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Extraction, properties and utilization potentials of African walnut (*Tetracarpidium Conophorum*) as an alternative to conventional vegetable oils

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Abstract
The extraction, physical and sensory properties, proximate composition, chemical properties, mineral and fatty acid composition of African walnut (*Tetracarpidium conophorum-Mull. Arg*) nuts and oil extracted using soxhlet extractor was evaluated in this work using standard analytical procedures. Physical properties of African walnut oil showed that the oil was liquid at room temperature (25°C), pale yellow colouration, odourless and tasteless. The proximate composition results showed the percentage (%) moisture, crude fibre, ash, crude protein, lipids and carbohydrate contents of the nut was 2.36, 1.6, 1.42, 14.5, 59.4 and 26.9 while the calorific values were 523.8 Kcal/100 g respectively. Chemical characteristics of the nut oil indicated the Specific gravity (SG) 0.94, Refractive index (PI) 1.445, Acid Value (AV) as 0.92 mg KOH g^{-1}, Free Fatty Acids (FFA) as 0.56 %, Peroxide Values (PV) as 1.02 meg O_2 kg^{-1}, Saponification Value (SV) as 170.1 mg KOH g^{-1}, Unsaponification Value as 4.1 g/ Kg^{-1}, Iodine Values (IV) as 91.3 (mg of $1g^{-1}$ of oil) Wijis. These results suggest that the African walnut examined may be nutritionally potent and also viable source of oil judging by its oil yield value. The data also showed that the oil is edible, a semi-drying inferring from the low AV and low FFA contents. Industrially, the results revealed the nut oil have great potentials in soap manufacturing industries because of the high SV. The main fatty acids identified by gas chromatography were palmtic (1.53 %), linoleic (13.05 %), linolenic (80.59 %), stearic (4.41 %) and eicosenoic (0.42 %) acids. The mineral contents of walnut were established by using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES). African walnut was highly rich in minerals such as K (3450.3 mg/kg), P (3021.7 mg/kg), Mg (1076.9 mg/kg), Ca (1008.5 mg/kg) with low amounts of B (52.5 mg/kg), Mn (38.7 mg/kg), Na (36.6 mg/kg), Fe (29.8 mg/kg), Zn (20.2 mg/kg), Se (13.6 mg/kg) and trace amounts of Cr (7.9 mg/kg), Ni (1.8 mg/kg) and Li (0.56 mg/kg). The results obtained for African walnut oil are compared with values reported for other conventional vegetable oils available commercially and discussed in terms of potential uses of the oil.

Keywords: African walnut (*tetracarpidium conophorum-mull. arg*). physical, sensory, proximate composition, chemical properties, mineral, fatty acid composition

Introduction

The sudden surge for the development of both the unknown and underutilized plant species across the globe lies on two important reasons. Firstly, is the need to increase our industrial raw materials as many underutilized plant species have specific properties which makes them very difficult to be substituted by either artificial or synthetic products. Secondly, no raw material, in the oilseeds is suitable for all purposes since each one has its unique properties.

Currently, many of the plant species that are now cultivated world-wide for food purposes were those initially neglected and underutilized (Magbagbeola *et al*., 2010) [17]. Some of these are potential industrial raw materials and can serve as precursor of new products. Oils derived from plant sources are obtained from plant seeds referred to as oilseeds. Oilseeds are a class of plants in which huge amount of lipids are stored in the seed tissue (Akanni *et al*., 2005) [2]. The amount of oil present in oilseeds such as soybean and corn ranged from 10-20% and 50% in sunflower and rapeseed (Obasi *et al*., 2012) [20]. Oilseeds are among the most ancient crops domesticated by mankind. For example, there is evidence that the cultivation of linseed oil bearing varieties in the eastern part of Nigeria has been on from many decades ago. However, for many years now, globally, there has been great increase in the demand for vegetable oils with up-trend in prices (Akanni *et al*., 2005) [2]. From the olden days, oilseed products were utilized for a variety of edible and non-edible applications. Seed oils have been extensively used for human consumption. Mostly, oils are used in food, both in cooking and as supplements. The nutritive and calorific values of oilseeds make them good sources of edible oils and fats in diets (Obasi *et al*., 2012) [20]. Besides been a vital component of human diet, oils also find importance in various industrial applications. The oilseeds containing unusual fatty acids are industrially important, as they are used in dispersants, pharmaceuticals, protective coatings, cosmetics, soap and a wide range of synthetic intermediates as stabilizers in plastic formulations (Ozcan, 2009). The significance of oilseeds nutritionally, economically and industrially is enormous. Thus an ever expanding market exists for oilseed crops from both nutritional and industrial perspectives.

