Phytochemical and biochemical compositions of African Walnut
(Tetracarpidium conophorum)

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Abstract:
The phytochemical and biochemical compositions of African walnut (Tetracarpidium conophorum) were determined for two samples of the variety - the boiled and mashed wet nuts, and the dried powdered nuts. Results of phytochemical analysis revealed the presence of the following expressed as percentage. For the mashed wet nuts: Saponins (8.37±0.1); flavonoids (3.20±0.2); phenols (1.90±0.1), tannins (0.51±0.0) and alkaloids (0.41±0.1). For the dried and powdered variety, the results are: Saponins (5.03±0.2); flavonoids (2.70±0.1), tannins (0.45±0.1), phenols (1.51±0.1) and alkaloids (0.350±0.1). Proximate compositional analysis expressed as percentage shows the following results: moisture (45.01±0.1)WM, (28.88±0.02)DP; Crude fat (13.81±0.01) WM, (32.21±0.01) DP; Crude protein (18.75±0.01) WM, (13.72±0.02 )DP; CHO (18.43±0.1) WM,(46.38±0.2) DP; Crude fiber (1.95±02) WM,(1.72±0.02 ) DP; Ash (1.91±0.01) WM,(3.11±0.01) DP, FE( 273.01±0.1) WM, (530.29±0.02) DP. The values of the vitamins are expressed in mg/ml as shown below: Vitamin A is expressed in µg/ml(1283.33±1.18)DP, (285.60±0.02)WM, Vit C (14.80±0.02)DP,(17.57±0.01)WM,riboflavin (0.12±0.02)DP,(0.12±0.02)WM, Thiamine (B1) (0.20±0.01)DP,(0.12±0.01)WM; Niacin(2.81±0.01)DP,(2.91±0.01).The mineral composition of the nut is very outstanding and not significantly affected by the processing methods. The values of the minerals are shown as follows: Mg (0.36±0.01) DP.,(0.31±0.01)WM; Ca (13.81±0.01) DP, (14.80±0.01)WM, (3.11±0.01) DP; CHO (18.43±0.1) WM, (46.38±0.2) DP; Crude fiber (1.95±02) WM, (1.72±0.02) DP; Ash (1.91±0.01) WM, (3.11±0.01) DP, FE (273.01±0.1) WM, (530.29±0.02) DP. The values of the vitamins are expressed in mg/ml as shown below: Vitamin A is expressed in µg/ml (1283.33±1.18)DP, (285.60±0.02)WM, Vit C (14.80±0.02)DP, (17.57±0.01)WM, riboflavin (0.12±0.02)DP, (0.12±0.02)WM, Thiamine (B1) (0.20±0.01)DP, (0.12±0.01)WM, Niacin (2.81±0.01)DP, (2.91±0.01). The mineral composition of the nut is very outstanding and not significantly affected by the processing methods. The values of the minerals are shown as follows: Mg (0.36±0.01) DP, (0.31±0.01)WM; Ca (2.10±0.2)DP, (1.88±0.1)WM; P (0.35±0.01)DP, (0.36±0.01)WM and others. The phytochemical and nutrient compositions of this tropical nut are expository and depicts the role of the seeds in nutrition and health.

Keywords: Biochemical composition, phytochemicals, African walnut.

Introduction:
One of the most unexpected nutritional discoveries of the 1990’s was that frequent eating of nuts greatly lower the risk of heart disease1]. Walnut is an edible seed of any tree of the genus, Juglans, especially , the Persian walnut. A study has suggested that consumption of walnut increases fat oxidation and reduces carbohydrate oxidation without affecting total consumption, suggesting that walnut consumption may improve the use of body lipids in overweight adults. Walnuts are shown to decrease endothelial dysfunction associated with high fat diets [2]. Scientists are not yet convinced whether walnuts act as chemoprotective agents, an effect which may show the result of the fruit’s high phenolic content [3], an antioxidant activity to be used in in vitro antiproliferative therapy[3]. Walnuts have been found in pre-historic deposits dating from the iron age in Europe. In the middle ages, walnuts were thought to ward off witchcraft, the evil eye and epileptic fits. Black walnuts are applied in certain skin conditions such as eczema, pruritus, psoriasis, warts, and parasitic skin conditions [4]. Extracts of black walnut are used to dye the hair, skin and cloths. Black walnut contain juglone (5-hydroxy-1,4-naphthoquinone, alpha(α)hydroxyjuglone and its glycoside, beta(β) hydrojuglone, caffeic acid, plumbagum, hyperin, kaempferol and tannin. Ellagic acid is also present.

