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RESEARCH ARTICLE

Varietal differences based on proximate, minerals and anti-nutritive composition in African walnut (*Tetracarpidium conophorum* Mull)

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Abstract

A research on proximate, minerals and anti-nutritive composition of African walnut collected from Nigeria and Cameroon was carried out at the Laboratory of Department of Biochemistry and Technology, Federal University of Technology, Owerri, Nigeria. Experimental design was completely randomized design with five replications. Proximate properties of the two lines did not show any significant difference ($P< 0.05$) except for carbohydrate. However, the line from Nigeria had higher values in protein (28.32%), crude fat (5.67%), ash (4.33%), and moisture (38,44%) content, while higher values for carbohydrate (20.87%) and crude fibre (9.32%) was obtained for the line from Cameroon. The mineral composition of the two lines showed significant difference ($P> 0.05$) in some mineral elements like manganese, sodium and chromium. Anti-nutritional composition of the two lines did not show any significant difference like the result on proximate analysis. However, higher values of phytate (6.27%), and oxalate (0.11%) was observed for the line from Nigeria while the line from Cameroon had higher content of tannin (0.36%) and saponin (0.27%). Apparently, the few significant differences observed between the two lines is not adequate to establish varietal difference. Further no single variety had higher values in all the evaluated biochemical (proximate, mineral and anti-nutritive) components, implying that the two lines are similar.

Keywords: African walnut, biochemical composition, properties, varietal differences

Introduction

Crops are grown primarily for the purpose of nutrition, to combat hunger which may include hidden hunger (deficiency in intake of essential vitamins and minerals collectively called micronutrients). The value placed on a crop is based on its nutrient content. It is the nutritional composition of crops that is used in their ranking in order of importance. Usually, crops with healthier nutritional quality are ranked higher than others. In recent times, analysis of nutritional quality of crops has enabled workers to establish intra and interspecific variation that exist between and within species, and the similarities and differences established has been fully utilized in the botanical classification of crops. It has helped to determine the relatives in crops, thereby facilitating simple classification.

In fact the nutritional value of a crop plays a vital role in policy making and the level of research a crop attracts. Invariably the optimum production and utilization of any crop is largely influenced by its nutritive composition. The ultimate realization of the full potentials of any crop is enhanced when its nutritional value has been well established.

African walnut (*Tetracarpidium conophorum* or *Plukenetia conophora*) is a shrub crop that is commonly found growing in semi wild areas in the forest zones of Africa and India. This climbing shrub that grows between 10-20 ft long is popular and cherished for its nutritious edible nuts that is cooked and consumed as snacks (Enujiugha, 2003; Srinivasan and Viraraghavan, 2008; Edem *et al*., 2009). African walnut has outstanding medicinal, nutritive, traditional, economic and industrial qualities. Medicinally leaves, bark, and fruit of *Tetracarpidium conophorum* can be used in treating rheumatism, syphilis, dysentery, diarrhoea, piles, cold, kidney pain, heavy menstrual bleeding, expelling worms, toothache, inflammation of the gums, mouth, throat and as an antidote to snake bite (Anderson *et al*., 2001; Odugbemi and Akinsulire, 2008; Nwaoguikp *et al*., 2012). In Southern Nigeria ethno-medicine, African walnut is used as a male fertility agent and in the treatment of dysentery (Ajaiyeoba and Fadare, 2006). Nutritionally, Ogunwusi and Ibrahim (2016) reported that immature fruit of African walnut is a good source of vitamin C. In addition the nuts of this crop have also been found to be a very good sources of vitamins A, B_1, B_2, B_6, E, folate, sodium, potassium, manganese, copper, chloride, iron and ascorbic acid (kanu and Okorie, 2015). Industrially, crushed dried walnut seeds can be processed to produce composite flour used in baking, and also in the production of milk used in preparing tea (Stevens and Domelam, 2003). Further the oil from the nut is used to produce wood vanish, stand oil, vulcanised oil for rubber and lather substances (Babalola, 2011), likewise the walnut shell has been found to be compactable with other materials and work well as filler in dynamite, and it is good when used as a rough agent in soap, and cosmetics production and as a dental cleaner (Oladiji *et al*., 2007). Economically, there is increase in the income of both farmers of African walnut and those who sale cooked walnut seeds (Akpauaka and Nwankwo, 2000; Ajaiyeoba and Fadare, 2006). The traditional uses of dry African walnut seeds include in the celebration of some festivals and preparation of some traditional recipes like dooya, chatani and dooya waer.