One of the underutilized plant species in Nigeria is the African walnut known as *Tetracarpidium conophorum- Mull. Arg*). African walnut belongs to the family Euphorbiaceae (Edem *et al*., 2009) [9]. However, Ayodeji and Aliyu (2018) [5]. stated that some walnut species are found in the family Olacaceae. African walnut is similar to *Juglans regia* (L.), known as the English walnut and belonging to the family Juglandaceae (Raja *et al*., 2012 [27]; Lamichhane *et al.*, 2016) [16]. African Walnut is a perennial creeping shrub that grows in temperate areas in Africa such as Cameroon, Gabon and Liberia including Nigeria (Ayoola *et al*., 2011) [7]. The immature fruits are usually green in colour but turn dark brown to black as they reach maturity (Ayodeji and Aliyu 2018) [5]. Walnuts are dry nuts which are encased in green pods. As walnut matures, the outer covering dries and falls off leaving the segment tough black shell and the white seed. The white seed nut is the edible nut (Ekwe and Ihemeje, 2013) [12]. Its range in Nigeria includes Uyo, Akamkpa, Akpabuyo, Lagos, Akure, Kogi, Ajaawa, Ogbomosho, Ibadan (Obianime and Uche, 2010 [21]; Ayodeji and Aliyu, 2018) [5], Ife, Ekiti and Ijeshaland. it is abundant in all cocoa-producing states in Nigeria and in the southern part of Nigeria (Udedi *et al*., 2014 [30]; Nwaichi *et al*., 2017 [18]; Ayodeji and Aliyu 2018) [5]. It is commonly called African walnut because of its West African origin. *T. conophorum* is often called by different names such as awusa (Yoruba), Ukpa in Igbo, kaso or ngak in Cameroon. Walnut tree is about 40m in length and are usually harvested between July to December (Ayoola *et al*., 2011) [7].

In Nigeria, ripe walnuts are mostly eaten whole often boiled, as dessert nuts or used in cakes, desserts and confectionery of all kinds and a large proportion is thrown away as a waste, whereas it is a rich source of nutrients and oils and can be used as a food (Ogunwusi and Ibrahim, 2016 [22]; Udedi *et al*. 2014) [30]. African walnut which are similar in shape and size to the English walnut (*Juglans regia L*) cultivated in Turkey, seems to have similar applications. However, there are very scanty reports on the properties of oil from African walnut, as most attention has been focused on the utilization of the nuts for dessert. Apart from its use for culinary purposes, local communities in Nigeria use oil extracted from African walnut for the treatment of infections caused by opportunistic, skin pathogenic microorganisms such as *Staphylococcus aureus, Candida albicans, Propionibacterium acnes and Pityrosporum ovale and Pseudomonas aeruginosa.*

While the African walnut plant is mostly maintained by cultural preferences and traditional practices, it remained inadequately characterized and neglected by both research and conservation. This made its high esteemed potentials to be underestimated, undervalued and underexploited. Conservatively, it exposes the plant in danger of genetic erosion and disappearance which have great potential to put a limit on its development options (Dansi *et al.*, 2012 [8]; Ogunwusi and Ibrahim, 2016) [22]. In addition, the constant focus on a few oilseeds that have gained cognizance to the detriment of most underdeveloped plant seeds has really reduced the number of oilseeds upon which, industrial, economic, global food security and agricultural development relies (Dansi *et al.*, 2012) [8]. This paper therefore assess the extraction, physical and sensory, proximate composition, chemical properties, mineral and fatty acid composition of African walnut oil, and to suggest possible uses for the oil as a prelude to an investigation into the scientific basis for its local use for the treatment of microbial skin infections, Comparison between African walnut oil and vegetable oils from other plant sources that are already enjoying a degree of utilization, popularity and acceptability are also made.

Materials and Methods

Collection and identification of African walnut

Fresh walnuts were collected from walnut trees growing in Ogun State in September through October 2016 and a final sample of about 3 kg was randomly taken. The nuts were taken to Olabisi Onabanjo University, Ago-Iwoye, Ogun State where they were authenticated by a taxonomist. Standard methods for sample processing and preparation and analytical procedures were used.

Sample preparation and processing

After harvest and identification, African walnuts were sorted, and damaged ones discarded. The nuts were washed in cold water to remove dirt adhering to the surface. After washing, manual separation of the black husk from the nuts was done. The nuts were then sundried in shade for 14 days (two weeks). Thereafter, the nuts were milled using MarlexExcella mixer/grinding machine (Amazon, UK), packed in air tight containers and kept in the refrigerator at 4°C for further processing.

