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PROXIMATE, AMINO AC[illegible] IPOSITION OF THE SEED OF *Cochlospermum tinctorium* A.RICH

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ABSTRACT

The proximate composition, amino acid profile and mineral constituents of the seed of *Cochlospermum tinctorium* A.Rich were evaluated. The proximate analysis revealed that the seeds contained 9.52 ± 0.07% moisture, 4.49 ± 0.04% ash, 15.92 ± 0.05 % crude protein , 33.27 ± 0.12% crude lipid, 6.01 ± 0.29% crude fiber and 40.22 ± 0.03% carbohydrates . The seeds also have high energy value (492. 15 ± 0.15 Kcal/100g dry weight). Amino acid analysis showed the presence of seventeen amino acids. Among the essential amino acids., leucine, phenylalanine and valine were predominant while glutamic acid,alanine and arginine were predominant among the non-essential amino acids. Mineral composition indicated the following results; K(21.65 ± 0.03 mg/100g), Na(22.79 ± 0.12 mg/100g), Ca(28.09 ± 0.07 mg/100), P(2.33 ± 0.02mg/100g), Mg(18.50 ± 0.01mg/100g), Cu(2.65 ± 0.03 mg/100g), Fe(12.53 ± 0.03 mg/100g), Mn(3.05 ± 0.02mg/100g) and Zn(1.06 ± 0.12 mg/100g). The mineral nutrient density, which is the index of nutritional quality was high (>100%) for Copper, Iron and Manganese, but low (<100%) for Potassium, Sodium, Calcium, Phosphorus, Magnesium and Zinc. This confirms that the seeds of *Cochlosperm tinctorium* A.Rich are a rich source of Copper, Iron and Manganese.

Keywords: *Cochlospermum tinctorium, Proximate, Minerals, Amino acids, Nutrient density.*

INTRODUCTION

Researchers, governments and other organizations charged with food and nutrition show great concern on the nutritional status of general populace more especially children, pregnant and lactating mothers habiting the developing countries (Andersen *et al., 2003;* Senna *et al., 2005).* In these countries, natural disasters, bad economic polices, political instability, population explosion, high price of food commodities, poor implementation of agricultural policies and restrictions in food importation are the major factors that contribute to the burden of inadequate food intake among average people (Adeboye and Philips, 2006). In these regions, starch-based foods are the main staples, which supply both energy and protein requirement. Thus, protein deficiency prevails among the population as recognized by food and Agricultural organization, FAO (Ladeji *et al.,* 1995). *Cochlospermum tinctorium* A. Rich with common name as Rose Imperial is an underutilized plant that belongs to the family *Cochlospermaceae.* In the northern part of Nigeria, it is known by various names such as Balagande and kyamba (Hausa) and Gombara (Nupe). It is also known as N'Tiribara in Mali. It is a shrub that can grow up to 10m high and the bark is used as rope. Leaves are alternate, palmately lobed with stipulates. Fruits are elongated 3-5 valved capsule containing seeds that are embedded in cotton foam. The seeds are beans shaped with brown to black colour. It contains oily endosperm with broad cotyledon (Abdullahi *et al.,* 2003).

This plant occurs in dry savanna, preferring devastated scrublands, rocky and annually burnt regions, at 300-1500m altitude. The plant has been reported to be used as traditional medicine in some Africa countries like Nigeria, Ivory coast, Ghana, Senegal and Mali (*Cochlospermum*, 2009). Despite the use of this plant for such purposes, the seed of *Cochlospermum tinctorium* A.Rich has not been given due research attention in terms of its nutritional content. Thus, the aim of this work is to bridge up the gap by providing information on the proximate, amino acids and mineral compositions of the seed of *Cochlospermum tinctorium* A.Rich with the hope that the information would be used in nutritional policy of the country.

