Scientia Africana, Vol. 18 (No. 1), June, 2019. Pp 38-46
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

OIL OBTAINED FROM *C. LEPIDOTA* SEEDS: EXTRACTION AND CHARACTERIZATION

C. Obi [1]*, F. N. Onyero[1] and M. U. Ibezim-Ezeani [1]

Department of Pure and Industrial Chemistry, Faculty of Science,
University of Port Harcourt, Rivers State, Nigeria
**Corresponding author: E-mail: chidi.obi@uniport.edu.ng*

Received: 08-04-19
Accepted: 22-04-19

ABSTRACT

The benign extraction of oil from monkey kola (Cola lepidota) seeds was carried out by means of solvent extraction using normal hexane as solvent. The percentage yield, specific gravity, viscosity, moisture content, color, acid value, peroxide value, iodine value, saponification value, and free fatty acid (FFA) were evaluated quantitatively. The oil obtained gave a percentage yield of 1.057 % and the color was light brown in nature. It has a specific gravity of 0.84 g/ml and viscosity of 24.63 $MPas^{-1}$ indicating that the oil was viscous. The free fatty acid (FFA), acid, iodine, peroxide and saponification values of the oil were 22.44%, 44.88mg/g, 57.19mg/g, 5.62mg/g, and 125.30mg KOH/g respectively. Results obtained revealed that the Cola lepidota oil belong to the category of non-drying oils. This classification recommends the oil for the production of cosmetics, soaps, plasticizers for lacquers and drying alkyd resins for surface coating. Its high saponification value makes it useful for biodegradable detergents. The oil has a rancid-free taste because of its low peroxide value.

Keywords: *Cola lepidota*, oil, extraction, characterization, applications

INTRODUCTION

Tropical African sub-regions are home to many valuable fruit species whose potentials have not been fully utilized. A good number of these fruit species are not yet domesticated; records show that plant foods represent the largest segment of dietary diversity that provide useful perspectives on a number of issues of contemporary scientific and public health importance including micronutrient deficiency and bioavailability, nutrition and disease, medicinal and functional activities (Essien and Udousoro, 2017).

The market of oil and fat is gradually expanding, probably at a rate slightly faster than the increase in population, and the demand for both domestic and industrial use is met by extracting the oil from plant and animal fats. The expansion of trade naturally puts pressure on the commodity and in the first instance; the increase in demand can be met by the simple expedience of growing more crops (Mclntosh and Miller, 2001).

Vegetable oils and fats are composed predominantly of triglycerides, which are long chain fatty acid esters of glycerol

Scientia Africana, Vol. 18 (No. 1), June, 2019. Pp 47-58
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

(Ibemesi, 2014). The predominant fatty acids present in vegetable oils and fats are saturated and unsaturated compounds with straight aliphatic chains. However, some proportions of other fatty acids may be present in the same vegetable sources, including a small amount of branched chain, cyclic and odd number straight chain acids (Zambiazi *et al*., 2007). The minor non-ester contents of vegetable oils and fats include phospholipids (or phosphatides), sterols, vitamins and their precursors. The non-ester portion is usually less than 2% of the total oil. The glycerides themselves contain about 95% fatty acids and 5% glycerol (Ibemesi, 2014). The amount of glycerol is the same in all vegetable oils; hence, the differences in the properties of different oils are largely determined by the variations on the fatty acid structure (Zambiazi *et al*., 2007; Ibemesi, 2014). Glycerides composed mostly of unsaturated fatty acids are oils (liquids) at room temperature, while those composed mostly of saturated fatty acids are fats (solid) at room temperature (Ibemesi, 2014).

Vegetable oils are one of the major components of human diets comprising as much as 25% of average caloric intake (Zambiazi *et al*., 2007). While high levels of saturated fatty acids are desirable to increase oil stability, on the other hand, nutritionally they become undesirable. At room temperature (25^{o}C), the saturated fatty acids from 12 to 24 carbon length have a waxy consistency, whereas unsaturated fatty acids of these lengths are only liquids (David and Michael, 2005). Because of the waxy consistency of the saturated fatty acids, diets rich in them result in the commonly known coronary diseases. When these oils are constantly consumed, they form solid fats deposit at the cardiac region, the arteries and the veins. These fats tends to block the free flow of blood in these arteries and veins and thus impede blood flow, which eventually results to high blood pressure, stroke and death if untreated (David and Michael, 2005).

