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Proximate and Anti-nutritional Constituents of *Abelmoschus esculentus* Grown in Fadaman Kubanni, Zaria, Kaduna State, Nigeria

Ekwumemgbo Patricia Adamma[1*], Sallau Mohammed Sani[1], Omoniyi Kehinde Israel[1] and Zubairu Safiya Yusuf[1]

[1]Department of Chemistry, Ahmadu Bello University, Zaria, Kaduna, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author SMS designed the study. Author EPA managed the literature searches wrote the protocol and the first draft of the manuscript. Author ZSY managed the experimental process and author OKI identified the species of plant. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: This work seeks to provide information on the proximate and anti-nutritional constituents of *Abelmoschus esculentus* (okra) grown in Fadaman Kubanni farms where effluents from Zaria Industrial Estate are discharged, and compare it with that grown in Rafin Yashi (control) in order to ascertain the effects of the effluent on the plant.
Study Design: Proximate and anti-nutritional constituent determination of *Abelmoschus esculentus* vegetative part and fruit samples.
Place and Duration of Study: This study was conducted between June, 2011 and May, 2013 in the Department of Chemistry, Ahmadu Bello University, Zaria, Nigeria.
Methodology: Standard AOAC method was employed in the determination of moisture, ash, fat, crude fibre and oxalate constituents of the samples; carbohydrate content was obtained by the standard anthrone method; protein content was obtained by standard Biuret protein assay; phytate content was evaluated as specified by Reddy and Love while saponin content was obtained by Rathod and Valvi method. Results were expressed as the mean±SD of five replicate determinations, compared using Student t-test and the level of significance determined at $P = 0.05$.
Results: The mean obtained for moisture, ash, crude protein, fat, fibre and carbohydrate

**Corresponding author: E-mail: pat_adamma@yahoo.com;*

of okra vegetative part from Fadaman Kubanni (FP) were 14.36±0.20%, 11.62±0.23%, 22.87±0.13%, 13.70±0.21%, 14.46±0.32% and 37.56±0.18% respectively, while the values for fruits (FF) samples were 18.63±0.12%, 7.35±0.10%, 13.15±0.04%, 9.73±0.12%, 29.76±0.23% and 51.13±0.20% respectively. The phytate, oxalate and saponin composition of (FP) were 0.66±0.01 mg/g, 0.83 mg/g ± 0.01 mg/g and 0.23 mg/g ± 0.03 mg/g respectively, while (FF) values were 0.66±0.03 mg/g, 0.859±0.02 mg/g and 0.28±0.01 mg/g respectively. Comparison of the values obtained with the control and the standards for consumable fruits indicate that the analyzed samples have good nutritional values.

Conclusions: The effluents discharged did not adversely affect the fruit quality. However, there must be strict adherence to effluent quality by industries before discharging into the environment.

Keywords: Chemical composition; contamination; fruit; industrial effluents; okra; vegetative part.

1. INTRODUCTION

Fruits and vegetables are commonly consumed by both rural and urban dwellers in Nigeria [1]. They play significant roles in human nutrition, especially as sources of vitamins, minerals, and dietary fibre [2,3]. Fruits are nature's gift to mankind; they are chief sources of vitamins, minerals and proteins. These constituents are essential for normal physiological well being and help in maintaining healthy state through development of resistance against pathogens. Vegetables are of special nutritional importance [4-6] because of their high protein components due to high quality content of essential amino acids [7]. Fruits and vegetables remain an important source of nutrients for human and animals consumption in many parts of the world, and offer advantages over dietary supplements because of low cost and wide availability.

Abelmoschus esculentus (L.) Moench (okra) could be consumed as both fruit and vegetable. It is known as Lady's finger, Bhindi or Gumbo, widely grown in the tropics, sub-tropics and warmer areas of the temperate zones [8]. Higher yields are obtained with hot weather (temperatures above 26ºC), especially in regions with warm nights (>20ºC). The five highest okra producing countries in the world are India, Nigeria, Sudan, Iraq and Côte d'Ivoire [9]. In several parts of the world, okra cultivation has gained place in urban and peri-urban areas partly because of the introduction of foreign high yielding varieties by seed companies [10].

