

Proximate Composition and Amino Acid Profile of Raw and Cooked Black Variety of Tiger nut (*Cyperus esculentus* L.) Grown in Northeast Nigeria

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Abstract

Tiger nut (*Cyperus esculentus* L.) is an underutilized tuber of family Cyperaceae which produces rhizomes from the base of the tuber that is somewhat spherical. The three varieties of yellow, brown and black tiger nut are grown in Nigeria. Proximate and amino acid compositions of raw and cooked black variety of *Cyperus esculentus* were investigated using standard analytical techniques. The respective proximate composition values (%) for the raw and cooked samples were: Moisture (3.73 and 4.66), ash (2.57 and 3.38), crude fat (8.94 and 9.92), crude protein (12.09 and 9.25), crude fibre (7.02 and 4.52), and carbohydrate (65.66 and 69.29). The calculated fatty acids and metabolizable energy for the raw and cooked samples were 7.15 and 7.94%; 1652.53 and 1702.22 kJ/100g, respectively. The values of metabolizable energy in this study showed that both samples have energy concentrations more favourable than cereals. The amino acid profile revealed that raw and cooked samples of *Cyperus esculentus* contained nutritionally useful quantities of most of the essential amino acids with total essential amino acid (TEAA) (with His) were 45.69 and 48.25%, respectively. The value of predicted protein efficiency ratio (P–PER) for the raw tuber was 2.77, the value is higher than the reported P–PER values of some legumes in the literature. However, essential amino acid supplementation may be required in Ile, Leu, TSAA and Val when comparing the EAA in this report with the recommended FAO/WHO provisional pattern. The limiting EAAs in the raw and cooked *Cyperus esculentus* tuber were Leu and TSAA, respectively.

Keywords: Proximate, amino acids, tigernut

1 Introduction

Tiger nut (*Cyperus esculentus*) belongs to the family *Cyperaceae* and the order, *Commelinalis*. It is found worldwide in warm and temperate zones, occurring in Southern Europe and Africa. This plant is cultivated for its small tuberous rhizome which is eaten raw or roasted, pressed for its juice to make beverage or milk, extracted of non-drying oil or used as hog feed (Ogbonna *et al.,* 2013). It can also be cooked, dried and powdered and may be used in confectionary to make biscuits with a delicious nut-like flavour. Mixing the ground tubers with cinnamon, sugar, vanilla and the cream, makes it a refreshing beverage. The roasted tubers are a substitute for coffee (Okafor *et al.,* 2003).

Tiger nuts *(Cyperus esculentus*) have long been recognized for their health benefits as they are rich in fibre, protein and natural sugars, minerals (phosphorus, potassium), and vitamins E and C (Belewu & Belewu, 2007; Adejuyitan, 2011). *Cyperus esculentum* is a popular plant seed in Nigeria and known by different names by different ethnic groups. In Hausa, it is known as “aya”, in Yoruba “mumu” and in Ibo “aki-Hausa” or “ofio”. It grows well in the middle belt of Nigeria (Okafor *et al.,* 2003), where three varieties are cultivated. These varieties include; the black, brown and yellow that has two varieties, the large and small. The yellow is larger in size, more attractive in color and has flesher body (Belewu & Belewu, 2007; Umerie *et al.,* 1997; Belewu & Abodurin, 2006). In Nigeria, the utilization of tiger nut is highly limited in spite of the fact that tiger nut is cultivated widely in the Northern part of the country (Ukwuru *et al.,* 2011).