Monkey kola is a common name given to a number of minor relatives of the Cola species that produce edible tasty fruits. The people of southern Nigeria and the Cameroons as well as some wild primate animals (especially monkeys, baboons) and other species relish the fruits. The plant belongs to the same botanical family Malvaceae and sub-family Sterculioideae as the popular West African plantation kola nuts (*Cola nitida*), grown for their masticatory and stimulating nuts (Okudu and Asumugha, 2018). Among the species commonly referred to as monkey kola are the *Cola pacycarpa, Cola lepidota* and *Cola lateritia* (Meregini, 2005). The pod of the yellow variety is roundish, while the white variety has a more cylindrical shape. Monkey kola is identified by various local names in southern parts of Nigeria such as “achicha” or “ohiricha” in Igbo and “ndiyah” in Efik, e.t.c. (Ogbu *et al*., 2007). Monkey kola is commonly found in southern parts of Nigeria between the months of June and November (Ogbu *et al*., 2007; Essien and Udousoro, 2017). Monkey kola has nutritional and medicinal values (Essien and Udousoro, 2017). Record shows that the yellow variety pulp is a good source of crude protein, crude fiber, crude fat, Ca, Mg, Zn, Cu, β-carotene and niacin while, *Cola lepidota* (the white variety) pulp is a good source of ash, starch, carbohydrate, potassium, phosphorus, and selenium (Ene-Obong *et al*., 2014; Okudu *et al*., 2015). Like most consumed fruits in Nigeria, only the pulp of the fruit is usually consumed.

The husks and seeds are normally discarded as waste. The consumption of monkey kola pulp or incorporating it into food may increase intake particularly those of micronutrients (Essien and Udousoro, 2017). Photographs of the monkey cola and the seeds used in this study are shown in Plates 1a and 1b.

Plate 1: Monkey kola a:fruits;b: seeds

Previous studies have shown marginal or no information on the extraction of oil from these fruits.Hence, it is hoped that from this study, valuable information will be added to the literature bank of monkey kola species.

This study is therefore aimed at the extraction and characterization of monkey kola seed oil.

MATERIALS AND METHODS

Collection of Sample and Preparation

The monkey kola fruits (sample) were bought from Mile One Market in Obio/Akpor Local Government Area, Rivers state, Nigeria. Impurities were removed by washing, and the hull of the fruits was also removed with a knife in order to isolate the seeds for the oil extraction. The seeds obtained were oven-dried at 105^{o}C to constant weight. The dried seeds were further stored in an air tight polythene bag for further processing. The seeds were initially pulverized using pestle and mortar and finally ground using an electric grinder. The chemicals or reagents employed in this study were all of analytical grade.

Extraction of Oil using Soxhlet Extractor

The ground monkey kola seeds, 10g each, were tied up in filter papers and put into the thimble. A reflux condenser and round bottom flask were fitted above and below the thimble respectively. This assembly of apparatus known as Soxhlet extractor was clamped firm into position and 500ml of n-hexane was poured into the round bottom flask. The soxhlet extractor was then heated electrically on a heating mantle. Continuous extraction was carried out for a period of 6hrs. The n-hexane extract was then heated to a constant weight on the heating mantle at 60^{o}C to ensure that there is no trace of n-hexane in the oil. After that, the oil was stored in a refrigerator.

The weight of the oil obtained was recorded and its percentage yield calculated as follows:

$$\% \text{ Yield} = \frac{\text{Weight of oil obtained} \times 100}{\text{Weight of sample}} \quad (1)$$

Scientia Africana, Vol. 18 (No. 1), June, 2019. Pp 47-58
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

Determination of Iodine Value

A 0.20g sample of the extracted oil was dissolved in 15ml of carbon tetrachloride in a 100ml glass stoppered bottle flask. Iodine (Wiji's) solution (25ml) was added and the flask was corked and allowed to stand for 2hrs in the dark at room temperature. 20ml of potassium iodide solution (10%) was added. The mixture was then titrated with 0.2N sodium thiosulphate ($Na_2S_2O_3$) using starch as indicator. A blank determination was carried out using 10ml of carbon tetrachloride and the iodine value calculated as follows:

$$\text{Iodine value} = \frac{12.69 \times N \times (V_2 - V_1)}{W_{sample}} \quad (2)$$

Where, W_{sample} = weight of the sample, V_1 = volume of thiosulphate used in the test, V_2 = volume of thiosulphate used in the blank and N = normality of thiosulphate.

Determination of Saponification Value

A 0.1g of the oil was dissolved in 20ml of ethanolic potassium hydroxide solution in a conical flask. The mixture was heated in boiling water for 2mins while shaking the flask frequently. Phenolphthalein (1.0ml) indicator was added and the hot soap solution was titrated with 0.5N H_2SO_4. A blank determination was carried out under the same condition and the saponification value was calculated as follows:

$$\text{Saponification value} = \frac{(V_{blank} - V_{sample}) \times N \times 56.1}{W_{sample}} \quad (3)$$

Where, V_{blank} == volume of H_2SO_4 used for the blank, V_{sample} = volume of H_2SO_4 used for the sample, W_{sample} = weight of sample, N = normality of the H_2SO_4 solution and 56.1 = conversion factor.

