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Fatty and amino acid compositions in dry (Brown) and fresh (Yellow) tiger nut (*Cyperus esculentus*) in the south western part of Nigeria

Ayoola P B
Department of Science Laboratory Technology (Chemistry option), Ladoke Akintola University of Technology, P.M.B. Ogbomoso, Oyo State, Nigeria

Abstract
Fresh (yellow) and dry (brown) *Cyperus esculentus* nuts are common nuts that most people eat indiscrimately without minding its contribution to the body system and the medicinal implications of the nuts to the society as a whole. The nutritional and medicinal uses of these nuts are scarcely evaluated and reported, therefore, the need for this research.
Fresh (yellow) and dry (brown) *Cyperus esculentus* nuts were obtained from a local market called "Aarada market" situated at Ogbomoso South Local Government Area, in Ogbomoso, Oyo State, Nigeria.
Fatty and Amino acid compositions were determined using standard methods of analysis. The fatty acids analysis result showed that the nuts contained saturated, mono unsaturated and poly unsaturated fatty acids, where the prominent saturated fatty acid was lauric acid with the value of 13.57% fresh (yellow) and 18.74% dry (brown) respectively. The prominent fatty acid in the mono unsaturated fatty acid was oleic acid with the values of 37.58% fresh (yellow) and 39.65% dry (brown) respectively. The prominent polyunsaturated fatty acid was linoleic acid with the values of 38.77% fresh (yellow) and 43.28% dry (brown) respectively.
The amino acids composition results revealed that dry (brown) *Cyperus esculentus* nut contained more of the essential and non essential amino acids than the fresh (yellow) type. Histidine (2.97±0.01 and 3.15±0.05%), leucine (8.15-8.57%), isoleucine (3.76 ± 0.01and 3.95 ± 0.01%), phenylalanine (7.45±0.02 and 7.64±0.02%) and valine (7.12 ± 0.02and 7.23±0.01%) were abundant in the dry tiger nut (essential amino acid) while tryptophan (0.57±0.08 and 0.72±0.02 %), threonine (0.49±0.01 and 0.68±0.01%) and methionine (1.26±0.01 and1.35±0.02 %) were in traces. Tyrosine (4.65±0.01 and 4.88±0.01%), cysteine (1.89±0.02 and 2.26±0.02%) and lysine (2.04±0.02 and 2.18±0.02 %) were abundant in the dry tiger nut (non-essential amino acid) while tryptophan (0.57±0.08 and 0.72±0.02%), threonine (0.49±0.01 and 0.68±0.01%), methionine (1.26±0.01 and1.35±0.02%) and arginine (1.08±0.01 and1.35±0.05%) were in traces.
The dry (brown) nut is more nutritious, stable and durable considering the amount of both essential and non-essential amino acid compositions in the nut and the unsaturated fatty acids present. However, apart from being nutritious, it could be used in drug formulation and treatment of cardiovascular diseases. Therefore, *Cyperus esculentus* (Tiger nuts) could be recommended as diet and medicine mostly for diabetes patient.

Keywords: *Cyperus esculentus***,** amino acids, fatty acids, cardiovascular, polyunsaturated

