



Functional groups determination and the production of biodiesel from *Garcinia Kola* seeds using trans-esterification reaction

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Abstract

Plants are important in our everyday existence. They provide our foods, produce the oxygen we breathe, and serve as raw materials for many industrial products such as clothes, foot wears and so many others. Plants also provide raw materials for our buildings and in the manufacture of biofuels, dyes, perfumes, pesticides and drugs. In this research, *Garcinia kola* seeds were purchased locally and the seeds were deshelled, washed and allowed to dry. The extraction of oil was done using soxhlet extraction technique (hot method). After the extraction, Biodiesel was then produced from the oil using the Trans-esterification process. The already produced Oil and biodiesel were characterized for its physicochemical properties and the result compared to standards. The bitter kola biodiesel was also exposed to FTIR analysis to determine the functional groups and organic compounds inherent in the biodiesel produced. From the experimental result obtained, the produced were found to meet the ASTM standards for biodiesel. The results obtained from analysis of biodiesel from *Garciniakola* include free fatty acid: 0.822%; acid value: 1.635mgKOH/g; specific gravity: 0.9; kinematic velocity: 1.714Cst; flash point: 45°C; pour point: 93°C; water content: 5.75%. Thus, the values obtained met with the ASTM standard. The percentage yield of oil from the Bitter kola seed is 34% and then the percentage yield from the bitter kola oil to the biodiesel is 62%. This shows that the poor yield of biodiesel from the seed would affect its usage as a Biofuel and this makes it not suitable to be used as a Biofuel. This research has shown that *Garcinia kola* seeds and oils are not good sources of Biodiesel. Therefore research should be on and going into more cheap and available materials in the production of biodiesel.

Keywords: *Garcinia Kola*, Biodiesel, Trans-esterification, percentage yield, physiochemical.

Introduction

The use of plants in traditional medical practice has a long drawn history, and remains the mainstay of primary health care in most of the third world. Traditional medicines are used by about 60% of the world population; in both developing and developed countries where modern medicines are predominantly used¹. While an estimated 60-80% Africa's population depends solely on herbal remedies for its primary health care needs. In diversity, plants are thought to be between 250,000 to 400,000 species spread across all continents from the Antarctic to the Arctic. They thrive in all in all environments from the flooded planes to the deserts, and from those who live on the seas and oceans to others that thrive on fresh water and ponds. For classification and easy identifications, plants were divided into different taxonomical groups known as kingdoms; these are further streamlined into phylum, class, order, family genus and species. Within the family of the *Clusiaceae* is found an amazing plant called the bitter kola (*Garcinia kola*)^{2,3}.

Kola cola is common name for a genus of about 125 species of evergreen trees (trees that certain foliage throughout the year). It is a native to tropical areas of the world. Kola trees are best known for their seeds or nuts which are rich in caffeine and used

in the manufacturing of carbonated soft drinks known as kola beverages. Kola trees belong to the cacao family *sterculiaceae*⁴. It is a tropical tree which is commonly known for the seeds containing caffeine called Bitter kolas. Bitter kola also scientifically called *Cola nitida*, is the plant species that belongs to *Malvaceae* family. It is also called Bitter kola, bitter kola, kola, cola, Gbanja kola and Bissy Nuts. Though being a lowland tree, it is available up to the altitudes of 300 metres (980ft) in the areas having deep as well as rich soils with evenly distributed rainfall. It is suitable for sandy, loamy or clay soils but does its best in well-drained soil. The tree could reach for about 3 meters for 4 years having the slow initial growth. When the trees reach 12 to 15 years of age, it could produce about 10 to 16 kilos of seeds annually for above 80 years. This plant is also cultivated in India, Brazil, Jamaica and Hawaii⁵.

The main species grown for their seed production are classified as *Kola nitida* and *Kola acuminata*. They are classified into these groups on the basis of the amount of cotyledons they have: *Kola nitida* is dicotyledonous while *Kola acuminata* has more than two cotyledons. These are two varieties of *Kola nitida* which are rubra and alba⁶.