The study of oil extract from Tetracarpidium conophorum seed showed the fatty acids and triacyl content with linoleic acid[4]. Walnuts have sufficiently higher amounts of omega-3 fatty acids as compared to other nuts[4]. Raw walnuts contain glyceryltriacylates of the n-3 fatty acid, alpha linolenic acid (AlA). Roasting reduces the antioxidant quality of the seeds. Many authors/researchers have identified some uses of the seeds and leaves of the plant. For example, an extract of the leaf is used to mitigate prolonged and constant hiccups, improvement of fertility in both men and women. It can also be used to improve spermatozoa count in men. It has equally been used to reduce the incidence of tumor and cancer cells [5]. It is used to regulate menstrual flow and to treat dysentery. Preparations of the leaf, stem bark, kernel and root extracts as well as the hexane, ethylacetate and methanol fractions of the leaf are able to inhibit the activities of gram negative bacteria such as Staphylococcus aureus , Bacillus subtilis, Pseudomonas aeruginosa and Escherichia coli [6]. The heavy metal content of the walnuts are within the WHO permissible limits of heavy metals. Cardiac glycosides were relatively absent. Compared to certain other nuts such as almonds, peanuts, and hazelnuts; walnuts, especially in their raw form, contain the highest total level of antioxidants, including both free antioxidants and antioxidants bound to fiber[7,8]. The research work is geared towards elucidating the phytochemical, biochemical, minerals and vitamins resident in this all important nut. We were intrigued by the avidity with which Nigerians purchase and consume the...
nuts during the harvest period especially people of the South Eastern region, as it is not always there throughout the year. The result of such research work would nonetheless, place this nut in its actual position of relevance in the nutritional and medicinal needs of Nigerians and by extrapolation to other people of other nations.

**Materials and methods:**

**Plant materials.**
The seeds of viable African walnuts were purchased from a local market within Owerri metropolis. Five hundred (500g) grams of recently plugged seeds removed from seed coat while fresh were prepared for the studies. Before hulling, the seeds were authenticated by a technologist, Mr. Francis Iwunze at the department of Forestry and Wild Life of the School of Agriculture and Agricultural Technology of the University.

**Sample preparation:**
The seed samples are into two groups of two hundred grams (200 g) each. Sample A consists of 200g of completely hulled seeds, boiled and sliced into small bits to expose the surface area and finally dried in an oven at 65 °C for six (6) hours. The oven dried seeds were ground to powder using a manually operated machine (LANDERA CORONA). The ground seeds sieved with a sieve of mesh, 30mm pore size to obtain flour of the seed. The second group B, consists of two hundred grams (200 g) of fresh seeds, mashed and sun-dried for two days. The macerated sun-dried seeds were powdered using a grinding machine (LANDERA CORONA), manually operated. The ground seeds were then sieved with a sieve of the same mesh size to obtain flour, which would then be used for analysis.

**Phytochemical Analysis:**
Phytochemical analysis is carried out with the two prepared samples – the mashed wet sample (B) and the dried powdered sample (A) respectively.

**Determination of alkaloids**
The analysis is done using the alkaline precipitation methods [9][10] . Five grams (5 g) of each of the samples are weighed into 250 ml beaker and 200 ml of 20 % acetic acid in ethanol was added. The flask was then covered and allowed to stand at room temperature. The mixture is shook for 30 minutes, using a magnetic shaker. The mixture was then concentrated using rotor evaporator maintained at 60 °C to obtain ¼ of the original volume. The extract is treated by drop wise addition of concentrated ammonium hydroxide solution until precipitation was complete. The solution is allowed to settle and filtered with Whatman filter paper (No 42). The precipitated alkaloid is dried at 60 °C and weighed.

**Determination of tannins**
The tannin content of the sample is estimated by the Folin–Dennis spectrophotometric method. The methods of [9] were also used for analysis.

**Determination of flavonoids**
Ten grams (10 g) of each of the samples was extracted repeatedly with 100 ml of 80 % aqueous methanol at room temperature. The entire solution is filtered through Whatman No 1 filter paper. The filtrate was then transferred into a crucible, evaporated to dryness over a water bath and weighed to a constant weight [11].

**Determination of phenol**
Determination of phenol content is done using the methods of the Association of Official Analytical Chemists [12] .

**Determination of the energy value**
The gross food energy value is estimated according to the method [13].

**Proximate analysis**
The proximate compositions of the wet and dry samples are analyzed for the moisture content, carbohydrate, crude lipids, protein, ash and crude fiber by the methods of the Association of Official Analytical Chemists [12].

**Determination of Vitamin A (β-Carotene)**
The determination of Vitamin A (β-Carotene) content of the samples is estimated using the methods described [14].

**Quantitative determination of vitamin b 1, (thiamine)**
Five grams (5 g) of samples are homogenized with 50 ml of ethanolic sodium hydroxide solution. This was filtered into a 100 ml flask. Ten milliliters (10 ml) of the filtrate was pipetted into a beaker and color developed by the addition of 10ml potassium dichromate . The absorbance is read at 360nm. A blank sample was also prepared and read at the same wavelength. The values are extrapolated from a standard curve [11].

