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Biochemical and Nutritional Composition of Two Perennial Crops Grown in Nigeria: A Comparison

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Authors' contributions

This work was carried out in collaboration between all authors. Author AMO conceived the study, wrote the study and performed the statistical analysis. Author AOD wrote the first draft of the study. Author ETA wrote the protocols. All authors read and approve the final manuscript.

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ABSTRACT

The nutritional composition, physicochemical properties and fatty acid composition of two perennial crops: *Tetracarpedium conophorum* (African walnut) and *Irvingia gabonensis* (African bush mango) were studied. The oils were extracted from the seeds using n-hexane in soxhlet extractor and were used to determine the physicochemical properties and fatty acid composition of the samples while the proximate analysis was done from the powdered form of the seeds. The results showed that both samples had high percentage oil yield with *I. gabonensis* having significantly higher oil yield than *T. conophorum* (p= .001). *I. gabonensis* have significantly higher protein content of 9.02% than 3.12% of *T. conophorum (p* =.006*)*. They both have high percentage of carbohydrate but *T. conophorum* has significantly higher percentage (p =.000). The fatty acids composition of the *I. gabonensis* are mainly saturated fatty acid (87.28%) while *T. conophorum* revealed high proportion of unsaturated fatty acid (99.95%) implying that, *T. conophorum* could provide the body with essential fatty acid. In conclusion, both samples have good nutritive values and can also be of

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economic importance in soap making, oil and domestic food industries since they are perennial plants with guaranteed availability during seasons. In addition, the consumption of *I. gabonensis* can be substituted for *T. conophorum* because of its high percentage of saturated fatty acid in order to avoid health risks associated with too much consumption of saturated fatty acid.

Keywords: Perennial crops; essential fatty acids; nutrition; I. gabonensis; T. conophorum.

1. INTRODUCTION

African walnut (*Tetracarpedium conophorum*) is a perennial plant grown in Africa and all over the world [1]. It belongs to the family *Juglandaceae* and the genus *Juglans*. It has high amount of protein and essential fatty acids. *T. conophorum* is cultivated principally for the nuts which are cooked and consumed as snacks [2]. It is called *'asala' or 'ausa'* by the Yorubas of the southwestern part of Nigeria.

It is one of the several high nutrient dense foods with the presence of protein, fiber, carbohydrate and vitamins [3]. The vitamin content is useful for the treatment of common cold and other diseases like prostate cancer [4]. *T. conophorum* is a rich source of mineral elements such as calcium, magnesium, sodium, potassium and phosphorus [5].

African bush mango (*Irvingia gabonensis*) is a species of African and South East Asian trees in the family *Irvingiacea.* It is commonly called wild mango, African mango, bush mango, dika or Ogbono by the Igbos of the southeastern part of Nigeria. It bears edible mango – like fruits which have a slight bitter taste and high fat and protein contents. The fruit is a large drupe with fibrous flesh and is valued for its nut which is aromatic and succulent. The nuts have high content of mucilage which enables them to be used as thickening agent for dishes such as ogbono soup. The nuts may also be pressed for vegetable oil. Many studies have reported the nutritional and medicinal values of *I. gabonensis* and *T. conophorum* [3,6-8].

These two plants are perennial crops which are mainly grown for their nuts and availability through seasons because of their perennial nature. The nut of *T. conophorum* is cooked and eaten while that of *I. gabonensis* is used as a thickening agent for soups. A closer look into the chemical nutritional and fatty acid properties of the oils and powdered form of these nuts is important since they have widespread use and consumption. In addition, they are available perennially for human use for various purposes.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Two bowls full of the seeds of *T. conophorum* used for this study were removed from matured pods collected from Odo-Omosuo farmland at Iworoko-Ekiti village in Irepodun/Ifelodun Local Government Area of Ekiti State, Nigeria and were identified at the Plant Science Department of Ekiti State University. The seeds were dehulled and were thoroughly washed with distilled water and dried under room temperature for two months before milling to a coarse powder and stored in an air tight bottle prior to analysis. Two bowls full of *I. gabonensis* seeds were bought from Erekesan market in Ado Ekiti, Ekiti State Nigeria. The seeds were dehulled, washed, dried and milled into powder.

