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Proximate, Anti-Nutrients and Elemental Constituents of *C. esculenta*

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Authors' contributions

This work was carried out in collaboration among all authors. Author OEC designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors AON and OEC managed the analyses of the study. Authors AIC and OEC managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Cocoyam (*C. esculenta*) is an aroid that is grown most importantly for edible corms. *C. esculenta* is used by the locals, it has been noted that when used as feed, it causes swelling of the lips, mouth and throat. The aim of this work was to investigate the proximate, anti-nutrients and elemental constituents of *C. esculenta*. Proximate, elemental and anti-nutrient constituents of *C. esculenta* were investigated. For the mineral composition Atomic absorption spectrometer was used, while a standard method of analysis by American Organization of Analytical Chemistry was adopted for the proximate analysis and anti-nutrient composition. After the experiment, the data obtained were presented as mean± SD and were subjected to statistical analysis using student T-test, to determine the level of significance at p≤0.05. The results showed that for the two samples, the flour processed with the bark has highest concentrations of carbohydrate and minerals, but the bark has a higher concentration of anti-nutrients which causes swollen lips, scratchiness of throat and astringent taste.

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Keywords: C. esculenta; proximate; antinutrient; mineral element; saponin; calcium; carbohydrate.

1. INTRODUCTION

Cocoyam (Colocasia esculenta) is a traditional root crop that can be found in different parts of the eastern state [1]. It belongs to the family Araceae [2]. Colocasia esculenta is used as vegetables for their corms, leaves and petioles [3].

According to Tagodoe and Nip [4], C. esculenta flours are low in fat, protein and ash, rich in starch and total dietary fibre. C. esculenta corm is composed of anthocyanins [5]. Generally cocoyam contains 20%-28% starch but C. esculenta contains 24.5% and its starch granules are 5-6 sided polygons [6]. Ramsden and Ling [7] reported about several abundance of watersoluble arabinogalactan proteoglycan in C. esculenta and the high content arabinogalactan contributes to the past like character peculiar to C. esculenta corms. De Castro et al. [8] and Monte Neschich et al. [9] observed the presence of two major but unrelated globulin families during the tuber development in C. esculenta. C. esculenta corm polyphenol oxidases have been characterized [10]. Raw C. esculenta corm is acidic [11]. C. esculenta is used as a raw material for brewing [12]. The high digestibility of the small size of C. esculenta granules makes it very suitable for processed baby foods [13].

The soft white-fleshed of *C. esculenta* are eaten boiled, fried or roasted as a side dish or are used for making fufu, and is used in the preparation of soups and puddings. It is said to reduce dental decay in children. The corms are processed into flour, used for biscuits and bread; they are also boiled and made into a paste that is left to ferment to produce 'poi' [14].

C. esculenta leaves and leaf stalks are used as a leafy vegetable and potherb for soups and sauces, or as relish. Leaves and leaf stalks of the dasheen type seem to be less acrid than those of the eddoe type. The stolons that are formed in some types are eaten too [15].

Medicinal uses of *C. esculenta* are few. In Gabon rasping from the corm are applied as a poultice to maturate boils, and to treat snakebites and rheumatism. In Mauritius, boiled young leaves are eaten to treat arterial hypertension and liver infections, whereas juice is applied externally to treat eczema. In Madagascar the corms are used to treat boils and ulcers. In Gabon the leaves in

combination with leaves of *Tephrosia* sp. are reportedly used in fish poison [16].

They are easily perishable due to its moisture composition which results to its short storage life. [17]. It contributes to about 20-48% of the total calories and about 7.1% protein to the diets of people of Nigeria and other parts of the world [18]. In the Southern part of Nigeria, they serve as the main source of calories which constitute over 50% of the calorie consumption of the people [19]. Cocoyam is fair with about 7-9% protein and calcium, which is why it has some nutritional advantages over the other root and tuber crops [20]. Although cocoyam having rare characteristics such as being rich in vitamins, minerals and small starch grains it remains a neglected food crop especially in malnourished rural households [21].

Although *C. esculenta* is used by the locals, it has been noted that when used as feed, it causes swelling of the lips, mouth and throat as well as astringent test. It is important to research on the anti-nutrient properties to find out which of its anti-nutrient property that causes the astringent taste and scratchiness in the mouth and throat when eaten raw or boiled. The aim of this work was to investigate the proximate, antinutrients and elemental constituents of *C. esculenta*.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

The specie of cocoyam (*Colocasia esculenta*) used for this work was obtained from a local farm in Awo Mberi Mbaitolu local government on the 21th of April 2017. It was identified by a Botanist in Biological Science Department Gregory University. The sample was washed and air dried, then it was divided into two- sample A and R

sample A: the cocoyam samples were cut into smaller pieces with the bark.