Bitter kolas have been used since ancient origins as a tea leaf or

coffee berry. West African cultures use it to chew for the restoration of vitality in a social setting or individually. These nuts have become a crucial part of traditional practice of religion and culture in Nigeria, Niger, Liberia and Sierra Leone. In capital of Niger during 1970, "Goro City" highlights the importance of Bitter kolas by Manu Hibango. It was used as a religious object or as an offering during ancestor veneration, prayers, funerals, weddings and naming ceremonies⁷.



Figure-1: Bitter kola seeds.

Garcinia kola is a medicinal plant grown in tropical rainforest in West Africa. The height of the plant is approximately 14m and it produces a reddish yellowish or orange colour fruits containing 2-4 seeds. *Garcinia kola* is endemic in the humid rain forest vegetation in the coastal areas and lowland plains up to 300m above sea level, average of 2500mm of rainfall per annum. Extract from the bark of the plant are used in traditional medicine for treatment of liver cirrhosis and hepatitis. *Garcinia kola* is a fruit from one of a family of many handsome, tropical evergreen trees and shrubs called mangosteens that are native to India and Southern Asia, southern Africa and Polynesia. The tree bears deep green, glossy, yellowish, pumpkin shaped fruit with a sour taste, primarily because of their high hydroxycitric acid content and they may contain thirty percent⁸. The tree, which may thrive in poor soils, has been known in Asia for many reasons. It produces brownish-yellowish gum resin (xanthone) that is used commercially as a pigment, and it has also had some value in the timber industry. The fruit has also been used in Indian cuisines to flavor curries, preserve fish and as a condiment⁹.

It has also occupied a place in an ancient Indian Ayurveda as a purgative and as an aid that activates digestion. The last property created interest in the herb, and in 1965, researchers identified a compound called hydroxycitric acid that had a chemical structure similar to that of citric fruits which may be of great value in weight loss programs and energy boosting regimens. Although most of the research into herb has been conducted in the laboratories, with no conclusive clinical trials to prove the herb efficacy, continuing test may hopefully reveal positive evidence. In Japan, *Garcinia* has been used to decrease

body fat for years. Some of the constituent induced in *Garcinia* are the all-important hydroxycitric acid, phenol, acetic acid, calcium, tartaric acid, succinic acid and carbohydrate. *Garcinia kola* is growing in popularity as a natural and effective way to help in weight loss programs. *Garcinia kola* is a medium sized forest tree found through west and central Africa. The seed are eaten as refresh past time in Nigeria and are known to contain high content of biflavonoid compound. *Garcinia kola* has been reported to contain a complex mixture of prephenylated benzophenones, xanthenes and biflavonoids. Antioxidants decline with age and such, requires nutritional supplements¹⁰.

This research was aimed at the extraction of oil from *Garcinia kola* through hot method and the production of biodiesel from the oil. The percentage yield of the biodiesel was calculated from the yield of oil produced as well as the properties of the biodiesel produced were compared with the ASTM standards¹¹.

Materials and methods

Sample collection: The Bitter kola seeds were purchased locally and the seeds were cleaned of adhering soil and unwanted materials were handpicked. The seeds were deshelled, washed and allowed to dry. The Bitter kolas were ground with a blender into fine particles and were ready for oil extraction.

Experimental Procedures: Extraction of Oil from the Bitter Kola Seed: The extraction of oil was done using soxhlet extraction technique (hot method) the powdered seeds (1800g) were packed into the extraction chamber and normal hexane poured into the round bottom flask of the soxhlet extractor. The mantle heater was set at about 64°C and the oil in the seeds was leached for 48 hours in each case until all the powdered seed was extracted. An exhaustive oil extraction was considered to be achieved when no more oil was obtained. The extracted oil was oven dried at 45°C for 48 hours. The seed oil was filtered through a filter paper to remove foreign particles. The pure oil was then preserved in cold storage.