Considerable interest has been generated by recent studies on the chemical composition of some fruits and vegetables in Nigeria, okra inclusive. Proximate determination is employed in the assessment of the composition of feeds in order to ascertain that their composition is within their normal compositional parameters. Although some fruits and vegetables have higher nutritional values however, some contain anti-nutritional factors such as phytate, saponin and oxalate that can affect the availability of nutrients required by the body. These factors have adverse effects on health by interfering with metabolic processes [11].

A lot of studied have been carried out on proximate and anti-nutritional compositions of seeds and vegetables for instance studies on the composition of some nutrients and anti-nutrients of sheanut (*Butyrospernum parkii*) [12]; assessment of the proximate compositions of okra seeds grown in two localities of Congo Brazzaville [13]; comparative analysis of the nutrient composition of cashew (*Anacardium occidentale*), apple and nut [14]; effect of

boiling on the nutrient and anti-nutrient composition of two vegetables *Solanum nigrum* and *Solanecio biafrae* [15] and analyses of the pulp of *Gardenia aqualla* fruit for nutritional and anti-nutritional composition [16].

Fadaman Kubanni is located very close to the Industrial Estate in Zaria, Kaduna State, Nigeria. Industries discharge their effluents into River Kubanni and these effluents contain some levels of pollutants such as cadmium, copper, lead and nickel. Okra is grown around this river throughout the year because of its wet nature. Therefore the aim of the present study is to determine the proximate and anti-nutritional constituents of okra grown in Fadaman Kubanni farms were effluents from Zaria Industrial Estate are discharged; compare these values with a control grown at Rafin Yashi were effluents are not discharged, in order to determine if the effluents discharge adversely affected okra which is widely consumed.

2. METHODOLOGY

Five samples each (vegetative part and fruits) of *Abelmoschus esculentus* (okra) were collected from five farms each in Fadaman Kubanni, Zaria Local Government Area of Kaduna State, Nigeria and coded FP and FF respectively. Zaria is the second largest city in Kaduna State, Nigeria and it is located between longitude 7º 36' to 7º 42' E and latitude 11º 00' to 11º 10' N of the Equator. It is found on the high plains of Northern Nigeria, in sub-Saharan Africa [17]. The control samples (vegetative part and fruits of okra) from Rafin Yashi, Giwa Local Government Area of Kaduna State, Nigeria coded CP and CF respectively were also collected. The samples were identified at the Herbarium, Department of biological sciences, Ahmadu Bello University, Zaria to be of the same species. The samples were air dried and then oven dried 45ºC to a constant weight. The dried samples were pulverized using mortar and pestle, passed through a 2.00 mm sieve and stored in air tight labelled polyethylene bottles until analyses.

2.1 Proximate Evaluation

Standard AOAC method [18] was employed for the determination of moisture, ash, fat and crude fibre constituent of the samples.

2.1.1 Carbohydrate

Carbohydrate content was obtained by the standard anthrone method [19]. Standard calibration curve was obtained by measuring into test-tubes 1.00 0.50, 0.25, 0.13 and 0.06 mg/cm^3 sucrose solution; 2.00 cm^3 anthrone solution was added; shaken for 15 minutes and boiled for 30 minutes, the compositions were then allowed to cool. Absorbance of the content of the test tubes was measured at 625 nm with the UV-Visible Spectrophotometer (Jenway 64050). The obtained absorbance values were plotted against concentrations which gave a linear standard calibration curve.