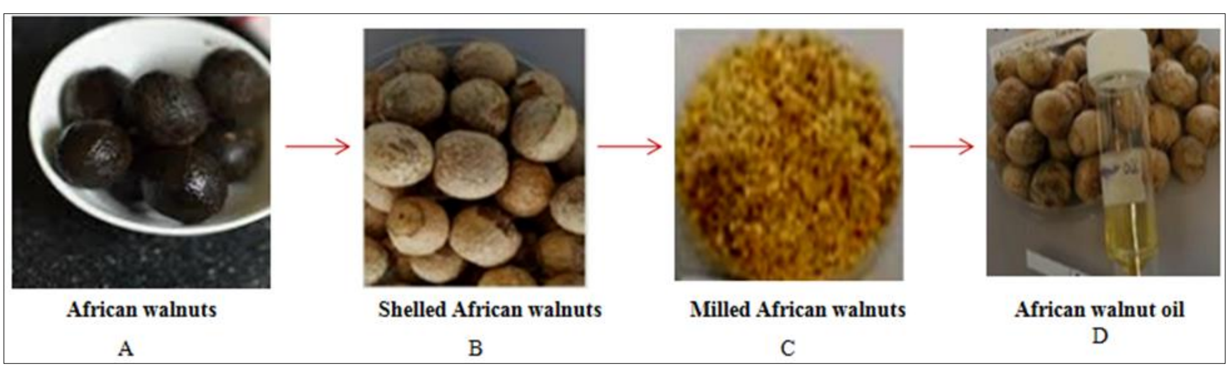

Fig 1

Extraction of oil from African walnut

Oil was extracted from the dried grounded nuts with petroleum ether in a process known as solvent extraction. Prior to extraction, the pulverized nut samples were kept in an oven at 105 ºC for 1 h to remove any moisture that may still be present. Twenty (20 g) of the dried sample was wrapped in a white muslin cloth and put into a porous thimble of the Soxhlet extractor. Then, 200 ml of petroleum ether of HPLC grade with boiling range of 40-60 ºC was added. The Soxhlet was coupled with condenser and flask already filled with the set up was heated in a heating mantle at 65 °C to allow solvent boiling. In the process the solvent vapour travels up a distillation arm and flowed into the chamber housing the sample material. The extract seeps through the pores of the thimble and fills the siphon tube where it flows back down into the round bottom flask. The process was allowed to continue for 8 h until a clear solvent was obtained in the thimble chamber. At the end of the extraction, the resulting mixture of the extracts was filtered with a 10mm Syringe-driven with a filter 0.45um to remove any impurities. The solvents were further removed completely with a rotary-evaporator (Model N-1, Eyela, Tokyo Rikakikal Co., Ltd., Japan). The extracts were stored in white bottles and tubes under nitrogen at 4 °C until analyzed. After extraction, the physical and sensory properties of the oil extract were recorded, and the amount of oil obtained was measured using an analytical balance and result obtained was used to calculate the percentage yield of the oil sample. Attempts were also made to extract oil from the seeds using the expeller pressed method (mechanical extraction). A simple press similar to the type used for pressing oil from smashed pulp of palm fruits in palm oil processing was used for extraction.

Physical and Sensory properties

The colour, smell, taste, sedimentation and other related physical characteristics of African walnut oil extracted were noted. A 10 man panel consisting of staff and students of Olabisi Onabanjo University, Yewa Campus was constituted to evaluate the smell and taste of the oil. Colour measurement was done with a Lovibond colorimeter.

Proximate compositions

African walnut fruit was assessed for various proximate compositions. Moisture, ash, crude fibre, crude oil, crude protein, carbohydrate and calorific energy value were determined using standard methods described by the Association of Official Analytical Chemists (AOAC, 2000) [6]. and Pearson, (1981) [25]. Moisture and ash contents of the processed nut sample were determined by gravimetric method. The crude fibre content was determined by the Weende method outlined in Pearson, (1981) [25]. Nitrogen was established by kjedahl analysis multiplied by 6.25 and determined as protein. The percentage crude fat (lipid) content of the oil sample was determined by the continuous soxhlet lipid extraction method using soxhlet reflux apparatus outlined by Horowitz, (1984) [13]. and AOAC, (2000) [6]. While the carbohydrate content was estimated as the Nitrogen free extract (NFE) as outlined in AOAC, (2000) [6]. In this method, the percentage carbohydrate was estimated as the difference between 100 and the total sum of the proximate composition of the sample. The nut weight of 100 fruits was determined.