Biodiesel Production (Trans-esterification): The oil produced was transferred to a round bottom flask and preheated to a temperature of about 60°C. Sodium hydroxide concentration of 0.3g was dissolved in 30ml of methanol of 3:1 ethanol oil mole ratio. The mixture of sodium hydroxide in methanol is added to the oil in the round bottom flask, while stirring. The mixture was allowed to stand for 1hr by maintaining a constant temperature to ensure homogeneity. After 1hr the mixture was poured in a separating funnel and was allowed to stand for 10mins to enable separation of glycerol from the biodiesel (Methyl ester). Two layers were observed of which the glycerol is the bottom layer while the diesel is the upper layer. The glycerol is discarded while the diesel was washed with hot water for about 5times to enable complete removal of the catalyst, unreacted methanol and soap. Then the washed biodiesel was heated in an oven to dry off the water in it at about 60°C temperature. The percentage conversion as calculated is

%conversion = volume of biodiesel ÷ volume of oil × 100

Physiochemical Properties of Fatty Methyl Esters (Biodiesel): Determination of Acid Value: The acid value was determined by American standard for testing material (ASTM) method. About 2g of the diesel produced was weighed into a conical flask and 25ml of methanol was added and 25ml of diethylether. The mixture was titrated against 0.1M of NaOH in the presence of 1ml of phenolphthalein indicator and a titre value was obtained. Then, the titre value obtained is used to calculate for both acid value and free fatty acid.

Observation: pink colouration was observed.

$$\text{Acid value} = \frac{\text{titre value} \times 5.61}{\text{wt of sample}}$$

$$\text{Free fatty acid} = \frac{\text{titre value} \times 0.0282}{\text{wt of sample}}$$

Determination of Specific Gravity: To determine the specific gravity of the biodiesel produced. A clean and dry cylinder of 25ml was weighed (w_0) and filled with water and reweighed (w_1). Thus, specific gravity is calculated as,

$$\frac{w_2 - w_0}{w_1 - w_0}$$

Where: W_2 = weight of cylinder + diesel, W_1 = weight of cylinder + water, W_0 = weight of empty cylinder.

Determination of Moisture Content: To determine the water content in the biodiesel produced from the *Garcinia kola* seed.

Procedure: weight of dried empty crucible was weighed as W_1 , 2g of the sample was weighed into the crucible using a spatula and the weight recorded as W_2 . The crucible containing the sample was placed in an oven at a temperature of 100°C for 3hrs, after which it was cooled and reweighed as W_3 .

Calculation;

$$\frac{w_2 - w_3}{w_2 - w_1}$$

Where: W_1 = weight of empty crucible, W_2 = weight of empty crucible and sample before drying, W_3 = weight of empty crucible and sample after drying.

Determination of saponification value: American standard for testing material (ASTM)¹¹ is used for the determining the saponification value of the biodiesel produced. 2g of the biodiesel was weighed into a conical flask containing 25ml of 12% alcohol potassium hydroxide. Attach a reflux condenser and heat the flask in boiling water for 60mins; shaking frequently. The resulting solution was subsequently titrated against 0.5M of HCl with phenolphthalein as an indicator, using same procedure for the blank. The titration continues until the

pink colouration turns colourless. Thus, it was calculated as;

$$\frac{(B - A) \times M}{\text{weight of sample}}$$

Where: B = ml of HCl required by blank, A = ml of HCl required by sample, M = Molarity of HCl

Determination of Iodine Value: About 0.3g of methyl ester produced was weighed into a conical flask and 20ml of carbon tetrachloride was added to dissolve the oil, 10ml of wigg reagent was added and left for 30mins, a stopper was inserted and vigorously swirled. At the end, 15ml of 10% of KI and 100ml of distilled water was added. And the content was titrated with 0.1M sodium thiosulphate using few drops of 1% starch indicator, in which the sodium thiosulphate was added drop by drop until colouration disappears completely after vigorously shaking. Same procedure was used for blank;

$$\frac{(B - A) \times 1.269}{\text{weight of sample}}$$

Where: B = volume of sodium thiosulphate used for blank, A = volume of sodium thiosulphate used for sample

Determination of Refractive Index: Refractive index of the biodiesel produced was determined using the value from iodine value, saponification value and acid value.