2.2 Extraction of Oil

150 g of the fine powdered of each sample was extracted using n- hexane in soxhlet extractor for 8 hrs at 60℃. The oil was then recovered by evaporating the solvent using rotary evaporator (Rotavapor R110).

2.3 Proximate Analysis

Standard methods of Association of Official Analytical Chemists [9] were used to determine the moisture, crude fat, crude fibre, crude protein and carbohydrate contents of the powdered form of each sample.

2.3.1 Determination of moisture content

The moisture content was determined by weighing 1.0 g of the powdered sample in a crucible of known weight. This was placed in an oven and dried at 105℃ for three hours. The sample was allowed to cool in a desiccator and then reweighed. The percentage moisture content was calculated by expressing the loss in weight on drying as a fraction of the initial weight of sample used.

$$moisture\ content(\%) = \frac{w_0}{w_i} \times 100$$

Wo= loss in weight (g) on drying
W_i = initial weight of sample (g)

2.3.2 Determination of crude protein

The crude protein was done using micro-Kjeldhal method. 1 g of the sample was weighed in triplicate and placed in digestion flasks. Few granules of anti bumps and about 3 g of copper catalyst mixture were added to each of the flasks (96% anhydrous sodium sulphate, 3.5% copper sulphate and 0.5% selenium dioxide). Digestion was then commenced by adding 20 cm^3 concentrated sulphuric acid to each flask and it was heated on a heating mantle. Digestion was continued until a clear solution was obtained and the flask was allowed to cool. This was then filtered and the filtrate was made up to 100 cm^3 with distilled water. 20 cm^3 of the diluted digest was used for distillation. 20 cm^3 of 2% boric acid with screened methyl red indicator was added to the samples in a round bottomed flask set on a heating mantle and connected to a beaker using a liebig condenser. 30 cm^3 of 40% sodium hydroxide was injected into the flask and distillation continued until there was a complete change in colour from purple to greenish-yellow. The boric acid mixture was then titrated with 0.1 N HCl to colourless end point. The total organic nitrogen was then calculated using the formula:

$$\%\,TON = \frac{TV \times N \times V}{M \times V_d} \times 100$$

Where

TV = Titre value
N = mg nitrogen equivalent to molarity of acid
V = total volume to which digest was diluted
M = mass of sample (g)
V_d = volume of digest distilled

$$\%\,crude\ protein = \%\,TON \times 6.25$$

2.3.3 Determination of crude fat

The crude fat was determined using soxhlet extractor. n hexane was used for the extraction. 50 g of the sample was extracted and allowed to dry. It was then reweighed. The percentage of crude fat was calculated using:

$$CF(\%) = \frac{M_e}{M_s} \times 100$$

Where

M_e = mass of extract
M_s= mass of sample used
CF = crude fat

2.3.4 Determination of crude fibre

2.0 g of the sample was weighed into 1 L conical flask 200 ml of boiling 1.25% H_2SO_4 was added and boiled gently for 30 min. the mixture was filtered through muslin cloth and rinsed well with hot distilled water. The sample was scrape back into the flask with spatula and 200 ml of boiling NaOH was added and allowed to boil gently for 30 minutes. It was filtered through muslin cloth and the residue washed thoroughly with hot distilled water and then rinsed with 10% HCl with industrial methylated spirit twice and then rinsed with distilled water and then drained. The residue was scrapped into a crucible, dried in the oven at 105℃, cooled in a dessicator and weighed again. The percentage crude fibre was determined using:

$$CFB(\%) = \frac{M_s}{M_d} \times 100$$

Where:

CFB = Crude fibre
M_s = Mass of sample used
M_d = Mass of dried sample

Total carbohydrate content of each sample was estimated by difference

Total carbohydrate (%) = 100 – (% moisture + % crude protein+ %crude fat + % crude fibre)

