INTERNATIONAL JOURNAL OF APPLIED SCIENCE RESEARCH
FEDERAL POLYTECHNIC OKO, ANAMBRA STATE

PROXIMATE, VITAMIN AND ELEMENTAL ANALYSES OF TIGER NUT (CYPERUS ESCULENTUS)

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ABSTRACT

Tiger nut is enjoyed by everyone who eats it, primarily due to its sweet taste. It is believed to have some nutritional benefits that are essential for the proper functioning of the body. The aim of this study was to determine the proximate, vitamin and some elemental compositions of tiger nut. Standard analytical, UV-visible spectroscopic and AAS techniques were used for the proximate, vitamin and elemental analyses of the sample. The results of the proximate analysis showed that the ash content was 5.395%, moisture content: 79.810%, crude fibre: 0.495%, crude fat: 3.433%, crude protein: 7.35% and carbohydrate 3.517%.Vitamin A was 17.619 mg/kg, vitamin B_1 (0.0552 mg/kg),vitamin B_2(0.552 mg/kg), vitamin B_3 (0.366mg/kg), vitamin B_6 (0.013 mg/kg),vitamin B_9 (14.790 mg/kg), vitamin B_{12} (1.538 mg/kg), vitamin C (13.3 mg/kg), vitamin D (11.712 mg/kg). Tigernut contains significant amount of Mg (10.326 ppm), K (2.520 ppm), Na (4.300 ppm), Zn (4.087 ppm), Fe (2.819 ppm), Hg (0.762 ppm), Cu (0.370 ppm) and Cd (0.219 ppm) but Pb and Ca were not detected. The implication of the results is that these vitamins and elements that are contained in tiger nut could be extracted by pharmaceutical industries and used to produce natural food and drug supplements but Hg and Cd should be selectively extracted to avoid their harmful effects in the body.

Keywords: Tiger nut, Proximate, Vitamin, Elemental Analyses

INTRODUCTION

Tiger nut *Cyperus esculentus lativum* is a grass-like plant of the Family *Cyperaceae*. They are not actually nuts but tubers found in the root of sedge plant (Takhatajah, 1992; Akuoma *et al*, 2000). Like other sedges, the plant is most frequently found inhabiting in wet marshes and edges of streams and ponds (Santos *et al*, 2015). In Nigeria, the Hausas call it "Aya", Yorubas "Imumu" the Igbos "Ofio", or "aki Hausa" (Oladele *et al.*, 2009). Tiger nut has been cultivated as a livestock feed and food for human consumption (Umerie *et al.*, 2007). According to Belewu and Abodunrin, (2006), three varieties are cultivated i.e. yellow, brown and black. But only the yellow and brown varieties are readily available in Nigeria market (Omode *et al.,* 2005). Sugar free tiger nut milk is suitable for diabetic patients (Anon, 2002). They are thought to be beneficial to those seeking to reduce cholesterol or lose weight (Beniwal, 2004; Reagor *et al*, 2002). It is good for arteriosclerosis (Malhotra, 1998). According to Stern *et al.* (2003), Paiute Indians pound tiger nut tubers with tobacco leaves use it for the treatment of athlete's foot.

Tiger nut can be used for the production of alcohol by fermentation (Des-Vries, 2001) and biodiesel (Nag, 2008). Roasted tubers are used as a coffee substitute (Oderinde and Tahir, 2008). It is rich in minerals, predominantly phosphorus and potassium and also vitamins C and E (Abaejoh *et al.,* 2006; Bamgbose *et al.*, 2003). Its content of vitamin E also collaborates against the cholesterol because it has an antioxidant effect over fats, which are ideal for coronary heart disease (Chukwuma *et al.,* 2010; Stern, 2003). It also finds uses as a flavouring agent for ice cream and biscuits (Cantalejo, 2007), and its flour does not lose any of its nutritional properties in the milling process (Sulaiman, 2008).

MATERIALS AND METHODS

Sample Collection: The Tiger nut sample that was used in this study was purchased at Eke Oko market, in Orumba North L.G.A, Anambra State, Nigeria.

