

## PROXIMATE COMPOSITIONS AND ANTIOXIDANT MINERAL LEVELS OF CHIA SEED AND TIGER NUT PROCURED FROM NNEWI, ANAMBRA STATE

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### ABSTRACT

*Salvia hispanica* (Chia Seed) also known as chia seed, is an annual herbaceous plant, originally from Southern Mexico. Chia seeds have become one of the world's most recognizable foods based on their nutritional properties and medicinal values. *Cyperus esculentus* L. (Tiger Nut) is a valuable food for nourishment with significant health benefits, they are rich in vitamins and minerals. This study was aimed at evaluating the proximate compositions and antioxidant mineral levels of chia seed and tiger nut procured from Nnewi, Anambra State were evaluated using standard methods. Proximate composition of *Salvia hispanica* and *Cyperus esculentus* were determined according to AOAC official method. Elemental analysis was conducted using Agilent FS240AA Atomic Absorption Spectrophotometer. Data obtained from the experiments were analyzed using the Statistical Package for Social Sciences (SPSS) version 26. Level of significance was set at  $p < 0.05$ . Results of proximate composition of chia seed and tiger nut seed samples used in this study revealed that chia seed and tiger nut samples used contain high percentage of carbohydrate and fat. It also revealed that chia seed had significantly higher ( $P < 0.05$ ) percentage of protein and fat than tiger nut. The result also revealed that chia seed contained significantly higher ( $P < 0.05$ ) concentrations of iron than tiger nuts, suggesting its usefulness in boosting blood parameters. Finally, the results of this study revealed that chia seed and tiger nut possess good quantity of antioxidant minerals and high percentage of carbohydrates and fats and therefore could be useful in energy generation and in the management of oxidative stress.

**Key words:** Antioxidant minerals, Proximate composition, Chia Seeds and Tiger nut.



## INTRODUCTION

### Chia Seed (*Salvia hispanica* L.)

Chia seeds, derived from *Salvia hispanica* L., are small edible seeds that have gained international recognition as a functional food due to their high content of omega-3 fatty acids, protein, and dietary fiber. Taxonomically, chia belongs to the Lamiaceae family, commonly known as the mint family, and is native to southern Mexico and Guatemala (Silva *et al.*, 2022). It is a flowering herbaceous plant with a short-day photoperiod and a growing interest due to its agronomic adaptability and nutritional value (Jamboonsri *et al.*, 2020).

The classification of chia has been supported by morphological, botanical, and molecular analyses. *Salvia hispanica* L. is classified within the order Lamiales, family Lamiaceae, and genus *Salvia*, which includes more than 900 species worldwide (Souza *et al.*, 2021). Modern molecular studies using DNA barcoding and simple sequence repeats (SSR markers) have been employed to distinguish chia from closely related species and to confirm its genetic stability (Silva *et al.*, 2022). The consistent seed morphology, coupled with molecular data, provides a solid framework for its classification.

Chia seed cultivation is expanding globally due to its ability to adapt to various climates and soils. Originally grown in Mesoamerica, it is now cultivated in South American countries like Argentina, Bolivia, and Peru, and has expanded to Australia and some regions of North America (Martínez-Cruz *et al.*, 2020). The plant thrives in subtropical and temperate regions with well-

drained soils, preferring a pH of 6.0 to 8.5 and elevations between 500 and 2,000 meters above sea level (Porrás-Loaiza *et al.*, 2023). It is sown during the spring when soil temperatures reach optimal levels for germination and can be grown under rainfed or irrigated conditions depending on the region.

Agronomic practices for chia cultivation include direct seeding at a depth of about 1–2 cm and row spacing of 60 to 80 cm to ensure adequate sunlight and air circulation. Chia is considered a low-input crop; it requires minimal fertilization and is relatively pest-resistant, which makes it suitable for organic farming systems (Souza *et al.*, 2021). However, weed control during the early stages is essential, as young chia plants are less competitive until they reach maturity. Crop rotation and minimal tillage are recommended to maintain soil fertility and reduce disease risk (Ramírez-Moreno *et al.*, 2021).