Exactly 5.00 g of each sample was weighed, mashed and 100.00 cm^3 of distilled water added and filtered. The filtrate was used for further analysis. Exactly 1.00 cm^3 aliquot of each filtrate was measured into test-tubes and 2.00 cm^3 anthrone solution added. These were shaken with Gallenkamp thermostated shaker for 15 minutes and boiled for 30 minutes. The compositions were allowed to cool and the absorbance was then was measured using UV-Visible Spectrophotometer (Jenway 64050), at 625 nm. The

carbohydrate concentrations of samples were obtained by reference to the standard calibration curve.

2.1.2 Protein

Standard Biuret protein assay [20,21] was adopted. Standard calibration curve was prepared by reading the absorbance of 1.00-10.00 mg/ cm^3 standard concentrations of bovine serum albumin (BSA) with the UV-Visible Spectrophotometer (Jenway 64050). The obtained absorbance values were plotted against concentrations which gave a linear standard calibration curve.

Sample aliquots (1.00 cm^3) of every test sample was placed in a test tube containing 4.00 cm^3 biuret reagent and incubated for 30 minutes. Absorbance of the content of the test tubes was measured at 540 nm with the UV-Visible Spectrophotometer (Jenway 64050). From the linear curve obtained, the concentration of protein in every test sample was calculated according to the standard curve of bovine serum albumin (BSA).

2.2 Anti-nutrients Evaluation

2.2.1 Oxalate

Oxalate content was obtained by the standard AOAC method [18]. Exactly 1.00 g of the oven dried sample was placed in a 250.00 cm^3 conical flask; distilled water (190.00 cm^3) and 6.00 M HCl were added. The mixture was warmed on a water bath at 90ºC for 4 hours and the digested sample was centrifuged at a speed of 2000 rpm for 5 minutes. The supernatant was diluted to 250.00 cm^3. Three 50.00 cm^3 aliquots of the supernatant were evaporated to 25.00 cm^3; the brown precipitate was filtered off and washed. The solution was titrated with concentrated ammonia solution in drops until salmon pink colour of methyl orange changed to faint yellow. The solution was heated on a water bath to 90^0C and the oxalate was precipitated with 5.00% calcium chloride solution (10.00 cm^3). The solution was allowed to stand overnight and centrifuged. The precipitate was washed into a beaker with hot 25% H_2SO_4 diluted to 125.00 cm^3 with distilled water and heated to 90ºC. It was then titrated against 0.05 M $KMnO_4$. The amount of oxalate was obtained by appropriate calculation.

2.2.2 Phytate

Exactly 4.00 g of the ground oven dried sample was soaked in 100.00 cm^3 of 2.00% HCl for 5 hours and filtered. Exactly 25.00 cm^3 of the filtrate was placed into a conical flask and 5.00 cm^3 of 0.30% ammonium thiocyanate solution was added. The mixture was titrated with a standard solution of iron (III) chloride until a brownish-yellow colour persisted for 5 minutes. The amount of phytate was obtained by appropriate calculation [22].

2.2.3 Saponins

Exactly 10.00 g of the ground oven dried sample was taken into 100.00 cm^3 of 20% aqueous ethanol in water and agitated with a magnetic stirrer for 12 hours at 55ºC. The solution was filtered using Whatman no 1 filter paper and the residue was re-extracted with 300.00 cm^3 of 20% aqueous ethanol. The extracts were reduced to about 40.00 cm^3 under vacuum using a rotary evaporator. The extract and 20.00 cm^3 diethyl ether was transferred into 250.00 cm^3 separatory funnel and shaken vigorously. The aqueous layer was discarded and the process of purification was continued until a colourless extract was obtained. The pH of the

remaining aqueous solution was adjusted to 4.50 by adding 4.00 g of NaCl and the solution shaken successively with butanol. The butanolic extract was washed twice with 10.00 cm^3 of 5% NaCl and evaporated to dryness in a fume cupboard to obtain the saponin which was weighed and expressed as a percentage [23].

All determinations were carried out in triplicates, and results were expressed as the mean ± SD of five replicate determinations. Means were compared using Student t-test and the level of significance was determined at $P = 0.05$.