Sample B: the cocoyam samples bark were first peeled and then cut into smaller pieces.

Both groups of samples were dried in an oven at the temperature of 40°C after which it was milled with a blender into flour and stored in air-tight labeled bottles and kept in a cool dry place for further analysis.



Fig. 1. C. esculenta tubers

2.2 Antinutrients

2.2.1 Oxalates concentration (titration method)

This determination involves three major steps digestion, oxalate precipitation and permanganate titration

2.2.2 Digestion

Sample (2 g) was suspended in 190 ml of distilled water in a 250 ml volumetric flask. HCl (10 ml) was added and the suspension digested at 100°C for 1 hour. Cooled and then made up to 250 ml mark before filtration.

2.2.3 Oxalate precipitation

Duplicate portions of 125 ml of the filtrate were measured into beakers and four drop of methyl red indicator added. This was followed by the addition of NH₄OH solution (drop wise) until the test solution changes from salmon pink color to a faint yellow color pH4-4.5. Each portion heated to 90°C, cooled and filtered to remove precipitate containing ferrous ion. The filtrate was again heated to 90°C and 10 ml of 5% CaCl₂ solution was added while being stirred constantly. After heating, it was cooled and left overnight at 25°C. The solution was then centrifuge at 2500 rpm for 5minutes and the supernatant was decanted and the precipitate completely dissolved in 10 ml of 20% (v/v) $\rm H_2SO_4$ solution.

2.2.4 Permanganate titration

At this point, the total filtration resulting from digestion of 2 g of flour was made up to 300 ml. aliquots of 125 ml of the filtrate was heated until near boiling and then titrated against 0.05 M standardized $KMNO_4$ solution to a faint pink color which persists for 30s and calcium oxalate content was calculated using the formula

$$\left(\frac{T \times (Vme)(Df) \times 105}{(ME) \times Mf}\right) \left((mg/100g)\right)$$

Where T is the titre of $KMn0_4$ (ml), Vme is the volume-mass equivalent (i.e. 1 ml of 0.05 m $KMn0_4$ solution is equivalent to 0.00225 g anhydrous oxalic acid). Df is the dilution factor Vt/A (2.4 where Vt is the total volume of titrate (300 ml) and A is the aliquot used (125 ml), ME is the molar equivalent of $KMn0_4$ in oxalate ($KMn0_4$ redox reaction) and Mf is the mass of sample used [22].

2.2.5 Saponins concentration

Exactly 5 g of the sample was put into 20% acetic acid in ethanol and allowed to stand in a water bath at 50°C for 24hours. This was filtered and the extract was concentrated using a water bath to one-quarter of the original volume. Concentrated NH₄OH was added drop-wise to the extract until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed. The saponin content was weighed and calculated in percentage [23].

Calculation:

%saponin content = ((weight of filter paper + residue) – (weight of filter paper))/ (Weight of Sample Analyzed) ×100

2.2.6 Cardiac glycosides concentration

Wang and Filled [24] method was used. To 1 ml of extract was added 1 ml of 2% solution of 3,5-DNS (Dinitro Salicylic acid) in methanol and 1 ml of 5% aqueous NaOH. It was boiled for 2 minutes (Until brick-red precipitate was observed) and the boiled sample was filtered. The weight of the filter paper was weighed before filtration. The filter paper with the absorbed residue was dried in an oven at 500 c till dryness and weight of the filter paper with residue was noted.

The cardiac glycoside was calculated in %.

Calculation:

% cardiacglycoside = ((weight of filter paper + residue) – (weight of filter paper))/ (Weight of Sample Analyzed) ×100

2.2.7 Tannins concentration (follins dennis titration)

The follins dennis titrating method as described by Pearson [25] was used. To 20 g of the crushed sample in a conical flask was added 100 mls of petroleum ether and covered for 24 hours. The sample was then filtered and allowed to stand for 15 minutes allowing petroleum ether to evaporate. It was then re-extracted by soaking in 100 ml of 10% acetic acid in ethanol for 4hrs. The sample was then filtered and the filterate collected.

25 ml of NH₄OH were added to the filter ate to precipitate the alkaloids. The alkaloids were heated with electric hot plate to remove some of the NH₄OH still in solution. The remaining volume was measured to be 33 ml. 5 ml of this was taken and 20 ml of ethanol was added to it. It was titrated with 0.1M Na₀H using phenolphthalein as indicator until a pink end point is reached. Tannin content was then calculated in % $(C_1V_1 = C_2V_2)$ molarity.