2.4 Physicochemical Analysis

The acid value, saponification value and iodine value were determined by standard methods as described by Association of Official Analytical Chemists [9]. The refractive index was determined with calibrated Abbey refractometer, viscosity was also determined by a calibrated viscometer (Stress Tech Rheological) and the pH was determined using a pH meter

2.4.1 Determination of the percentage oil yield

50 g of the sample was placed in the thimble and about 150 ml of normal hexane was poured into the round bottom flask. The oil was extracted for 8 hrs at 60℃ using soxhlet apparatus. The oil extracted was poured in an empty conical flask

which weight was known and then was reweighed with the sample oil inside. The mass of oil extracted was calculated by difference and the percentage oil yield was calculated using:

$$Percentage\ \ oil\ yield = \frac{m}{m_o} \times 100$$

Where:

m = mass of oil extracted (mass of conical flask + oil – mass of empty conical flask)
m_o= mass of the sample

2.4.2 Determination of acid value

1 g of each oil sample was weighed into a conical flask mixed with 1 ml diethyl ether and 20 ml ethanol. 1 ml of 1% phenolphthalein indicator was added to the mixture. The solution was titrated with 0.1 M sodium hydroxide. Acid value was calculated.

$$Acid\ value = \frac{56.1 \times N \times V}{W}$$

Where:

N= Normality of sodium hydroxide
V= Volume of sodium hydroxide in the test
W= Weight of sample.

2.4.3 Determination of saponification value

1 g of each oil sample was weighed into a conical flask and 5 ml of alcoholic potassium hydroxides solution was added. The flask with its content was attached to a reflux condenser and heated on a boiling water bath for one hour with occasional shaking; 1 ml of phenolphthalein indicator was added to the solution and filtered while hot with 0.5 M hydrochloric acid. Analysis was carried out on a blank solution which contained all the reagents without sample.

$$Saponification\ value = \frac{56.1(V_1 - V_2)N}{W}$$

where:

N = Normality of hydrochloric acid
V_1 = Volume of hydrochloric acid used in the test
V_2 = Volume of hydrochloric acid used in the blank
W = Weight of sample.

2.4.4 Determination of iodine value

Oil samples of (0.25 g) each accurately weighed on a small square foil and this was dropped into a dry 250 ml conical flask. 15 ml of chloroform were added with dry measuring cylinder and the flask was shaken to dissolve the oil. 25 ml of anus reagent was pipette with a pipette filler into the fume cupboard and corked. At the end of 30 minutes 10 ml of 15% potassium iodide and about 25 ml of distilled water were added. The iodine was back titrated with sodium thiosulphate equivalent of the iodine absorbed by the oil.

$$Iodine\ value = \frac{(b-a)1.26}{w}$$

Where:

a = titre value
b = blank titre value
w =weight of sample

2.4.5 Determination of peroxide value

This test was carried out in the dark, 1g of each oil sample was weighed into a clean drying boiling tube and 20 ml solvent mixture (acetic acid+ diethylether) were added into the tube and boiled for 60 second. The content was poured into the titration flask containing 20 ml of 5% potassium iodide. The content was therefore titrated with 0.002 M thiosulphate using starch indicator.

$$Peroxide\ value = \frac{1000(V_1 - V_2)N}{W}$$

N = Normality of thiosulphate
V_1 = Volume of thiosulphate used in the test
V_2 = Volume of thiosulphate used in the blank
w = weight of sample

2.5 Determination of Fatty Acid

The fatty acid was determined by transesterification and derivatization of the methyl ester as described briefly. 50 mg of the extracted fat was saponified for five minutes at 95℃ with 3.4 ml of 0.5 M KOH in dry methanol. The mixture was neutralised by using 0.7 M HCl. 3 ml of 14% boron triflouride in methanol was added. The mixture was heated for 5 min at 90℃ to achieve complete methylation process. The Fatty Acid Methyl Esters (FAME) was extracted thrice from the mixture with redistilled n- hexane. The content was concentrated to 1 ml for gas chromatography analysis.

2.6 Statistical Analysis

Obtained data were statistically analyzed for statistical significance using Chi – square test with the significant level set at $p < 0.05$.