3. RESULTS AND DISCUSSION

3.1 Proximate Composition

The proximate composition determination is a scheme for routine description of materials devised in 1865 by Henneberg and Stohmann of the Weende Experiment Station in Germany [24]. It is often referred to as the Weende System and was mainly devised to separate carbohydrates into two broad classifications: crude fibre and nitrogen free extract (NFE). Currently, the system consists of determinations of water (moisture), ash, crude fat, crude protein and crude fibre.

3.1.1 Moisture

The moisture content of Fadaman Kubanni vegetative part (FP) is 14.36±0.20% and that of Fadaman Kubanni fruit (FF) is 18.63±0.12% as presented in Fig. 1. This result shows that the fruits have higher moisture content than the vegetative parts. When compared with Rafin Yashi vegetative part (CP) which is 12.24±0.15% and Rafin Yashi fruits (CF) with value 13.61±0.33% as also depicted in Fig. 1, the result shows that moisture is higher in the samples obtained from Fadaman Kubanni. There is no significant difference ($P = 0.05$) in the moisture content of the vegetative parts of okra of Fadaman Kubanni and control site but no significant difference was observed in the fruits of okra of both Fadaman Kubanni sites and the control site. Moisture content is a measure of stability and the susceptibility to microbial contamination [25]. Therefore, the low moisture content remains an asset in storage and preservation of nutrients because higher moisture content could lead to food spoilage through increasing microbial action [26].

3.1.2 Ash content

The ash content was 11.62±0.23% and 18.76±0.03% for FP and CP respectively and 7.35±0.10% and 7.04±0.01% for FF and CF respectively as shown in Figs. 1 and 2 respectively. This result shows that the vegetative part has more ash content than the fruits of Fadaman Kubanni samples. When compared with CP and CF, the ranges of ash are close to that of FP and FF. Ash content was high in both Fadaman Kubanni and control sites when compared to fresh okra and other legumes. Ash content is an indication of the mineral content [27], these values indicate that the fruits contain some nutritionally important minerals. There is significant difference ($P = 0.05$) in the ash contents of both the vegetative parts and fruits of okra of Fadaman Kubanni and control sites. This means that the effluents discharge had no effect on the ash content.