The harvesting of chia seeds occurs when the plant reaches physiological maturity, typically between 90 and 120 days after sowing. The plant is ready for harvest when its flowers dry and the seed capsules turn brown or gray, indicating full seed maturity (Porrás-Loaiza *et al.*, 2023). Manual harvesting is still common in traditional farming regions; however, mechanical harvesting is increasingly adopted in large-scale operations for efficiency. After harvesting, the seeds are separated using threshing and sieving techniques, followed by cleaning and drying processes to reduce moisture levels and prevent fungal contamination.

Proper post-harvest handling is critical to maintain chia seed quality. After separation from plant

material, seeds are dried to a moisture content of less than 10%, which helps extend their shelf life and reduce the risk of spoilage (Ramírez-Moreno *et al.*, 2021). They are then stored in cool, dry conditions, ideally in airtight containers to preserve their nutritional properties. Mechanical cleaners and gravity separators are often used to remove impurities such as stones, dust, and broken seeds.

Identification of chia seeds involves both morphological and biochemical methods. Morphologically, chia seeds are small, oval, and typically measure around 2 mm in diameter. They exhibit a mottled appearance with colors ranging from black and brown to white and gray, depending on the variety (Silva *et al.*, 2022). Biochemically, chia seeds are rich in  $\alpha$ -linolenic acid, dietary fiber, antioxidants, and proteins, which serve as biochemical markers for seed verification and quality assurance (Souza *et al.*, 2021). These traits are also used in authentication to distinguish genuine *Salvia hispanica* seeds from adulterants or similar-looking seeds of lower nutritional value.

Recent advances in molecular biology have enhanced the identification process through the application of DNA-based tools. Techniques such as SSR markers and next-generation sequencing are used to study the genetic diversity of chia, identify superior cultivars, and trace geographic origins (Jamboonsri *et al.*, 2020). These tools are critical for conservation programs, breeding efforts, and the protection of genetic resources. Additionally, researchers have started using metabolomic profiling to identify distinct

biochemical compounds that help differentiate chia seeds from other *Salvia* species.

### **Taxonomic Classification of Chia Seed (*Salvia hispanica* L.)**

<b>Kingdom:</b>	Plantae
<b>Subkingdom:</b>	Tracheobionta (Vascular plants)
<b>Superdivision:</b>	Spermatophyta (Seed plants)
<b>Division:</b>	Magnoliophyta (Angiosperms / Flowering plants)
<b>Class:</b>	Magnoliopsida (Dicotyledons)
<b>Subclass:</b>	Asteridae
<b>Order:</b>	Lamiales
<b>Family:</b>	Lamiaceae (Mint family)
<b>Genus:</b>	Salvia
<b>Species:</b>	Salvia hispanica L.



**Plate 1:** Chia seed

### **Tiger Nut: Cultivation, Identification, and Classification**

Tiger nut (*Cyperus esculentus*) is a perennial plant belonging to the sedge family (Cyperaceae), widely cultivated for its nutritional and medicinal benefits. It is commonly known as yellow nutsedge, chufa, or earth almond, and is native to Africa, Southern Europe, and parts of Asia (*Cyperus esculentus* - Wikipedia, 2023). The plant produces small, tuberous structures that are rich in fiber, healthy fats, and essential minerals, making it a valuable functional food (Parker *et al.*, 2018).

## Identification of Tiger nut

Tiger nut plants grow up to 90 cm (3 feet) tall, with triangular stems and slender leaves measuring 3–10 mm wide (*Cyperus esculentus* - Wikipedia, 2023). The plant produces distinctive spikelets, which contain flat, oval seeds surrounded by four hanging bracts positioned at 90-degree angles (Teoh *et al.*, 2018). The tubers, which are small, round, and hard, vary in color from yellow to brown or black, depending on the variety (Marcinek & Krejpcio, 2019).