Calculation;

$$\frac{.4643 - 0.0000665 - 0.0096A}{S + 0.00011711}$$

Determination of Kinematics Viscosity: The sample was charged in to the viscometer at 40°C and 100°C respectively and its dynamic viscosity was recorded.

$$\frac{\text{Dynamic viscosity}}{\text{density of sample}}$$

Determination of Flash Point: The flash point was determined using a crucible, thermometer and hot plate. About 5ml of the methyl ester produced was poured in the crucible and placed uncovered on the hot plate. The thermometer was inserted into the sample and the temperature rise was observed carefully. The temperature at which the diesel started to burn while on the red-hot filament of the hot plate was immediately recorded and taken as the flash point. This process was done twice and its average was recorded.

Determination of Fire Point: This was also done using similar method to flash point and the temperature at which the ester gives off enough vapour which ignites and burns continuously for at least 5sec was recorded as the fire point.

Results and discussion

The free fatty acid present in the Bitter kola according to AOCS⁶ should be less than 1% and the experimental value of the *Garcinia kola* oil produced was 0.822%. This shows the value lies within the specified range. The lower the amount of fatty acid is, the higher the amount of Biodiesel that would be produced. The acid value of the *Garcinia kola* oil produced was 1.635 which is also within the range of that specified by AOCS⁶. It should be noted that the acid value is directly proportional to the free fatty acid present. The bitter kola oil failed the test with respect to Specific gravity. It was noted that the specific gravity gotten (0.949) is higher than that specified (0.91–0.915). The refractive index (1.46-1.47) was also within the range specified. The Saponification value as well as the Iodine value according to Table-1 was above and below the specification standard respectively. A high saponification value would result to the presence of soap which would lead to a decrease in biodiesel formation while the decrease in the iodine value shows a decrease in the unsaturation of the bitter kola oil. Therefore, it can be said that the bitter kola oil produced because the bulk of its properties lies within the specified range can be used for the biodiesel production.

Table-1: Measured physicochemical properties of the Bitter kola oil.

Parameters	Unit	AOCS Standard ⁶	Experimental values
Free fatty acid	%	<1%	0.822
Acid value	mgKOH/g	0.72-3.0	1.635
Specific gravity	-	0.91-0.915	0.949
Kinematic viscosity	Cst	-	2.073
Refractive index	-	1.46-1.47	1.4642
Smoke point	°C	-	165
Fire point	°C	-	120
Moisture content	%	-	0.39
Saponification value	mgKOH/g	188-195	285
Iodine value	mgKOH/g	84-100	54.30
Peroxide value	mEq/kg	-	8
Cloud point	°C	-	48

Table-2: FTIR analysis of the Bitter kola Biodiesel.

Name of the sample	Wave number(cm ⁻¹)	Functional group present
Bitter kola Biodiesel	3701.62363	-OH
	3440.76815	-NH ₂ , -OH
	3295.10661	-OH
	3021.57136	-C-H, -C≡H
	2855.73312	-C-H
	2609.47704	-COOH,
	2102.44109	N≡C, C≡N
	1870.20895	-NH ₂ , C=O, C=C
	1631.27493	C=C
	1301.76853	C=S,
	839.208697	-C-H, C=C

Table-3: Measured physicochemical properties of the Bitter kola Bio-diesel.