MATERIALS AND METHOD

Sample collection and sample treatment: The sample of *Cochlospermum tinctorium* A.Rich used in this study was collected from the bushes behind New York area of Bosso, Bosso local

government area of Niger state, Nigeria. The chemicals used were manufactured by M&B and BDH Chemicals of England.

Prior to analysis, the seed pod was separated from the branches of the plant after harvesting. The pod was broken and the seeds separated from the woolly portion in the pod. The seeds were washed with ordinary water then rinsed with distilled water and dried at room temperature. Thereafter the seeds were oven dried at 55°c to constant weight. The dried seeds were then ground in porcelain mortar, sieved through 2 mm mesh sieve and stored in polyethylene bags till when needed (Umar *et al.,* 2007). The powdered sample was used for all the analysis. Moisture content was however, evaluated using fresh seed.

Proximate analysis: The moisture content of the seeds were determined by drying 5g of the seeds(in triplicate) in a Gallenkamp oven at 105°c until constant weight was attained (AOAC,1990). Ash content was determined according to the method described by Idris *et al.,*(2009) which involved dry ashing in lenton muffle furnace at 600°c until grayish white ash was obtained. Crude protein content was calculated by multiplying the value obtained from kjeldahl's nitrogen by a protein factor of 6.25 and nitrogen content was determined using titration method described by AOAC,1990 . Crude lipid was quantified by the method describe by AOAC(1990) using the soxhlet apparatus and n-hexane as a solvent. Crude fiber was determined by the method of AOAC,1990 which involved defatting 1g of the sample and boiled under reflux using 200 cm^3 of 1.25% H_2SO_4. Available carbohydrates were calculated by difference i.e., total sum of Crude protein, Crude lipid, Crude fibre and Ash deducted from 100% dry matter (AOAC,1990).

The sample calorific value was estimated using the following equation:

Calorific value (kcal/100g) = (Crude lipid x 9) + (Crude protein x 2) + (carbohydrate x 4) (Hassan *et al.,* 2008).

Amino acid analysis: 3.0g of ground sample was extracted with petroleum ether (40 – 60 ^{0}C) using soxhlet extractor for six hours (Cooper *et al*, 2000). 30 mg of the defatted samples was weighed into a glass ampoule and 7.0 cm^3 of 6.0 mol/dm^3 hydrochloric acid was added. Oxygen was expelled by passing nitrogen into the ampoule (to avoid possible oxidation of some amino acids during hydrolysis). The ampoule was sealed with Bunsen flame and put in an oven preset at 105 ^{0}C for 22 hours, after which it was allowed to cool, broken at the tip and the content filtered. The filtrate was evaporated to dryness at 40 ^{0}C under vacuum in a rotary evaporator. The residue was dissolved with 5.0 cm^3 of acetate buffer (pH 2.0), stored in plastic bottle and kept in deep freezer for 24 hours.

Five to ten microlitres of the hydrolysate was loaded on the Technicon Sequential Multi-Sample (TSM) amino acid analyzer (DNA 0209) made by Technicon (Ireland) Ltd. This was dispensed into the cartridge of the analyzer and the analysis lasted for 76 minutes (Sparkman *et al*, 1958).

Sample preparation for mineral analysis: Six (6) gram of the powdered sample was weighed into a crucible and gently heated over a Bunsen burner until it charred. The charred sample with the crucible was transferred into a lento muffle furnace at about 600°C and content ashed until grayish white ash was obtained. It was cooled first at room temperature and then in a desiccator. 5 cm^3 of conc. HCl was added and heated for 5 minutes on a hot plate in a fume cupboard. The mixture was then transfered into a beaker and the crucible washed several times with distilled water. The mixture was made up to 40 cm^3 and boiled for 10 minutes over a Bunsen burner. This mixture was then cooled, filtered and rinsed into a 100 cm^3 volumetric flask made up to the 100 cm^3 mark with distilled water (Idris *et al.,* 2009). The solution prepared in triplicates.

