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Evaluation of Nutritive, Antinutritive and Mineral Content of *Tetracarpidium conophorum* (African Walnut) Seed Oil at Different Stages of Fruit Maturation

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Abstract: Nutritional and industrial processes have increased the demand of oils and this in turn has led to the search for oils from different types of seeds for possible development and use. It is in this vein that the *Tetracarpidium conophorum*fruit nuts were extracted with n-hexane (soxhlet extraction at 65^0C). The proximate composition,antinutrient and mineral content of freshly harvested *Tetracarpidium conophorum*(African walnut)seed from 4 weeks after anthesis (WAA) to fruit maturation were assessed in this study. Data obtained for the proximate composition at matured stage of fruits developmentrevealed significant high amount of fat(43.4±0.82) %; moisture(36.7±1.39) %; crude protein(30.1±1.38) %; carbohydrate (16.91±1.07) %;low crude fibre (2.59±0.64) %, and ash content(7.3±0.07) %[which contained higher amount ofminerals such as of Fe (130.81 ± 3.8) ppm, Mn (40.11 ± 1.00) ppm, Ni (2.92 ± 0.81) ppm, and Cu (12.01 ± 0.71) ppm, decrease inCa (1.082 ± 0.08) %, Cr and Cd were not detected throughout the period] at 20 WAA compare to immature stages of 4, 6, 8, 10, 12, 14 and 16 WAA. The level of antinutrientfactors are oxalate (0.46±0.05) mg/100g; Phytate (29.2±0.44) mg/100g and cyanogenic glycosides (0.17±0.07) recorded lower content, except tannins (90.5 ±3.93) mg/100gwhich recorded significant ($p < 0.05$) higher content at 20 WAA when compared with immaturestages in 4, 6, 8, 10, 12, 14 and 16 WAA. This study revealed the nutritional profile of the fruitnut as good sources of plant protein, carbohydrate and fat, with reduction in the level of some anti-nutrients in matured fruits which are potentials that could be exploited by food and pharmaceutical industries.

Keywords: *Tetracarpidium conophorum*,proximate, antinutrient, n-hexane, anthesis

INTRODUCTION

In Africa, fruits are on high demand, and this is because they are complemented with food to ensure balanced diet. Fruits serve as sources of fat, carbohydrate, vitamins and minerals hence, they also become important when the functions of these nutrients are being considered in the body [1]. Fat and oil are used in a variety of ways, for food texturing, baking, and frying and also used industrially, in the manufacture of soap, detergent, cosmetics and oil paints. In plants, oil is deposited in the seeds mostly in the endosperm along with carbohydrates where they jointly nourish the embryo [2]. It is also found in some plants mesocarp (palm fruits) [2]. Nutritional and industrial processes have increased the demands for oils and this in turn has led to the search for oils from different types of seeds.

African walnut is an edible seed of any tree of the genus *Euphorbiaceae*, especially the *Tetracarpidium conophorum*, found in Nigeria and Cameroon. It is a climbing shrub of 10-20ft long, cultivated principally for the nuts which are cooked and consumed as snacks or dessert [3]. It is known in the South-East Nigeria as *ukpa* (Igbo), Western Nigeria as *awusa* or *asala* (Yoruba), South-South Nigeria as *Okhue* (Edo) [2, 4].

Previous study on*Tetracarpidium conophorum* has shown that consumption of its seeds increases protection against proliferous diseases, oxidative stress and endothelial dysfunction [5]. *Tetracarpidium conophorum* leaves and roots extracts are applied in certain skin conditions such as eczema, psoriasis, warts, and parasitic skin conditions [6]. Studies have shown that extracts of the leaves are 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 [7]. It is used to regulate menstrual flow and to treat dysentery, inhibit the activities of gram negative bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Escherichia coli* [8] .

It has been reported that African 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 [6]. The oil extract from *Tetracarpidium conophorum* seed showed the fatty acids and triacyl content with linoleic acid [6].Walnuts have sufficiently higher amounts of omega-3-fatty acids as compared to other nuts [1]. Raw walnuts contain glyceryltriacylates of the n-3 fatty acid, alpha linolenic acid (ALA) [1].

