., Rajagopal K., (2008). Experimental Studies on the Combustion and Emission Characteristics of a Diesel Engine Fuelled with Used Cooking Oil Methyl Ester and its Diesel Blends. International Journal of Engineering and Applied Sciences, **4**:64-70.

Rashid, U., Anwar F., Moser., B.R, Knothe G., (2008). Moringa oleifera oil: a possible source of biodiesel. Bioresource Technology. **99 (17)**:8175-9.

Refaat, A.A., (2009). Correlation between the chemical structure of biodiesel and its physical properties. Int. J. Environ. Sci. Technol. 6: 677-694.

Schut, M., Slingerland M., Locke A., (2010). Biofuels development in Mozambique. Update and analysis of policy, potential and reality. Journal of Energy Policy. **38**:5151-5156.

Sharma, Y.C., Singh B., (2008). Development of biodiesel from karanja, a tree found in rural India. Journal of Fuel. **87**:1740-1742.

Srivastava, A., Prasad R., (2000). Triglycerides-based diesel fuel. Renewable and ustainable. Oil Energy Review. **4**:111-133.

Stidham, W.D, Saeman D.W., Danzer M.F., (2007). Method for preparing a lower alkyl fatty ester product from vegetable oil. US patent No. 6. 127:560.

Taufiq-Yap, Y.H., Lee, H.V.R. Yunus and Juan, J. C. (2011). Transesterification of non-edible Jatropha curcas oil to biodiesel using binary Ca-Mg mixed oxide catalyst: effect of stoichiometric composition. Chemical Engineering Journal. **178**:342–347.

Ullman, T., Mason R., Montalvo D., (1990). Study of cetane number and aromatic content effects on regulated Emission from a heavy-duty engine. Southwest Research Institute Report 08-2940.

Van Gerpen, J., (2005). Biodiesel processing and production. Fuel Processing Technology. 86(10):1097-1107.

Van Gerpen, J.H., Shanks B., Pruszko R., (2004). Biodiesel Analytical Method". Iowa State University. National Renewable Energy Laboratory. **42(5)**:3041-3393.

Warra, A.A., Wawata I.G., Gunu S.Y., Aujara K.M., (2011). Extraction and Physicochemical Analysis of Some Selected Northern Nigerian Industrial Oils. Archives of Applied Science Research, **3**:536-554.

Weiss, E.A., (1971). Castor, Sesame and Sunflower. Leonard Hill, London, pp. 235.

Wilson, R., Van Schie B.J., Howes D., (1998). "Overvew of the preparation, use and biological studies on polyglycerol polyrecinoleate. Food Chem. Toxicol., **36(9-10)**: 711-8.

World Bank, (2010). Rising Global Interest in Farmland. Can it Yield Sustainable and Equitable Benefit. The World Bank.

Yahaya, A. T., Taiwo O., Shittu T.R., Yahaya L.E., Jayeola C.O., (2012). Investment in cashew kernel oil production; Cost and return analysis of three processing methods. American Journal of Economics, **2(3)**:45-49.

Zhang, Y.,. Dubé M. A, McLean D. D., Kates M., (2003). Biodiesel production from waste cooking oil: Process design and technological assessment. Bioresource Technology. **89(1)**:1-16.