Mineral quantification: Sodium (Na) and Potassium (K) were analyzed by flame atomic emission spectrophotometer using NaCl and KCl used to prepare the standards. Phosphorus (P) was determined with Jenway 6100 spectrophotometer at 420nm using Vanadium Phosphomolybdate (Vanadate) Colorimetric method with KH_2PO_4 as the standard (Idris *et al.,* 2009). The concentrations of Calcium(Ca), Magnesium(Mg), Copper(Cu), Iron(Fe), Manganese(Mn) and Zinc(Zn) in the solutions were determined using computer controlled Atomic Absorption spectrometer AAS 969(Bulk scientific,MODEL 210VGP) (AOAC,1990).

Nutrient density (ND): The sample nutrient densities were calculated using the equation reported in Hassan *et al.*(2008), which is:

$$ND(\%) = \frac{[Np/Ep]}{[Nr/Er]} \times 100$$

Where Np = nutrient concentration (mineral element in the food),
Ep = energy supplied by food,
Nr = recommended daily intakes of nutrient and
Er = recommended energy intake (3000 Kcal/day for an adult male given by WHO/FAO (cole 1980).

$$\% \text{ Amino acid} = \frac{\text{Amino acid in the sample}}{\text{Standard reference pattern}} \times 100$$ (Hassan and Umar, 2008).

$$\text{Contribution to RDA (\%)} = \frac{\text{concentration of the element}}{\text{RDA}} \times 100$$

RDA = recommended dietary allowance (NRC,1989)

Data Analysis: Data were generated in triplicates and the mean standard deviation determined according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

Proximate composition: The proximate composition of the seed of *Cochlospermum tinctorium* A.Rich was presented in table 1. The moisture content of the seed of *Cochlospermum tinctorium* A.Rich was found to be 9.50 ± 0.07% wet. This value is low compared to the 42.51± 0.74% wet weight reported for the seed of Sugar Apple *(Annona squamosa)* (Hassan *et al.,* 2008) where as the ash content of the seed (4.49 ± 0.04%) was high compared to 2.78 ± 0.02% indicated in the seed of Sugar Apple (*Annona squamosa*) (Hassan *et al.,* 2008). The ash content of a plant material is an index of total mineral content. This finding indicated that the seed of *Cochlospermum tinctorium* A.Rich may contain nutritionally important mineral elements.

Table1: Proximate compoxition of the seed of *Cochlospermum tinctorium* A.Rich.

Parameter	Concentration (%Dry weight)
Moisture content[a]	9.52± 0.07
Ash	4.49 ± 0.04
Crude protein	15.92 ± 0.05
Crude lipid	33.27 ± 0.12
Crude fiber	6.01 ± 0.29
Available carbohydrate	40.22 ± 0.03
Colorific (Energy) value (Kcal/100g)	492.15 ± 0.15

The data are mean values ± standard deviation (SD) of triplicate determination.
[a]Value expressed as % wet weight.

The crude protein content of the seed of *Cochlospermum tinctorium* A.Rich (15.92 ± 0.05% dry matter) was high compared to 2.4% reported for the seed of *Annona muricata* (Onimawo, 2002) and 4.43 ± 0.15% obtained in the seed of *Annona squamosa* (Hassa *et al.,* 2008). The protein content of this seed shows that the plant can make significant contribution to dietary intake.

The Crude lipid content of the seed (33.27 ± 0.12%) was relatively high placing the seed in the group of oil seeds. The lipid content of the seed was higher than that revealed in the seed of *Annona muricata* (20.5%) (Onimawo, 2002), but lower than the 48.2% for benni seed (Egbekun and Ehieze, 1997).

The Crude fiber content was found to be 6.01 ± 0.29% dry matter. This value is low compared to 8% reported for the seed of *Annona muricata* (Onimawo, 2002) and 36.33 ± 1.17% indicated in the seed of Sugar Apple (*Annona squamosa*) (Hassa *et al.*, 2008). Fiber can be of benefit in human diet on the muscles of large and small intestive (Lanza and Butrum, 1986). This indicates that the seed could be a source of bulk roughage.