Introduction

Cyperus esculentus (Tiger nut) belongs to the family *Cyperaceae* and the order, *Commelinalis*. It is found widely in warm and temperate zones occurring in Southern Europe and Africa. *Cyperus esculentus* is a popular edible nut in Nigeria and it is known as Aya in the Northern part (Hausa), ofio in the South western part (Yoruba) and aki-Hausa in the Eastern part (Ibo). It grows well in the middle belt of Nigeria (Okafor, Mordi, Ozumba, Solomon and Olatunji, 2003) [15]. *Cyperus esculentus* is good to be taking by diabetics because of its content of sucrose and starch and its high content of arginine which stimulates the production of insulin in human system (Belewu and Belewu, 2007) [4]. Plant seeds such as the *Cyperus esculentus* are important sources of nutrients and can serve as high quality dietary protein sources to meet nutritional requirements (Perumal, Klaus, and Harinder, 2001) [16]. Therefore, one of the least expensive ways of increasing protein levels in the diets of low income families is by encouraging the consumption of local indigenous edible seeds which have been found to be rich in protein (Sigh, Rao, Subrahmanuam, and Saxena, 1993) [19]. The nut contained saturated and unsaturated fatty acids which are prophylactic and therapeutically in action.
Fatty acids are molecules that differ in the length of their carbon chain and the number of double bonds within them. Each of the resulting" families" has its own peculiarities as regards energy generation, structure and metabolism. Passing from the short, saturated chains to the very long polyunsaturated ones produces an increasing degree of specialization in metabolic and structural functions important for the body's development and general homeostasis. Fatty acids are the main ingredient of the tissue components known as the lipids (triglycerides, cholesterol esters and phospholipids). They are thus virtually ubiquitous in living organisms, where they exhibit structural, energy-yielding and metabolic functions. Fatty acids are therefore the lipid fraction par excellence for infant's functions and requirements (Carlo, 1992).
Most plants whose seed oils had been analyzed for fatty acid composition are rich in long chain fatty acids (Ayodele, 2003) [2]. Those with short or medium chain acids are rare (Wolf *et al.*, 1983). It has been reported that lauric acid

predominates in species of *laureaceae* and *palmae* families while several *ulmaceae* are rich in capric acid (Hilditch and William, 1964). The fatty acid analysis of the oil showed that linolenic acid constituted 66% of the fatty acids. Polyunsaturated fatty acids accounted for over 80% of the fatty acids (Ogunsua and Adebona, 1983). The fatty acids compositions of fresh (yellow) and dry (brown) Tiger nuts (*Cyperus esculentus*) found in Ogbomoso South western Nigeria are being reported in literature for the first time.

This research work was designed to determine the fatty and amino acid compositions present in the locally sold *Cyperus esculentus* nuts in Ogbomoso, Southwestern Nigeria in order to assess their nutritional potentialities and medicinal values.

Materials and Methods

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Sample collection

Fresh *Cyperus esculentus* nuts (fresh and dry) were purchased at Aarada market which is located at Okin alapa/Ejigbo road at Ogbomoso South Local Government Area, Oyo State, Nigeria (Plate 1 and Plate 2).

Plate 1: Fresh (Yellow) *Cyperus esculentus*

Plate 2: Dried (Brown) *Cyperus esculentus*

Sample Preparation

The nuts were thoroughly checked to remove dirt, stones and other visible particles that could be present. The nuts were rinsed with distilled water and allowed the water to dry off.

Five hundred grammes (500 g) each of the fresh and dry nuts were weighed separately into different containers and air dry for 7 days, thereafter, ground to coarse powder using mechanical grinder. The powdered samples were air dried by spreading on a white cardboard in the laboratory for seven (7) days then transferred into a desicator separately in order to be certified that all water molecules had dried off, thereafter, the samples were transferred into air tight plastic sample bottles and kept for analysis.

Determination of Fatty acids compositions

Isolation of oils

The powdered samples of both nuts were weighed (100 g) into separate 2.5 L bottles and 1.5 L of n-hexane was added into each of the bottle and the mixtures were left for 72 h with intermittent shaking (extraction by maceration). The mixtures were filtered using glass wool in funnel and the filtrates were exposed to air where all the n-hexane present in the filtrates evaporated. The yellow residues (oil) were weighed to calculate the percentage yield. The oil had distinct characteristic pleasant odour.

Gas chromatography

The oils were subjected to GC analyses on GC 2010 gas chromatograph. Column oven temperature is 60 ºC injection temperature of 250 ºC, split injection mode, at 100, 2k Pa; Column flow of 1.61 ml/min and total flow of 6.2 ml/min; 1.0 split ratio; oven temperature programming is 60ºC for 5 min and at the rate of 5 ºC / min till 140 ºC, 15 º/min till 280 º C.'