Recently, there is awareness for increased utilization of tigernut (Belewu & Abodunrin, 2006; Belewu & Belewu, 2007; Ade-Omowaye *et al*., 2008; Ukwuru *et al*., 2008). Tigernuts are valued for their highly nutritious starch content, dietary fibre and carbohydrate (Umene & Enebeli, 1997) and are rich in sucrose (17.4- 20.0%), fat (25.5%), protein (8.0%) (Kordyias, 1990; Temple *et al.*, 1990). Tigernut is also rich in mineral content such as calcium, potassium, magnesium, zinc and traces of copper (Omode *et al*., 1995; Oladele & Aina, 2007). The dietary fibre content of tiger nut is effective in the treatment and prevention of diseases such as colon cancer, coronary heart diseases, obesity, diabetes and gastro-intestinal disorders (Anderson *et al*., 1994). Tiger nut tubers are diuretic and can be used as stimulant and tonic and in the treatment of flatulence, indigestion, diarrhea, dysentery and excessive thirst (Chopral *et al*., 1986). In addition, tiger nut has been demonstrated to contain higher essential amino acids than those proposed in the protein standard by FAO/WHO (1995) for satisfying adult needs for protein (Bosch & Alegna, 2005). Researchers have developed phyto milk of acceptable quality from tiger nut tubers (Abaejo *et al*., 2006; Ukwuru *et al*., 2008). Possible industrial application of tiger nut tubers has also been investigated (Oderinde & Tahir, 1988). Tiger nut tubers can be processed in different ways to obtain different products such as milk, flour, oil, cake, cream cheese, chocolate, biscuits, cookies, etc.

Tiger nut milk originated from Spain where it is known as *chufa de horchata,* but it is commonly called *kunnu aya* in Northern Nigeria (Bamishaiye & Bamishaiye, 2011). It is a healthy and rich source of nutrients such as carbohydrates, vegetable fat, protein, fibre, vitamins, minerals, energy and some digestive enzymes such as catalase, lipase and amylase (Adejuyitan, 2011). Tiger nut milk has been reported to contain more iron, magnesium and carbohydrate than cow milk. In addition, it has the advantage of not containing sodium, lactose sugar, casein protein, gluten, cholesterol and therefore ideal for people who are hypertensive or do not tolerate gluten or lactose and its derivatives present in cow milk (Belewu & Abodunrin, 2006). The nut is higher in oil content and the oil was implicated as a lauric acid grade oil which was non-acidic stable, non-drying and of very low unsaturated. The oil remained uniformly liquid at refrigeration temperature. This makes the oil suitable for salad making (Umerie and Enebeli, 1997).

There are no across board reports on the quality parameters of amino acids of black variety of tiger nut (*Cperus esculentus*). The present study examines the proximate composition, metabolizable energy, amino acid profiles, isoelectric point, predicted protein efficiency ratio and amino acid scores of black variety of raw and cooked *Cperus esculentus* tuber grown in north east Nigeria.

2 Materials and Methods

2.1 Collection of samples

The black variety of tiger nut (*Cperus esculentus*) tubers were purchased from a local market in Wukari, Taraba State, Nigeria. The tubers were thoroughly sorted to remove the stones, pebbles and bad ones before washing with tap water.

2.2 Preparation of samples

2.2.1 Raw tubers

Three hundred grams of the cleaned nuts were oven dried at 50^oC for 36 h to a moisture content of about 12%. The dried nuts were ground into powder with a food blender and sieved through 600 µm aperture size. The powdered sample was packed and sealed in polythene bags and kept at 4^oC prior to analyses.

2.2.2 Cooked tubers

The cleaned nuts (300 g) were also cooked for 1 h 15 min in distilled water at 100^oC. The cooked nuts were drained using a perforated basked, and then dried in an oven at 50^oC for 48 h. powdered sample was obtained as described earlier and stored at 4^oC prior to analyses.

2.3 Proximate analysis

The ash, moisture, crude fat, crude protein (N x 6.25), crude fibre and carbohydrate (by difference) were determined in accordance with the methods of AOAC (1995). All proximate analyses of the sample flour were carried out in triplicate and reported in percentage. All chemicals were of Analar grade.

2.4 Amino analysis

The amino acid analysis was by Ion Exchange Chromatography (IEC) (FAO/WHO, 1991) using the Technico Sequential Multisample (TSM) Amino Acid Analyzer (Technicon Instruments Corporation, New York). The period of analysis was 76 min for each sample. The gas flow rate was 0.50 $mLmin^{-1}$ at 60°C with reproducibility consistent within ±3%. The net height of each peak produced by the chart recorder of the TSM (each representing an amino acid) was measured and calculated. Amino acid values reported were the averages of two determinations. Nor–leucine was the internal standard. Tryptophan was not determined.