The available carbohydrate content of the sample (40.22 ± 0.03%) was high compared to the range of 32.93% to 34.38% reported as available carbohydrate content of African locust been seeds (Lockeett *et al.*, 2000., Hassan and Umar, 2004) and 12.45 ± 2.76% indicated in the seed of *Annona squamosa* (Hassan *et al.*, 2008). Carbohydrates provide the body with a source of fuel and energy that is required to carry out daily activities and exercise. Carbohydrates are also important for the correct functioning of vital physiological systems of the body.

The Calorific (energy) value for the seed of *Cochlospermum tinctorium* A.Rich was found to be 492.15 ± 0.15 Kcal/100g dry matter. This value is high compared to 463.55 ± 4.50 Kcal/100g obtained for the seed of *Annona squamosa* (Hassan *et al.*, 2008). The high energy value of the sample could be due to relatively higher crude lipid content (Hassan and Umar,2008).

Amino acid profile: The amino acids content of the seed of *Cochlospermum tinctorium* A.Rich is presented in Table 2. Twenty standard amino acids are commonly found as components of proteins. However, in this study only seventeen amino acids were detected possibly due to conversion of the amide glutamine and asparagine to their corresponding amino acids and the complete destruction of tryptophan during acid hydrolysis. The results indicated that the percentage of non-essential amino acids was higher than essential amino acids, which were 59.95% and 40.05% respectively (Table 2), when compared to the total amino acids analyzed in the sample. Among the essential amino acids., leucine, phenylalanine and valine were predominant while glutamic acid,alanine and arginine were predominant among the non-essential amino acid.

Table 2: Amino acid composition of the seed of *Cochlospermum tinctorium* A.Rich

Amino acid	Abbreviation	g/100g protein
Isoleucine	Ile*	3.14
Leucine	Leu*	6.26
Lysine	Lys*	3.17
Methionine	Met*	1.15
Cysteine	Cys	0.79
Phenylalanine	Phe*	4.06
Tyrosine	Tyr	3.22
Threonine	Thr*	2.30
Valine	Val*	3.72
Histidine	His	2.13
Alanine	Ala	4.48
Arginine	Arg	4.08
Aspartic acid	Asp	2.30
Glutamic acid	Glu	9.03
Glycine	Gly	3.60
Proline	Pro	2.97
Serine	Ser	3.02
Essential amino acids	23.8 (40.05%)	
Non-essential amino acids	35.62 (59.95%)	

*Essential amino acids.
Values in paratheses are their corresponding percentages when compared to the amino acids analysed.

To evaluate the nutritional quality of the seed of *Cochlospermum tinctorium* A.Rich, the percentages of the essential amino acids in the samples were tabulated against those of reference standard amino acid profile established for both adults and children by WHO/FAO/UNU (1985) in Table 3. This indicate that all essential amino acids exceeded the reference value for adults, Leucine, Lysine, Threonine and total Sulphur containing amino acids are below the standard requirement for children.

Table 3: Amino acid score for the seed of *Cochlospermum tinctorium* A.Rich

Amino acid	Concentration g/100g protein	WHO/FAO/UNU standard reference pattern Children (g/100g protein)	Adult (g/100g protein)	% Amino acid Children	Adult
Ile	3.14	2.8	1.3	112	262
Leu	6.26	6.6	1.9	95	329
Lys	3.17	5.8	1.6	55	198
Total Sulphur AA	1.94	2.5	1.7	78	114
Total Aromatic AA	7.28	6.3	1.9	116	383
Thr	2.30	3.4	0.9	68	256
Val	3.72	3.5	1.3	106	286
His	2.13	1.9	1.6	112	133