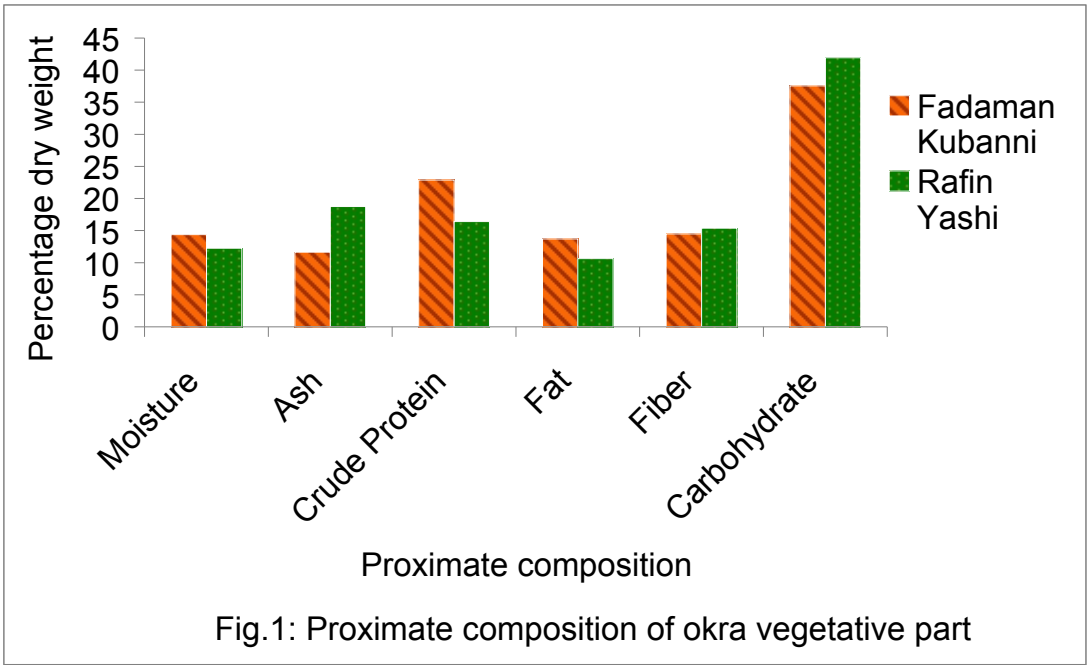


Fig.1: Proximate composition of okra vegetative part

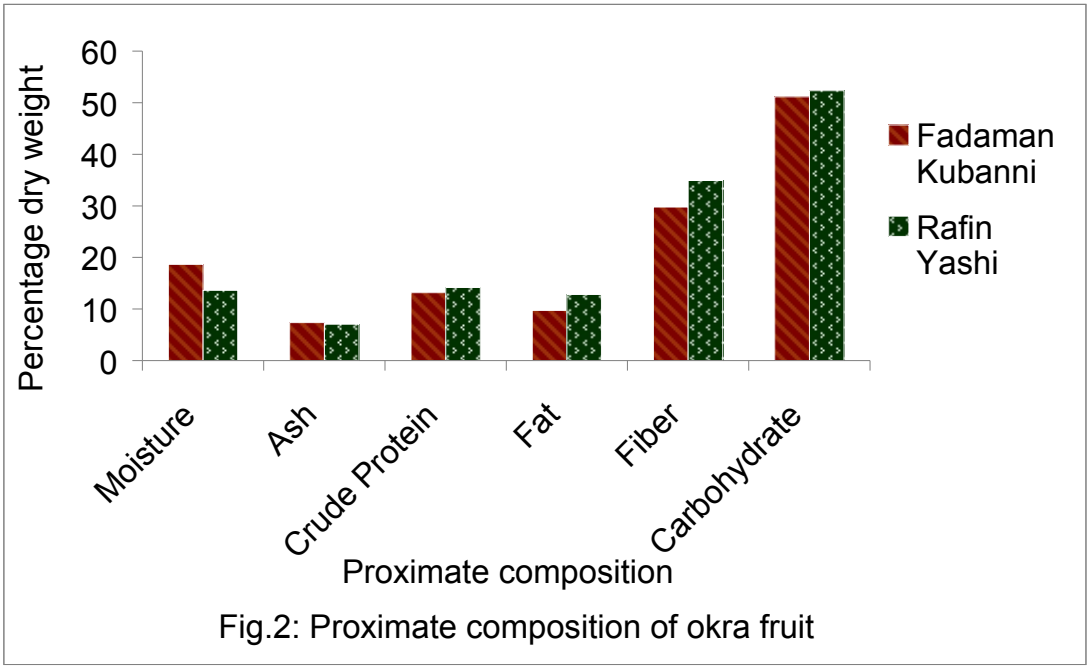


Fig.2: Proximate composition of okra fruit

3.1.3 Crude protein

Crude protein content of FP and FF was 22.87±0.13% and 13.15±0.04%; 16.41±0.00% and 14.18±0.04% for CP and CF as presented in Figs. 1 and 2 respectively. The result is low when compared to the range of 23.73% - 25.48% for crude protein content of Brazillian

varieties of okra [17] but close to the reported protein content that decrease from 14.54% to 12.68% with storage time [28] but also within the reported range of13.61% - 16.27% [29]. There is significant difference (P = 0.05) in the protein content of the fruits of Fadaman Kubanni and control sites but the difference could be attributed to the difference in geographical and climatic factors [30] and assimilation of nutrients from effluent discharge from the Industrial Estate. Okra can be considered a high protein vegetable when compared with *Talinum triagulare, Amarantus hybridus and Celosia argentia* [31] but the protein content of the samples analysed is in agreement with the report that protein content of okra is generally low [32].