2.5 Determination of isoelectric point (pI), quality of dietary protein and predicted protein efficiency ratio (P–PER)

The predicted isoelectric point was evaluated according to Olaofe and Akintayo (2000):

$$\text{pIm} = \sum_{i=1}^{n=1} pI_i X_i$$

Where:
pIm = the isoelectric point of the mixture of amino acids;
pI_i = the isoelectric point of the i^{th} amino acids in the mixture;
X_i = the mass or mole fraction of the amino acids in the mixture.

The quality of dietary protein was measured by finding the ratio of available amino acids in the sample protein compared with the needs expressed as a ratio. Amino acid score (AAS) was then estimated by applying the FAO/WHO (1991) formula:

$$\text{AAS} = \frac{mg\ or\ amino\ acid\ in\ 1\ g\ of\ test\ protein}{mg\ of\ amino\ acid\ in\ reference\ protein}\ x\ \frac{100}{1}$$

The predicted protein efficiency ratio (P–PER) of the seed sample was calculated from their amino acid composition based on the equation developed by Alsmeyer *et al.* (1974) as stated thus; P–PER = –0.468 + 0.454 (Leu) –0.105 (Tyr).

2.6 Statistical analysis of the samples

The fatty acid values were obtained by multiplying crude fat value of each sample with a factor of 0.8 (i.e. crude fat x 0.8 = corresponding to fatty acids value (Paul and Southgate, 1978). The energy values were calculated by adding up the carbohydrate x 17kJ, crude protein x 17kJ and crude fat x 37kJ for each of the samples (Kilgore, 1987). Errors of three determinations were computed as standard deviation (SD) for the proximate composition. The grand mean, SD and coefficient variation (CV%) were also determined.

3 Results and Discussion

The mean proximate composition, energy values and calculated fatty acids of *Cyperus esculentus* are presented in Table 1. The moisture content values were 3.73 ± 0.38% (raw) and 4.66 ± 0.22% (cooked) though higher than those reported for kersting's groundnut (1.7 ± 0.12%) and cranberry bean (1.7 ± 0.51%) (Aremu *et al.,* 2006a) but comparable to those reported for *Luffa cylindrical* (5.8%) (Olaofe *et al.,* 2008) and fermented *Brachystegia eurycoma* (4.32%) (Aremu *et al.,* 2014). The high moisture content may make the *Cyperus esculentus* tuber highly susceptible to microbial attack. The ash content (2.38 – 2.57%) is comparable to the value of raw sample of pinto bean (2.5%) (Audu & Aremu, 2011) but much lower than the total ash obtained for *Tilapia quinnesis* (8.2%) (Aremu *et al.,* 2007c). The crude fat value (8.94 ± 0.11%) of the raw tuber did not qualify *Cyperus esculentus* as an oil–rich tuber when compared with soybean (22.8 – 23.5%) (Elias *et al.,* 1976; Salunkhe *et al.,* 1985) or other seeds such as pumpkin (Asiegbu, 1989; Fagbemi & Oshodi, 1991) and *Citrullus vulgaris* Schrad (47 – 51.1%) Olaofe *et al.,* 1994; Ogungbenle *et al.,* 2005; Ige *et al.,* 1984) grown in Nigeria. Crude protein values (9.25 – 12.09%) are very low when compared with those of protein–rich foods such as soyabean, cowpeas, kersting's groundnut, pigeon peas, bambara groundnut and some oil seeds (Ferrao *et al.,* 1987; Apata & Ologhobo, 1994; Olaofe *et al.,* 2006; Aremu *et al.,* 2006a). Crude fibre is a significant component in the body. It increases stool bulk, and decreases the time that waste materials spend in the gastrointestinal tracts. Crude fibre in the diet consists mostly of the plant polysaccharides that cannot be digested by human dietary enzymes such as cellulose, hemicelluloses and some materials that make up the cell wall (Southland, 1975). The fibre content values obtained in the raw (7.02 ± 0.66%) and cooked 4.52 ± 0.22%) samples of *Cyperus esculentus* are comparable with the values of crude fibre content obtained for some plant foods in the literature (Aremu *et al.,* 2012; Audu & Aremu, 2011). This suggests that black variety of *Cyperus esculentus* tuber would provide additional dietary fibre in the diet. The value of carbohydrate obtained in this study compares favourably with those reported for some varieties of cowpeas (Oke *et al.,* 1985; Aremu *et al.,* 2006a)), cranberry bean (Aremu *et al.,* 2010) and African yam bean (Adeyeye & Aye, 1998). The calculated fatty acids and metabolizable energy values for the raw *Cyperus esculentus* tuber were 7.15% and 1652.53 ± 4.41 kJ/100g, respectively. The fatty acids value in the present study suggests that the oils may be both edible and suitable for industrial purposes while that of the energy showed that the sample had an energy concentration more favourable than cereals (Paul & Southgate, 1978). Despite the effect of processing (cooking), the coefficient of variation (CV%) levels were relatively close ranging from 1.48 in calculated metabolizable energy to 21.66 in crude fibre.