Chemical properties

Chemical analysis was carried out on African walnut oil to determine the Saponification values, Unsaponifiable matter content, acid value, peroxide values, free fatty acids (FFA), iodine, Refractive index (RI) and Specific gravity (SG). The procedures of Egan *et al*., (1981) [10]. and AOAC, (2000) [6]. were adopted for the estimation of Saponification values, Unsaponifiable matter content, acid value, peroxide value, free fatty acids (FFA) and iodine content, Refractive index (RI) and Specific gravity (SG) of the oil samples. The saponification value was determined by the estimation method. About 100 mg of the extracted oil was mixed with 0.5 M ethanolic potassium hydroxide solution and boiled under reflux on a water bath for 30 minutes. The solution was then titrated with 0.5 M HCl, using phenolphthalein solution as indicator. The saponification value was calculated using the titration values. For the determination of unsaponifiable matter, about 100 mg of the extracted oil was heated under reflux and saponified with 5 mL of ethanolic potassium hydroxide solution (20 % w/v) for 2 h. The unsaponifiable matter was extracted thrice with 15 mL of petroleum ether and the extracts were combined and evaporated at 40 °C under reduced pressure. The unsaponifiable residue was weighed. For peroxide determination, a known weight of African walnut oil was dissolved in a mixture of acetic acid/chloroform (3:2 v/v) and a saturated solution of KI (1 mL) was added. The liberated iodine was thereafter titrated with sodium thiosulphate solution (0.05 m) in the presence of starch as indicator. For the free oil acidity (acid value), the titration method was used. For this determination, a known weight of African walnut oil was dissolved in a mixture of ethanol (95 %) and ether, previously neutralized with 0.1 M potassium hydroxide solution to phenolphthalein solution. The mixture was then titrated with 0.1 M potassium hydroxide solution as indicator until the solution remained faintly pink after shaking for 30 seconds. Free fatty acid (FFA) determination was by colorimetric method, while iodine value was determined using Wij's (iodine monochloride) method. In this method, a weighed amount of oil sample was dissolved in carbon tetrachloride and added to iodine monochloride. The resulting solution was kept in the dark for 30 minutes and then titrated with 0.1 M sodium thiosulphate, using starch solution as the indicator. Refractive index (RI) was measured with a Refractometer (RFM342, Bellingham + Stanley, England) while the specific gravity (SG) of the oil sample was determined gravimetrically. Both were determined using the methods of Egan *et al*., (1981) [10]. The chemical properties of African walnut oil were further compared with those of commercially available vegetable oils.

Determination of fatty acids

Fatty acid composition of African walnut oil was determined using a modified fatty acid methyl ester method. The oil was extracted three times from 2 g air-dried seed sample by homogenization with petroleum ether. The oil sample (50-100 mg) was converted, that is transesterified to its fatty acid methyl esters (FAME). Transesterification (Boron trifluoride methanolysis-BF3-MeOH) of the walnut oil sample was done in accordance with the guidelines of International Olive Oil Council (IOOC, 2006). Briefly (60 mg) sample was placed in a vial with screw cover and 2 mL of 0.5 mol/L NaOH-methanol solution was added. The mixture was heated at 100 °C for 7 minutes and allowed to cool. After cooling, 3 mL of 14% BF_3-MeOH reagent was added and the vial was sealed and heated for another 5 min at 100 °C. After cooling, 3 mL of hexane and 7 mL of saturated NaCl solution were added and shaking vigorously. The resulting hexane layer (3 mL) sample was dried with anhydrous sodium sulphate and was left in a rack at room temperature (25 °C) to settle. The upper hexane layer that was clear containing methyl esters was decanted into amber Agilent vials and stored at -20 °C for GC analysis. A trans-esterified sample of African walnut oil was sent to GlycoAnalytics Services, San Diego, United States for accurate analysis. African walnut methyl ester sample was pre-analysed by injecting 1µL and profiling of fatty acids were done using an Agilent 6890N Gas Chromatography device (Agilent Technologies, Wokingham, United States). Profiling and quantification of fatty acids were achieved using Restek-5MS (30m x 30mm x 0.25µm) column. Column temperature was programmed at 80°C for 3 min, and then raised to 220 °C at 4°C min^{-1} and then held for 3 min. The carrier gas was helium with a column flow rate of 1.12 cm^3 and the Mass detector transfer line temp: 280 °C.

A standard fatty acid methyl ester mixture known as Supelco 37 component FAME mix (Supelco, Merck, Bellefonte, UK) containing methyl esters of fatty acids ranging from C8 – C24 including monounsaturated and polyunsaturated fatty acids was used for the experiment as reference to aid in the identification of the relative retention times of the fatty acids. Quantitative analyses of the fatty acids were performed using the heptadecanoic acid methyl ester as internal standard. The results presented are mean values of three replicate readings.