3.1.4 Fat content

The fat content of FP and FF was 13.70±0.21% and 9.73±0.12%; 10.63±0.02% and 12.82±0.11% for CP and CF as presented in Figs. 1 and 2 respectively. The results agree with the reported range of 6.94% -9.75% of fat in the mycoflora of okra which decreased with storage time [28]. Results show no significant differences (P = 0.05) in vegetative parts and fruits of okra of Fadaman Kubanni and control site and this difference could be attributed to the discharge from the Industrial Estate into the Fadaman Kubanni site.

3.1.5 Fibre content

Fibre contents of FP and FF were 14.46±0.32% and 29.76±0.23%; 15.36±0.12% and 34.93±0.12% for CP and CF as presented in Figs. 1 and 2 respectively. The fruits contain more fibre than the vegetative part. According to previous report, fibre content for okra fruit of local varieties ranged from 10.15% -11.63% [29]. Present results agree with the range of 25.71% to 29.31% [17]. However, the fibre content of okra can be considered high when compared with *Amarantus hybridus* (1.66%) and *Laurea taraxifolisa* (2.0%) [31]. Significant difference was observed in the fibre content of fruits of Fadaman Kubanni and the control site.

The fibre content is relatively high which may suggest that consumption of okra will aid digestion. Interest in fibre evaluation has increased due to the recent information on the potential role of dietary fibre in human nutrition [33]. Evidences from epidemiological studies suggest that high fibre consumption may contribute to a reduction in the incidence of certain diseases like diabetes, coronary heart disease, colon cancer, high blood pressure, obesity and various digestive disorders [34]. Dietary fibre is known to alter the coronary environment in such a way as to protect against colorectal diseases. It provides protection by increasing faecal bulk, which dilutes the increased colonic bile that occurs with high fat diet [35]. When found in excess, it may bind some essential trace elements leading to deficiency of some minerals such as iron and zinc [36].

3.1.6 Carbohydrate content

The carbohydrate content of FP and FF was 37.56±0.18% and 51.13±0.20%; 41.94±0.23% and 52.34±0.37% for CP and CF as presented in Figs. 1 and 2 respectively. Previous research reported 31.84% carbohydrate in okra seed samples indicating that the samples in the present study had relatively high carbohydrate content [13]. The fruits contain more carbohydrate than vegetative part. There was no significant difference (P = 0.05) in the carbohydrate content of the fruits of Fadaman Kubanni and the control but significant difference was observed in the vegetative parts of Fadaman Kubanni and control.

3.2 Anti-nutritional Constituents

3.2.1 Phytate content

The phytate content of FP and CP did not differ (0.66±0.02 mg/g) between plant organs and the control as shown in Fig. 3 and 0.66±0.03 mg/g and 0.66±0.01 mg/g for FF and CF respectively. There was no significant difference (P = 0.05) in the phytate content of the analysed samples and the control.

