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<sup>1</sup>Department of Pure and Industrial Chemistry, Federal University Birnin Kebbi, Kebbi State, Nigeria

<sup>2</sup>Department of Energy and Applied Chemistry, Usmanu Danfodiyo University, Sokoto, Sokoto State, Nigeria

<sup>3</sup>Department of Polymer and Textile Engineering, Ahmadu Bello University, Zaria, Kaduna State, Nigeria

<sup>4</sup>Kebbi State Polytechnic Dakingari, Kebbi State, Nigeria

<sup>5</sup>Department of Science Technology, Waziri Umaru Federal Polytechnic, Birnin Kebbi, Kebbi State, Nigeria

<sup>1</sup>Affiliation of Author 1, e.g. Department of Chemistry, ABC University, Kwara State, Nigeria.

\*Corresponding author's email:

[aminuabbajega@gmail.com](mailto:aminuabbajega@gmail.com)

+2347066903764

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## Comparative Nutritional Composition of Three Varieties of *Abelmoschus esculentus* L. Seed

Aminu Abba<sup>1\*</sup>, Kabiru J. Umar<sup>1</sup>, Lawal G. Hassan<sup>2</sup>, Mohammed K. Yakubu<sup>3</sup>, Faruk Usman<sup>1</sup>, Abdullahi Umar<sup>1</sup>, Yahaya U. Dan-Ilela<sup>4</sup> and Sarajo B. Dandare<sup>5</sup>

The nutritional and industrial potentials of three varieties of *Abelmoschus esculentus* (Okra) seeds were evaluated using the AOAC method. Proximate analysis revealed significant varietal differences among Clemson Spineless, NHAE-74, and Ex-Samaru seeds. While residual moisture (4.67–5.33%), crude fibre (8.33–9.83%), and available carbohydrates (440.17–447.00 mg/100g) showed no significant differences ( $p > 0.05$ ), Ex-Samaru exhibited significantly ( $p < 0.05$ ) higher ash content (6.17%) compared to Clemson Spineless (4.83%) and NHAE-74 (4.67%). The crude lipid content in NHAE-74 (25.67%) was significantly ( $p < 0.05$ ) higher than that of Clemson Spineless (24.33%) and Ex-Samaru (23.50%), with corresponding variations in energy values. Elemental analysis demonstrated varietal-specific mineral profiles: potassium content was significantly ( $p < 0.05$ ) higher in Clemson Spineless (350 mg/100g) than NHAE-74 (323 mg/100g), while Ex-Samaru showed elevated sodium (198.42 mg/100g) and copper (0.75 mg/100g) levels compared to other varieties. Iron content remained consistent ( $p > 0.05$ ) across varieties, whereas calcium and phosphorus exhibited significant differences ( $p < 0.05$ ). Manganese content in Clemson Spineless (6.47 mg/100g) significantly ( $p < 0.05$ ) surpassed Ex-Samaru (3.48 mg/100g) but not NHAE-74 (5.15 mg/100g). Anti-nutritional analysis revealed no significant differences ( $p > 0.05$ ) in phytate or tannin content, though oxalate levels varied significantly ( $p < 0.05$ ). Bioavailability assessment using molar ratios (anti-nutrient to mineral) indicated that all varieties are safe for consumption. However, Clemson Spineless and Ex-Samaru exhibited a distinct oxalate-to-calcium ratio compared to NHAE-74. The results confirm that all three okra varieties possess favourable nutritional profiles, high mineral bioavailability, and suitability for dietary and industrial applications.

**Keywords:** Okra Seed, Ex-samaru, Bioavailability, Minerals, and Antinutrients.

## 1. Introduction

Okra is a dicotyledonous crop and considered among important vegetables in Nigeria, due to its rich nutrient content, and the whole crop is edible (Liu *et al.*, 2019). The pod (including the seeds) is used in making soup (Elkhalifa *et al.*, 2021). Studies have shown that the seeds contain high levels of minerals (Ca, K, Cu, Fe, P, Mg, Zn, and Mn),  $\alpha$ -tocopherol (vitamin E), and various bioactive compounds (Dantas *et al.*, 2021). According to Chaemsawang *et al.* (2019) and Elkhalifa *et al.* (2021), the seed extract includes isoquercitrin, which has chemo-protective activities against oxidative stress, cardiovascular disorders, diabetes, allergic reactions, cancer, and anti-fungal capabilities. Additionally, it is said to slow the spread of pancreatic and urinary bladder cancer (Valentova *et*

*al.*, 2014). Okra is often referred to as the perfect villagers' vegetable' due to its rich nutritional profile, providing an excellent source of essential nutrients. In addition to being a significant source of fibre, the seed protein included the amino acids *tryptophan* and *lysine*, which are uncommon in most seed proteins. Its high-quality protein and necessary amino (aspartic) acids, which are used in the production of other amino acids, make it an important part of the human diet (Gemede *et al.*, 2015; Liu *et al.*, 2019). Okra seeds can be used as a decaffeinated coffee substitute after being baked and ground into a powder (Liu *et al.*, 2019). Okra seed oil contains between 20 and 40 % (Oferi *et al.*, 2020). According to Elkhalifa *et al.* (2021), okra seeds are being used for commercial oil extraction