This present study investigated the proximate, antinutritive and mineral composition of *Tetracarpidium conophorum* seednutsat differentstages of fruitdevelopment.

MATERIALS AND METHODS

Plant materials

Matured fruits of *Tetracarpidium conophorum* were collected from private farm land in Ondo Town, Ondo State, Nigeria. The fruits were authenticated by the Department of Botany, University of Medical Sciences, Ondo. A voucher specimen of each plant was there after deposited in the herbarium of the same Department.

Preparation of Sample

Forty fruits were collected randomly from each of the studied trees at biweekly intervals starting from the fourth week after fruit set until senescence. The collected fruits were cleaned with a moist soft cotton wool and then the seeds were carefully separated from the fruits. Part of the separated nuts were immediately used for oil extraction, while the remaining part was dried at 65^0C for 4 hrs in an oven, crushed with a laboratory mortar and pestle and kept in a well labelled air tight polythene bags or screw-capped bottles at 4^0C for subsequent biochemical analysis.

All reagents used were of analytical grade purchased from Sigma Chemicals Co, London, and BDH Chemicals Ltd., England.

Extraction of Oil

The soxhlet extraction method was employed. The sample (5g) was weighed into a weighed filter paper and folded neatly. This was placed inside the pre-weighed thimble (W_1). The thimble with the sample (W_2) was inserted into the soxhlet apparatus and extraction under reflux was carried out with the n-hexane (40-60^0C boiling range) for 6 hours. At the end of extraction, the thimble was dried in the oven for about 30minutes at 100^0C to evaporate off the solvent and was cooled in a desiccator and later weighed (W_3). The fat extracted from a given quantity of sample was then calculated.

Calculations

% Crude Fat (W/W) = [Loss in Weight Sample/Original Weight of Sample] x100

Proximate analysis

The proximate compositions of the wet and dry samples are analyzed for the moisture content, carbohydrate, crude lipids, protein, ash and crude fibre by the methods of the Association of Official Analytical Chemists AOAC [9].

Antinutrient Analysis

Quantitative phytochemical analyses of anti-nutrients were determined using the methods of Sofowora [10]. The mineral content was according to methods as described by [9]. All determinations were done in duplicates.

Statistical analysis

The mean, standard deviation, analysis of variance (ANOVA) of the data obtained from the study and separation of means using least significant difference test (LSD) were computed using statistical package for social sciences (SPSS) version 13. Significant difference was judged at $p<0.05$.

RESULTS

The proximate composition of *Tetracarpidium conophorum*oil seeds are shown in figure 1. The seeds had the highest amount of lipid and protein content at the matured stage of fruits development.

Table 1: Proximate composition of *Tetracarpidium conophorum* Seeds from 4 WAA till maturation

Moisture content %	Ash content %	Fibre content %	Protein content %	Lipid content %	Carbohydrate Content %
88.7 ± 1.36	6.0 ± 0.11	1.22 ± 1.10	11.7 ± 0. 62	9.6 ± 1.7	71.58 ± 1.64
83.0 ± 1.28	6.1 ± 0.12	1.39 ± 0.70	20.1 ± 0.83	10.3 ± 0.78	62.71 ± 1.22
81.1 ± 0.89	6.0 ± 0.21	1.28 ± 0.47	18.9 ± 0.162	16.9 ± 1.00	57.0 ± 2.88
63. 8 ± 0.69	7.0 ± 0.10	1.25 ± 042	20.8 ± 0.67	19.5 ±0.80	51.85 ±1.24
55.8 ± 1.14	8.0 ±0.11	1.82 ± 0.31	20.3 ± 1.48	36.7 ± 0.38	34.18 ± 1.89
42.4 ± 0.82	7.2 ± 0.10	2.41 ± 0.22	22.3 ± 0.27	35.6 ±1.16	32.59 ± 1.39
41.7 ± 1.30	7.6 ± 0.08	2.27 ± 0.09	26.0 ± 0.63	39.1 ±0.97	25.13 ± 0.97
37. 2 ± 1.79	7.1 ± 0.06	2.55 ± 0.15	28.6 ± 0.77	42.4 ± 0.13	19.65 ± 1.37
36.7 ± 1.39	7.3 ± 0.07	2.59 ± 0.64	30.1 ± 1.38	43.4 ± 0.82	16.91 ± 1.07