**Determination of riboflavin (Vitamin B 2)**
Five grams (5 g) of each of the samples was extracted with 100 ml of 50 % ethanol solution shaken for 1 hr. This was filtered into a 100 ml flask. Ten milliliters (10 ml) of the filtrate was pipetted into a beaker and color developed by the addition of 10ml potassium dichromate . The absorbance is read at 510 nm in a spectrophotometer [11].

**Determination of niacin**
Five grams (5 g) of the sample is treated with 50 ml of 1N sulfuric acid and shaken with magnetic shaker for 30 mins. Three drops of ammonia solution added to the mixture and filtered. Ten milliliters (10 ml) of the filtrate was pipetted into a 50 ml volumetric flask, and 5 ml potassium cyanide added . The mixture acidified with 0.02 MH2SO4, and the absorbance read at 470 nm in a spectrophotometer (Unicam Spectronic 20 ).

**Determination of Vitamin C**
The ascorbic acid content of the samples were estimated by the Barakat titrimetric method. Twenty (20 g) grams of the samples homogenized in 100 ml EDTA/TCA extracting solution by bleaching for 5 mins. The homogenate filtered and the filtrate analyzed by the methods of [11].

**Determination of Vitamin E**
The vitamin E content of the samples is quantifiable using the method described [11].

**Determination of mineral composition**
The determinations of the mineral compositions of the samples done using the methods [13] involving the determination of sodium and potassium by flame
Results:

Table 1. Phytochemical composition of both the dried and the wet samples of *Tetracarpidium conophorum*. Values are expressed as percentages.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Flavonoids</th>
<th>Alkaloids</th>
<th>Saponins</th>
<th>Tannins</th>
<th>Phenols</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dried and Powdered</td>
<td>2.70±0.1(^a)</td>
<td>0.35±0.01(^b)</td>
<td>5.03±0.01(^a)</td>
<td>0.45±0.01(^b)</td>
<td>1.51±0.01(^a)</td>
</tr>
<tr>
<td>Wet and Mashed</td>
<td>3.20±0.00(^a)</td>
<td>0.41±0.01(^a)</td>
<td>8.37±0.12(^a)</td>
<td>0.51±0.00(^a)</td>
<td>1.90±0.01(^b)</td>
</tr>
</tbody>
</table>

The values in the table are the Mean± SD from triplicate determinations. Values with the same superscript are significantly related along the row at p≤0.05 and different from others.

Table 2. Proximate composition of the Seed of *Tetracarpidium conophorum*. Values are expressed as percentage (%). composition.

<table>
<thead>
<tr>
<th>Samples</th>
<th>MC (g/100g)</th>
<th>Crude Fat (g/100g)</th>
<th>CF (g/100g)</th>
<th>Ash (g/100g)</th>
<th>CP (g/100g)</th>
<th>CHO (g/100g)</th>
<th>FE (J)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dried and Powdered</td>
<td>2.88±0.02(^a)</td>
<td>32.21±0.01(^a)</td>
<td>1.72±0.02(^a)</td>
<td>3.11±0.01(^a)</td>
<td>13.72±0.02(^a)</td>
<td>46.38±0.02(^a)</td>
<td>530.29±0.02(^a)</td>
</tr>
<tr>
<td>Wet and Mashed</td>
<td>45.01±0.01(^a)</td>
<td>13.81±0.01(^b)</td>
<td>1.95±0.02(^a)</td>
<td>1.91±0.01(^b)</td>
<td>18.75±0.01(^b)</td>
<td>18.43±0.01(^b)</td>
<td>273.01±0.02(^b)</td>
</tr>
</tbody>
</table>

The values in the table are the Mean± SD from triplicate determinations. Values having the same superscript are significantly related along the columns at p≤0.05.

Table 3. Vitamin composition of the seeds of *Tetracarpidium conophorum*. Results are expressed in mg/100g or µg/100g.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Vitamin A</th>
<th>Vitamin C</th>
<th>Riboflavin</th>
<th>Thiamin</th>
<th>Niacin</th>
<th>Vitamin E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dried and Powdered</td>
<td>1283.33±1.18(^a)</td>
<td>14.80±2.43(^a)</td>
<td>0.12±0.01(^a)</td>
<td>0.20±0.01(^a)</td>
<td>2.81±0.13(^a)</td>
<td>0.22±0.02(^a)</td>
</tr>
<tr>
<td>Wet and Mashed</td>
<td>285.60±9.95(^a)</td>
<td>17.57±0.02(^b)</td>
<td>0.13±0.01(^a)</td>
<td>0.12±0.01(^a)</td>
<td>2.91±0.10(^b)</td>
<td>0.27±0.02(^b)</td>
</tr>
</tbody>
</table>

The values in the table above are the Mean± SD from triplicate determinations. Values with the same superscript are significantly related at p≤0.05 across the rows and columns.