by Hunan QiyiXinye Cultural Media, Hunan, China, and Uttarabala Exports Private Limited, New Delhi, India. With a primary content of 32.22–43.07 % linoleic acid, which is indicative of polyunsaturated fatty acids, 6.79–12.34 % linolenic acid, and 4.31–6.98 % oleic acid, it is a good source of unsaturated fatty acids (Liu *et al.*, 2019). Antioxidants are abundant in the oil (Graham *et al.*, 2017). Therefore, it is crucial to boost okra production globally. However, the decline in okra productivity is not solely due to a shortage of land (Ghimire *et al.*, 2018). Furthermore, Dahal *et al.* (2021) report that the continued use of traditional outdated cultivars and low-quality seeds results in reduced yield and diminished productivity. One effective approach to address these challenges involves developing improved okra varieties through gamma-ray-induced mutagenesis. This technique generates extensive genetic variability, which can be exploited to enhance productivity and quality of okra crops (Fayad *et al.*, 2020). While agricultural research institutes have recently developed numerous improved okra cultivars, literature reviews indicate that academic researchers have largely overlooked comprehensive nutritional profiling of these varieties. This study aimed to evaluate the nutritional composition of three improved okra seed varieties: Ex-Samaru, Clemson Spineless, and NHAE-74.

## 2. Methodology

One kilogram each of dried seeds from three improved okra (*Abelmoschus esculentus*) varieties – Clemson spineless, NHAE 74, and Ex-samaru were procured from the Institute of Agricultural Research, Ahmadu Bello University in Zaria, Nigeria. Following procurement, the seeds were sun-dried for seven days, then homogenized using a porcelain mortar and pestle. The resulting powder was sieved (500 µm mesh) and stored in airtight polyethylene containers at ambient temperature pending analysis (Ndangui *et al.*, 2010). All analytical procedures were conducted using reagent-grade chemicals. Distilled water was employed throughout the experimental work. All glassware underwent a standardized cleaning protocol involving detergent washing with laboratory-grade liquid soap, triple rinsing with distilled water and oven-drying at 105°C for 2 hours (Ndangui *et al.*, 2010).

### 2.1 Proximate Analysis

The proximate composition analysis of the samples (residual moisture, ash, crude protein, crude lipid, crude fibre and available carbohydrates) was conducted as follows:

#### 2.1.1 Determination of Residual Moisture

Three clean, dried, and pre-weighed crucibles were each charged with 2 g of sample and dried at 105 °C for 24 hours in an oven. After cooling in a desiccator for 30 minutes, the samples were reweighed. The procedure was repeated hourly until a constant weight was achieved. The moisture content was calculated using equation 1.

$$\text{Moisture Content (\%)} = \frac{\text{Loss in Weight}}{\text{Weight of the sample}} * 100 \quad \text{eqn. 1}$$

#### 2.1.2 Determination of Ash Content

Triplicate samples (2 g each) were weighed into pre-cleaned, dried, and weighed crucibles and ashed at 600°C for 3 hours in a muffle furnace. After cooling in a desiccator to room temperature, the crucibles were reweighed. Ash content was calculated using equation 2.

$$\text{Ash (\%)} = \frac{\text{Weight of Residue}}{\text{Weight of the sample}} * 100 \dots\dots\dots 2$$

#### 2.1.3 Determination of Crude Fibre

The crude fibre content was determined through sequential acid-base digestion (Ilodibia *et al.*, 2014) wherein 2.0 g samples underwent reflux extraction with 150 cm<sup>3</sup> of 1.25% H<sub>2</sub>SO<sub>4</sub> (30 min), followed by hot distilled water rinsing and filtration through muslin cloth and Whatman No. 1 filter paper, then repeated with 1.25% NaOH solution; the residue was washed with 98% ethanol, transferred to pre-weighed crucibles, dried at 105°C to constant weight, ashed at 600°C in a muffle furnace, and quantified using Equation 3.

$$\text{Crude Fibre (\%)} = \frac{\text{Lost in weight}}{\text{Weight of the Sample}} * 100. \quad \text{eqn. 3}$$

#### 2.1.4 Determination of crude protein content (Micro-Kjeldahl Method)

The crude protein content was quantified using the Micro-Kjeldahl method (Yerima & Kanawa, 2018). Briefly, 2 g samples were digested in 20 cm<sup>3</sup> distilled water with 20 cm<sup>3</sup> concentrated H<sub>2</sub>SO<sub>4</sub> and a selenium catalyst tablet at 100°C for 4 hours until a clear digest was obtained. After cooling, digests were diluted to 50 cm<sup>3</sup> with distilled water, and 10 cm<sup>3</sup> aliquots were distilled with 20 cm<sup>3</sup> 20% NaOH into 20 cm<sup>3</sup> 2% boric acid containing bromocresol green-methyl red indicator. Liberated ammonia was titrated against 0.01 M H<sub>2</sub>SO<sub>4</sub> until the endpoint transition (green-pink). Nitrogen content was calculated (Equation 4) and converted to crude protein using a 6.25 conversion factor (Equation 5).

$$N(\%) = \frac{\text{Titre value} * 0.01M H_2SO_4 * 0.014 * \text{Dilution factor}}{\text{Weight of Sample}} * 100 \quad \text{eqn. 4}$$

$$\text{Crude Protein (\%)} = N(\%) * 6.25 \quad (\text{equ. 5})$$

### 2.1.5 Determination of Crude Lipid

The crude lipid content was quantified by continuous solvent extraction using a Soxhlet apparatus (Bouanga-Kalou et al., 2011). Briefly, 50.0 g samples of okra seed powder were extracted with light petroleum ether (BP 40-60°C) in a 5.0 L Soxhlet extractor for 8 hours. The solvent was then evaporated under reduced pressure using a rotary evaporator (40°C, 175 rpm), and the residual oil was pre-weighed. Lipid content was calculated according to Equation 6

$$\text{Crude Lipid(\%)} = \frac{\text{Weight Crude Lipid}}{\text{Weight of Sample}} \times \frac{100}{1} \quad \text{eqn. 6}$$