Data are the average of 3 replicates ± SE

Table 2: Elemental content of *Tetracarpidium conophorum* seeds from 4 WAA till maturation

WAA	Fe (ppm)	Mn (ppm)	Cr (ppm)	Ni (ppm)	Pb (ppm)	Cd (ppm)	Cu (ppm)	Ca (%)
Week 4	192.17 ± 0.04	32.04 ± 1.8	Nd	Nd	3.10 ± 2.9	1.23 ± 0.7	31.77 ± 2.81	0.341 ± 0.04
Week 6	181.48 ± 2.4	31.8 ± 1.3	Nd	1.07 ± 0.09	4.07 ± 2.0	1.04 ± 0.4	34.04 ± 0.21	0.568 ±0.07
Week 8	184.33 ± 3.6	32.65 ± 2.8	Nd	1.13 ± 0.04	4.26 ± 1.0	Nd	31.12 ± 0.41	1.004 ± 0.08
Week 10	166.29 ± 1.8	38.17 ± 1.7	Nd	Nd	6.05 ± 0.4	Nd	25.31 ± 0.61	1.311 ± 0.02
Week 12	148.09 ± 3.8	46.81 ± 2.1	Nd	3.44 ±1.3	8.04 ± 1.7	Nd	33.91 ± 0.23	1.051 ± 0.08
Week 14	168.42 ± 3.4	53.21 ± 1.3	Nd	3.07 ± 0.8	4.22 ±1.2	Nd	13.62 ± 0.44	1.077 ± 0.01
Week 16	131.09 ± 4.8	43.11± 1.07	Nd	2.87± 0.08	3.08 ± 0.2	Nd	14.28 ± 0.46	0.797 ± 0.90
Week 18	125.4 ± 2.4	32.04 ± 1.07	Nd	3.01 ± 0.05	3.16 ± 0.4	Nd	13.17 ± 0.43	0.971 ±0.03
Week 20	130.81 ± 3.8	40.11 ± 1.00	Nd	2.92 ± 0.81	4.01 ± 0.2	Nd	12.01 ± 0.71	1.082 ± 0.08

Data are the average of 3 replicates ± SE

Table 3: Anti-nutrient content of *Tetracarpidium conophorum* seeds from 4 WAA till Fruits Maturation

WAA	Phytate (mg/100g)	Oxalate (mg/100g)	Tannins (mg/100g)	Hydrocyanic (mg/100g)
4	32.6 ±0.23	0.75±0.01	20.5±0.98	0.29± 0.04
6	32.8±0.14	0.71±0.17	26.1±1.23	0.31± 0.01
8	32.61± 0.04	0.66±0.06	20.0± 1.41	0.27± 0.09
10	32.4±0.21	0.54±0.02	50.1±2.81	0.27 ± 0.06
12	32.0±0.14	0.57±0.10	58.3±3.18	0.24± 0.01
14	30.2±0.11	0.57± 0.02	60.5±2.48	0.25± 0.03
16	30.6±0.31	0.51±0.06	84.5±3.41	0.14 ± 0.02
18	28.7±0.13	0.44±0.01	85.0± 3.11	0.17± 0.05
20	29.2±0.44	0.46±0.05	90.5±3.93	0.17± 0.07

Data are the average of 3 replicates ± SE

The levels of Iron (Fe), Manganese (Mn), Chromium (Cr), Nickel (Ni), Lead (Pb), Cadmium (Cd) and Copper (Cu) in the seed nuts shown in figure 2. The results showed that Fe and Mn have the highest levels while Cr and Cd were not detected from immature to matured fruits.