Calculation:

Data

 C_1 = conc. of Tannic Acid C_2 = conc. Of Base

 V_1 = Volume of Tannic acid V_2 = Volume of Base

Therefore $C_1 = C_2V_2/V_1$

% of tannic acid content = $C_1 \times 100$ / Weight of sample analyzed

2.2.8 Phytates concentration

Phytate contents were determined using the method of Young and Greaves (1940) as adopted by lucas Markakes [26]. 0.2 g of each of the differently processed *colocasia* sample was weighed into different 250 ml conical flasks. Each sample was soaked in 100 ml of 2% concentrated HCL for 3hr, the sample was then filtered. 50 ml of each filtrate was laced in 250 ml beaker and 100 ml distilled water added to each sample. 10 ml of 0.3% ammonium thiocynate solution was added as indicator and titrated with standard iron (iii) chloride solution which contained 0.00195 g iron per 1 ml.

2.2.9 PhenoIs concentration

The quantity of phenol was determined using the spectrophotometer method as described by AOAC (2005) [27]. The sample is boiled with 50 ml of (CH $_3$ CH $_2$) $_{20}$ for 15min. 5 ml of the boiled sample was then pipette into 50 ml flask, and 10 ml of distilled water is added. After the addition of distilled water, 2 ml of NH $_4$ OH solution and 5 ml of concentrated CH $_3$ (CH $_2$) $_3$ CH $_2$ OH was added to the mixture. The samples was made up to the mark and left for 30min to react for color development and was measured at 505 nm wavelength using spectrophotometer.

2.2.10 Cynogenic glycosides concentration by AOAC (2005) [27] acid titration method

Samples (10 -20 mg) is placed, ground to pass N0.20 sieve into 800 ml kjeldahl flask 100 ml H_2O is added and macerated at room temperature for 2hours. H_2O (100 ml) is added, steamed and distilled, collecting distillate in 20 ml 0.02N AgNO₃ acidified with 1 ml HNO₃. Before distillation adjusts appropriately, so that tip of condenser dips below surface of liquid in receiver. When 150 ml is passed over, filtered and distillate is passed through a gooch wash receiver and gooch with little H_2O . Excess AgNO₃

is titrated in combined filtrate and washed with 0.02N KCN, using Fe alum indicator. 1 ml 0.02N $AgNO_3 = 0.54$ mg HCN.

2.3 Proximate Analysis

2.3.1 Moisture content [27]

A petri-dish was washed and dried in the oven. Approximately 1-2 g of the sample was weighed into petri dish. The weight of the petri dish and sample was noted before drying. The petri-dish and sample were put in the oven and heated at 100 °C for 1hr the result noted and heated another 1hr until a steady result is obtained and the weight was noted. The drying procedure was continued until a constant weight was obtained

% moisture content =
$$\frac{W1 - W2 \times 100}{Weight \text{ of sample}}$$

Where W_1 = weight of petri-dish and sample before drying

 W_2 = weigh of petri-dish and sample after drying.

2.3.2 Ash content

[27]. Empty platinum crucible was washed, dried and the weight was noted. Approximately 1- 2 g of wet sample was weighed into the platinum crucible and placed in a muffle furnace at 100°C for 3 hours. The sample was cooled in a desiccator after burning and weighed.

Calculations

% Ash content = $W_3 - W_1 / W_2 - W_1 \times 100/1$

Where

W₁ = weight of empty platinum crucible

W₂ = weight of platinum crucible and sample before burning

 W_3 = weight of platinum and ash.

2.3.3 Crude fiber [27]

Sample (2 g) was defatted with petroleum ether and boiled under reflux for 30 minutes with 200 ml of a solution containing 1.25 g of H_2SO_4 per 100 ml of solution. The solution was filtered through linen and washed with boiling water until the washings are no longer acidic.

Transferred the residue to a beaker and boiled for 30 minutes with 200 ml of a solution containing 1.25 g of carbonate free NaOH per 100 ml. Filtered the final residue through a thin

but close pad of washed and ignited asbestos in a Gooch crucible. Dried in an electric oven and weighed. Incinerated also, cooled and weighed.

The loss in weight after incineration x 100 is the percentage of crude fiber.