### 2.1.6 Determination of Available Carbohydrate (CHO)

CHO was obtained by difference as showcased in Equation 7.

$$\text{CHO (\%)} = 100 - (\text{Ash} + \text{Crude Protein} + \text{Crude Lipid} + \text{Crude Fibre}) \quad \text{eqn. 7}$$

### 2.1.7 Determination of energy values

The gross energy values were calculated from proximate composition data using the physiological fuel coefficients (4 kcal/100g protein, 9 kcal/100g lipid, and 4 kcal/100g carbohydrates) as established by Dangoggo et al. (2011) in Equation 8.

$$\text{Energy (kcal/100g)} = [(\% \text{CHO} \times 4) + (\% \text{Crude protein} \times 4) + (\% \text{Crude lipid} \times 9)] \dots \text{eqn. 8}$$

## 2.2 Mineral Analysis

### 2.2.1 Mineral Digestion and Preparation for AAS Analysis

The powdered samples were oven-dried at 105°C for 1 hour and cooled in a desiccator. For digestion, 2 g each of the sample's was put into digestion flasks, followed by addition of 27 cm<sup>3</sup> of a freshly prepared 2:1 (v/v) HNO<sub>3</sub> (65%)-HClO<sub>4</sub> (70%) acid mixture. The samples were heated gradually on a hot plate in a fume chamber until the evolution of brown fumes ceased and the digest became clear. After cooling to room temperature, the digest was diluted with distilled water and filtered through Whatman No. 1 filter paper into pre-cleaned 50 cm<sup>3</sup> volumetric flasks. The filtrate was diluted to the mark with water and transferred to properly labelled polyethylene bottles for analysis by atomic absorption spectrophotometry (AAS; Model 210VGP, Buck Scientific). The method quantified the sodium, magnesium, potassium, calcium, manganese, iron, copper, and zinc in the digested samples (Uddin et al., 2016).

### 2.2.2 Determination of phosphorus. (Bray No. 1 Method)

Phosphorus content was determined spectrophotometrically following the Bray No. 1

method (Gaikwad et al., 2018). A primary standard solution (1.0 P/cm<sup>3</sup>) was prepared by dissolving 0.1000 g anhydrous KH<sub>2</sub>PO<sub>4</sub> (dried at 110°C for 2 h) in 25 cm<sup>3</sup> 3.5 M H<sub>2</sub>SO<sub>4</sub> and diluting to 100 cm<sup>3</sup>. Working standards (0–5 ppm P) were prepared by serial dilution, with each 10 cm<sup>3</sup> aliquot reacted with 2.0 cm<sup>3</sup> acidified ammonium molybdate [(NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O] solution and 1.0 cm<sup>3</sup> freshly prepared SnCl<sub>2</sub> reductant solution. After 10 min incubation at ambient temperature, the phosphomolybdenum blue complex absorbance was measured at 882 nm (1 cm pathlength). Sample P concentrations were determined against a 5-point calibration curve with blank correction.

## 2.3 Determination of Anti-nutrients

### 2.3.1 Determination of Cyanide

A 10 g sample was transferred to an 800 cm<sup>3</sup> distillation flask and 200 cm<sup>3</sup> distilled water was added and allowed to stand for 3 hours. The flask was connected to a micro-Kjeldahl distillation apparatus, steam distilled, and 150-160 cm<sup>3</sup> distillate collected in a 250 cm<sup>3</sup> volumetric flask containing 50 cm<sup>3</sup> of 2.5% (w/v) NaOH solution. The solution was diluted to the mark with distilled water. A 100 cm<sup>3</sup> aliquot was transferred to a 250 cm<sup>3</sup> conical flask and 2 cm<sup>3</sup> of 5% (w/v) KI solution was added as indicator. The content was then titrated with 0.02 M AgNO<sub>3</sub>(aq) standard solution to the endpoint determined by the appearance of permanent, slight turbidity (AgI formation). Hydrogen cyanide concentration was calculated using Equation 9 (FSSAI, 2015).

$$1 \text{ cm}^3 \text{ of } 0.02 \text{ M AgNO}_3 \equiv 1.08 \text{ mg HCN} \quad \text{eqn. 9}$$

### 2.3.2 Determination of Nitrate (IITA Method, 1998)

Powdered samples (1 g each) were put into separate 15 cm<sup>3</sup> centrifuge tubes and 10 cm<sup>3</sup> distilled water each were added. The tubes were incubated at 45°C for 1 hour in a water bath, cooled and the mixture centrifuged at 5,000 rpm for 15 minutes. The clear supernatants were transferred to sterile test tubes, sealed, and refrigerated until analysis.

A stock solution (5,000 ppm NO<sub>3</sub><sup>-</sup>) was prepared by dissolving 0.1 g KNO<sub>3</sub> in 20 cm<sup>3</sup> distilled water. Standard series (0-5 ppm) were prepared by appropriate dilution in 20 cm<sup>3</sup> volumetric flasks.