Determination of Acid Value

The acid value was determined by adding 25ml of diethyl ether and 25ml of ethanol to 0.20g of the extracted oil sample. The mixture was titrated with 0.1N sodium hydroxide (NaOH) with phenolphthalein as indicator. The acid value was calculated using the following formula:

$$\text{Acid value} = \frac{(56.1 \times N \times V)}{W_{sample}} \quad (4)$$

Where, N = normality of the NaOH solution, V = volume of NaOH used, W_{sample} = weight of the oil sample.

Determination of Free Fatty Acid Value

The free fatty acid is usually calculated as oleic acid (0.1 M sodium hydroxide ≡ 0.0282 g of oleic acid) in which case the value = 2 × % FFA (Onwuka, 2005).

Determination of Viscosity

The Ostwald-type viscometer (Figure 1) was used to determine the oils viscosity. The viscometer, which was previously washed and completely dried, was filled with the oil sample under examination through tube L to slightly above the mark G, using a long pipette to minimize wetting the tube above the mark. The viscometer was placed vertically in a water bath maintained at a constant temperature of 30^{o}C and was allowed to stand for 30mins to allow the contents to reach equilibrium temperature. The volume of the sample was adjusted so that the bottom of the meniscus settled at the mark G. The oil sample was sucked to a point about 5mm above the mark E.

After releasing pressure or suction, the time taken for the bottom of the meniscus to fall from the top edge of mark E to the top edge of mark F was measured.

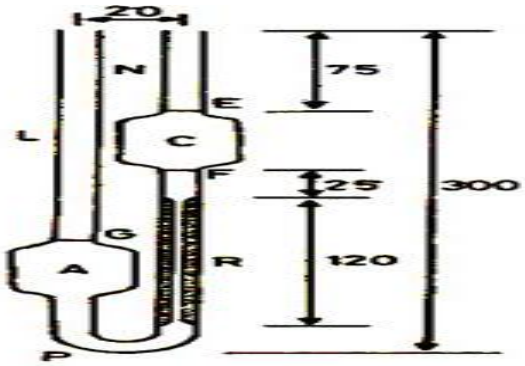

Figure 1: Ostwald – type viscometer

Calculations:

Kinematic viscosity (V) in square millimetres per second (mm^2s^{-1}) was calculated from the expression:

$$V = kt \qquad (5)$$

Dynamic viscosity (h) in millipascal seconds (mPas) was calculated from the expression:

$$\eta = k\rho t \qquad (6)$$

where t = outflow time (in seconds) for the meniscus to fall from E to F,

Density, $\rho = \frac{mass}{volume}(gcm^{-3})$ obtained by multiplying the relative density of the liquid under examination by 0.998203.

The viscometer constant (k) of the instrument was determined using a liquid of known viscosity.

Peroxide Value - AOCS Method

The sample (5.0g) was weighed into a 250ml glass stoppered Erlenmeyer flask. 30ml of the acetic acid-chloroform (1:2) solution was added into a graduated cylinder. The mixture was swirled vigorously and allowed to stand in the boiling water for 30s. The mixture was then poured into another flask containing 0.5ml of saturated potassium iodide solution which was corked and swirled for exactly1min. 30ml of de-ionized water was immediately added and shook vigorously to liberate the iodine from the chloroform layer. The mixture was titrated with 0.1N sodium thiosulphate solution using starch indicator until the blue gray color disappeared in the aqueous (upper) layer. The procedure was repeated for the blank in the absence of the oil sample.

Calculations:

The peroxide value (PV) is given by the following equations:

$$\text{Peroxide value} = \frac{(S \times B) \times N \text{ thiosulfate} \times 100}{\text{Weight of Sample}} \qquad (7)$$

or

$$(S - B) \times N \text{ thiosulphate} \times 20 \qquad (8)$$

Where S = volume of titrant for oil sample and B = volume of titration for blank

Scientia Africana, Vol. 18 (No. 1), June, 2019. Pp 47-58
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

Determination of Specific Gravity

A clean 50ml specific gravity bottle was weighed empty (W_1) and filled to the brim with water and its stopper was inserted. The water on the stopper was carefully wiped off and the bottle reweighed (W_2). The same process was repeated, but using the oil sample instead of water, and weighed again (W_3). The specific gravity of the oil sample was calculated using the following formula:

Specific gravity of the sample = $\frac{W_2-W_1}{W_3-W_1}$(9)

Determination of Moisture Content (Air – Oven Method)

Moisture content of oils and fats is the loss in mass of the sample on heating at 105±10^{o}C under operating conditions specified. A 5.0g sample of the oil was weighed in a previously dried and tared dish which was thoroughly mixed by stirring. The dish was loosely covered with a lid and heated in an oven at 105±10^{o}C for 1hr. The dish was removed from the oven and the lid was closed. The dish was cooled to room temperature in a desiccator and re-weighed. The process was repeated every 1hr until change in weight between two successive observations did not exceed 1mg. The determination was carried out in duplicate. The percentage moisture in the oil was calculated from the formula:

Moisture and volatile matter (% wt) = $\frac{W_1}{W} \times 100$(10)

Where W_1 = loss in grams of the material on drying and W = weight in grams of the material before drying.

RESULTS

The results of the characterized oil obtained from *Cola lepidota* seeds and the bar charts generated comparing other fruits and seeds oils with the present study were shown in Table 1 and Figures 2-5.

Table 1: Physicochemical Properties of Monkey Kola (*Cola lepidota*) Seed Oil

Parameter	Values obtained
% Yield	1.057
Color	Light brown
Specific gravity	0.840
Moisture content (% wt)	0.51
Viscosity (MPa.s^{-1})	24.63
Acid value (mg/g)	44.88
Saponification value (mgKOH/g)	125.30
Iodine value (mg/g)	57.19
Peroxide value (meq/kg)	5.62
Free fatty acid (%)	22.44