Several studies have been carried out in the assessment of proximate, mineral and phytochemical composition of African walnut (Ajaiyeoba and Fadare, 2006; Isong *et al*.,2013; Apeh *et al*., 2014; Akpoghelie *et al*., 2016; Igara *et al*., 2017; Akin-Osanaiye *et al*., 2018), there is paucity of information on the comparative evaluation of the biochemical constituents of lines from different agro-geographical areas. The primary target of this study is to characterize the lines based on biochemical compositions. Such a study is important in the establishment of varietal differences in African walnut and assessment of evolutionary status of the crop..

Materials and methods

Seeds of African walnut crop grown in Nigeria and Cameroon were used for the study and designated as lines. In total two lines were used in the present study collected from two regions.

Sample preparation

Evaluated seeds of African walnut were first sorted and cleaned. The seeds were then soaked in water for 20 hours and dehulled and then dried. The testa was subjected to grinding in mortar.

The dehulled seeds were also subjected to grinding in a mortar. Both the ground testa and the ground cotyledons were kept for analysis.

Proximate analysis

This was carried out to determine the macronutrient in nut samples. The purpose was to evaluate the crude protein, moisture content, fat content, crude fiber, ash and carbohydrate content in the nut.

Crude protein

The crude protein was determined by micro kjeldahl method according to standard procedure A.O.A.C. (2000). This analysis was carried out in duplicate.

Crude fibre

Crude fibre was determined according to standard procedure (A.O.A.C. 2000), it was carried out in duplicate and calculated as follows

Crude fibre (%) = $\frac{F1 - F2}{W}$ x 100

Where

F_1 = weight before ignition

F_2 = weight after ignition

w = weight of the sample before the analysis

Crude fat

Crude fat was determined by soxhlet extraction according to the standard procedure (A.O.A.C 2000), it was also carried out in duplicate and calculated as follows

Fat (%) $\frac{Sb - Sa}{W}$x 100

Where

S_a = weight of flask before extraction

S_b = weight of the flak + oil after extraction

W = weight of the sample

Moisture content

The moisture content was determined according to the standard procedure (A.O.A.C. 2000), it was carried out in duplicate and calculated as follows

Moisture content (%) = $\frac{Ms - Mt}{W}$ X 100

Where

M_s = weight of the moisture can + sample before drying

M_t = weight of the moisture can + sample after drying

W = weight of the sample used.

Carbohydrate

The nitrogen free method by A.O.A.C (2000) was used to determine the carbohydrate content. It was calculated as weight difference between 100% and the summation of other proximate parameter as nitrogen free extract (NFE).

% carbohydrate (NFE) =100- (M+P+F+A+F2)

M = moisture, P= protein F1=fat, A=Ash and F2 crude fibre.

Ash

The ash content was determined according to the standard procedure (A.O.A.C 2000). It was carried out in duplicate and calculated as follows

Ash contents (%) = $\frac{A2 - A1}{W}$ x 100

Where

A_2 = weight of crucible + sample before ashing

A_1 = weight of crucible + sample after ashing

W = weight of the sample used.

Anti-nutritional (Phytochemical) analysis

This was carried out to extract, screen and identify the medicinally active substances found in African walnut.

Oxalate

Two grams (2g) of the samples placed in 250 ml volumetric flask suspended in 190ml distilled water. 10ml of 6N HCL solution was added to the samples were then cooled and made up to 250ml mark of the flask with distilled water. The sample were filtered and the duplicate portion of 125ml of the filtrate was measured into the beaker and four drops of methyl red indicator was added, followed by the addition of cone ammonia solution drops wise until the solution changed from pink to yellow. The solution was then heated to 90 ^{0}C, cooled and filtered to the precipitate containing ferrous ion. The filtrate was again heated to 90 ^{0}C and 10ml of $CaCL_2$ solution was added to the samples with consistent stirring and dissolved in 10ml of 20% H_2SO_4 and diluted to 200ml with distilled water. Aliquots of 125ml of the filtrate was heated to near boiling and filtrated against 0.05m $KMAO_4$ solution to a pink colour which persisted for 30 secs. The oxalate contents of each sample were calculated.