Total Sulphur amino acid = Met + Cys
Total Aromatic amino acid = Phe + Tyr
AA = amino acid

Mineral content: The mineral composition of the seed of *Cochlospermum tinctorium* A.Rich was presented in Table 4. The Potassium content in the seeds was found to be 21.65 ± 0.03 mg/100g dry matter. This value is low compared to 22.00 ± 0.58 mg/100g reported for the seed of Sugar Apple (*Annona squamosa)* (Hassan *et al.,* 2008). The Sodium content (22.79 ± 0.12 mg/100g) was low compare to 30.00 ± 1.15 mg/100g reported for the seed of *Annona squamosa* (Hassan *et al.,* 2008). Sodium is associated with potassium in the body in maintaining proper acid – base balance and nerve transmissions (Idris *et al.,*2009).

Table 4: Mineral composition of the seed of *Cochlospermum tinctorium* A.Rich

Mineral elements	Concentration (mg/100g dry matter)
K	21.65 ± 0.03
Na	22.79 ± 0.12
Ca	28.09 ± 0.07
P	2.33 ± 0.02
Mg	18.50 ± 0.01
Cu	2.65 ± 0.03
Fe	12.53 ± 0.03
Mn	3.05 ± 0.02
Zn	1.06 ± 0.12
Cal/P	12.06

The data are mean value ± standard deviation (SD) of three replicates.

Calcium content was found to be 28.09 ± 0.07 mg/100g dry matter. This value is low compared to 650 ± 4.36 mg/100g indicated in *Annona squamosa* seed (Hassan *et al.,* 2008). Calcium is important for bone and teeth formation, the transmission of nerve impulses, for muscle contraction and blood clothing (Idris *et al.,* 2009).

The concentration of phosphorus in the seed of *Cochlospermum tinctorium* A.Rich (2.33 ± 0.02 mg/100g) was low compared to 21.00 ± 0.05 mg/100g showed in *Annona squamosa* seed (Hassan *et al.,* 2008). Phosphorus is important for healthy bones and teeth. It is important for the utilization of nutrient in the body and in order to release energy inside the cells (Idris *et al.,* 2009). According to Guil - Guerrero *et al.* (1998), for good calcium and phosphorus intestinal utilization, Ca/P ratio must be close to unity. The seed of *Cochlospermum tinctorium* A.Rich had a high ratio (12.06), indicating that diet based on this seed required to be supplemented in favour of phosphorus.

Magnesium is an important mineral in connection with circulatory diseases and calcium metabolism in bone (Ishida *et al.,* 2000). The magnesium content of the seed of *Cochlospermum tinctorium* A.Rich (18.50 ± 0.01 mg/100g) appeared to be low when compared to the 50 .00 ± 1.53 mg/100g in the seed of *Annona aquamosa* (Hassan *et al.,* 2008).

Copper is known for the role its plays in haemoglobin formation and also contribution to iron and energy metabolism (Cabrera *et al.,* 1996; Adeyeye, 2002). The concentration of copper in the seed of *Cochlospermum tinctorium* A.Rich was found to be 2.65 ± 0.03 mg/100g. The value is higher than the 0.03 ± 0.01 mg/100g reported for seed of *Annona squamosa* (Hassan *et al.,* 2008). From the result, the seed of *Cochlospermum tinctorium* A.Rich have good amount of copper relative to its recommended dietary allowance (RDA) set by the United States of America National Research Council, NRC (1989), which are 1.5 – 3 mg/day for adult male and female, pregnant and lactating mothers and 1 – 3 mg/day for children (7 – 10 years) respectively.