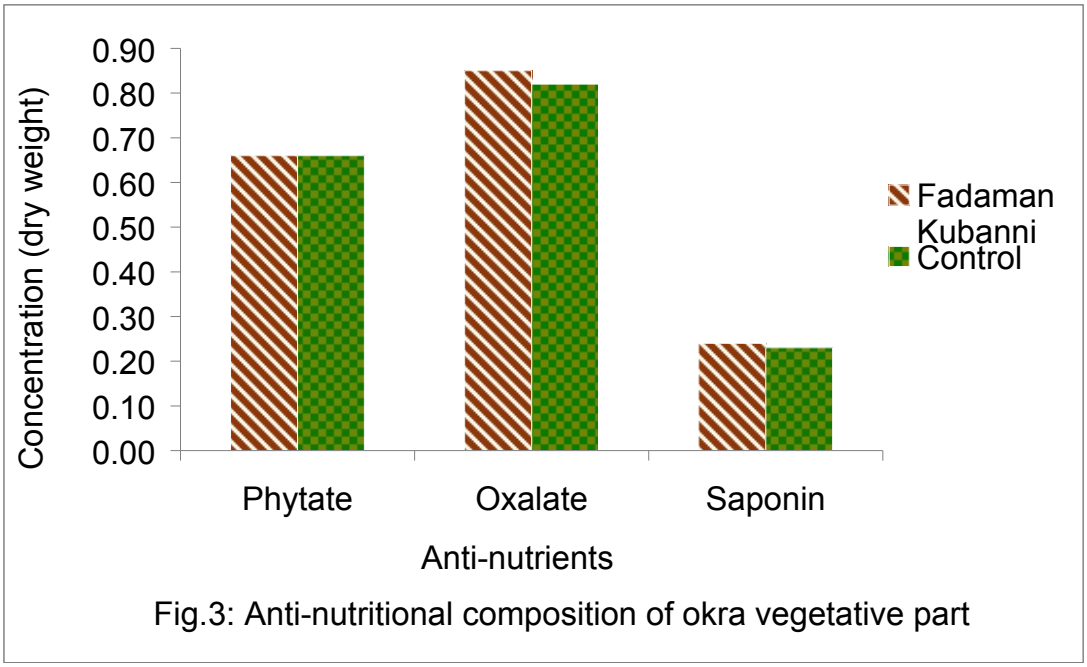


Fig.3: Anti-nutritional composition of okra vegetative part

Phytate is the salt form of phytic acid known as inositol hexakisphosphate (IP6). Phytin refers specifically to the calcium or magnesium salt form of phytic acid [37]. It is the principal storage form of phosphorus in many plant tissues, especially bran and seeds. Phytate is not digestible by humans or nonruminant animals, so it is not a source of either inositol or phosphate if eaten directly.

It has the ability to chelate that is to form complexes with proteins and inhibits the enzymatic digestion of injected protein. Phytic acid chelates, makes unabsorbable certain important micronutrients such as zinc and iron, and to a lesser extent, also macronutrients such as calcium and magnesium [37]. Then phytic acid has some anti-nutritional properties due to its ability to lower the bioavailability of some essential minerals such as zinc and iron, and to a lesser extent, the calcium and the magnesium in the digestive tract resulting in mineral deficiencies [38]. The phytate composition of the samples analyzed is low and might not pose any health hazard [39].

3.2.2 Oxalate content

The oxalate content of FP and CP was 0.83 mg/g ± 0.01 mg/g and 0.82±0.02 mg/g respectively as shown in Fig. 3. This is comparable to already reported range [29]. Samples

FF and CF contained 0.859±0.02 mg/g and 0.830±0.01 mg/g oxalate respectively as shown in Fig. 4. There was significant difference (*P* = 0.05) in the oxalate content of the analysed samples and the control.