Determination of mineral contents

Mineral content **of** African walnut oil was determined by the method described by Ozcan, (2009). About 0.3-0.6 g of milled African walnut fruit was put into burning cup and 15 mL of pure nitric acid (NHO_3) was added. The sample was heated in a MARS 6 microwave oven (CEM Corporation Manufacturer) at 200 °C. Distilled deionized water and commercial acids high purity was used to prepare all reagents, standards, and walnut samples used for the analysis. After digestion treatment, samples were filtrated through whatman filter paper No 42 with a diameter of 12.5 cm and pore size 2.5 µm. The filtrates were collected in 50 mL Erlenmeyer flasks and analyzed by ICP-AES (Varian) using optimized instrumental conditions. The mineral contents of the samples were quantified against standard solutions of known concentrations of boron, calcium, chromium, lithium, iron, potassium, magnesium, manganese, sodium, phosphorus, selenium, nickel and zinc prepared from stock solution (1000 mg/L) (Fisher Scientific, UK). Both the sample mineral contents and that of the standard solutions were analyzed concurrently.

Statistical analysis

All extractions and analysis were performed in triplicates. Results are expressed as Mean ± Standard Deviation (SD).

Results and Discussion

The two methods used for the extraction of oil from African walnut seem effective. The recovery rate using the expeller method involving the use of mechanical extractor was however less effective compared to the use of Solvent extraction. A large amount of seeds will be required for the mechanical press designed for extraction of oil from African walnut. Use of mechanical press (clamp) to squeeze oil out of the nut is easy to use as no special skill is required, but the recovery rate is slow (20.34%). Yield of oil extracted with Soxhlet extractor was 59.4%. In view of the advantages of solvent extraction, as observed in this study, the oil used for this analysis was extracted with solvent using a Soxhlet extractor.

The physical and sensory analysis of the colour, taste, sedimentation properties of African walnut oil indicated that it was pale yellow in colour, clear, tasteless without impurities. The oil is liquid at room temperature 30 °C (Table 1). The method of extraction of oil usually reflects on the oil's quality, colour and taste. This informed the need to pay some attention to the various possible methods of extracting oil from African walnut. Although no major differences were observed in the quality of oil extracted using mechanical press methods, extraction with solvent seems more efficient and economical for the extraction of oil from African walnut. This is in conformity with the observations of Akpuaka and Nwankwor, (2000). Use of mechanical press methods could be suitable for domestic purposes; it may be adopted for use by farmers who are familiar with a similar process which is commonly used for palm-oil extraction.

Table 1: Physical and sensory attributes of African walnut oil

Attributes	Characteristics / Properties
Colour	Pale yellow, clear and transparent ($5^1/_4$) on Lovibond meter)
Taste	Neutral, free of bitter taste, free of after tastes.
Smell	Neutral, free of smell coming from the plant material.
Sediments	Free of sediments. No impurities
State at room temperature	Liquid.

Source: Laboratory analysis, (2019).

The proximate composition of African walnut is shown in Tables 2. In Table 2, the moisture, ash, crude fibre, crude protein, crude oil, total carbohydrate contents of the nut were established. The results show that African walnut is rich in protein (13.5 %), carbohydrate (26.9 %) but low in moisture (2.36 %), ash (1.42 %) and fibre (1.6 %) content. It also has a good energy value (523.8 kcal/100g) and a rich source of oil (59.4 %). The moisture content of African walnut (2.36 %) obtained in this study is low and falls within the acceptable limit expected of edible nuts (Ozcan, 2009). The moisture content correlates with 2.36 % reported by Akin-Osanaiye, (2018) [3]. The low moisture content of the nut is an advantage when the shelf-life of the nut is considered. Crude protein content of African walnut was 14.5 % which is within the range recorded by other researchers (Kanu *et al*., 2015 [15]; Ayodeji and Aliyu, (2018) [5]. They reported protein values of