To the standards, blank and sample extracts (0.2 cm<sup>3</sup> of the aliquot in a separate 20 cm<sup>3</sup> volumetric flasks), 0.8 cm<sup>3</sup> of 5% (w/v) salicylic acid in H<sub>2</sub>SO<sub>4</sub> was added to each flask, mixed thoroughly, and allowed to stand for 20 minutes for complete reaction. The contents were adjusted to pH 12 by adding 2 mol dm<sup>-3</sup> NaOH solution, diluted to the 20 cm<sup>3</sup> mark with distilled water and cooled to room temperature. The concentrations of the standards, blank and sample extracts at 410 nm were measured using a spectrophotometer. The actual

nitrate concentration in the samples was calculated Equation 10.

$$\text{NO}_3^- (\text{mg} / 100\text{g}) = \frac{X (\text{ppm}) * \text{Volume of Solution (cm}^3\text{)}}{\text{Aliquot (cm}^3\text{)} * \text{Sample Weight (g)}} * 100 \quad \text{eqn.10}$$

Where X is the sample concentration as determined by the spectrophotometer.

### 2.3.3 Determination of Total Oxalate

The titration method described by Kayode *et al.* (2013) was used to determine the samples' oxalate content. The samples (2g each) were digested with 6 M HCl by boiling for 1 hour, followed by iron removal through addition of concentrated ammonia solution dropwise until the solution's pink hue turned to a faint yellow, then filtrated. The oxalate was then selectively precipitated as calcium oxalate at 90°C using 5% CaCl<sub>2</sub>, cooled to 5°C for 24 hours to ensure complete crystallization, and subsequently dissolved in H<sub>2</sub>SO<sub>4</sub> (1:4 v/v) for titration against standardized 0.05 M KMnO<sub>4</sub>, to the persistent pink endpoint (>30 sec). Blank determination was carryout in similar manner. The concentration of oxalate in the samples was calculated using Equation 11

1 cm<sup>3</sup> 0.05 (KMnO<sub>4(aq)</sub>) ≡ 2.25 mg anhydrous oxalic acid eqn. 11

### 2.3.4 Determination of Phytate Content

The phytate content was determined following Kayode *et al.* (2013) through an iron-precipitation method. The samples (4.0 g each) phytate was extracted in 100 cm<sup>3</sup> of 2% (v/v) HCl for 3 hours, followed by filtration through Whatman No. 1 filter paper. A 25 cm<sup>3</sup> aliquot of the filtrate was mixed with 53.50 cm<sup>3</sup> distilled water and 5 cm<sup>3</sup> of 0.3% (w/v) ammonium thiocyanate indicator in a conical flask, then titrated against standardized FeCl<sub>3</sub> solution (0.00195 g Fe/cm<sup>3</sup>) until a persistent brownish-yellow endpoint (≥5 min duration) was achieved, with parallel blank correction. Phytate-phosphorus

content was calculated using the titration factor (1 cm<sup>3</sup> FeCl<sub>3</sub> ≡ 1.19 mg phytate-phosphorus) and converted to total phytate by multiplying by 3.55 (Ola and Obah, 2000).

### 2.3.5 Determination of Tannins Content (Folin-Denis Method)

The Folin-Denis approach, as reported by Elgailani and Ishak (2014), was used to estimate the tannins extracted from the sample, which was based on the procedure outlined by Santo *et al.* (2013). The tannin content was quantified by initially extracting 0.1 g of dried seed powder with 50 cm<sup>3</sup> distilled water in a 100 cm<sup>3</sup> conical flask at 80–90°C for 1 hour, followed by cooling, filtration, and thorough residue washing. For analysis, 10 cm<sup>3</sup> of sample extract and standard tannic acid solutions (0–5 cm<sup>3</sup>) were separately reacted with 2.5 cm<sup>3</sup> Folin-Denis reagent and 10 cm<sup>3</sup> of 20% (w/v) Na<sub>2</sub>CO<sub>3</sub> solution in volumetric flasks, diluted to mark with distilled water, and incubated for 20 minutes after vigorous shaking to develop the blue chromogen. Absorbance was measured at 725 nm using a spectrophotometer, and tannic acid equivalents were calculated using Equation 12 using a calibration curve constructed from the standard series, with results expressed as mg tannic acid equivalents per gram dry weight.

### 2.4 Minerals Bioavailability

Equation 13 was used to compute the bioavailability of minerals by dividing the mole of anti-nutrient by the mole of minerals (Gemede *et al.*, 2016), and Equation 14 was used to calculate the ratio of the product of calcium moles and phytate moles: zinc moles ratio (Fekadu *et al.*, 2013).

$$\text{Tannic Acid} = \frac{X (\text{ppm}) * \text{Extracted Volume (50 cm}^3\text{)}}{\text{Aliquot (10 cm}^3\text{)} * \text{Sample Weight (0.1g)}} \quad \text{eqn. 12}$$

$$\frac{\text{mg of Anti - nutrient}}{\text{Mol. Wt of Anti - nutrients}} / \frac{\text{mg of Element}}{\text{Mol. Wt Element}} \quad \text{eqn.13}$$

$$\left( \frac{\text{mg of Ca}}{\text{Mol. Wt of Ca}} * \frac{\text{mg of Phytate}}{\text{Mol. Wt of Phytate}} / \frac{\text{mg of Zn}}{\text{Mol. Wt of Zn}} \right) \quad \text{eqn. 14}$$

## 2.5 Statistical Analysis

The analysis's findings were presented as mean ± standard deviation of triplicate values, and a two-way ANOVA was used to determine whether the means differed significantly at a 5% level of confidence.

## 3. Results and Discussion

### 3.1 Proximate Analysis

The proximate composition (ash, residual moisture, crude lipid, crude protein, and available carbohydrate) of the three okra seed types analysed are displayed in Table 1. The amounts of available carbohydrates, residual moisture, and crude fibre in

the seeds did not differ significantly ( $p > 0.05$ ). Both Clemson spineless and NHA-E-74 had considerably different ash contents ( $p < 0.05$ ) from ex-samaru, although their crude lipid and crude protein levels do not differ significantly ( $p > 0.05$ ) from NHA-E-74.