Figure 3 revealed that the levels of phytate, oxalate and hydrocyanic acid were very low in concentration and tannin was higher when compared to the other anti-nutritional factors assessed.

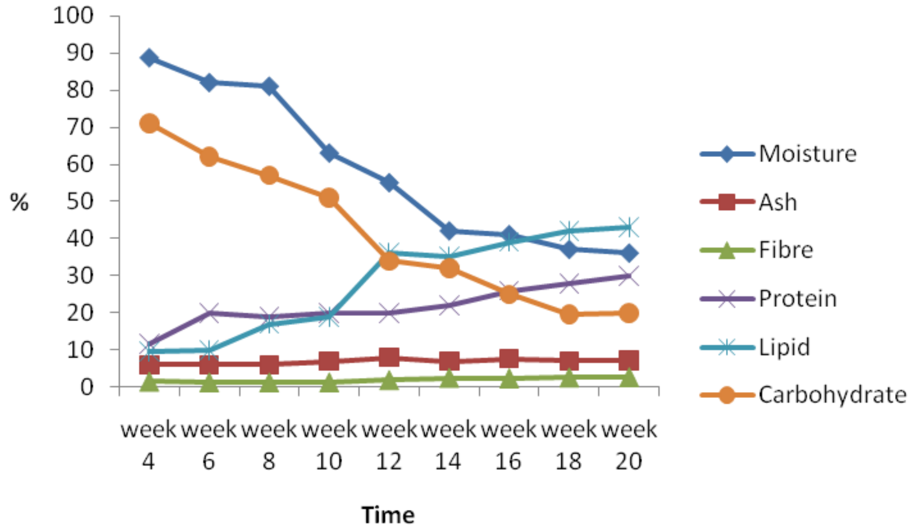

Fig-1: Proximate Composition of *Tetracarpidium Conophorum* 4-20 WAA of Fruits Development. Values are mean ± SEM. (* = P<0.05)

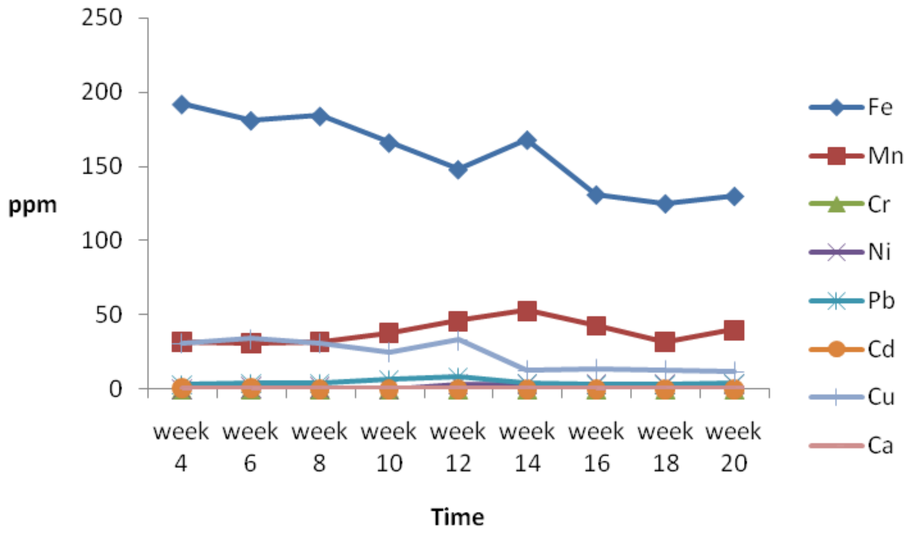

Fig-2: Elemental content of Tetracarpidium conophorum 4-20WAA of fruits Development. Values are mean ± SEM (* = P<0.05)