Sample Preparation: Fresh Tiger nuts were visually inspected. Defective tubers were manually removed and discarded. Hence, only healthy tiger nut tubers were selected. One kilogram of Tiger nut was weighed out in portion, washed thoroughly in two changes of clean water and drained properly. Five hundred grams of tiger nut was packed in a nylon bag, kept in an air tight container and stored in a refrigerator.

Proximate Analysis Determinations (AOAC, 2006)

Ash Content

Empty platinum crucible was washed, dried and the weight was noted. Two grams of wet sample was weighed into the platinum crucible and heated in a muffle furnace at 500°C for 3hours. The sample was cooled in a desiccators after burning and weighed.

$$\% \text{ Ash content} = \frac{W3-W1}{W2-W1} \times \frac{100}{1}$$

Where W1= weight of empty platinum crucible, W2 = weight of platinum crucible and sample before burning, W3 = weight of platinum and ash.

Moisture Content

A petri dish was washed and dried in the oven. Two grams of the sample was weighed into petri-dish, and the weight of the petri dish and sample was noted before drying. The petri-dish and sample were put in oven, heated at 100°C for 1hour and allowed to cool. The weight was noted. It was further heated for another 1 hour until a steady weight was obtained and the weight noted. The drying procedure was repeated until a constant weight was obtained.

$$\% \text{ Moisture content} = \frac{W1-W2}{Weight\ of\ sample} \times \frac{100}{1}$$

Where W1= weight of petri-dish and sample before drying, W2= weight of petri-dish and sample after drying.

Crude Fat Content

Five grams of the sample was dried in a flask in an oven at 105-110°C for about 30 minutes and transferred into a dessicator and allowed to cool. The flask was filled with 300 mL petroleum ether (boiling point 40-60°C). The extraction thimble was lightly plugged with cotton wool and soxhlet apparatus was assembled and allowed to reflux for about 6 hours. The thimble was carefully removed and petroleum ether

at the top of the set up was allowed to drain into a container for re-use. When the flask was almost free of petroleum ether, the sample was removed and dried at 105-110°C for 1 hour. It was transferred from the oven into a dessicator and allowed to cool, and then weighed.

Crude Fibre

Two grams of the defatted sample from crude fat analysis was boiled under reflux for 30 minutes with 200 mL of a solution containing 1.25g of H_2SO_4 per 100 mL of solution. The solution was filtered through linen on a fluted funnel and further washed with boiling water in a beaker until the washings are no longer acidic which was confirmed by testing with litmus paper. The residue was transferred to another beaker and boiled for 30 minutes with 200 mL of a solution containing 1.25g of carbonate free NaOH per 100 mL. The final residue was filtered through a thin but close pad of washed and ignited asbestos in a Gooch crucible, and further dried in an electric oven and weighed. It was finally incinerated, cooled and weighed.

The loss in weight after incineration × 100 is the percentage of crude fibre.

$$\%\text{Crude fibre} = \frac{Weight\ of\ fibre}{Weight\ of\ Sample} \text{ X } 100.$$

Crude Protein

This was done by Kjeldahl method described by Chang (2003). The total nitrogen was determined and multiplied with factor 6.25 to obtain protein content.

Two grams of the sample was put into Kjeldahl flask and added 25 mL of concentrated H_2SO_4. Half of cupper sulphate, five grams of sodium sulphate and a speck of selenium catalyst was added to it before it was heated under a fume cupboard until a clear solution was obtained (the digest). The digest was diluted to 100 mL in a volumetric flask and used for the analysis.

The 10 mL of the digest was mixed with equal volume of 45% NaOH solution in a Kjeldahl distillation apparatus. The mixture was distilled into 10 mL of 40% boric acid containing 3 drops of mixed indicator (bromo cresol green/ methyl red). A total of 50 mL of distillate was collected and titrated against 0.02 N EDTA from green to a deep red end point. A reagent blank was also digested, distilled and titrated. The nitrogen content and hence the protein content was calculated using: 1mL of 1N H_2SO_4 = 14mg.

$$\%\ N = \left(\frac{14\ x\ VA\ x\ 0.1\ x\ W}{1000\ x\ 100}\right) \text{ X } 100;$$

VA = volume of acid used, W = Weight of sample, % crude protein = % N X 6.25.