% crude fiber =
$$\left(\frac{\text{Weight of fiber x 100}}{\text{Weight of sample}}\right)$$

2.3.4 Crude fat (soxhlet fat extraction method) [27]

Clean boiling flasks (250 ml) were dried in oven at $105 - 110^{\circ}\text{C}$ for 30 minutes after which they were transferred into a desiccator and allowed to cool. The cooled boiling flasks were weighed correspondingly and labeled. Filled the boiling flasks with 300 ml of petroleum ether (boiling point $40 - 60^{\circ}\text{C}$) and plugged the extraction thimble lightly with cotton wool. Assembled the soxhlet apparatus and allowed to reflux for about 6 hours.

Removed the thimble with care and collected petroleum ether in the top container of the set – up and drain into a container for re – use. When flask was almost free of petroleum ether, it was removed and dried at 105°C- 110°C for 1hour. Transferred from the oven into a desiccator and allowed to cool before weighing.

% fat =
$$\left(\frac{\text{wt of flask + oil - wt of flask } \times 100}{\text{Wt of sample}}\right)$$

2.3.5 Crude proteins (AOAC, 2005) [27]

Exactly 0.5 g of sample was weighed into a 30 ml kjehdal flask and then 20 ml of sulphuric acid and 0.5 g of the kjedahl catalyst was added. The mixture was heated cautiously in a digestion rack on the digester until a clear solution appeared. The clear solution was then allowed to stand for 30 minutes and allowed to cool. After cooling was made up to 100 ml with distilled water to avoid caking and then 5 ml was transferred to the kiedahl distillation apparatus, followed by 5 ml of 40% sodium hydroxide. A 100 ml receiver flask containing 5 ml of 2% boric acid and indicator mixture containing 5 drops of Bromocresol blue and 1 drop of methlene blue was placed added under a condenser of the distillation apparatus so that the tap was about 20 cm inside the solution and distillation commenced immediately until 50 drops gets into the receiver flask, after which it was titrated to pink color using 0.01N hydrochloric acid.

Calculations

% Nitrogen =Titre value x 0.01 x 14 x 4 % Protein = % Nitrogen x 6.25

2.3.6 Carbohydrate determination (differential method) [27]

100 – (%Protein + %Moisture + %Ash + %Fat + %Fibre)

2.4 Elements

Heavy metal analysis was conducted using Varian AA240 Atomic Absorption Spectrophotometer according to the method of APHA 1995 [28] (American Public Health Association).

2.4.1 Working principle [29]

Atomic absorption spectrometer'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 their own characteristic absorption wavelength, a source lamp composed of that element is used, making the method relatively free from spectral interferences. The amount of energy of the characteristic wavelength absorbed in the flame is proportional to the concentration of the element in the sample. Obtained and diluted the digest with distilled water to the 50 ml mark.

2.4.2 Statistical analysis

After the experiment, the data obtained were presentated as mean± SD and were subjected to statistical analysis using student T-test, to determine the level of significance at p≤0.05.

3. RESULTS

After the experiment; the following results are obtained.

3.1 Proximate Analysis

this result Fig. 2a, shows the ash content of sample A and B are almost on the same level, the protein content of sample A is slightly higher than that of sample B, the fibre content of sample A is slightly higher than B, the fat content of sample A and B are also almost on the same level, the moisture content of sample A is slightly higher than that of B and the carbohydrate content of sample A is obviously lower than that of B. Although most of the component assessed tends to be higher in sample A. The observed differences between the two samples were not statistically significant p≤0.05.

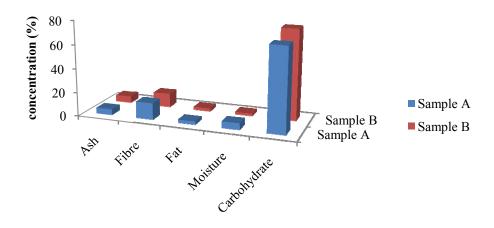


Fig. 2a. Result for proximate analysis



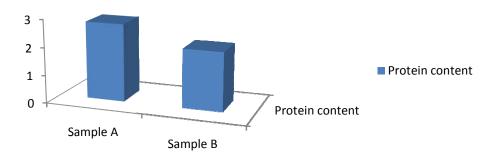


Fig. 2b. Result for protein analysis

3.2 Anti-Nutrient

In Fig. 3A, sample A shows higher saponin content than that of B, while tannin, phytate and cardiac glycoside shows only slight variation in between both sample. There appears to be no significant difference in tannin, phytate and cardiac glycoside of both sample; but sample A saponin content is significantly higher than that of sample B at p≤0.05. in Fig. 3B, the presence of oxalate in Sample A and Sample B are observed

to be within the same range. There is no significant difference observed between the oxalate content of both sample. the result in Fig. 3C, showed that sample B has a higher cyanogenic glycoside content than that of sample A. Cyanogenic glycoside content of sample B is significantly higher than that of sample A at p \leq 0.05. it was observed that the phenol content of sample A is significantly higher than that of sample B at p \leq 0.05 (Fig. 3D).