African walnut to be 15.6 %, and 14.9% respectively. Any plant food that provides more than 12% of its energy from protein is considered a good source of protein (Kanu *et al*., 2015) [15]. Therefore, the protein content of African walnut as recorded in this study meets this requirement and as such is considered a rich source of protein. The crude fibre content of 1.6 % obtained for African walnut was within the acceptable limit 0.8-6.29 % reported for most seeds and nuts in Africa (Obasi *et al*., 2012) [20]. This indicate that adequate intake of African walnut can lower cholesterol level, risk of coronary heart diseases, hypertension, diabetes, colon and breast cancers (Ayodeji and Aliyu, 2018) [5]. Thus the nut could be regarded as valuable source of dietary fibre in human nutrition. Ash content which indicates the presence of mineral element is low (1.42 %). The recorded ash value is far less than those reported for some non –conventional oil seed and nuts (Akanni *et al*., 2005) [2]. The carbohydrate content of 26.9 % is higher than 23.0 %, 16.9 % and 15.38 % reported by other researchers (Udedi *et al*., 2013; Kanu *et al*., 2015 [15]; Ayodeji and Aliyu, 2018) [5]. The estimated calorific value 523.8 kcal/100 g in African walnut is also relatively higher than values reported for *Telferia occidentalis* (60.53 kcal/100g) and *Mucuna ureans* (381.5 kcal/100g) reported by Ekop, (2007) [11]. However, the value was in agreement with those reported for some dietary and medicinal wild plants in the tropical Africa (Ekpo, 2007). Lastly the whole nut weight of African walnut was 10.2 g.

Table 2: Proximate properties of African walnut

Fruit properties	Values (%)
Moisture	2.36±0.67
Ash	1.42±0.02
Crude fibre	1.6±0.4
Crude protein	14.5±0.09
Crude oil (lipid)	59.4±2.0
Total carbohydrate	26.9±3.2
Calorific energy value (kcal/100 g)	523.8±1.10
Whole nut weight/a walnut (g)	10.2±1.0

Each data is mean of three replicate readings ± Standard Deviation (SD)

Source: Laboratory analysis, (2019)

The chemical properties of oil extracted from African walnut are shown in Table 3. The data (Table 3) showed the saponification values, unsaponifiable matter content, acid value, peroxide values, free fatty acids (FFA), iodine, Refractive index (RI) and Specific gravity (SG) of the oil was 170.1 mg KOH g^{-1}, 4.1 g/kg^{-1}, 0.92 mg KOH g^{-1}, 1.02 meq O_2 kg^{-1}, 0.56 %, 91.3 Wij's, 1.445 and 0.94. Based on the result presented in Table 3, a high saponification value of 170.1 mg KOH g^{-1} indicates that the oil has low molecular weight fatty acids. This attribute is of importance in soap making as well as in shampoo making (Ajiwe, 1994; Akanni *et al*., 2005) [2]. The value obtained is the range of values (174.84 - 192.45 mg KOH g^{-1}) obtained for rapeseed oil and soyabean oil that have gained much market priority as reported by Obasi *et al*., (2012) [20]. Unsaponification value was found as 4.1 g/kg^{-1}, and similar to the value of 4.7 g/kg^{-1}reported for terebinth fruit and English walnut (*Juglan regia*) by Ozcan, (2004) [24]. Obviously, this attribute of the oil can be linked with the cholesterol content it contained. Acid value was 0.92 mg KOH g^{-1}, lower than those of soya bean (4.30 mg KOH g^{-1}) and turkey (5.61 mg KOH g^{-1}) reported by Obasi *et al*., (2012) [20]. Iodine value was 91.3 Wij's, in the range of cotton seed oil and sun flower oil iodine values (90-119 Wij's) reported by Popoola and Yangomodou, (2006) [26]. This value which is in the middle range shows that the oil is semidrying and unsaturated. This further shows that the oil could be used in liquid soap formulation, just as the low acid value recorded for the oil could be of significance in paint and varnish manufacturing (Popoola and Yangomodou, 2006) [26]. Also, refractive index and specific gravity values of 1.445 and 0.94 compares favorably well with that of *Juglans regia* (1.446 and 0.97) as reported by Ozcan, (2009). The specific gravity of 0.94 at 25 °C of African walnut oil and the observed melting point of 17.8 °C, which is really lower than the average tropical room temperature 25 °C, explains why the oil is liquid at room temperature. The peroxide value was 1.02 meq O_2 kg^{-1} lower than generally recommended value for commercial edible crude vegetable oil. This is an indication that African walnut oil can resist lipolytic hydrolysis and oxidative deterioration (WHO, 2002).

Table 3: Chemical properties of African walnut oil

Oil properties	Values
Specific gravity	0.94±0.01
Refractive index	1.445±0.002
Acid value (mg KOH/g)	0.92±0.12
Free fatty acid (%)	0.56±0.10
Peroxide value (meq O_2/ kg^{-1})	1.02±0.19
Saponification number (mg KOH/g)	170.1±5.12
Unsaponifiable fraction (g/kg^{-1})	4.1±0.65
Iodine (mg of $1g^{-1}$ of oil) (Wiji's)	91.3±1.09

Each data is mean of three replicate readings ± Standard Deviation (SD)

Source: Laboratory analysis, (2019).