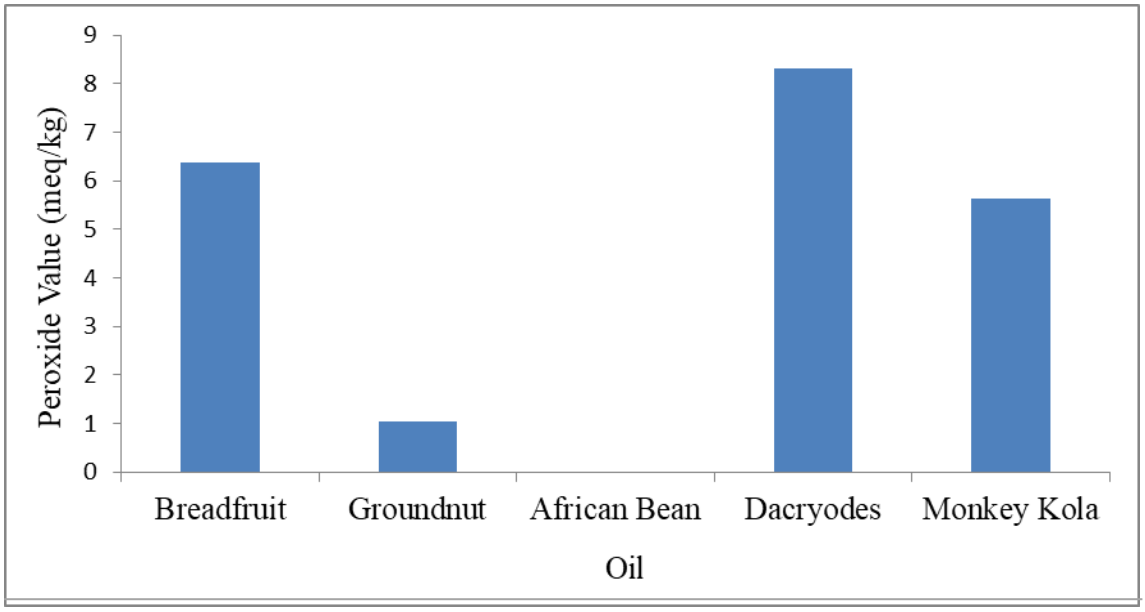

Figure 2: Peroxide Values of Monkey Kola seed and other Vegetable Oils

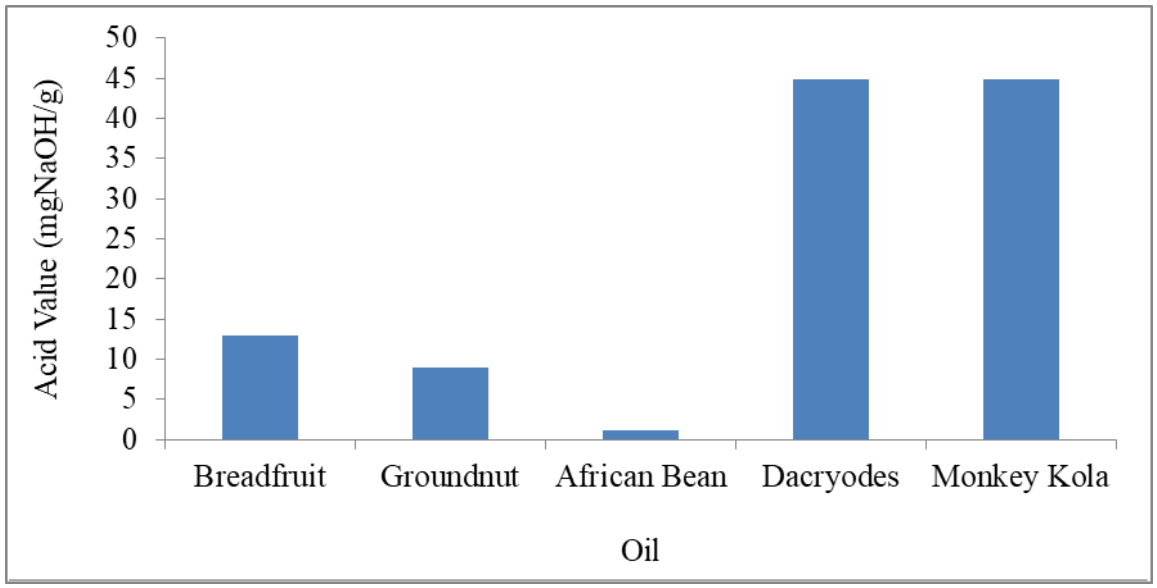

Figure 3: Acid Values of Monkey Kola seed and other Vegetable Oils

Scientia Africana, Vol. 18 (No. 1), June, 2019. Pp 47-58
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