Parameters	Unit	ASTM ¹¹	Experimental values
Acid value	mgKOH/g	-	1.635
Specific gravity	-	0.87-0.9	0.9
Kinematic viscosity	Cst	1.9-6.0	1.714
Flash point	°C	130 min	45
Pour point	°C	-	93
Saponification value	mgKOH/g	-	65.637
Iodine value	mgKOH/g	-	70.047
Peroxide value	mEq/kg	-	2.9
Calorific value	Joules	-	1826
Free fatty acid	%	<1%	0.822

The percentage yield of oil from the Bitter kola seed is 34% and then the percentage yield from the bitter kola oil to the biodiesel is 62%. This shows that the poor yield of biodiesel from the seed would affect its usage as a biofuel and this makes it not suitable to be used as a biofuel.

From the FTIR analysis conducted as shown in Table-2, it is seen that the biodiesel produced contains the following functional group: $-\text{CONH}_2$, $-\text{NH}_2$, $-\text{OH}$, $-\text{C}-\text{H}$, $-\text{C}\equiv\text{H}$, $-\text{COOH}$, $\text{N}\equiv\text{C}$, $\text{C}=\text{O}$, $\text{C}=\text{C}$, $\text{C}=\text{S}$, SO_2 . The FTIR technique is an important tool to identify the characteristic functional groups, which are instrumental in the determination of functional groups and organic compounds inherent in the biodiesel produced from the bitter kola biodiesel. Result of the FTIR spectra is shown in the table above. From the table of results, the peak value at 839.208697cm^{-1} was assigned to $-\text{C}-\text{H}$, $\text{C}=\text{C}$ compounds. The peak value around 1301.76853cm^{-1} , 1631.27493cm^{-1} and 1870.20895cm^{-1} were assigned to $\text{C}=\text{S}$, $\text{C}=\text{C}$, and $(-\text{NH}_2, \text{C}=\text{O}, \text{C}=\text{C})$ respectively. The absorption around 2102.44109cm^{-1} was due to $\text{N}\equiv\text{C}$, $\text{C}\equiv\text{N}$ compound. The absorption around 2609.47704cm^{-1} was assigned to COO stretching vibration of acid compound whereas the medium band at 2855.73312cm^{-1} was assigned to $-\text{C}-\text{H}$. The broad band around 3295.10661cm^{-1} , 3701.62363cm^{-1} were assigned to OH hydroxyl compounds of primary, secondary and tertiary alcoholic compound present in the *Garcinia kola* biodiesel.

The Specific gravity of the Biodiesel as seen in Table-3 is within the specified range according to ASTM D6751-10¹¹ and therefore the biodiesel can be said to have passed the Specific gravity test. The standard kinematic viscosity required for biodiesel is higher than that produced. This shows that the rate of flow of the biodiesel is very high.

It should also be noted that the Iodine value of the bitter kola oil produced is less than that of the Biodiesel. This shows that the degree of un-saturation increased during the process of transesterification. It should also be noted that the Acid value as well as the free fatty acid of the bitter kola oil as well as the biodiesel produced is the same.

Conclusion

The results obtained from analysis of the biodiesel from *Garcinia kola* include free fatty acid: 0.822%; acid value: 1.635mgKOH/g; specific gravity: 0.9; kinematic velocity: 1.714Cst; flash point: 45°C; pour point: 93°C; water content: 5.75%. Thus, the values obtained met with the ASTM standard¹¹. Most of the properties of the Biodiesel as well as *Garcinia kola* oil produced from the *Garcinia kola* seeds conformed to the stated standards but the percentage yield of oil from the Bitter kola seed is 34% and then the percentage yield from the bitter kola oil to the biodiesel is 62%. This shows that the poor yield of biodiesel from the seed would affect its usage as a biofuel and this makes it not suitable to be used as a biofuel. This research has shown that biodiesel can be gotten from the seeds of *Garcinia kola* but it also showed that *Garcinia kola* seeds and oils using the Hot process of extraction as well as Trans-esterification process of Biodiesel production are not good sources of Biodiesel since the yield produced is small and wouldn't serve the needs of the consumers if Industrial production should be embarked upon. I recommend that, other

processes such as the cold process of Oil extraction and Biodiesel production other than Trans-esterification should be embarked upon since the raw material used is readily available to determine processes that would produce a higher yield.

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