## Classification of Tiger nut

Tiger nut belongs to the kingdom Plantae, order Poales, and family Cyperaceae. Its binomial name is *Cyperus esculentus* L., and it is classified as a monocotyledonous angiosperm (*Cyperus esculentus* - Wikipedia, 2023). The species is hermaphroditic, meaning it has both male and female reproductive organs, and is primarily pollinated by wind (PFAF Plant Database, 2023).



Plate 2: Tiger nut

## MATERIALS AND METHODS

**Equipment:** Standard equipment and materials were used in this study and were procured from the reputable manufacturers and their major distributors.

**Reagents:** All the reagents used in this study were of analytical grade and were procured from the manufacturers and their major distributors.

### Collection and Identification of Plant Materials

*Salvia hispanica* seeds used for this study was procured from Roban Shopping Mall, Nnewi, Anambra State while dried *Cyperus esculentus* was procured from Nkwo market, Nnewi. Both samples were identified by a taxonomist.

### Preparation of ethanol extract of the samples

The seeds were washed and air-dried at room temperature. The dried seeds were pulverized into powder using Corona manual grinding machine. Then 1 kg of the ground seed powder of the samples were soaked in 5 L of 80% ethanol for 24 h for complete extraction. The ethanol extraction were sieved using a muslin cloth and filtered using Whatman number 1(125 mm) filter paper. The filtrate were evaporated to dryness using a rotary evaporator. The extracts were stoppered in a universal bottle and preserved in the refrigerator for use.

### Proximate Analysis

This was done in accordance with Official Method of Analysis of AOAC (1990).

### Determination of Moisture Content

A 5.0g of each fresh sample was dried to constant weight in an oven (Gen. Lab Oven MINO/30 WIDNES, Cheshire England) at 75°C for 6 hours. The loss in weight obtained after cooling and

reweighing represented the moisture content and was expressed in percentage.

$$\text{Moisture Content} = \frac{(W_2 - W_1) 100}{W_2 - W_1}$$

- Weight of the crucible as  $W_1$
- Weight of crucible and plant part as  $W_2$
- Dry weight of sample and crucible as  $W_3$

### Determination of Ash Content

A 1.0g oven dried sample was weighed into a previously dried and weighed porcelain crucible. The crucible and its contents were placed in a muffle furnace (Vestar Furnace type EF3 Chesterfield U.K) and the temperature of the furnace was allowed to rise slowly to 450°C. This temperature was maintained for 4 hours. The crucible was then transferred to a desiccator, cooled to room temperature and weighed. The percentage loss on ignition from weight loss during combustion was calculated.

$$\% \text{ Ash (dry basis)} = \frac{(W_3 - W_1) 100}{W_3 - W_1}$$

Where:

$W_1$  is weight of crucible

$W_2$  is weight of crucible and sample before ashing

$W_3$  is weight of crucible and ash

### Determination of the Crude Protein Content

The crude protein content of the samples was determined by the micro Kjeldahl method. A 1.0g of the dried finely ground sample was weighed into a 500ml round bottom Kjeldahl flask. About 2.0g of digestion catalyst ( $K_2SO_4$  and  $H_2O$  mixture) was added followed by 20ml of concentrated

$H_2SO_4$ . The flask was gently heated until frothing subsided. The heat was increased until a colorless or pale green digest was obtained. The digest was allowed to cool and then diluted to 5ml with distilled water. Then 20ml of the diluted digest was placed in the distillation flask with addition of 25ml of 4M NaOH solution followed by distillation. The distillate was collected in the receiver containing 10ml of boric acid of indicator solution. A 0.1M HCl solution was used to titrate to pale end point. The crude protein was calculated using a conversion factor of 6.25 to multiply the nitrogen content from the titre value.