Clemson spineless (5.33%), ex-samaru (4.83%), and NHA-E-7 (44.67%) had moisture contents that were comparable to (4.27%) Azhari *et al.* (2014) but lower than the 9.27 to 12.70% described by Habtamu *et al.* (2018). The study's moisture content falls between 4.27 and 5.63 percent, this is within Petkova and Antova's (2015) bounds. This suggests that all of the seeds can be kept for a respectable amount of time without running the risk of spoiling or microbial deterioration (Nasreen, 2000).

The ash content of the three seeds falls between 4.67 and 6.17%, similar to the ash content of three Egyptian okra cultivar seeds which ranges between 5.76 and 10.84% (Hassan and Ali's, 2015), and ash content of the native Ethiopian okra seed accession which falls between 4.53 and 6.05 percent (Habtamu *et al.*, 2018). However, the findings are higher than the reported 1.2% ash concentration of okra grown in Peshawar (Bangash *et al.*, 2011). A plant material's ash content has been said to be proportionate to its mineral content (Lillian *et al.*, 2013; Ur Rehman *et al.*, 2014).

The fibre content of the okra seeds (8.33–9.83%) is comparable to the 9.46% reported for okra pods (Ndamitso *et al.*, 2012), although it is greater than the 1.94–5.96% reported by Habtamu *et al.* (2018). Fibre-rich diets have been shown to reduce the incidence of high blood pressure, obesity, diabetes,

coronary heart disease, colon cancer, and a number of digestive problems (Adamma *et al.*, 2014). Fibre is also linked to normal intestinal peristaltic movement (Oloye *et al.*, 2014). The fibre content (8.33 to 9.83%) is less than the 20 to 35 g/day that epidemiological data suggests adults should consume (Adegoke *et al.*, 2006; Orisa *et al.*, 2020).

The crude lipid content of the examined okra seeds (23.50–25.67%) is higher than the published 15.05% for cotton seed oil (Adelola and Ndudi, 2012), but it is equivalent to the values for olive oil (25–30%) and linseed oil (33.5%) reported by Gunstone (2002). As per Ndangui *et al.* (2010), Sorapong (2012), and Gemedé *et al.* (2014), the values obtained are between 20 and 40 %. The seeds were classified as high oil yielding seeds based on their oil yield (Warrier and Tripathi, 2011; Singh *et al.*, 2014).

The crude protein content of the okra seeds ranges from 8.11 to 8.69%, which is less than the 22.51 to 34.25% recorded for the eight native Ethiopian okra seed accessions (Habtamu *et al.*, 2018). Despite having a low protein concentration, the seeds could be used as an additional source of protein to help people in rural regions who are protein deficient (Sonawane and Arya, 2018).

The seeds' accessible carbohydrate (46.47–48.89%) is lower than 58.15% for *Abelmoschus esculentus* (okra) pods (Ndamitso *et al.*, 2012) but greater than 37.56% for okra grown in Fadaman Kubanni, Zaria, Kaduna state, Nigeria (Adamma *et al.*, 2014). The seeds' high carbohydrate content suggested that they would be a valuable source of energy.

**Table 1** Proximate Composition of three varieties of Okra seed (% DW)

Parameter	Okra Varieties		
	Clemson spineless	Ex-samaru	NHA-E-74
Residual Moisture	5.33±0.29 <sup>a</sup>	4.83±0.29 <sup>a</sup>	4.67±0.29 <sup>a</sup>
Ash	4.83±0.29 <sup>b</sup>	6.17±0.58 <sup>a</sup>	4.67±0.29 <sup>b</sup>
Crude Fibre	8.50±0.50 <sup>a</sup>	8.33±0.29 <sup>a</sup>	9.83±0.58 <sup>a</sup>
Crude Lipid	24.33±0.29 <sup>b</sup>	23.50±0.50 <sup>b</sup>	25.67±0.58 <sup>a</sup>
Crude Protein	8.11±0.05 <sup>b</sup>	8.31±0.09 <sup>b</sup>	8.69±0.13 <sup>a</sup>
Available Carbohydrate	48.89±0.46 <sup>a</sup>	48.85±0.98 <sup>a</sup>	46.47±1.39 <sup>a</sup>
Energy Value (Kcal/100g)	447.00±1.32 <sup>b</sup>	440.17±1.26 <sup>c</sup>	451.57±1.93 <sup>a</sup>

The result is expressed as the mean ± standard deviation of the triplicate results. Values in the same row with different superscript (a, b, c) differ significantly at  $p < 0.05$ .

Seeds have energy levels between 440.17 to 451.57 kcal/100 g, which is greater than the 368.80 kcal/100 g found in Habtamu *et al.* (2018). According to Ndangui *et al.* (2010), okra seeds are a significant source of energy for both humans and animals due to their high energy content of 379 to 440 kcal/100 g. The variances in plant type, cultivation climate, ripening stage, and seed harvesting timing may all contribute to the variability in the proximate compositions of seeds (Hassan and Ali, 2015).