Table 1: Proximate composition (%)[a] of tiger nut

Parameter	Raw	Cooked	Mean	SD	CV%
Moisture	3.73 ± 0.38	4.66 ± 0.22	4.20	0.74	17.54
Ash	2.57 ± 0.61	2.38 ± 0.22	2.48	0.10	3.84
Crude fat	8.94 ± 0.11	9.92 ± 0.23	9.43	0.49	5.20
Crude protein	12.09 ± 0.74	9.25 ± 0.95	10.67	1.42	13.31
Crude fibre	7.02 ± 0.66	4.52 ± 0.22	5.77	1.25	21.66
Carbohydrate[b]	65.66 ± 2.49	69.29 ± 1.38	67.48	1.82	2.69
Fatty acid[c]	7.15 ± 1.05	7.94 ± 2.35	7.55	0.40	5.23
Energy[d]	1652.53 ± 4.41	1702.22 ± 3.62	1677.38	24.85	1.48

[a]Mean values ± standard deviations of triplicate determinations
[b]Carbohydrate percent calculated as the (100 – total of other components)
[c]Calculated fatty acid (0.8 x crude fat)
[d]Calculated metabolizable energy (kJ/100g) (protein x 17 + fat x 37 + carbohydrate x 17)

The result of amino acid composition of the samples of *Cyperus esculentus* is shown in Table 2. Glutamic acid was the most concentrated (10.22 – 19.70 g/100g crude protein, cp) non–essential amino acid in the samples. The raw sample value obtained in this study is higher when compared with that of glutamic acid value (13.3 g/100g cp) obtained for *Prosopis Africana* flour (Aremu *et al.,* 2007a), *Anarcadium occidentale* protein (13.6 g/100g cp) (Aremu *et al.,* 2007b), *Luffa cylindrical* kernel (13.0 g/100g cp) (Olaofe *et al.,* 2007). Arginine constituted the highest single essential amino acid (EAA) in the raw sample (9.32 g/100g cp). Arg is an essential amino acid for children growth (Aremu *et al.,* 2006a). Phe with its sparring partner Tyr had concentrations of 5.06 and 3.18 g/100g cp, respectively. Tyr in this report is comparable with Tyr (3.2 g/100g cp) reported for *Anarcadium occidentale* (Aremu *et al.,* 2006b). Tryptophan concentrations could not be determined in the samples. Cys was found to be the least concentrated amino acid (1.06 – 1.32 g/100g cp) in all the samples. The calculated isoelectric point (pI) was 4.45 in the raw sample. This is useful in predicting the pI for protein in order to enhance a quick precipitation of protein isolate from biological samples (Olaofe & Akintayo, 2000). The predicted protein efficiency ratio (P–PER) is one of the quality parameters used for protein evaluation (FAO/WHO, 1991). The P–PER in this report for the raw sample (2.77) is higher than the reported P–PER values of some legumes flours/concentrates: *Phaseolus coccineus* (1.03) (Salunkhe & Kadam, 1989; Olaofe *et al.,* 2008; Aremu *et al.,* 2006c); however, it is in close agreement with that of pinto bean (Audu & Aremu, 2011). It can be said that the tuber of *Cyperus esculentus* under investigation could satisfy the FAO/WHO (1991) requirements.