Gas chromatography- Mass spectrometry

The GC-MS analyses were performed on GC-MS QP2010 Plus ion, Source temperature 200 ºC; interface temperature 250 ºC; solvent cut time 2.5 min; with relative detector gain mode and threshold 3000; scan MS ACQ mode; detector FTD; mass range of m/z 40-400.

Identification of components

Identification of the oil components were based on their retention indices (determined with a reference to a homologous series of n-alkanes), along with comparison of their mass spectral fragmentation patterns in computer matching against in built data and commercials such as Joulain and Koenia (1998) [9], Adams (1995) [1] and Massada (1976) [12] Libraries as well as in-house "Baser Library of Essential oil constituents" built up by genuine compounds and components of known oils.

Determination of Fatty Acids Profile of the Fresh and Dry tiger nut Using Spectrophotometric Method.

Two grammes (2 g) each of the samples was weighed into 100 ml conical flask and 20 ml of benzene were added, shaking thoroughly to extract all the fatty acids. The mixture was transferred into a 250 ml separating funnel to separate the benzene extract from the aqueous extract. Then 5 ml aliquot of the benzene extract was pipette into a 15 ml test tube and 2 ml of 10 % copper acetate added to develop a blue colour. Standard solutions of each fatty acid were

prepared in the range 0-10 ppm from 100 ppm stock solution of each fatty acid. Absorbance or optical density of sample extract as well as standard solutions of different concentrations were read on a spectrophotometer at a wavelength defined for each fatty acid as listed below:
Lauric acid (640 nm), Stearic acid (650 nm), Palmitic acid (630 nm), Arachidonic acid (690 nm), Oleic acid (670 nm), Linoleic acid (660 nm), Linolenic (680), Ricinoleic acid (610 nm) and Dihydroxystrearic acid (655 nm) (AOAC, 2005).
The % of each fatty acid was obtained using the formula:

$$\% \text{ fatty acid} = \frac{\text{Absorbance of sample x gradient factor of a specific fatty acid x Dilution Factor}}{\text{Weight of sample x 10000}}$$

Determination of Amino acids

The free amino acids concentrations were determined with ninhydrin reagent using phenylalanine as standard and reading the developed color at 570 nm and extrapolating the values from a standard curve of phenylalanine. Ninhydrin in acetone (0.1%) was diluted with distilled water in the ratio 1:4. Exactly, 20µL each of the diluted extracts was added to 4ml portions of the diluted ninhydrin. The resulting solutions were heated to boiling for 5 min, cooled and the absorbance read in a spectrophotometer at 570nm using distilled water as blank (Schroeder, Kay and Mills, 1990; Edem, D.O., Amugo, C.I., and Eka, 1990) [18, 6].
The major amino acids constituents of the samples were carried out with the aid of Technicon Sequential Multi sample Amino acid Analyzer (TSM) (Yemm and Cooking, 1954) [20].

Results and Discussion

Table 1 shows the saturated fatty acids present in the nuts. The amount in the dry nut was higher than in the fresh nut. These are indications that the oil of the nuts could be used for other purposes than the stated uses. The same thing happened in the values of the monounsaturated fatty acids in both nuts where the dry nut has higher values than the fresh nut (Table 2).

Table 1: Saturated Fatty Acids Composition of Fresh and Dry *C. esculentus* (Tiger) nut

Fatty Acid	Double bond	Fresh (%)	Dry (%)
Caproic acid	6:0	0.89	1.23
Caprylic acid	8:0	1.56	1.89
Capric acid	10:0	1.34	1.95
Lauric acid	12:0	13.57	18.74
Myristic acid	14:0	0.95	1.18
Palmitic acid	16:0	1.83	2.09
Stearic acid	18:0	4.27	6.78
Behenic acid	22:0	0.00	0.05
Lignoceric acid	24:0	0.06	0.11
Total		24.47	34.02

Table 2: Mono unsaturated Fatty Acids Composition of Fresh and Dry *C. esculentus* (Tiger) nut