### 3.2 Mineral Content

Table 2 shows the elemental composition of the three okra seed varieties that were examined. The calcium, iron, and phosphorus content of the three

seeds varied significantly ( $p < 0.05$ ). There was no significant difference ( $p > 0.05$ ) in the copper, magnesium, and zinc content of the seeds between Clemson spineless and NHA-E-74, but there was a significant difference ( $p < 0.05$ ) with ex-samaru. The manganese content of Clemson spineless and ex-Samaru differs significantly ( $p < 0.05$ ). Clemson spineless has a substantially higher sodium level ( $p < 0.05$ ) than both NHA-E-74 and Ex-samaru, while NHA-E-74 has significantly lower potassium content ( $p < 0.05$ ) than both.

Ex-samaru seeds have the highest potassium concentration (350 mg/100g), followed by Clemson spineless seeds (330 mg/100g) and NHA-E-74 seeds (323 mg/100g). The potassium content of Clemson

spineless and ex-samaru, as well as Clemson spineless and NHAE-74, did not differ significantly ( $p > 0.05$ ). However, the difference between NHAE-74 and ex-samaru is significant ( $p < 0.05$ ). Although the potassium content in the samples is higher than the 97.70–187.92 mg/100 g reported by Habtamu *et al.* (2018) and lower than the 4080–5480 mg/100 g in okra seed flour (Hassan and Ali, 2015), and comparable to the values reported in okra pod accessions (318.2–360 mg/100 g) (Ndamitso *et al.*, 2012; Gemede *et al.*, 2016). The average daily intake of potassium that is deemed sufficient for infants (7–11 months), children (15–17 years), and adult men and women is 3,500, 800–3500, and 750 mg, respectively (Turck *et al.*, 2016; Hankey and Fracp, 2017). Consequently, the seed could help these groups meet their potassium needs.

Clemson spineless (8.57 mg/100 g) and ex-samaru (7.92 mg/100 g) did not differ significantly ( $p > 0.05$ ) in sodium content, nor did ex-samaru (7.92 mg/100 g) and NHAE-74 (7.33 mg/100 g). However, NHAE-74 (7.33 mg/100 g) and Clemson spineless (8.57 mg/100 g) differed significantly ( $p < 0.05$ ). This result's range of Na (7.33 - 8.57 mg/100 g) is higher than the 6.9–7.02 mg/100 g found in okra pods

(Gopalan *et al.*, 2007; Ndamitso *et al.*, 2012), but lower than the 15.06–27.98 mg/100 g found in seven native Ethiopian okra seeds (Habtamu *et al.*, 2018). In The WHO's (2012) recommendation for a maximum daily intake of 2 g of sodium, suggest the safety of consuming any of the Okra seeds under investigation.

The calcium content of the seeds varied significantly ( $p < 0.05$ ) from one another. NHAE-74 (188.90 mg/100 g) and ex-samaru (189.07 mg/100 g) are lower than Clemson spineless (191.77 mg/100 g). The samples' calcium concentration is lower than the 250–350 mg/100 g reported by Hassan and Ali (2015) in okra seed flour, but greater than the 66.37–103.66 mg/100 g reported by Habtamu *et al.* (2018) and 2.41 mg/100 g reported by Ndamitso *et al.* (2012). According to Babu *et al.* (2016), calcium is an essential mineral for the body's physiological and biochemical processes; Cell adhesiveness, mitosis, blood coagulation, muscular contraction, glandular secretion, and the development of strong bones (Arunkumar *et al.*, 2015). According to Heaney *et al.* (2012), the calcium content of the samples is low when compared to the recommended daily intake of 700–1300 mg/day, depending on age.

**Table 2** Minerals Composition of three varieties of the Okra seeds (mg/100 g)

Element	Clemson spineless	Ex-samaru	NHAE-74
Potassium	330.00±10.00 <sup>ab</sup>	350.00±10.00 <sup>a</sup>	323.33±5.77 <sup>b</sup>
Sodium	8.57±0.40 <sup>a</sup>	7.92±0.14 <sup>b</sup>	7.33±0.14 <sup>b</sup>
Calcium	191.77±3.0 <sup>b</sup>	189.07±5.2 <sup>a</sup>	188.90±10 <sup>c</sup>
Magnesium	201.17±10.47 <sup>a</sup>	198.42±2.89 <sup>b</sup>	201.31±10.17 <sup>a</sup>
Phosphorus	4.23±0.02 <sup>a</sup>	4.32±0.03 <sup>b</sup>	4.50±0.02 <sup>c</sup>
Copper	0.49±0.03 <sup>b</sup>	0.75±0.07 <sup>a</sup>	0.45±0.42 <sup>b</sup>
Iron	5.26±0.51 <sup>a</sup>	5.38±1.13 <sup>a</sup>	5.21±0.43 <sup>a</sup>
Manganese	6.47±0.48 <sup>a</sup>	3.48±166 <sup>b</sup>	5.15±0.55 <sup>ab</sup>
Zinc	1.01±0.15 <sup>a</sup>	0.66±0.15 <sup>b</sup>	1.02±0.62 <sup>a</sup>

The result is expressed as the mean ± standard deviation of the triplicate results. Values in the same row with different superscript (a,b, c) differ significantly at  $p < 0.05$ .

Ex-samaru's magnesium concentration (198.42 mg/100 g) is substantially lower ( $p > 0.05$ ) than those of Clemson spineless and NHAE-74 (201.17 and 201.31 mg/100 g, respectively). Less than 440 to 780 mg/100 g of okra seed flour (Hassan and Ali, 2015). People suffering with Asthma can breathe more easily because magnesium helps relax the muscles that run along the airways to the lungs (Dangoggo *et al.*, 2011). Depending on age, the recommended daily intake of magnesium in the diet ranges from 30 to 420 mg (6 months to over 51 years) (Hu *et al.*, 2022).