Crude protein = % Nitrogen x converting factor

And % Nitrogen =

$$\frac{\text{Titre value} \times M \times \text{mass} \times DF}{\text{Sample wt/volume}}$$

Sample wt/volume

### Determination of Total Lipids

A 5.0g of sample was weighed into a soxhlet extraction thimble. The thimble was transferred into a 60ml capacity soxhlet extractor. A clean dry 250ml flat-bottomed flask (boiling flask) containing black beads was accurately weighed. Twenty milliliters (20ml) of ether were added to the flask which was connected to the extractor and extraction was continued for 4-6 hours.

At the end of extraction, the flask was removed and placed in a water bath, and the ether was evaporated off using a stream of nitrogen. The flask was left in a vacuum oven at 40°C for 30 minutes and cooled in desiccators and reweighed the total lipid was then calculated.

$$\% \text{ Fat} = \frac{(\text{weight of fat}) 100}{\text{Weight of sample}}$$

## Determination of Crude Fibre Content

A 1.0g of the digested sample was weighed into a 200ml Pyrex flask and 1.25% H<sub>2</sub>SO<sub>4</sub> was added. The beaker was covered with a watch glass and gently boiled on a hot plate for 30 minutes. The residue decanted under a sintered glass crucible into a beaker. Beaker was covered with a watch glass and gently boiled with 200ml of a solution containing 1.25g of carbonate free NaOH per 100ml on a hot plate for 30 minutes. The alkali was removed and the sample washed twice with 50ml boiling water. The content of the beaker was washed into a sintered glass crucible and was then dried and then incinerated, cooled and weighed to constant weight. The difference divided by the sample weight expressed in percentage gave the fibre content.

## Determination of Carbohydrate Content

The carbohydrate content was calculated as a difference of 100 from the others i.e.  $100 - \text{Moisture Content} + \text{Ash} + \text{Protein} + \text{Fat} + \text{Fibre} = \% \text{CHO}$ .

## Analysis of Minerals

### 3.5.1 Methods for the elemental analysis of samples

Elemental analysis was conducted using Agilent FS240AA Atomic Absorption Spectrophotometer according to the method of APHA, 1998 (American Public Health Association).

**Working principle:** Atomic absorption spectrophotometer's working principle is based on the sample being aspirated into the flame and atomized when the AAS's light beam is directed through the flame into the monochromator, and onto the detector that measures the amount of light absorbed by the atomized element in the flame.

Since metals have their own characteristic absorption wavelength, a source lamp composed of that element is used, making the method relatively free from spectral or radiational interferences. The amount of energy of the characteristic wavelength absorbed in the flame is proportional to the concentration of the element in the sample.

### Sample Digestion (Adrian, 1973)

Two grams (2g) of the dried sample was weighed out into a digestion flask and 20ml of the acetic acid mixture (650ml conc HNO<sub>3</sub>; 80ml perchloric acid; 20ml conc H<sub>2</sub>SO<sub>4</sub>) The flask was heated until a clear digest was obtained. The digest was diluted with distilled water to the 100ml mark. Appropriate dilutions were then made for each.

**Procedure:** The sample was thoroughly mixed by shaking, and 100ml of it was transferred into a glass beaker of 250ml volume, to which 5ml of conc. nitric acid was added and heated to boil till the volume is reduced to about 15-20ml, by adding conc. nitric acid in increments of 5ml till all the residue was completely dissolved. The mixture was cooled, transferred, and made up to 100ml using metal free distilled water. The sample was aspirated into the oxidizing air- acetylene flame. When the aqueous sample was aspirated, the sensitivity for 1% absorption is observed.