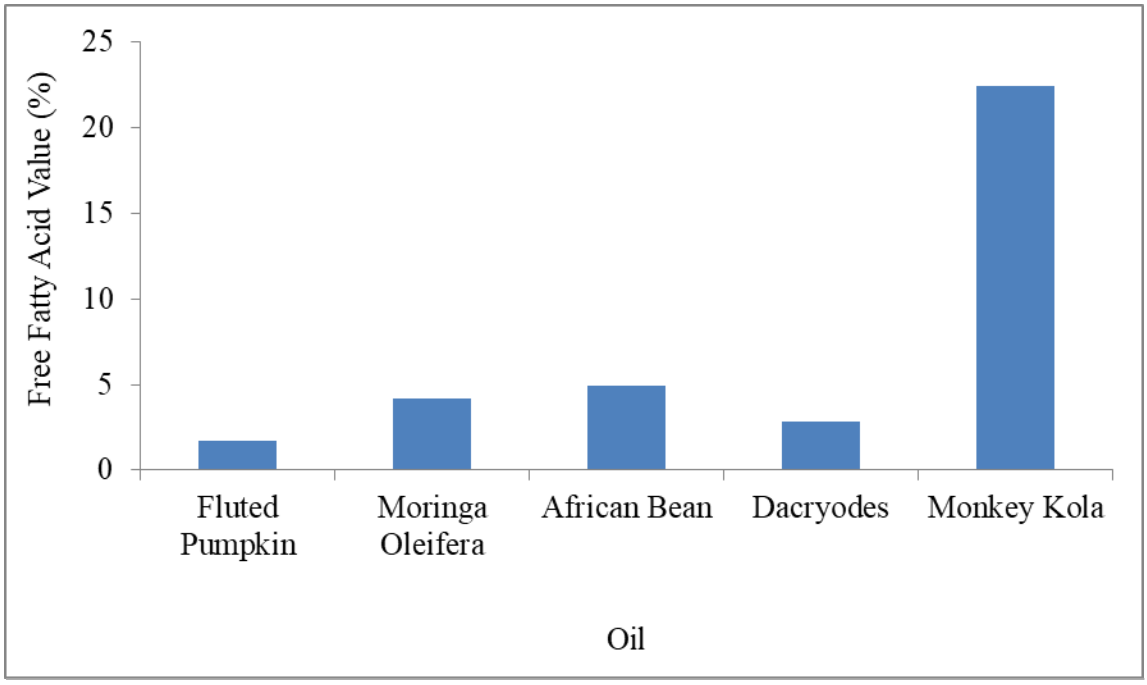

Figure 4: Free Fatty Acid Values of Monkey Kola seed and other Vegetable Oils

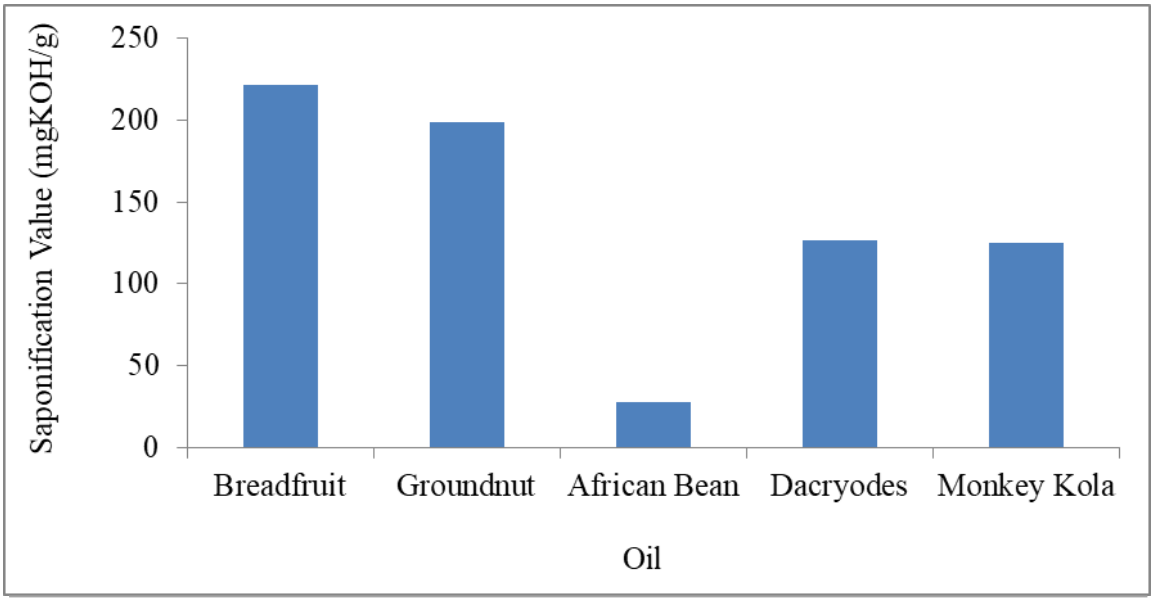

Figure 5: Saponification Values of Monkey Kola seed and other Vegetable Oils

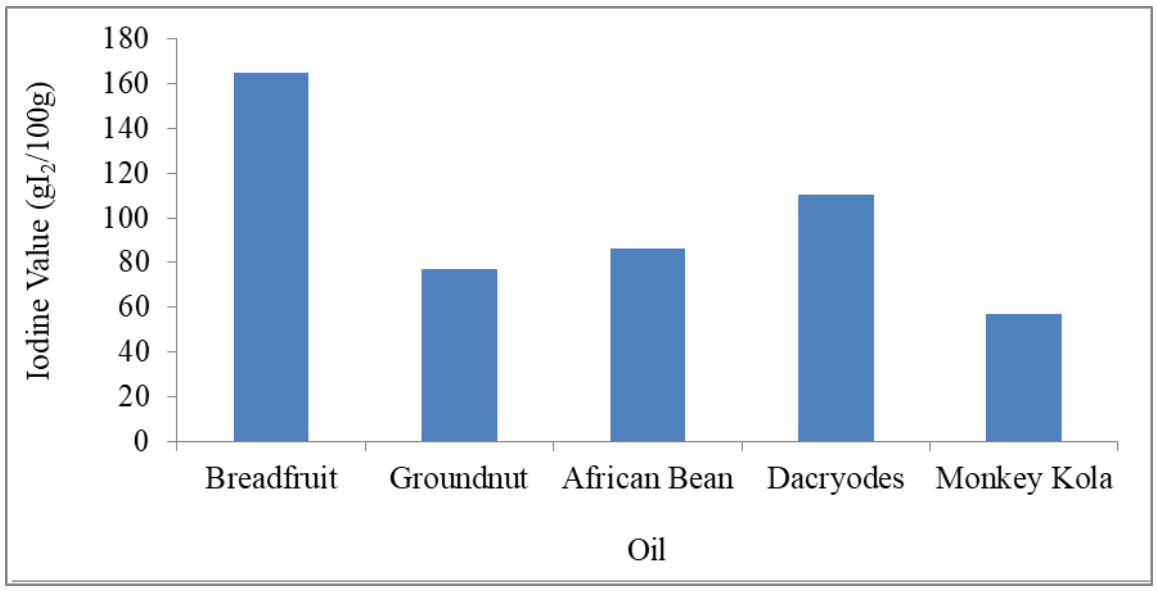

Figure 6: Iodine values of Monkey Kola seed and other Vegetable Oils

DISCUSSION

The use of normal hexane in the soxhlet extraction gave percentage yield of 1.057. However, monkey kola seed oil can be refined to increase its yield by the use of sophisticated extraction technique for commercial purposes (Sabinus, 2012). It should be noted that the mode of extraction and the type of solvent contribute significantly on the quality and yield of oil. Singh and Saroj (2009) reported that the best available method for extraction, especially for castor oil at present, was by the use of hydraulic press.