The phosphorus content revealed a significant difference ( $p < 0.05$ ) among the three varieties. The largest concentrations are seen in NHAE-74 (4.50 mg/100 g), ex-samaru (4.32 mg/100 g), and Clemson spineless (4.23 mg/100 g). The result exceeds the 0.80–1.31 mg/100 g that Hassan and Ali (2015) reported. Age determines how much phosphorus the body needs. Adults aged 19 and older require 700 mg per day, children aged 9 to 18 require 1250 mg per day, children aged 1 to 3 and 4

to 8 require 460 and 500 mg per day, and infants aged 0 to 6 and 7 to 12 months require 100 and 275 mg per day, respectively (DRI, 1997). The seeds have a low phosphorus concentration, but can used to complement other sources.

Compared to Clemson spineless (0.49 mg/100 g) and NHAE-74 (0.45 mg/100 g), Ex-samaru (0.75 mg/100 g) has a substantially higher copper content ( $p < 0.05$ ). The average value of Ex-samaru is marginally higher than the WHO limit, the values of Clemson spineless and NHAE-74 is in agreement with the WHO limit for Cu (0.50 mg/100 g). This is acceptable because copper is essential for many physiological processes, including the body's use of iron, the removal of free radicals, the growth of bones and connective tissue, and the synthesis of melanin, the pigment found in skin and hair (Lillian *et al.*, 2013). Dietary copper deficiencies have been linked to problems with the neurological system, cardiovascular system, anemia, and bones (Dangoggo *et al.*, 2011).

The iron content of the okra seeds did not differ significantly ( $p > 0.05$ ). In native Ethiopian okra seed accessions, the finding is smaller than the 8.33–20.29 mg/100 g reported by Habtamu *et al.* (2018) but greater than the 0.41 mg/100 g reported by Ndamitso *et al.* (2012). Iron is a crucial part of *myoglobin* in muscles and *hemoglobin* in red blood cells. It facilitates the production of blood and the movement of carbon dioxide and oxygen between tissues. In order to prevent anemia, it is therefore a crucial component of the diets of elderly people, pregnant women, nursing mothers, and newborns that are convulsing (Oluyemi *et al.*, 2006). Considering the required daily intake of 2 to 5 mg of iron (NRC, 1989), the seeds are rich source of iron.

Clemson spineless has substantially greater manganese content (6.47 mg/100 g) than Ex-samaru (3.48 mg/100 g) ( $p < 0.05$ ). The immune system, blood sugar regulation, energy synthesis, and cell division are all aided by manganese. Manganese intakes between 1 and 10 mg/day were established by the Scientific Committee for Food in 1993 (EFSA, 2013). On the basis of the manganese contents, the seeds might be potentially a good source of manganese.

The zinc content of NHAЕ-74 (1.02 mg/100 g) and Clemson spineless (1.01 mg/100 g) differs considerably ( $p > 0.05$ ), but it is significantly greater ( $p < 0.05$ ) than that of Ex-samaru (0.66 mg/100 g). The levels are comparable to the findings of Ndamitso *et al.* (2012) (0.95 mg/100 g), but lower than the report of Habtamu *et al.* (2018). Because the fetus's cells divide quickly during pregnancy, zinc becomes even more crucial for growth and the immune system. It also helps prevent night blindness. Taking into account the zinc dietary requirement recommendations of 15 mg per day (Pathak and Agrawal, 2014). Okra seeds can only supplement other sources of zinc because the body may not receive enough of it from them.

### 3.3 Anti-nutrient Compositions

Table 3 displays the anti-nutritional characteristics of the okra seeds: NHAЕ-74, Ex-samaru, and Clemson spineless. The following parameters were examined: tannins, cyanide, nitrate, oxalate, and phytate. The varieties differed significantly ( $p < 0.05$ ) in all the parameters under investigation.

Cyanide levels in the samples ranged from 6.81 to 16.82 mg/100g, and there were notable variations between the three types ( $p < 0.05$ ). The range of results exceeds the *gari* readings (0.21–0.91 mg/100g) found in five separate market places in Nigeria, Ekiti state (Omolar, 2014). However, values in tapioca (12.50 mg/100), cassava chips (7.40 mg/100), fufu (13.20 mg/100), and cassava grit (10.97 mg/100g) were recorded by Ikediobi *et al.* (1980). Additionally, flax seed (39.00 mg/100g) and apple seed (69.00 mg/100g) had greater levels, according to Ikediobi *et al.* (1980). Taking into

account the death dose of cyanide (200–300 mg) to adult humans calculated by Akiyama *et al.* (2012) and the acute lethal dose of hydrogen cyanide for humans (0.5–3.5 mg kg<sup>-1</sup> body weight) provided by Burns *et al.* (2012), shows that the seeds are for consumption.

The nitrate concentration of NHAЕ-74 was the greatest at 122.81 mg/100g, followed by Ex-samaru at 70.64 mg/100g and Clemson spineless at 56.42 mg/100g. The nitrate content of the seeds differed considerably ( $p < 0.005$ ). The samples' nitrate concentration is less than the 3.7 mg/kg daily permissible intake (Elinge *et al.*, 2012), suggesting the safety of consuming the Okra seeds. Furthermore, cooking and boiling can reduce total nitrate by 16–62% and 75%, respectively (Du *et al.*, 2007; Gorenjak and Cencič, 2013).