3. RESULTS AND DISCUSSION

The result of the proximate analysis of the samples is presented in Table 1. Both samples have high percentage protein but *I. gabonensis* has significantly higher percentage of 9.02% compared to 3.12% from *T. conophorum*. *I. gabonensis* also has higher percentage of crude fibre of 7.16%, against 5.72% for *T. conophorum*. Crude fibre provides the viscous nature characteristics of some local African diets and this justifies the use of *I. gabonensis* as a thickening agent and also as condiments in some African traditional diets such as Ogbono soup which is popular delicacy among the Igbos and Yorubas of the South Eastern and Western parts of Nigeria respectively. Both samples were rich in fat but the *I. gabonensis* had a higher fat content compared to 35.32% recorded from the *T. conophorum*. The carbohydrate content of *T. conophorum* was 51.03% and this was higher than that of *I. gabonensis* (12.74%). This shows that these samples could be good sources of protein, fat and carbohydrate the main classes of food nutrients and further justifying their good nutritional and economic values. The values of the nutrients` content obtained for *I. gabonensis* in this work are higher when compared to 4.38% for crude fibre and 7.47% for protein reported by Okoronkwo et al. [10] for *I. gabonensis* seed oil but are close to the 7.70%, 10.23%, 65.46% and 10.93% for crude protein, crude fiber, crude fat and carbohydrate respectively reported by Oniwawo et al. [11] for *I. gabonensis*. The observed differences between this study and that of Okoronkwo et al. [10] may be due to differences in the geographical areas. Okoronkwo et al. [10] sourced their samples from South Eastern Nigeria while the samples for this work and those of Oniwura et al. [11] were sourced from South Western Nigeria. This is confirmed the facts that nutrients contents of plants and seeds may vary from place to place depending on the climatic and soil conditions [12].

Table 2 shows the physicochemical properties of the samples. The percentage oil yields of both samples were 64.10% and 32.11% for *I. gabonensis and T. conophorum* respectively. Both samples had acid values of 7.48 (mg KOH/g of oil) and 7.05 (mg KOH/g of oil) for *T. conophorum* and *I.gabonenesis* respectively. The lower acid values of both samples justify their edibility and safety for human. In addition, oils with lower acid values have good storage ability and cannot easily go rancid. Hence, they could be preserved for use in the period of scarcity and this is more important in the developing world where there is lack of high technology storage facilities thus further affirming the suitability of their use for various purposes in these countries. Although these oils have slightly higher acid values than the codex recommended value of 6.6 (mg KOH/g of oil) for virgin oils [13]; they can still be considered as having good storage ability and therefore the oils cannot go rancid easily. These values are higher when compared with cashew nut seed oil (0.82 mgKOH/g) as reported by Aremu et al. [14].

T. conophorum has iodine value of 110.40 (mg/g of oil) while *I. gabonensis* has iodine value of 99.55 (mg/g of oil). Oils with iodine values less than 100 can be classified as non drying oil [15], since both oils have iodine value close to 100 (mg/g of oil), they can be classified as semi drying and thus can be of importance in leather industry, dressing candle and in the manufacture of lubricants and hydraulic brake fluid [16].

Table 1. Proximate analysis of the *Tetracarpedium conophorum* and *Irvinga gabonensis*

Parameters	*Tetracarpedium conophorum*	*Irvingia gabonensis*	X^2	P
Moisture content	4.78	5.62	0.07	0.79
Protein (%)	3.12	9.02	7.50	**.006***
Crude fibre (%)	5.72	7.16	0.16	0.68
Crude fat (%)	35.32	65.46	9.01	**.003***
Carbohydrate	51.06	12.74	23.31	**.000***

**significant difference at p. <0.05*

Table 2. Physicochemical parameters of *Tetracarpedium conophorum* and *Irvingia gabonensis*