Phytate

2g of each sample was weighed into 250ml conical flask. 100ml of 2% Hcl was used to soak the samples in the conical flask for 3hrs, then filtered with white man fitter paper No1, 50ml of each sample filtered was placed in 250ml beaker and 10ml was added to improve the acidity. 10ml of 0.3% ammonium thiocyanate solution was added to each sample's solution as colour that persisted for 5 mins was obtained. The phytate was calculated from the titre value.

Saponin

To determine the saponin content, 20 grams of each samples was dispensed in 200 ml of 20% ethanol, the suspension was heated on a water bath for 4 hours with continuous stirring at about 55 ^{0} C. The mixture was filtered, and residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90 ^{0}C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ethanol layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chlorides. The remaining solution was heated in a water bath. After evaporation the sample was oven dried to a constant weight. The saponins content was calculated in percentage as difference in weight.

Tannin

This was carried out by weighing out five hundred milligrams of sample into 100 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50ml volumetric flask and made up to mark. Then 5ml of the filtrate was pipetted out into a tube and mixed with 3ml of 0.1 M FeCL3 in 0.1N HCL and 0.008 M potassium ferricyanide added. The absorbance was measured in a spectrophotometer at 120 nm wavelength within 10 minutes. A blank sample was prepared and the colour also developed and read at the same wavelength. A standard was prepared using tannin acid to get 100 ppm and measured.

Mineral composition analysis

The amount of K, Na and Ca in African walnut seed were determined by flame photometry according to the standard procedure. Iron content was determined by DREL/5 spectrophotometer according to the standard procedure, likewise Magnesium content was determined by EDTA method according to the standard procedure. Determination of the Manganese was done using the colorimetric method while Zinc was by zincon method by the adaptation of the standard methods for the examination of water and waste water at a

wavelength of 575 nm. Copper content was determined according to the standard procedure (A.O.A.C. 2000).

Results and discussion

Fruits and nuts play a very important role in the nutrition and diet of rural dwellers. They are the major source of vitamins and minerals for most people living in the rural areas. These fruits and nuts are usually gotten from wild and semi-wild plants, which generally is believed to be better sources of vitamins and minerals than the genetically improved species. The nutritional value placed on food from these natural sources attract people living in cities to rural areas. In this study, seeds of African walnut tree crop found in semi wild areas of Nigeria and Cameroon was used to assess varietal differences in this crop based on proximate, mineral and anti-nutritional composition.

Cameroon except for carbohydrate. However, moisture content of the line sourced from Nigeria (38.44%) was higher than that from Cameroon (36.21%), also the crude protein (28.32%) and ash (4.33%) content of the line from Nigeria was higher than that of the line from Cameroon (crude protein; 0.11%, ash 3.63%). Contrarily the crude fiber (9.32%) and crude fat (4.73%) content of the line from Cameroon was higher than that from Nigeria (crude fiber 8.78%, crude fat 4.73%).

Mineral composition results of the African walnut lines collected from Nigeria and Cameroon (Table 3) showed that some mineral elements like Magnesium, Sodium and Chromium had significant differences between the lines. The result further showed that the line from Nigeria had higher content of Calcium (26.07mg/l), Potassium (120.00mg/l), Zinc (0.83mg/l) and Copper (1.01mg/l) than the line from Cameroon (Calcium 8.87mg/l, Potassium 100.00, Zinc 0.558mg/l , Copper 1.013mg/l). On the other hand, the iron (0.0140mg/l) and Copper (1.76) content of the line sourced from Cameroon was significantly higher than that from Nigeria (Iron 0.120mg/l, Copper 1.01). Proximate analysis result of the two lines of African walnut investigated did not show any significant difference except for carbohydrate. Invariably the nutrient content of the line from Nigeria was the same with that from Cameroon except for their carbohydrate content. Hence varietal difference cannot be established between the two lines.