There are notable differences ( $p < 0.05$ ) in the oxalate content of the seeds (0.005–0.01 mg/100g). The oxalate concentration is higher than the  $9.9 \times 10^{-4}$  mg/100g reported by Munro and Bassir (1989), but lower than the range of 0.74 to 0.75 mg/100g observed in eight distinct indigenous Ethiopian okra seed genotypes (Habtamu *et al.*, 2018). The oxalate concentrations fall below the 250 mg/100 g threshold that is considered safe for human consumption (Oguchi *et al.*, 1996). Therefore, the nutritional qualities of the seeds are unaffected by the oxalate levels in the samples.

Significant variations ( $p < 0.05$ ) were observed in the phytate levels of the three types of okra seeds. Phytate concentrations in the Clemson spineless, Ex-Samaru, and NHAЕ-74 samples were 33.80, 7.54, and 23.10 mg/100, respectively. The finding is greater than the 0.39–0.46 mg/100g reported by Habtamu *et al.* (2018), but lower than the 66 mg/100 g of okra grown in Fadaman Kubanni, Zaria, reported by Adamma *et al.* (2014). The results of obtained are comparable to the phytate found in other Thai fruits that diabetic patients frequently eat, such as dragon (37 mg/100 g) and longan (39 mg/100 g) (Bello and Olawore, 2007). The samples' phytate content may not be harmful to health (Shamsuddin, 2002) and can be utilized to treat diabetic patients.

The tannin content of the okra seeds varied significantly ( $p < 0.05$ ). The tannin content of the samples reported in this study (34.83 to 48.20 mg/100g), is less than 89.0 mg/100g for *Moringa oleifera* seed (Mohammed and Abd Manan, 2015) but greater than 4.4 – 26.68 mg/100g for 11 varieties of rambutan (*Nephelium lappaceum* L.) seed (Chai *et al.*, 2018). Tannins are known to bind proteins irreversibly and form insoluble complexes. The formation of the insoluble complexes renders the protein indigestible by intestinal enzymes and prevents their bioavailability (Talabi *et al.*, 2016). Hence, causes growth depression (Chai *et al.*, 2018). The adult human's daily permissible consumption of tannic acid is 560 mg/100g, and as such the tannin concentration have no significant

impact (Fekadu *et al.*, 2013). Depending on the fertility of the soil, genetic variations, and climatic conditions, different plant species have different concentration of tannin (Gonzalez-Hernandez *et al.*, 2003; Okonwu and Ugiomoh, 2015).

### 3.4 Minerals Bioavailability

[oxalate]: [calcium], [phytate]: [calcium], [phytate]: [iron], [phytate]: [zinc], and [(phytate) \* [Calcium]: [Zn]] are the results of anti-nutrient to element(s) mole ratios that are displayed in Table 4. Both Clemson spineless and Ex-Samaru have [oxalate]: [calcium] molar ratios that are considerably ( $p > 0.05$ ) lower than NHA-E-74's. Other molar ratios varied significantly ( $p < 0.05$ ).

Mineral bioavailability is the term used to describe the body's capacity to digest minerals from meals and absorb them (Fekadu *et al.*, 2013). The samples' [phytate]: [Ca] molar ratio range (1.07–7.41) is below the threshold value of 0.24, indicating good calcium bioavailability (Woldegiorgis *et al.*, 2015). According to Hurrell *et al.* (2003), phytate has no influence on iron absorption because the samples' [Phytate]: [iron] molar ratios range (0.01 to 0.05) is less than 1.0 (the critical value) in the samples. The [phytate]: [zinc] ratio ranges from 0.11 to 0.33, foods having a [Phytate]: [zinc] molar ratio of less than 10 demonstrated appropriate zinc bioavailability, while a value larger than 15 indicated inadequate zinc bioavailability, according to Morris and Ellis (1989).

According to Fekadu *et al.* (2013), the [Phytate]\*[Ca]/[Zn] ratio is a more accurate measure of zinc bioavailability than the [Phytate]/[Zn] molar ratio. When the [Ca][phytate]/[Zn] ratio is more than 0.5 mol/kg, high calcium content in food can exacerbate the phytate-induced reduction in zinc bioavailability (Hailu and Addis, 2016). Both NHA-E-74 (1.06 mol/kg) and Clemson spineless (1.58 mol/kg) had values over the threshold values, suggesting that calcium interference is more likely to impact zinc bioavailability (Hailu and Addis, 2016). All the seeds had good bioavailability of the elements on the basis of their anti-nutritional-element molar ratio.

## 4. Conclusion

Proximate analysis revealed no significant differences ( $p > 0.05$ ) among the three varieties in iron content (Fe), carbohydrates, crude fibre, and residual moisture levels. However, significant varietal differences ( $p < 0.05$ ) were observed in Energy values, Crude protein and lipid contents (with Clemson Spineless and Ex-Samaru differing significantly from NHA-E-74), Elemental mineral composition, Anti-nutritional factors and Mineral bioavailability ratios. The seeds demonstrated excellent nutritional potential, characterized by high protein (22–26%) and

carbohydrate (440–447 mg/100g) content, providing substantial dietary energy. They are low residual moisture (4.67–5.33%), ensuring good storage stability, considerable ash content (4.67–6.17%), indicating rich mineral composition, elevated crude fibre (8.33–9.83%), offering potential health benefits for disease prevention and substantial crude lipids (23.50–25.67%), suggesting value for both culinary and industrial oil extraction.

Anti-nutritional analysis confirmed that conventional cooking methods (boiling/processing) effectively mitigate any potential negative nutritional impacts, making all varieties safe for consumption. The combined nutritional profile positions these okra seeds as valuable functional food ingredients and potential industrial raw materials.

## Conflict of Interest

The authors declare no conflict of interest.

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