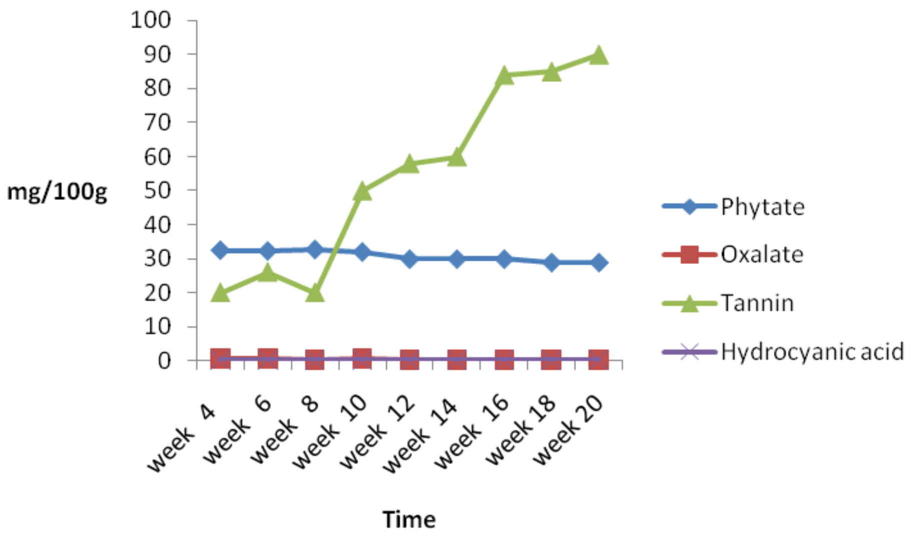

Fig-3: Anti-nutritional content of *Tetracarpidium conophorum* 4-20WAA of Fruits Development. Values are mean ± SEM (* = P<0.05)

DISCUSSION

Determination of the proximate composition of plants is important because it predict the profitability of a given plants as potential source of nutrients. High oil content in plant seeds implies that processing them for oil would be economical [11]. The oil yield found in the studied plants compares favourably well with the oil yields reported for some commercial plant oils such as cotton seed (36%), sesame(44%), olive (17%), ground nut (40%), sunflower (44%), soybean (18%), oil palm (22%) and corn oil (3.4%) [2, 12].

The fat content (within the 4th– 10thWAA) showed slight variation but increased rapidly as the week increases. This suggests a slow formation of the chemical constituents during the fruit development [13], but a prominent increase in fat content from the (10th-20thWAA) was observed which suggest full maturity.

It has also been reported that *Tetracarpidium conophorum* nut yield an average of 47.7% Ndie *et al.*, [14], which is consistent with our findings (43.4% average).This result supports the fact that *Tetracarpidium conophorum* nut is relatively richer source of lipids than other conventional sources and can replace some in culinary uses.

The moisture content (on wet weight basis) of the seeds decreases with maturation and was consistent with earlier studies [5, 13]. The decrease in the moisture content and the concomitant increase in the fat content in the studied plants demonstrated a close negative trend and showed that the two constituents remained negatively constant for fruits widely differing in oil content [15].

The crude protein content of *Tetracarpidium conophorum* extracts (4 -20 WAA) significantly increased ($p < 0.05$). The protein content of *Tetracarpidium conophorum* agrees with the values previously reported by other researchers at the time of harvest [15]. In contrast, higher than the range values (24.0 – 60.0%) reported by the authors [16, 17]. The variation in the present study and those reported by earlier studies could be attributed to the difference in the methods of analysis employed, genetic makeup and racial origin of the fruit. This equally emphasised the rich source of the fruits in plant proteins with high content of available lysine (27.0 –39.0mg/100g protein, [17].

The crude fibre content significantly increased ($p<0.05$) with increase in development but had a slight variation (within 4 – 12 WAA) suggesting a period of slow formation of the indigestible carbohydrate. The fibre content within 16–20 WAA of *Tetracarpidium conophorum*, were lower than that previously reported [13]. The state of development at the time of harvest and geographical growth location of the fruit plant could influence variation [18].