Fatty Acid	Double bond	Fresh (%)	Dry (%)
Palmitoleic acid	16:1	0.05	0.14
Oleic acid	18:1	37.58	39.65
Erucic acid	22:1	0.15	0.39
Total		37.78	40.18

Polyunsaturated fatty acids (omega-3 and omega-6) are known for their health functions and uses in the preventive and treatment of cardiovascular diseases. The dry nut has higher concentrations of the polyunsaturated fatty acid than the fresh nut (Table 3). Polyunsaturated fatty acids have some medical importance such as anti inflammatory effect, benefit in rheumatoid arthritis (Oleic acid). α –linolenic acid (omega-3) prevents cardiovascular disease, other members of the omega-3 (EPA+ DHA) also have ability to prevent obesity, brain development rheumatoid, arthritis and type 2 diabetes (Katalin and Loana-Daria, 2017) [10].

Table 3: Poly unsaturated Fatty Acids (PUFA) Compositions of Fresh and Dry *C. esculentus* (Tiger) nut

Fatty Acid	Double bond	Fresh (%)	Dry (%)
Linoleic acid	18:2	38.77	43.28
Linolenic acid	18:3	3.21	3.87
Arachidonic acid	20:4	2.03	3.94
Total		44.01	51.09

Cyperus esculentus are good sources of essential amino acids such as; leucine, phenylalanine and valine, while that of the non essential amino acids is tyrosine. Other amino acids were present in traces. The dry *Cyperus esculentus* has the highest amount of leucine, phenylalanine and valine (Table 4 and 5). Leucine promotes the healing of muscle tissue, skin and bones. Leucine is recommended for those recovering from surgery, lower blood sugar level and aids in increasing growth hormone. Deficiency in the essential amino acids may hinder healing recovery process (Mat Jais AM, Mc Culloh, and Croft, 1994) [13].

Table 4: Essential Amino acids Composition of Fresh and Dry *C. esculentus* as a percentage of total protein

Amino Acid	Fresh (%)	Dry (%)
Histidine	2.97±0.01	3.15±0.05
Tryptophan	0.57±0.08	0.72±0.02
Leucine	8.15±0.02	8.57±0.01
Isoleucine	3.76±0.01	3.95±0.01
Lysine	2.04±0.02	2.18±0.02
Methionine	1.26±0.01	1.35±0.02
Phenylalanine	7.45±0.02	7.64±0.02
Threonine	0.49±0.01	0.68±0.01
Valine	7.12±0.02	7.23±0.01

Values are means (±SD) of triplicate determinations

Phenylalanine is used by the brain for the production of epinephrine, a chemical that transmits signals between nerve cells in the brain. It promotes alertness and vitality, elevates mood, decreases pain, aids memory and learning, used to treat arthritis, depression, menstrual cramps, migraine obesity, Parkinson's diseases and schizophrenia. Valine is needed for muscle metabolism and coordination, tissue repair and for the maintenance of proper nitrogen balance in the body. It is used as an energy source by muscle tissue, helpful in treating liver and gall bladder disease. Valine promotes mental vigor and calm emotions (Guide for Amino acids, 2011) [17].
The non-essential amino acids composition result shows that tyrosine in the dry tiger nut has the highest concentration and all other amino acids are present in traces (Table 5). It is a precursor of adrenaline, nor epinephrine and dopamine, which regulate mood and stimulates metabolism and the nervous system, acts as a mood elevator, suppresses the

appetite and helps reduce body fat, aids in the production of melanin (the pigment responsible for hair and skin color) and in the function of the adrenal, thyroid and pituitary glands, has been used to help chronic fatigue, narcolepsy, anxiety, depression, low sex drive, allergies and headaches. Histidine aids in sexual arousal, removal of heavy metals from the body, lower blood pressure and protect body from radiation damage. Isoleucine aids in the healing, repair of muscle tissue, skin, and bone. It is also needed for haemoglobin formation, stabilizing, regulating blood sugar and energy (Nwaoguikpe and Reginald, 2010) [14].