Table 4. The Mineral compositions of Samples A and B OF *Tetracarpidium conophorum*. Values are expressed as percentage of sample.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Mg (%)</th>
<th>Ca (%)</th>
<th>K (%)</th>
<th>Na (%)</th>
<th>P (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dried and Powdered</td>
<td>0.36±0.01(^a)</td>
<td>2.10±0.20(^a)</td>
<td>1.02±0.02(^a)</td>
<td>0.26±0.01(^a)</td>
<td>0.35±0.01(^a)</td>
</tr>
<tr>
<td>Wet and Mashed</td>
<td>0.31±0.01(^a)</td>
<td>1.88±0.11(^b)</td>
<td>0.87±0.01(^a)</td>
<td>0.39±0.01(^a)</td>
<td>0.36±0.01(^a)</td>
</tr>
</tbody>
</table>

The values in the table are the Mean± SD from triplicate determinations. Values with the same superscript are significantly related at p≤0.05 along the rows and columns. The minor difference observed in the data was due to the effect of heat on drying, leading to concentration effect on the different minerals present in the samples.

Discussion:

The findings or results from the analyses of some samples revealed fascinating observation. The results of phytochemical analyses revealed high preponderance of phytochemicals especially saponins and flavonoids in both the dried and wet samples. The high level of antioxidants in this nut is reported in the study of many workers\(^{[15]}\). Many researchers have equally reported on the level of polyphenolic compounds such as Ellagic and Gallic acids; apart from these, other phenolic acids have been found in African walnuts such as phenylacetic acid, a strong antiseeking agent\(^{[16]}\), protocatechic acid, syringic, vanillic acid and caffeic acid. These phenolic acids found in foods have been associated with astringency, discoloration and inhibition of some enzyme activity; they are also known to provide the human body with extra line of defense against bacterial and viral attacks, thereby, boosting the immune system\(^{[16-18]}\).

The high protein content vis-à-vis, the high carbohydrate and food energy values are reminiscent of high rate of consumption during the harvest period. From our findings, it can be seen in Table 2, that the nuts are rich in protein with values-13.27 % for the dried and powdered sample to 18.75% for the wet and mashed sample. This finding is in line with what other workers reported for other nuts like Hazel nuts\(^{[19]}\), Almonds and peanuts respectively. The nut is also rich in nutrients which lower the risk of gaining unwanted weights\(^{[20]}\). For example, some workers have reported the presence of resveratrol\(^{[20-22]}\), a phytochemical found in grapes and walnuts, which activates STRT 1 gene, which in turn increases the body’s metabolic rate, also implicated in positive correlation with longevity\(^{[23,24]}\).

Several studies have highlighted or unveiled a gamut of substances present in walnuts\(^{[25]}\). Several vitamins are resident in the African walnuts, whose compositions agree with our findings. Some of the vitamins and other nutrients reported by other workers and their values include—
vitamin A (β-Carotene) 380 IU/100g, thiamin 0.48-0.53mg /100g, riboflavin 0.11-0.14mg/100g, nicotinic acid 0.6-1.2mg/100g; when compared with our findings/results ,the dried and powdered /wet and mashed results are shown :vitamin A 1283.3/285.6 IU per 100g; vitamin C :14.80/17.57 per 100g;it has been found that this value of vitamin C is lower than that reported for some nuts like Hazel nuts and peanuts[22,23], but results of Vitamin A estimation, showed higher than what some workers reported . Essential amino acids are present in walnuts, such as Arginine 2.3mg/100g; Histidine 0.4mg/100g; Isoleucine 0.73mg/100g; others include-leucine1.3, lysine 0.3, methionine 0.25, phenylalanine 0.8, threonine 0.5, tryptophan 0.18 and valine 0.9, all expressed in mg/100g. The mineral content of Tetracarpidium conophorum is very outstanding. The presence of Mg, Ca, K, Na and P cannot be under rated because of the role of these metal ions in health and nutrition. Magnesium is a cofactor for many enzymes. Calcium and phosphorus are very essential for bone metabolism. Sodium and potassium are very important for nerve transmission and osmolality of body fluids and moreover Zinc is an antioxidant, as it is a cofactor for many antioxidant enzymes like catalase. The phytochemical and nutrient composition of the walnut is worth investigating. The results from our work have far reaching importance as this could support the numerous nutritional and medicinal roles attributed to this all important nut[24,27]. It is reviewed extensively, the roles of this nut. The arginine content of the nut has been attributed the role of an antihypertensive drug by producing Nitric oxide in vivo, whose function is to act as a vasodilator of the smooth muscles and blood vessels[12].

References:

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