## RESULT

### Proximate Compositions of the Chia Seed and Tiger Nut used in the Study

Result in Table 1 revealed that Chia seed and tiger nut samples used contain high percentage of carbohydrate and fat. It also revealed that Chia seed had significantly higher ( $P < 0.05$ ) percentage of protein & fat than tiger nut. Antioxidant mineral

compositions of Chia Seed and Tiger nut used in this study

The result in Table.2 revealed that Chia seed and Tiger nut samples used contain moderate concentrations of Zinc, Manganese, Selenium and Cupper. Result in Table 2 also revealed that Chia seed contain significantly higher ( $p<0.05$ ) concentration of Iron than Tiger nut



**Table 1: Proximate Compositions of the Chia Seed and Tiger Nut**

	<b>Chia Seed</b>	<b>Tigernut</b>
Moisture (%)	6.23 ± 0.00	13.39 ± 0.01*
Ash (%)	3.67 ± 0.00	3.83 ± 0.00
Fat (%)	19.98 ± 0.01*	14.57 ± 0.01
Fibre (%)	7.39 ± 0.01	4.63 ± 0.01
Protein (%)	9.10 ± 0.01*	4.90 ± 0.01
Carbohydrate (%)	53.63 ± 0.02	58.68 ± 0.02*

*\*=denote significant increase ( $p<0.05$ )*

**Table 2: Some antioxidant mineral compositions of Chia Seed and Tiger nut used in this study**

<b>Minerals</b>	<b>Chia Seed</b>	<b>Tigernut</b>
Zinc (ppm)	0.68 ± 0.00	0.92 ± 0.00
Manganese (ppm)	0.18 ± 0.00	0.48 ± 0.00
Iron (ppm)	1.48 ± 0.00*	0.68 ± 0.00
Selonioum (ppm)	0.15 ± 0.00	0.13 ± 0.00
Cupper (ppm)	0.17 ± 0.00	0.47 ± 0.00

*\*=denote significant increase ( $p<0.05$ ) .*

## DISCUSSION

In this study the proximate compositions and antioxidant mineral levels of chia seed and tiger nut procured from Nnewi, Anambra State were evaluated. Results of proximate composition of chia seed and tiger nut seed samples used in this study (Table 3.1) revealed that chia seed and tiger nut samples used contain high percentage of carbohydrate and fat. It also revealed that chia seed had significantly higher ( $P < 0.05$ ) percentage of protein and fat than tiger nut. This study is in agreement with the work of Ashoush *et al.* (2024) which also revealed that chia seed contain high fiber, protein and lipid content which contribute to their blood sugar- lowering effects., The result of this study is also in agreement with the work of Iboyi *et al.* (2021) which investigated the nutritional and in vitro antioxidant activities of tiger nut and revealed high crude fiber, protein and carbohydrate content, along with significant amounts of magnesium, potassium, calcium, selenium, copper, zinc and sodium. The findings presented in Table 3.1 is also align with those of Mohammed *et al.* (2018).

Furthermore, results of antioxidant mineral compositions of chia seed and tiger nut used in this study (Table 3.2) revealed that chia seed and tiger nut samples used contain moderate concentrations of zinc, manganese, selenium and copper. The result in Table 3.2 also revealed that chia seed contain significantly higher ( $P < 0.05$ ) concentrations of iron than tiger nuts, suggesting its usefulness in boosting blood parameters. The result of this study is in agreement with the work of Ani *et al.* (2021) who analyzed the mineral, vitamins and phytochemical content of tiger nut

reporting the presence of high level of minerals, antioxidant vitamins and phytochemicals. The result of this study is also in agreement with the study by Mmuo and Okoli (2021) who reported that tiger nut is rich in vitamins A, B-complex, C and D, along with essential minerals such as magnesium, potassium, sodium and zinc, which contribute to its functional food properties. The result of this study is also in agreement with the work of Shrestha *et al* (2022) who revealed that chia seed contains high levels of phosphorus, magnesium, zinc and manganese, supporting bone health and enzymatic functions.

## CONCLUSION

Finally, the results of this study revealed that chia seed and tiger nut possess good quantity of antioxidant minerals and high percentage of carbohydrates and fats and therefore could be useful in energy generation and in the management of oxidative stress.

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