The fatty acid composition of African walnut oil was determined as corresponding methyl esters, using gas chromatography (Table 4). The main fatty acids methyl esters identified included saturated, monounsaturated and polyunsaturated fatty acids. The saturated fatty acids identified include palmitic acid and stearic acid. Only one monounsaturated fatty acid eicosenoic acid was identified. Also, the polyunsaturated fatty acids were predominantly linoleic (C18:2) and linolenic acid (C18:3) otherwise called α linolenic acid (alpha linolenic acid). Linolenic acid 80.59 % was present in the highest concentration, followed by linoleic acid 13.05 % and stearic acid 4.41 %. Palmitic (C16:0) and Eicosenoic acid were only present in minor amounts (1.53 % and 0.42 %). To date, this is the first study to give detailed fatty acid composition of African walnut oil fractions from Nigeria In a previous study on the fatty acids of African walnut oil, it was established that palmitic (C16:0), α-linolenic (C18:3), linoleic (C18:2) were the main components of the oil (Nkwonta, 2015) [19]. Tchiegang *et al*., (2001) [29]. also reported same types of saturated and polyunsaturated fatty acids in walnut cultivars grown in Cameroon. Although different capillary columns were used by both authors, they still noted that the most abundant fatty acid was the conjugated C18:3 - linolenic acid (70 %) followed by C18:2 (linoleic acid) and C18:0 (palmitic acid) fatty acids. These findings are in good correlation with the results obtained in this present study and suggest a strong similarity between the oil of walnuts grown in Nigeria and Cameroon despite different ecological factors that possibly influence the growth of the plants. In a study reported by Ozcan, (2009), *Juglans regia* was found to have linoleic acid present in highest composition 55.3%, followed by oleic acid (13.4 %), linoleic 8.7%, palmitic acid (6.4 %) while minor amounts of stearic acid (1.7 %), palmitoleic acids (0.1 %) were obtained. This probably shows a difference between the fatty acid composition of African walnut oil and the English walnut oil. However, as observed in this study, the fatty acid composition shows high content of both linolenic and linoleic acids which are beneficial to human health and linoleic especially linolenic acid play vital roles for human health regarding cardio vascular system (Tchiegang *et al*., 2001 [29]; Ozcan, 2009; Nkwonta, 2015) [19]. All the fatty acids present in African walnut oil are important for dietary purposes and therefore confirms the usefulness of the oil in diets. In addition, nuts and oils intended to be cooked for food purposes are expected to have low polyunsaturated fatty acid content, as polyunsaturated fatty acids have a higher tendency to char (Ozcan, 2009).

Table 4: Fatty acid composition of African walnut oil

Oil properties	Values (%)
Palmitic acid (C16:0)	1.53±0.1
Linoleic acid (C18:2)	13.05±0.3
Linolenic acid (C18:3)	80.59±1.2
Stearic acid (C18:0)	4.41±0.5
Eicosenoic acid (C20:0)	0.42±0.12

Each data is mean of three replicate readings ± Standard Deviation (SD)
Source: Laboratory analysis, (2019)

The mineral content of African walnut was determined by ICP-AES as shown in Table 5. African walnut was found to have thirteen minerals such as boron (B), calcium (Ca), chromium (Cr), lithium (Li), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P), selenium (Se), nickel (Ni) and zinc (Zn). Interestingly, African walnut was highly rich in some minerals such as K (3450.3 mg/kg), P (3021.7 mg/kg), Mg (1076.9 mg/kg), Ca (1008.5 mg/kg). Low amounts of B (52.5 mg/kg), Mn (38.7 mg/kg), Na (36.6 mg/kg), Fe (29.8 mg/kg), Zn (20.2 mg/kg), Se (13.6 mg/kg) and trace amounts of Cr (7.9 mg/kg), Ni (1.8 mg/kg) and Li (0.56 mg/kg). In *Juglans regia* kernel, Ozcan, (2009) reported K (4627.6 mg/kg), P (3621.9 mg/kg) and Mg (1089.9 mg/kg) as minerals highly available. Mineral contents of K, P and Mg obtained in this study were found to be lower compared with those reported by Ozcan, (2009). Also, K, P and Mg values of African walnut were higher than those of *P. terebinthus* reported by Ozcan, (2004) [24]. Values for K, P and Mg in *P. terebinthus* were K (1364.19 mg/kg), P (801.88 mg/kg), Mg (318.39 mg/kg). Ca value was higher in African walnut. All other minerals were also available in lower amounts and compares favorably well to those of *Juglans regia* kernel and *P. terebinthus*. Calcium is the major component of bone and teeth development. African walnut appears to contain inorganic elements such as cadmium and lithium which are essential for biological processes.