Parameters	*Tetracarpedium conophorum*	*Irvingia gabonensis*	X^2	P
Percentage oil yield (%)	32.11	64.10	10.63	.001*
Iodine value (mg/g oil)	110.40	99.53	0.56	0.45
Saponification value (mgKOH/g oil)	190.42	189.40	0.002	0.96
Peroxide value (meqO^2/Kg oil)	10.69	11.55	0.03	0.85
Unsaponifiable matter (%)	0.81	0.81	0.00	1.00
Acid value (mgKOH/g of oil)	7.48	7.05	0.01	0.90
Specific gravity	0.90	0.92	0.002	0.98
Refractive index	1.47	1.47	0	1.00
Kinetic viscosity (mm^2/sec)	42.5	43.02	0.003	0.95
pH	3.69	4.04	0.016	0,89

** Significant difference at p< 0.05*

The saponification values of the samples are 189.40 and 190.42 mg/KOH/g for *I. gabonensis* and *T. conophorum* respectively. These values are lower when compared with 194 mg KOH g^{-1} of liquid red palm oil [17] and 213 mg KOH g^{-1} of neem seed oil [18] and high when compared to 179.87 mgKOH g^{-1} reported by Isong et al. [19] for *T. conophorum*. This shows that oils from both samples can be used in soap making industry. The percentage of oil yield for *I. gabonensis* is 63.75% while that of *T. conophorum* is 34.00. This value is lower when compared with 57.50% reported by Isong et al. [20] for *T. conophorum*. The relatively high percentage yield of oil of these samples showed that they can be good sources of oil.

The fatty acid composition of the samples shows that *I. gabonensis* has high percentage of saturation with lauric acid being the most predominant (26.48%) followed by myristic acid (24.58%) while Oleic acid and Nervonic acid have percentage composition of 7.18% and 5.54% respectively. Other saturated fatty acid constituted 28.97% of the total fatty acid content. The fatty acid composition of *T. conophorum* showed that it has very high percentage of linoleic acid (85.79%), palmitoleic acid (11.03%) and linolenic acid (3.17%) (Table 3); which are essential fatty acids. The result of the fatty acid composition shows that *T .conophorum* composed mainly of unsaturated fatty acid while *I. gabonensis* is composed of mainly saturated fatty acid. This indicates that *T. conophorum* can provide the body with essential fatty acids (Omega 6 and 3) which are very important for healthy state and neuronal development [20]. This affirms the beliefs that consumption of walnut leads to improve higher functions of the brain and this further highlights the health benefit of walnut [21].

Although *I. gabonensis* has high level of protein, carbohydrate, crude fibre and crude fat, its consumption or use as condiment should be controlled because of the high percentage of saturated fatty acid. Saturated fatty acids have been associated with heart diseases [22].

Table 3. Fatty acid composition of *I. gabonensis* and *T. conophorum* oil

Fatty acid	*I. gabonensis (%)*	*T. conophorum (%)*
Myristic (C14:0)	24.58	ND
Palmitic (C16:0)	7.25	ND
Oleic (C18:1)	7.18	ND
Nervonic (C24:1)	5.54	ND
Lauric (C12:0)	26.48	ND
Palmitoleic (C16:1)	ND	11.03
Linoleic (C18:2)	ND	85.75
Linolenic (C18:3)	ND	3.17
Other Saturated acid	28.97	0.05
Total saturated fatty acid	87.28	0.05
Total unsaturated fatty acid	12.72	99.95

ND – not detected

The proximate, fatty acid composition and physicochemical properties of these two nuts showed that they have the same range of protein, crude fibre and carbohydrate. Also, their physicochemical properties are quite similar but there is a wide discrepancy between their fatty acid composition therefore consumption of *I. gabonensis* may be substituted with *T. conophorum* for people prone to the risk of high cholesterol associated diseases.