Clinical biochemical and pathological observations in experiments conducted in humans and laboratory animals showed that high leucine in the diet impairs the metabolism of tryptophan and niacin and it is responsible for niacin deficiency in sorghum eaters (Ghafoorunisa & Narasinga, 1973). High leucine is also a factor contributing to the pellagragenic properties of maize (Belvady & Gopalem, 1969). These studies suggest that the leucine/isoleucine balance is more important than dietary excess of leucine alone. The Leu/Ile ratios in the samples (1.09 – 2.06) were low (Table 2). The processing method (cooking) reduced the concentrations of all the amino acids except Ala and Leu which were enhanced by 22.75 and 55.30%, respectively.

Table 2: Amino acid profiles of tiger nut (g/100g crude protein)

Amino acid	Raw	Cooked	Mean	SD	CV%
Lysine (Lys)[y]	6.24	4.11	5.18	1.07	20.56
Histidine (His)[y]	3.03	2.40	2.72	0.10	31.50
Arginine (Arg)[y]	9.32	5.52	7.42	1.90	25.61
Aspartic (Asp)	10.12	8.50	9.31	0.81	8.70
Threonine (Thr)[y]	4.09	3.04	3.57	0.53	14.71
Serine (Ser)	3.37	2.66	3.02	0.35	11.50
Glutamic acid (Glu)	19.70	10.22	14.96	4.74	31.68
Proline (Pro)	3.66	2.24	2.95	0.68	23.07
Glycine (Gly)	4.30	3.65	3.98	0.33	8.17
Alanine (Ala)	3.34	4.10	3.72	0.38	10.22
Cystine (Cys)	1.32	1.06	1.19	0.13	10.92
Valine (Val)[y]	4.20	3.65	3.93	0.28	7.00
Methionine (Met)[y]	1.85	1.15	1.50	0.35	23.33
Isoleucine (Ile)[y]	3.55	2.92	3.24	0.32	9.72
Leucine (Leu)[y]	3.87	6.01	4.94	1.07	21.66
Tyrosine (Tyr)[y]	3.18	2.70	2.94	0.24	8.16
Phenylalanine (Phe)[y]	5.06	3.96	4.51	0.55	12.20
Isoelectric point (pI)	4.45	3.79	4.12	0.33	8.01
P–PER	2.77	1.98	2.38	0.40	16.60
Leu/Ile	1.09	2.06	1.58	0.49	30.70

[y]Essential amino acid; **P–PER =** Predicted protein efficiency ratio

Transamination and deamination reactions might be responsible for the changes in the amino acid profiles of raw and cooked *Cyperus esculentus* tubres. Despite the effect of cooking method, the CV% levels were relatively close with hot spot at 31.68 in Glu whereas others ranged from 7.00 in Val to 31.50 in His.

Evaluation report on amino acids based on classification of raw and cookd *Cyperus esculentus* tuber is shown in Table 3 because the nutritive value of a protein depends primarily on its capacity to satisfy the needs for nitrogen and essential amino acids (EAA) (Oshodi *et al.,* 1993). Total amino acids (TAA), total essential amino acids (TEAA) with His and total sulphur amino acids (TSAA) of raw and cooked tubers were 90.20 and 67 67.89; 41.21 and 32.76; 3.17 and 2.21 g/100g cp, respectively. The concentrations of TAA, TEAA and TSAA of the cooked tubers were reduced by 24.73, 20.50 and 30.28%, respectively. The TSAA which ranged from 2.21 – 3.17 g/100g cp are lower than the 5.8 g/100g cp recommended for the infants (FAO/WHO/UNU, 1985). The values of EArAA (3.96 – 5.06 g/100g cp) are also lower than the ideal range suggested for infant protein (6.8 – 11.8 g/100g cp) (FAO/WHO/UNU, 1985). Table 3 also shows TAAA values which were found to be greather than the TBAA, indicating that the protein is probale acidic in nature (Aremu *et al.,* 2012). The percentage ratios of TEAA (with His) to TAA in the samples were 45.69 and 48.25% for the raw and cooked samples, respectively. These values are comparable to that of egg (50%) (FAO/WHO, 1991), *Vigna subterranea* L. Verdc concentrate (49.7% (Aremu *et al.,* 2008), scarlet runner bean (48.31%) (Aremu *et al.,* 2006) and some selected spices (47.30 – 49.95%) (Aremu *et al.,* 2011). However, the TEAA contents (%) in this report are well above the 39% considered to be adequate for ideal protein food for infants, 26% for children and 11% for adults (FAO/WHO/UNU, 1985).