Carbohydrate

The carbohydrate content was determined by subtraction method (Olaoye, and Onilude, 2008)

(%Total Carbohydrate = [100 - %(Moisture + Protein + Fat + Ash + Fibre)].

Vitamin Analysis

The analysis was carried out using Official Methods of Analysis by Association of Analytical Chemists (AOAC, 2005).

Vitamin A

One gram each of the sample and the standard were mixed with 30 mL of absolute alcohol and 3 mL of 50 mL KOH solution was added to it and boiled gently for 30 minutes under reflux. After washing with distilled water, vitamin A was extracted with 3 X 50 mL of diethyl ether. The extract was evaporated to dryness at room temperature and was then dissolved in 10 mL of isopropyl alcohol. One millilitre of standard vitamin A solution prepared and that of the dissolved extract were transferred to separate curvettes and their respective absorbance were read in a spectrophotometer meter at 325 nm with a reagent blank at zero.

Calculation: = $\frac{Absorbance\ of\ Sample \times Concentration\ of\ Standard}{Absorbance\ of\ Standard}$

Vitamin B_1 and B_2

One gram of sample was weighed into a conical flask, the sample was dissolved with 100mL of deionized water, it was shake thoroughly and heated for 5 minutes and allowed to cool and filtered. The filtrate was poured into a curvette and their respective wavelength for the vitamins set to read the absorbance using spectrophotometer.

Vitamin B1 = 261 nm, Vitamin B2 = 242 nm. Calculations: Concentration (mg %) = $\frac{A \times DF \times Volume\ of\ curvette}{5}$. Where A = Absorbance, E = extinction coefficient = 25 for B_1 and B_2, DF = dilution factor

Vitamin B_3 (Nacotinamide)

Five grams of sample was dissolved in 20 mL of anhydrous glacial acetic acid and warmed slightly. Five millilitres of acetic anhydride was added and mixed. Two drops of crystal violet solution was added as indicator and 0.1M perchloric acid was added to titrate to a greenish blue colour. Calculation: Vitamin B_3 = $\frac{Titre\ value \times 0.0122}{0.1}$

Where, 0.1 is the volume of perchloric acid and 0.0122 is a factor

Vitamin B_6

Five grams of sample was dissolved in a mixture of 5 mL of anhydrous glacial acetic acid and 6 mL of mercury (II) acetate solution. Two drops of crystal violet was added as indicator and 0.1M perchloric acid was used to titrate to green colour end point. Calculation: each mL of 0.1M perchloric acid is equivalent to 0.02056 g of $C_8H_{11}NO_3HCl$.

Vitamin B_{12}

Twenty five milligram of sample was dissolved in 250 mL of deionized water.

The absorbance was read at 361nm.

Calculation: Concentration (mg %) = $\frac{A \times DF \times Volume\ of\ curvette}{E}$

Where A =Absorbance, E = Extinction coefficient =25, DF = dilution factor =5

Vitamin C (Ascorbic Acid)

This was determined by the titrimetric method. A weighed sample was homogenized in 6% EDTA/TCA solution. The homogenate was filtered and used for the analysis, with 20 mL of 30% KI solution and it was added to the homogenate followed by 10 mL of distilled water. One millilitre of 1% starch solution was added to it and it was titrated against 0.1M $CuSO_4$ solution. The end point was marked by a black coloration, and reagent blank was also titrated. Vitamin C content was calculated based on the relationship below.