Table 5: Non Essential Amino acids Composition of Fresh and Dry *C. esculentus* as a percentage of total protein

Amino Acid	Fresh (%)	Dry (%)
Glutamic acid	1.79±0.01	1.98±0.01
Cysteine	1.97±0.02	2.26±0.02
Cystine	1.89±0.2	1.97±0.01
Alanine	0.25±0.01	0.41±0.02
Aspartic acid	0.84±0.02	1.15±0.01
Ornithine	0.17±0.01	0.29±0.02
Tyrosine	4.65±0.01	4.88±0.01
Glycine	0.58±0.02	0.67±0.01
Serine	0.79±0.01	0.8±0.01
Arginine	1.08±0.01	1.35±0.05

Lysine aids calcium absorption and maintain a proper nitrogen balance in adults. Also aids in the production of antibodies which have the ability to fight cold sores and herpes outbreaks, helps form collagen, which makes up cartilage and connective tissue. Methionine is a powerful antioxidant and a good source of sulphur which prevents disorders of the hair, skin and nails. It helps to detoxify harmful agents such as lead and other heavy metals. Beneficial for women who take oral contraceptives because it promotes the excretion of estrogen, reduces the level of histamine in the body which can cause the brain to relay wrong messages (Guide for Amino acids, 2011) [17].

Tryptophan is a natural relaxant, helps alleviate insomnia by inducing normal sleep, reduce anxiety and depression and stabilizes mood, helps in the treatment of migraine, headache, helps the immune system function properly, aids in weight control by reducing appetite, enhances the release of growth hormones, helps control hyperactivity in children. Threonine helps maintain proper protein balance in the body, important for the formation of collagen, elastin and tooth enamel, aids liver and lipotropic function when combined with aspartic acid and methionine, prevents the build-up of fat in the liver. Alanine aids in the metabolism of glucose, a simple carbohydrate that the body uses for energy; guards against the buildup of toxic substances that are released into muscle cells when muscle protein is broken down quickly to meet energy needs, such as what happens with aerobic exercise; strengthens the immune system by producing antibodies. Amino acids like aspartic acid, glycine and glutamic acid are known to play a key role in the process of wound healing (Chyun and Griminger, 1984) [5].

Aspartic acid increases stamina and is good for chronic fatigue and depression. Cysteine functions as a powerful antioxidant in detoxifying harmful toxins. It protects the body from radiation damage, protects the liver and brain from damage due to alcohol, drugs and toxic compounds found in cigarette smoke. It is used in the treatment of rheumatoid arthritis and hardening of the arteries. Promotes the recovery from severe burns and surgery, promotes the burning of fat and the building of muscle. Slow down aging process. Glycine retards muscle degeneration, improves glycogen storage thus freeing up glucose for energy needs, promotes a healthy prostrate, central nervous system and immune system, useful for repairing damaged tissue and promotes healing (Heimann, 1982; Witte, Thornton, Tantry and Barbul, 2002) [8].

Glutamic acid is important in the metabolism of sugars and fats. It aids in the transportation of potassium into the spinal fluid, acts as fuel for the brain, helps correct personality disorders and is used in the treatment of epilepsy, mental retardation, muscular dystrophy and ulcers (Guide for Amino acids, 2011) [17].

Serine is needed for the proper metabolism of fats and fatty acids, the growth of muscle and the maintenance of a healthy immune system. It is important in RNA and DNA function and cell formation aids in the production of immune globulins and antibodies. Proline improves skin texture by aiding the production of collagen and reducing the loss of collagen through the aging process, helps in the healing of cartilage and the strengthening of joints, tendons and heart muscles, works with vitamin C to promote healthy connective tissues (Gaby and Singh, 1991) [7].

Conclusion

This study shows that the dry tiger nuts are more nutritious and medicinal than the fresh tiger nuts and people are advised to always eat the dry nut. However, more research could be embarked upon to analyze both nuts to establish bioactive compounds that could be useful in formulation of drugs and feeds for animals.

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