Table 3: Concentrations of essential, non–essential, acid, neutral, sulphur, aromatic, etc. (g/100g crude protein) of tiger nut

Amino acid description	Raw	Cooked
Total amino acid (TAA)	90.20	67.89
Total non–essential amino acid (TNAA)	48.99	35.13
% TNAA	54.31	51.75
Total essential amino acid (TEAA)		
With histidine	41.21	32.76
Without histidine	38.18	30.36
% TEAA		
With histidine	45.69	48.25
Without histidine	42.33	44.72
Essential alphatic amino acid (EAAA)	15.71	15.62
Essential aromatic amino acid (EArAA)	5.06	3.96
Total neutral amino acid (TNAA)	26.72	26.03
% TNAA	29.62	38.34
Total acidic amino acid (TAAA)	29.82	18.72
% TAAA	33.06	27.57
Total Basic amino acid (TBAA)	18.59	12.03
% TBAA	20.61	17.72
Total sulphur amino acid (TSAA)	3.17	2.21
% cystine in TSAA	41.64	47.96

The EAA scores of the raw and processed samples based on the provisional amino acid scoring pattern (FAO/WHO, 1991) are displayed in Table 4. With the exception of Lys, Phe + Tyr and Thr in the raw tuber of *Cyperus esculentus*, and only Phe + Tyr in the cooked tuber, the contents of essential amino acids are greater than FAO/WHO (1991) recommendation. The reason for these changes is that early in the cooking process there is loss of toxic activity, particularly of the pertinacious toxins, trypsin inhibitors and haemagglutinion (Liener, 1980). As heating proceeds, protein quality increases to a maximum before declining again with continued heating; thus, reduction is likely to be related to increasing Mallard browning causing lysine to be rendered unavailable (Aremu *et al.,* 2010; Audu and Aremu, 2011).

Table 4: Amino acid scores of tiger nut based on FAO/WHO standards

EAA	PAAESP g/100g protein	Raw		Cooked	
		EAAC	AAS	EAAC	AAS
Ile	4.0	3.55	0.89	2.92	0.73
Leu	7.0	3.87	0.55	6.01	0.86
Lys	5.5	6.24	1.13	4.11	0.75
Met + Cys (TSAA)	3.5	3.17	0.91	2.21	0.63
Phe + Tyr	6.0	8.24	1.37	6.66	1.11
Thr	4.0	4.09	1.02	3.04	0.76
Try	1.0	nd	na	Nd	na
Val	5.0	4.20	0.84	3.65	0.73
Total	**36.0**	**33.36**	**6.71**	**28.60**	**5.57**

nd = not determined; **na =** not applicable

Therefore, for a healthy diet based on raw and cooked samples of *Cyperus esculentus*, the samples will require supplementation with essential amino acids such as Ile, Leu, TSAA and Val for the raw; Ile, Leu, Lys, TSAA, Thr and Val for the cooked. Try was not determined. It has been reported that the essential amino acids most often acting in a limiting capacity are Met, Cys, Lys and Try. However, Leu was the limiting AA for raw while Met + Cys (TSAA) served as first LAA for the cooked sample.

4 Conclusion

The studies conducted on the proximate and amino acid compositions of raw and cooked black variety of tiger nut (*Cyperus esculentus*) flour revealed that crude fat and crude protein contents are far below that of oil and protein rich foods, respectively such as soybean and groundnut. Though the samples under investigation contained nutritionally useful quantities of most of the essential amino acids but dietary formual based on the tubers may require some essential amino acids supplementation such as Ile, Leu, TSAA and Val.

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