1mL of 0.1 mole $CuSO_4$= 0.88mg Vit C; Vitamin C mg/ 100= $\frac{100\ x\ 0.88\ x\ Titre\ value\ -Blank}{W}$

Vitamin D and K

Total vitamin D and K were estimated by the method described by Dutta, (2011). The experiment was carried out in the dark to avoid photolysis of vitamin D once the saponification was completed. The sample (0.5g) was homogenized and saponified with 2.5 mL of alcoholic potassium hydroxide in a water bath at 60^0C for 30 minutes. The saponified extract was transferred to a separating funnel containing 10-15 mL of petroleum ether and mixed well. The lower aqueous layer was then transferred to another separating funnel and the upper petroleum ether layer containing the carotenoids was then collected. The extraction was repeated until the aqueous layer became colourless. A small amount of anhydrous sodium sulphate was added to the petroleum ether extract to remove excess moisture. The final volume of the petroleum ether extracted was noted. The absorbance of the yellow colour was read in a spectrophotometer at 450nm and 503nm respectively for vitamin D and K using petroleum ether as blank. The amount of the total vitamin D and K was calculated thus:

Amount of total Vitamin D = $\frac{\text{A450} \times Volume\ of\ Sample \times 100 \times 4}{Weigt\ of\ \ Sample}$

Amount of total Vitamin K = $\frac{\text{A503} \times Volume\ of\ Sample \times 100 \times 4}{Weigt\ of\ the\ Sample}$

Vitamin E (Tocopherol)

One gram of the sample was mixed with 10 mL of ethanolic sulphuric acid and boiled gently under reflux for 30 minutes. It was transferred to a separating funnel and treated with 3x30 ml diethyl ether and recovering diethyl ether layer each time. The ether extract was transferred to a dessicator and dried for 30 minutes and later evaporated for dryness at room temperature. The dried extract was dissolved in 10 mL of pure ethanol. One milliliter of the dissolved extract and equal volume of standard vitamin E were transferred to separate tubes. After continuous addition of 5 mL of absolute alcohol and 1 mL of conc. nitric acid solution, the mixtures were allowed to stand for 5 minutes and the respective absorbance measured in a spectrometer at 410 nm with blank reagent at zero.

Determination of the Some of The Elemental Components of the Tiger nut

Na, K, Ca, Fe, Mg, Zn, Cu, Pb, Hg and Cd were determined in AAS according to (AOAC, 2003).

RESULTS AND DISCUSSION

The results of the proximate composition of tiger nut in Table 1 showed that the yellow type of fresh tiger nut has a substantial quantities of ash; 5.395 %, moisture; 79.810 %, crude fat: 3.433 % crude protein; 7.350 % and carbohydrate; 3.517 % and fibre with a value of 0.495 %. Apart from crude protein, the other parameters of the proximate composition of the fresh yellow tiger nut were not in agreement with each other. Ekeanyanwu and Ononogbu (2010), Mahammed *et al.* (2018) and Gambo and Da'u (2014) in their various analyses reported the following results: crude protein; (8.07 %, 8.51 % and 7.15 %), fat: (24.3 %, 17.00 % and 32.13 %), fiber: (24.3 %, 13.10 % and 6.26 %), carbohydrates: (30.00 %, 17.82 % and 46.99 %) and ash contents (1.80 %, 1.18 % and 3.97 %) respectively.This comes to disagree with the belief that what is usually left after chewing tiger nut is all fibre. The results as presented in Table 1 has however, shown that fresh tiger nuts are fairly good sources of protein with a value of 7.350%.

Table 1: The results of the Proximate Composition of Tigernut

Parameters	Values (%)
Ash Content	5.395
Moisture Content	79.810
Crude Fibre	0.495
Crude Fat	3.433
Crude Protein	7.350
Carbohydrate Content	3.517

The results of the UV-visible analyses of tiger nut in Table 2 showed that the tiger nut contained preventive or protective nutrients. Its vitamin A content was 17.6190 mg/kg. Ekeanyanwu and Ononogbu (2010) and Mohammed *et al.* (2018) reported 0.21mg/100g and 0.87mg respectively for vitamin A content of fresh yellow tiger nut. Vitamin A is a vital vitamin for vision and sight. In Table 2, the amount of vitamin B complexes of tigernut indicated that vitamin B_1 (0.0552 mg/kg), vitamin B_2 (0.5520 mg/kg), vitamin B_3 (0.3660 mg/kg), vitamin B_6 (0.0130 mg/kg), vitamin B_9 (14.7900 mg/kg), vitamin B_{12} (1.538 mg/kg). Vitamin B complexes are important vitamins in the body that provide the necessary nutrients needed by the body for rapid growth and tissue development, (Anon, 2002). The result of vitamin C content of fresh yellow tiger nut as shown in Table was 13.3 mg/kg. Mohammed *et al*(2018), obtained 30.70 mg per 100 g of sample and Ekeanyanwu and Ononogbu (2010) reported 7.30 mg/100 g. Vitamin C helps in the formation of collagen. It is an anti-oxidant and important in building body immunity against diseases (Chukwuma *et al.* 2010).Vitamin D from the results in Table 2 was 11.7120 mg/kg, but Ekeanyanwu and Ononogbu (2010) reported 0.42mg/100 g. Vitamin