Tetracarpidium conophorum nut ash content within 4–20WAA also increased with increase in fruit maturity. This results agrees with the values (4.6 – 6.9%) reported by Nwaoguikpe *et al*., [5]. The carbohydrate content significantly decreased ($P<0.05$) with maturation. The metabolism of the polysaccharides in the cell starch hydrolysis which contribute to the increase in the total sugars observed in climacteric fruits could have been responsible for this decrease [19]. Also, the decrease may be attributed to the changes in the quantity of cell wall materials during ripening [13].

Tannins are plant phenols, which have ability to form complexes with metal ions and with macro-molecules such as protein and polysaccharides [20]. Saponins are glycosides, which include steroids saponins and tri-terpenoid saponins [20]. A high level of saponins in feeds affects feed intake and growth rate in animals. Reduction in feed intake has been ascribed to the bitter taste of saponins. Saponins (in excess) causes hypocholesterolemia because it binds cholesterol making it unavailable for absorption [6, 21].

The most common antinutritional factors in fruits are oxalate, tannins, phytic acid and hydrocyanic acid [22]. A daily intake of 450mg/100g of oxalic acid has been reported to reduce the bioavailability of such metal as calcium. Phytic intake of (4.00 – 9.00mg/100g) reduces Iron (Fe) absorption by 4-5 folds in humans [23]. The anti-nutrient compositions were generally low, in matured seeds of *Tetracarpidium conophorum*, phytate and cyanic levels were low. These are in agreement with the results obtained from previous work [22]. But it should be noted that the concentration of anti-nutrients are reduce during processing and as such, there might be a reasonable concentration of anti-nutrients in raw fruits that make consumption of the raw fruits harmful to health. It is therefore safe to consume the fruits when cooked or boiled [22, 23]. As seen in the results, the immature fruits contain higher levels of these anti-nutrients than the matured. It may be unsafe to consume immature seeds of *Tetracarpidium conophorum.* The oxalate concentrations in and *Tetracarpidium conophorum* were within normal range as stipulated by WHO throughout the maturation period [24].

In the elemental study, the results revealed that the fruits of *Tetracarpidium conophorum*nut did not contain chromium (Cr) and cadmium (Cd) throughout the fruits maturation, but traces of lead (Pb) and nickel (Ni) were detected in the studied plants which were below the maximum permissible level. According to WHO the permissible limit of lead is (10ppm), cadmium (3.0ppm), chromium (2ppm) WHO, 1991. Iron (Fe) content was prominently higher than all the metals analysed, followed by manganese (Mn), copper (Cu) and calcium (Ca).

Cu plays an important role in the metabolism of Fe and as cofactor in the enzymatic systems. Cu deficiencies lead to impairment of Fe absorption. In severe cases of copper deficiency, the development of anemia has been documented [25]. Mn in fruits shows that *Tetracarpidium conophorum* can be used to treat bones disease [26]. On the basis of these results, *Tetracarpidium conophorum* fruits are rich sources of Fe, Cu and Mn. Hence, this nut having the higher amount of these trace elements is helpful in maintaining various functions of human body.The presence of heavy metals in fruits can cause serious diseases to the consumers. Pb cause adverse effects on physiological and behavioural activities in living beings. Its chronic toxicity causes kidney dysfunction, osteomalacia and obstructive lung disease [27]. Cadmium is another carcinogen associated with the risk of serious health hazard IARC, 1994. Liver and kidney are considered as the main target organs in acute and chronic Cd exposure [27]. On the basis of the low heavy metal content of the studied plants, this makes the fruits safe for consumption.

CONCLUSION

Tetracarpidium conophorum seeds oil is consumed to a limited extent in Nigeria but of which no large scale use is of, partly because there is little information on their nutritive values. These oil-seeds can be exploited as sources of edible and industrial oils. Furthermore, this study has shown that the seeds oil is safe for consumption and comparable to other currently used vegetable oils, and has satisfactory nutritional value.

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