The results of physicochemical properties of monkey kola seed oil presented in Table 1showed that the oil was light brown in color. The moisture content (%wt) was found to be 0.51, which was higher than the AOAC acceptable limit. The relatively low moisture content of the sample is of advantage since high moisture content is associated with increase in bacterial action during storage (Akintayo *et al*., 2002). The specific gravity was found to be 0.84g/ml, indicative of the fact that monkey kola seed oil was less dense than water. The viscosity value of 24.63MPas^{-1} revealed that the monkey kola seed oil was viscous.

The peroxide value of monkey kola seed oil was 5.62meq/kg and this was found to be within the range of AOAC standard for vegetable oils. The low value of PV was indicative of low level of oxidative rancidity of the oil and also suggests strong presence or high levels of antioxidants. Akpambang *et al*. (2008) observed a PV of 1.72 ± 0.01 and 1.42 ± 0.01meq/kg for *C. citrullus* and *C. edulis* respectively. The peroxide values for breadfruit oil, groundnut oil, and dacryodes were 6.38, 1.03, and 8.3meq/kg (Sabinus, 2012). The peroxide value was low and a pointer to the fact that the oil may not be easily susceptible to deterioration. The value of PV obtained in this study was compared with the values of other seed oils produced in the previous studies as presented in Figure 2.

Scientia Africana, Vol. 18 (No. 1), June, 2019. Pp 47-58
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

Acid value represents free fatty acid (Figure 3) content due to enzymatic activity, and is usually indicative of spoilage. Its maximum acceptable level is 4mg NaOH/g oil (AOAC, 1990). The result obtained from this study indicate that the acid value of the monkey kola seed oil was 44.88mg NaOH/g. Higher acid value was due to free fatty acid present in the oil. The free fatty acid of monkey kola was 22.44%. This value was relatively high when compared with other vegetable oils (Figure 4). However, high acid values have been found for dacryodes(44.88), palm kernel (19.04±0.41mg KOH/g oil) and breadfruit (12.903mg KOH/g oil) (Sabinus, 2012). This parameter can be used to check the level of oxidative deterioration of the oil by enzymatic or chemical oxidation. The acid value is expected to range from 0.00 to 3.00mg KOH/g for the oil to have application in cooking. This high acid value found for monkey kola seed oil can be remedied by subjecting it to refining and this may also improve its quality for industrial purposes (Oderinde *et al*., 2009).

The saponification value (SV) (Figure 5) of the monkey kola seed oil was 125.30mg KOH/g. This shows that more alkali would be required to enable it neutralize the free fatty acid liberated by the oil. Sabinus (2012) reported that the saponification values of palm kernel, breadfruit, groundnut, coconut, soybean, and dacryodes oils were found to be in the range of 195 to 261mg KOH/g sample. Kyari (2008) reported that SV for palm oil was 200mg KOH/g, groundnut oil was 193mg KOH/g, and coconut oil was 257mg KOH/g. The differences observed might be as a result of the differences in the methods of extraction (Singh and Saroj, 2009). The monkey kola seed oil with SV of 125.30mg KOH/g may be used for making soaps, shampoos and lather shaving creams (Oderinde *et al*., 2009). Saponification values had been reported to be inversely related to the average molecular weight of the fatty acids in the oil fractions. Oil fractions with saponification values of 200mg KOH/g and above have been reported to possess low molecular weight fatty acids (Abayeh *et al*., 1998).

The result obtained for the iodine value (Figure 6) of monkey kola seed oil was 57.19mg/g. However, higher values show increase in the average degree of unsaturation of the oil, as such, the amount of iodine which can be absorbed by unsaturated acids would be higher. The oil may be classified as non-drying oil; since its iodine value is lower than 100mg/g (Abayeh *et al*., 1998). Oils whose values are less than 100 gI_2/100 g sample could be used as lubricants and hydraulic brake fluids production. The iodine value obtained here was comparable to the literature value of castor oils and olive oils, both of which are non-drying oils. A good drying oil should have iodine value of 180 mg/g (Abayeh *et al*., 1998). Thus, the oil of monkey kola seed is not suitable as resins for paint formulation or in varnishes; it may, however, find application in conjunction with amino resins as finishes for certain appliances, and in this case, the oil can also act as plasticizers (Abayeh *et al*., 1998).