D is a group of fat soluble steroids responsible for increasing intestinal absorption of Ca, Mg, sulphate and phosphate, and multiple other biological effects (Oladele, and Aina, 2000). Vitamin D is plays important roles in immunity, body metabolism, sexual development and reproduction (Dutta, 2011). Vitamin E was 3.3100 mg/kg. It is a fat-soluble vitamin. It which contains antioxidant properties. The amount of Vitamin K from the result was1.2660mg/kg. Vitamin K is a group of structurally similar, f at soluble vitamins that the human body requires for complete synthesis of certain proteins that are prerequisites for blood coagulation and which the body also need for controlling and binding of calcium in bones and other tissues. These nutrients could significantly contribute to the body's metabolic processes.

Table 2: Results of the Vitamin Contents of Tiger nut.

Vitamins	Concentration (mg/kg)
A	17.6190
B_1	0.03360
B_2	0.0552
B_3	0.3660
B_6	0.0130
B_9	14.7900
B_{12}	1.5380
C	13.2000
D	11.7120
E	3.3100
K	1.2660

Tiger nuts can supply the body with adequate Zn (4.087ppm), Fe (2.819ppm), Na (4.300ppm), K (2.520ppm) and Mg (10.326ppm) as shown in Table 3. In their separate researches Gambo and Da'u (2014), Ekanayanwu and Ononogbu (2010) and Mohammed *et al.* (2018) reported the following results: Ca (155mg/100g, 100.0mg/100g and 43.36mg), Na (245mg/100g, 34.13mg/100g and 17.02mg), K (216mg/100g, 486mg/100g, and 267.18mg), Mg (51.2mg/100g, 94.4mg/100g, and 118.14mg), Fe (0.65mg/100g and 2.82mg), Zn (0.01mg/100g and 1.39mg) and Cu (0.02mg/100g and 0.54mg) respectively. Zinc is important in many metabolic reactions and plays important role in immunity, alcohol metabolism, and reproduction (Hays, and Swenson, 1985). Copper aids in iron metabolism. Fe is the functional component of hemoglobin (Chandra, 1990). Magnesium provides bone strength, aids enzyme, nerve and heart functions. This shows that tiger nut could be a better alternative in providing this element to the body. Also in Table 3, the results showed that tiger nut does not contain Ca to the detectable level in AAS. This indicates that those that are in need of formation of bone and teeth like infant and adult cannot get this element from tiger nut. However, tiger nut should be taken by everybody because of the presence of vitamins which nourishes the body system (Wills, 2002).

But in moderation especially by adults because of the presence of Cd and Hg which are heavy metals that are carcinogenic. Its high content of K and Na is beneficial to the young ones but not to the aged to avoid uncontrollable supply of these elements which poses the danger of high blood pressure and stroke to the body.

Table 3: Results of the Elemental Composition of Tiger nut

Elements	Concentration (ppm)
Cd	0.219
Cu	0.370
Pb	0.000
Ca	0.000
Hg	0.762
Fe	2.819
Zn	4.087
Na	4.300
K	2.520
Mg	10.326.

CONCLUSION

This study on proximate, vitamin and elemental component analyses of tiger nut has provided information on its nutritive values. The result showed that tiger nut is rich in H_2O, K, Na, Mg and preventive or protective nutrients (vitamins, Fe, Cu and Zn). It does not have AAS detectable amount of Ca and Pb, but contain Hg and Cd which are carcinogenic.

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