Table 5: Mineral contents of African walnut

Minerals	Values (mg/kg)
B	52.5±14.3
Ca	1008.5±11.6
Cr	7.9±0.5
Li	0.56±0.04
Fe	29.8±3.9
K	3450.3±21.6
Mg	1076.9±19.8
Mn	38.7±5.7
Na	36.6±6.5
P	3021.7±10.3
Se	13.6±1.8
Ni	1.8±0.4
Zn	20.2±2.4

Each data is mean of three replicate readings ± Standard Deviation (SD)
Source: Laboratory analysis, (2019)

Table 6 shows the properties of lipid in Africa walnut oil are comparable in quality to those of processed vegetable oils that are commercially available. Africa walnut oil was found to have physical and chemical properties that compares well with those of processed groundnut oil, cotton-seed oil, soybean oil, corn oil, rapeseed oil and sunflower-seed oil.

Table 6: Comparison of physico-chemical properties of African walnut oil to some recorded values of other commercially available vegetable oils in Nigerian market.

Properties	African walnut oil	Groundnut oil	Soya bean oil	Cotton seed oil	Corn oil	Sunflower oil	Rapeseed oil
Colour	Pale yellow	Light yellow	Light yellow	Light yellow	Light yellow	Light yellow	Bright yellow
Smell	Neutral	Neutral	Neutral	Neutral	Neutral	Neutral	Neutral
Taste	Neutral	Peanut flavor	Neutral	Neutral	Neutral	Neutral	Neutral
FFA (%)	0.56	6.26	0.10	0.10	0.10	0.10	0.10
Unsaponifiable matter	4.1	1.5	1.5	1.5	1.5	1.5	1.5
Iodine (Wij's)	91.3	99-111.9	120-142	99-111.9	105-130	118-144	90-120
Refractive index (RI)	1.444	1.4620-1.4640	1.467-1.470	1.464-1.468	1.465-1.468	1.466-1.469	1.464-1.469
Peroxide value (meq O_2 Kg^{-1})	1.02	2.5	2.5	2.5	2.5	2.5	2.5
Saponification value (mg/KOH/g)	170.1	188-210	189-195	189-198	187-195	189-198	166-198
Specific weight (25 °C)	0.940	0.912-0.920	0.919-0.925	0.915-0.921	0.917-0.925	0.918-0.926	0.906-0.914

Values for African walnut oil are as obtained in this investigation. Values for other oils except (RI) are those for commercially sold edible oils made by Cidersan Vegetable and Olive Oil Company of Turkey (online:htp://www.cirdersan.com.tr/physical). The values are therefore for processed oils. Source: Food industry manual (1997). (Ranken and Kill, 1997).

Conclusions

The extraction, properties and utilization potentials of African walnut (*Tetracarpidium conophorum*) as an alternative to conventional vegetable oils was evaluated in this study. African walnut examined was found to be nutritionally potent with appreciable high levels of nutrients and energy. Thus, the nut could be good source of food supplement for human nutrition. The nut also contain high level of good quality oil whose characteristics compared favourably with most conventional vegetable oils sold commercially in the Nigerian markets. The walnut oils had agreeable colour and taste and was liquids at room temperature (25 °C).

The lipid indices of African walnut oil showed that the oil is edible and judging by its low acid value and the corresponding low free fatty acid (FFA) level suggested low hydrolytic and lipolytic activities in the oil. Industrially, the chemical composition results revealed that African walnut oil has great potentials in soap manufacturing industries because of the high saponification value and as such, the nut oil could be commercialized for such purposes. African walnut oil is a semi-drying due to its low iodine value, however, this also suggests that the oil contain few unsaturated bonds and therefore have low susceptibility to oxidative rancidity as confirmed by the peroxide value.

The physical and chemical properties of African walnut oil as enumerated in this work, makes it a good substitute to some convectional oils. The oil has good values as edible oil and may also find industrial applications in soap, shampoo and other related cosmetic industries. As the properties of lipid extracted from African walnut compares well with those of processed groundnut oil, cotton-seed oil, soybean oil, corn oil, rapeseed oil and sunflower-seed oil, it is suggested that further efforts should be made to discover other possible relevance of lipid from African walnut. African walnut oil has promising potentials which could be tapped for domestic and industrial uses.

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