Table 1: Result on anti-nutritional composition of the two African walnut lines

Treatments	Phytate	Oxalate	Tannin	Saponin
Nigeria	0.066	0.034	0.361	0.275
Cameroon	0.270	0.113	0.314	0.235
LSD (0.05)	Ns	ns	ns	ns

Expressed in %

Table 2: Result on proximate analysis of the two African walnut lines

Treatments	Protein	Crude fibre %	Fat	Ash	Moisture	Carbohydrate
Nigeria	28.38	8.78	5.67	4.33	38.44	14.46
Cameroon	25.33	9.32	4.73	3.63	36.24	20.87
LSD (0.05)	Ns	ns	ns	ns	ns	ns

Expressed in %

Table 3: Result on mineral composition of the two walnut lines

Treatments	Magnesium	Calcium	Iron	potassium	Manganese	Sodium	Copper	Chromium
Nigeria	2.27%	26.07	0.12	120.00	3.70	2.43	1.013	0.71
Cameroon	8.87%	25.23	0.14	100.00	1.30	0.40	1.765	1.76
LSD (0.05)	2.44	ns	ns	ns	ns	2.007	ns	0.43

All expressed in %

This result may have some evolutionary implication. Further the details of the proximate analysis result of the evaluated two lines of African walnut was in agreement with result of previous studies carried out by other workers on African walnut (Browsher *et al*., 2008; Ekwe and Ihemeje, 2013; Arinola *et al*., 2014; Oyekale *et al*., 2015; Akpoghelie *et al*., 2016; Chikezie, 2017; Udedi *et al*. 2013; 2014). Apparently, the result of this study showed that the nut of this plant is a rich source of protein, carbohydrate and fat. Previous studies have reported that any food plant that can provide 12% or more of its energy from protein is considered as a good source of protein (Dosunumu *et al*., 2008; Kanu and Okorie 2015). Some workers reported that African walnut is an energy rich food substance, because it is an excellent source of poly-saturated fatty acids such as alpha linoleic acid and contains omega- 3- essential fatty acids. In addition, it provides more omega 3 per pound than any other food, hence can play a vital role in providing food security to people living in rural areas (Kalu, 2010; Udedi *et al*., 2013; 2014).

The result on mineral composition showed significant differences in few elements like Magnesium, Sodium, and Chromium between the two lines. The composition of other minerals (Calcium; Iron, Potassium, Manganese and Copper) in the two lines were statistically the same. Summarily from this result the composition of more mineral elements was not significantly different between the two lines. Therefore, this result is not adequate to be used to establish varietal difference between the lines. Further the result showed that African walnut contains essential vitamins and minerals like magnesium, iron, calcium, copper, Zinc and Manganese. Previous studies have also reported a similar result on the mineral composition of African walnut (Edem *et al.,* 2009; Kanu and Okorie, 2015), and these mineral elements play a very important role in boasting the immune system of the body as well as in preventing anaemia (Ojobor *et al*., 2015).

Analysis of composition of anti-nutritive content of the two African walnut lines did not show any significant difference in their anti-nutritive composition unlike the result on proximate and mineral composition that showed little significant differences. Invariably the two lines are the same; there is no varietal difference between them based on their anti-nutritive composition. This result suggests that the evolution of this crop into diverse types is very slow. Further the result showed low content of tannins (0.31%), phytate (0.06%), oxalate (0,03%), and saponin (0.27%) in the two lines.

Previous study on the examination of phytochemical composition of boiled nuts of this crop reported a similar result of moderate concentration of tannins (Chikezie, 2017). The low content of anti-nutrients in African walnut implies that it is good for human consumption. Udedi *et al.,* (2014) suggested serving the nut of this crop as a food supplement in school children feeding programmes.

A detailed examination on the result of this investigation showed that neither the line from Nigeria nor the one from Cameroon consistently had higher values in the composition of proximate, minerals and anti-nutrient content. Invariably varietal differences between the two

lines cannot be established based on analysis of their biochemical constituents. Further the observed similarity in the biochemical composition of the two lines suggests that the development of African walnut into diverse types through evolutionary processes is very slow. The result generated by our study cannot be used to elucidate lineage or predict the ancestor of walnut in the two lines. These two lines of African walnut has been in existence in these areas for many years (Daziel, 1937; Ajaiyeoba and Fabaro, 2006) and should have significantly developed into different forms which can be established to a reasonable extent through the biochemical content of the crop. However, this study is opening a new area of research to breeders to see if they can establish varietal difference in this crop with other methods. According to Singh *et al.,* (1996) the production of certain biochemical components is a reflection of genetic variation which may be responsible for variation in some morphological features like leaflet size and colour.

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