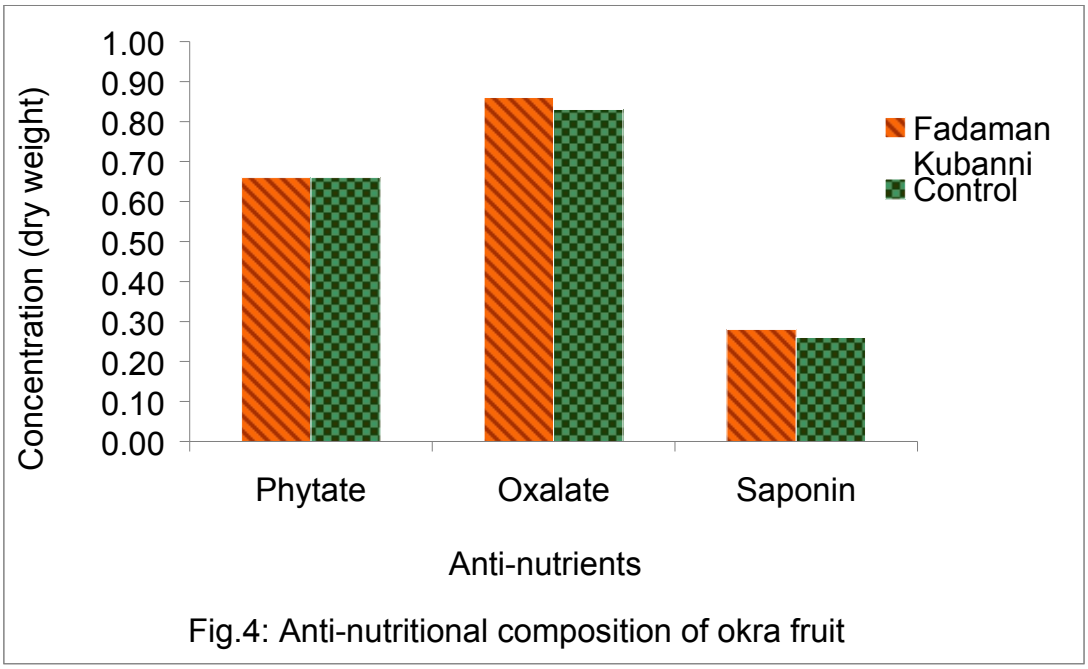


Fig.4: Anti-nutritional composition of okra fruit

Oxalic acid is an organic acid produced in animals and plants when sugar, carbohydrates and other carbon sources are metabolized. Oxalic acid does not circulate freely in the body; it links to sodium or potassium in a soluble oxalate-salt form. But when oxalic acid finds itself in the presence of calcium, it has the ability to link to it too, forming a particular insoluble salt crystal known as calcium oxalate.

Human could obtain oxalates in two ways, some of it is absorbed from the diet and some manufactured in the body primarily in the liver. In humans, about 20-50% is thought to come from diet. Oxalates do not appear to have any necessary function in the body; they are just a metabolic end-product destined to leave the body through urine as urea. A distinctive property of oxalic acid that makes it so dangerous is that once it has linked with calcium, it is practically insoluble at the acidic pH normally found within the body. An unusual characteristic of calcium oxalate is that nothing can dissolve it and that makes it such an exasperating problem. Over consumption of diet with high concentration of oxalate causes kidney stones [40]. The level of oxalate in the samples analyzed is not high to pose any health treat [41].

3.2.3 Saponin content

The saponin content of FP and CP is 0.23 mg/g ± 0.03 mg/g and 0.22±0.01 mg/g respectively as shown in Fig. 3 while FF and CF contained 0.28±0.01 mg/g and 0.26±0.02 mg/g respectively as shown in Fig. 4. Higher levels were observed in fruits than in vegetable in both sites. Saponin levels were generally low compare with results from other researchers [42] but higher than the tolerable level of saponins in ruminants which is 1.50 to 2.00% [43].

There was no significant difference (P = 0.05) in the saponin content of the analysed samples and the control. Saponins are naturally oily glycosides occurring in wide variety of plants. They are dangerous when they get into the blood stream because they quickly haemolyse red blood cells [11,44].

4. CONCLUSION

The study reveals that the moisture content of the samples analysed was low which is advantageous because low moisture content remains an asset in storage and preservation of nutrients. Ash content was high which symbolises the presence of mineral that is indicative of the fact that the fruits contain some nutritionally important minerals. Crude protein content was low which is in agreement with the report that protein content of okra is generally low. Fat content was within the normal ranged presented in literature. Fibre content was relatively high which may suggest that consumption of okra will have some health benefits such as digestion enhancement while carbohydrate content was relatively high with values in the fruits higher than that of the vegetative part. The anti-nutritional analysis showed that all the samples contained phytate, oxalate and saponin. However, values obtained are lower than the established toxic level. Hence they can be consumed without any restriction. However, consumption of large amounts of fruits with higher levels of anti-nutrients should be avoided.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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