The percentage oil content of *Cola lepidota* seeds whose yield was low indicates that satisfactory result could not be achieved by solvent extraction process, using normal hexane as the solvent or by using the laboratory soxhlet apparatus. This study reveals that some of the values of the

physical and chemical properties obtained complied with the standards specified by AOAC for vegetable oils. The oil was of good quality and due to its relatively high saponification value could be recommended as suitable for industrial applications, especially in the making of soap, shampoos, etc. As non-drying oil, it can also be used for lubricant and hydraulic brake fluid production. The oil may need to be refined if it is to be used for other purposes.

REFERENCES

Abayeh, O. J., Aina, E. A and Okuonghae, C. O. (1998).Oil content and oil quality characteristics of some Nigerian oil seeds. *Journal of Pure and Applied Sciences,* 1, 17-23.

Akintayo, O., Adebayo, E. A and Arogundade, L. A. (2002).Chemical composition, physical and functional properties of Akee (B. Sapid) pulp and seed flours.*Food Chemistry,* 77, 333-336.

Akpambang, V. O. E., Amoo, I. A and Izuagie, A. A. *(2008).* Comparative compositional analysis on two varieties of melon (Colocynthis citrullus and Cucumeropsis edulis) and a variety of almond (Prunusamydalus). *Research Journal of Agriculture, Biology and Science,* 4(6), 639-642.

Association of Official Analytical Chemists (AOAC) (1990).Official Methods of Analysis"14 ed., Arlington, V.A.,67, 503-515.

David, L. N and Michael, M. C. (2005). A practical method for determining the oil content of avocado. *Citrus Subtropical Journal,* 84-92.

Ene-Obong, H. N., Okudu, H. O and Asumugha, V. U. (2014). Nutrient and phytochemical compositions of two varieties of monkey kola (Cola parchycarpa, Cola lepidota): An underutilized fruit. *Journal of Food Chemistry,* 193, 154-159.

Essien, E. E and Udousoro, I. I. (2017).Cola parachycarpa K. Schum: Chemical evaluation of amino acids, vitamins and other nutritional factors on seed, fruit mesocarp and epicarp. *Journal of Pharmaceutical and Biosciences,* 5(4), 23-29.

Ibemesi, J. A. (2014).From studies in polymers and vegetable oils to sanitization of the academic system.19th Inaugural lecture, University of Nigeria, Nsukka, pp. 21-47.

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

McIntosh, M and Miller, C. (2001).A diet containing food rich in soluble and insoluble fiber improves glycemic control and reduces hyperlipidemia among patients with type 2 diabetes mellitus. *Nutrition Review*, 59(2), 52-55.

Meregini, A. O. A. (2005).Some endangered plants producing edible fruits and seeds in South Eastern Nigeria. *Fruits,* 60, 211-220.

Oderinde, R. A., Ajayi, I. A and Adewuyi, A. (2009).Characterization of seed

Scientia Africana, Vol. 18 (No. 1), June, 2019. Pp 47-58
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

and seeds oil of HuraCrepitans and the kinetics of degradation of the oil during heating. *Electronic Journal of Environmental, Agricultural and Food Chemistry,* 8(3), 201- 208.

Ogbu, J. U., Essien, B. A and Kadurumba, C. H. (2007).Nutritional value of wild Cola spp. (Monkey kola) fruits of Southern Nigeria. *Nigeria Journal Horticultural Science,* 12, 113-117.

Okudu, H. O., Ene-Obong, H. N and Asumugha, V. U. (2015).The chemical and sensory properties of juice developed from two varieties of monkey kola (Cola parachycarpa, Cola lepidota). *African Journal Food Science and Technology,* 6(5), 149-155.

Okudu, H. O and Asumugha, V. U. (2018).Nutrient potentials and phytochemical composition of two varieties of monkey kola (Cola parachycarpa, Cola lepidota) seed flour. *CPQ Nutrition,* 1(1), 01-10.

Onwuka, G. I. (2005).Food analysis and instrumentation theory and practices. Napthali Prints, Lagos, pp. 140-176.

Sabinus, O. O. E. (2012).Physicochemical properties of oil from some underutilized seeds available for biodiesel preparation. *African Journal of Biotechnology,* 11(42), 100-107.

Singh, R. K and Saroj, K. P. (2009).Characterization of Jatropha oil for the preparation of biodiesel. *Indian Journal of Natural Products and Resources,* 8(2), 127-132.

Zambiazi, R. C., Przybylski, R., Zambiazi, M. W and Mendonca, C. B. (2007).Fatty acid composition of vegetable oils and fats. *B. CEPPA, Curitiba,* 25(2), 111-120.