Iron is required for haemoglobin formation and its deficiency leads to anaemia (Turan *et al.,* 2003). The assay indicated that the seed of *Cochlospermum tinctorium* A.Rich contain 12.53± 0.003 mg/100g of Iron. This value is high compared to 2.05 ± 0.02 mg/100g in *Annona squamosa* seed (Hassan *et al.,* 2008). When comparing to the RDA for iron which are 10 mg/day for adult male and children (7 – 10 years), 13 mg/day for pregnant and lactating mothers and 15 mg/day for adult female (NRC,1989), it can be concluded that the seeds of *Cochlospermum tinctorium* A.Rich are good source of iron.

Manganese is a mineral element that is nutritionally essential. The manganese content of the seed of *Cochlospermum tinctorium* A.Rich is 3.05 ± 0.02 mg/100g. The RDA for manganese are 2 – 5 mg/day for adult male and female, pregnant and lactating mothers and 2 -3 mg/day for children (7 – 10 years) (NRC,1989). Based on the RDA, it is clearly indicated that the seeds of *Cochlospermum tinctorium* A.Rich are good sources of manganese.

Zinc is known to play a role in gene expression, regulation of cellular growth and it also participates as a co – factor of enzymes responsible for carbohydrates, protein and nucleic acid metabolism (Camara and Amaro, 2003). The concentration of this element was found to be 1.06 ± 0.12 mg/100g which is low compared to 2.05 ± 0.02 mg/100g in the seed of *Annona squamosa* (Hassan *et al.,* 2008).This value is low compared to the RDA for Zinc which are 10 mg/day for children (7-10 years), 12 mg/day for adult female, 15 mg/day for adult male and 19 mg/day for pregnant and lactating mothers (NRC,1989).

The contribution of the seed of *Cochlospermum tinctorium* A.Rich to the dietary intake of essential elements was evaluated as described by Hassan *et al.,* (2005) and presented in Table 5. The seeds were rich sources of Copper, Iron and Manganese, moderate source of Zinc and poor source of Potassium, Sodium, Calcium, Phosphorus and Magnesium when compared with their respective recommended dietary allowances. This indicated that the seeds supplement other dietary sources of Copper, Iron, Manganese and Zinc.

Table 5: Contribution to the dietary intake and nutrient density of some essential mineral elements by the seed of *Cochlospermum tinctorium* A.Rich.

Minerals	RDA(mg)	Contribution to RDA(%)	ND%
K	2000	1	7
Na	500	5	28
Ca	1200	2	14
P	1200	0.2	1
Mg	350	5	32
Cu	1.5 – 3	88 – 177	538 – 1077
Fe	10 – 15	84 – 125	509 – 764
Mn	2 – 5	61 – 153	372 – 930
Zn	12 – 19	21 – 9	34 - 54

RDA = recommended dietary allowance (NRC, 1989).
ND = Nutrient density.

The nutrient density is the index of nutritional quality used to evaluate the nutritional significance of minerals elements (Hassan *et al.,*2005).Food materials with nutrient density of 100% supply the nutrient needed in the same proportion as the caloric needed. ND of 100% or more indicates that the food material, if consumed in sufficient quantities, contributes substantially to the intake of that particular element (Amaro – Lopez *et al.,* 1998). Those with ND less than 100% will not provide proportionate amount of the nutrient. For all the elements analysed except for Copper,

Iron and Manganese, the percentage nutrient density of other elements were lower than 100% and this results as shown in Table 5, confirmed that the seed of *Cochlospermum tinctorium* A.Rich is a rich source of Copper, Iron Manganese.

CONCLUSION

The results of the nutritional analysis show that the seeds of *Cochlospermum tinctorium* A.Rich are good sources of plant Protein, Crude lipid, Carbohydrate and Energy. The results also revealed that the seeds are good source of essential and non – essential amino acids. The seeds are good source of mineral elements such as Copper, Iron and Manganese, Moderate source of Zinc, which meet the recommended daily allowances. Their nutrient densities suggest that if consumed in sufficient amount, could contribute to the intake of the tested element. Based on these findings, the seed of *Cochlospermum tinctorium* A.Rich can be recommended as a good source of nutrients to supplements othe major sources.

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