4. CONCLUSION

It can be concluded from the results that *I. gabonensis* and *T. conophorum* have good nutritional values therefore they can find applications in various domestic and nutrition purposes in addition to their applications in different industries like soap making, oil producing and food industry. *T. conophorum* has higher percentage of unsaturated fatty acid than *I. gabonensis* and this makes consumption of the oil of *T. conophorum* preferable to that of *I. gabonensis.*

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Caglarimak N. Biochemical and physical properties of some walnut genotypes (*Juglan regia*). Nahrung/ Food. 2003; 47(1):28.
2. Oke OL. Leaf protein research in Nigeria. University of Ibadan Press, Ibadan, Nigeria; 1995.
3. Savage GP, McNiel DL, Dutta PC. Some nutritional advantages of walnuts. Acta Hort. 2001;544:557-563.
4. Okwu DE, Okele O. Phytochemical screening and mineral composition of chewing sticks in South Eastern Nigeria. Global J. Pure Appl. Sci. 2003;9:235–238.
5. James NR. Volatile components of green walnut husks. J. Agric. Food Chem. 2000; 48(7):2858-2861.
6. Adamson I, Okafor C, Abu-Bakare A. A supplement of dikanut (*Irvingia gabonesis*) improves treatment of type II diabetics. West Afr J Med. 1990;9(2):108-115.
7. Okolo CO, Johnson PB, Abdurahman EM, Abdu-Aguye I, Hussaini IM. Analgesic effect of *Irvingia gabonensis* stems bark extract. J Ethnopharmacol. 1995; 45(2):125-129.
8. Ngondi JL, Oben JE, Minka SR. The effect of *Irvingia gabonensis* on body weight and blood lipids of obese subjects in Cameroon. Lipids in hlth and Disease. 2005;4:12.
9. Association of Official Analytical Chemists (AOAC). Official methods of analysis.14th edn, Arlington VA; 1995.
10. Okoronkwo CU, Agoha EE, Ogodo AC, Nwachukwu NO. Physical and chemical characteristics of the African bush mango (*Irvingia gabonensis var gabonensis*) seed oil. IJAEM. 2014; 1(6):28-31.
11. Oniwawo I, Oteno F, Orokpo G, Akubor PI. Physicochemical and nutrient evaluation of African bush mango (*Irvingia gabonensis*) seeds and pulp. Plant Foods for Human Nutr. 2003;58(3):1-6.
12. Olatunya AM, Olatunya OS, Adegoke S, Obafemi A, Akintayo ET. Analysis of phenolic compounds, phytosterols, lignans and stilbenoids in garlic and ginger oil by gas chromatography. J. Food Chem. Nutr. 2014;02(02):53-60.
13. FAO. Codex standard of fats and oils from vegetable source.
    Available:www.fao.org
    (Last Modified 1999, retrieved 04/09/2015)
14. Aremu MO, Olonisakin A, Otene IW, Atolaiye BO. Compositional studies and physicochemical characteristics of cashew nut (*Anarcadium occidentale*) flour. Pak. J Nutr. 2005;5(4):328-333.
15. Dawodu FA. Physico – chemical studies on oil extraction processes from some Nigerian grown plant seeds. EJEAFChe. 2009;8(2):102-110.
16. Apiamu A, Igunbor CO, Even UF, Ugbebor G, Osanebi O. Comparative assessment of lipids and physicochemical properties of African locust beans and Shea nut oil. J. Natural Sci. Res. 2013;3(11):25-31.
17. Osita BU. Physicohemical properties of “Eketeke” (hard red palm oil). Nig. J. Nutr Sci. 2007;1:157-162.
18. Akpan UG. Extraction and characterization of neem seed oil. In: Eyo AA, Aloku PO, Garba SA, Ali UD, Lamai SL and Olufeagba SO (editors) Biotechnology and sustainable development in Nigeria.

Proceedings of the 12th Annual Conference of the Biotechnology Society of Nigeria; 2000.
19. Isong N, Alozie YE, Ekwere Y. Physicochemical properties of African walnut oil and its suitability for domestic and industrial uses. Nig. J. Agric, Food and Environ. 2013;9(3):12-15.
20. Needlman RD. Growth and development In: Nelson Textbook of Paediatrics. 17th ed. Elsevier Imprints, Philadephia, USA; 2004.
21. Nanditha PR. Why walnuts are the ultimate brain food. Available:www.naturalnews.com (Last Modified 2013. Retrieved 07/12/2015)
22. Stuchli M, Zak S. Vegetable lipids as components of functional foods. Biomed Papers. 2002;146(2):3-1.

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