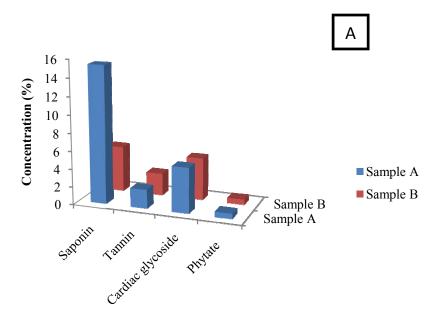


Fig. 3a. Results for anti-nutrient analysis

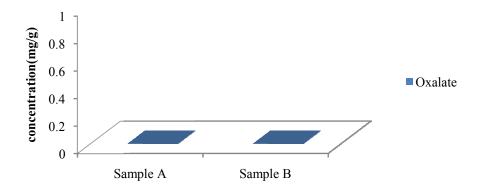


Fig. 3b. Oxalate content

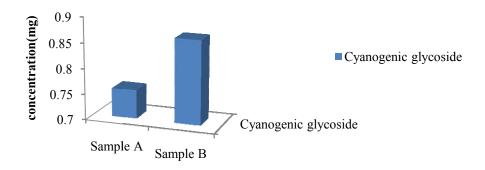


Fig. 3c. Cyanogenic glycoside content

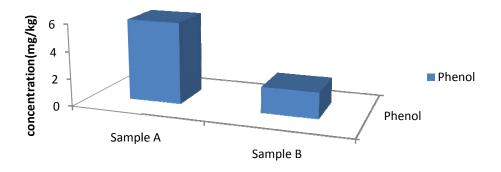


Fig. 3d. Phenol content

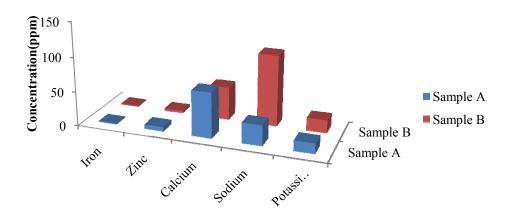


Fig. 4. Result for mineral element analysis

3.3 Mineral Analysis

it can be observed that the level of iron in sample A and B are the same, level of zinc in sample A is slightly higher than that of sample B, level of calcium in sample A is higher than that of sample B, the level of sodium in sample B is higher than that of sample A and the level of potassium in sample B is slightly higher that of sample A. of all the variation observed only sodium and calcium shows statistical difference in their content in both samples at p \leq 0.05.

4. DISCUSSIONS

From the results obtained from proximate analysis, the protein content of the *C. esculenta* flour processed with the bark (2.80%) is higher than that of the flour processed without the bark (2.10%). This exhibited a significant difference when compared with the protein content of raw *C. esculenta* grown in Ethiopia (6.44%) and could be as a result of different weather conditions and soil composition amongst other factors [30]. However, the value (i.e. 6.44%) is higher than the values reported by [31]. On the other hand, the low protein content of my result is in agreement with the works of Tagodoe and Nip [4] that *C. esculenta* flours are low in protein.

The fat content of the flour processed with the bark (2.65%) is higher than that of the flour processed without the bark (2.50%). The values used in the study is relatively higher than the values of 0.83% reported by Nwanekezi et al, 2010 [32] and 0.47% reported by Eze and Okorji,

2003 [33]. It can be deduced from the results above that flour are not excellent sources of fat and should be recommended for obese patients [34].

The fibre content of flour processed with the bark (13.80%) is higher than that of the flour processed without the bark (11.70%). The values of the present study strongly disagrees with the works of Perssini and Sensidoni [35], who reported *C. esculenta* to have a relatively low yield of dietary fibre with values that range from 0.4-2.9% as well as the value of 0.20% reported by Nwanekezi et al. [32].

The ash content of the *C. esculenta* flour processed with the bark (5.85%) is slightly higher than that of the flour processed without the bark (5.10%). The result of this analysis falls under the ranges of the total ash content *C. esculenta* of flours (4.4-5.44%) but higher than the ash contents reported by Moy et al., 1989 [36]. The differences in the ash contents can be as a result of soil type, climatic factors and varietal difference.

The moisture content of *C. esculenta* flour processed with the bark (5.40%) is higher than the flour processed without the bark (2.00%). The result of the analysis totally disagrees with the previous work of Bradbury [37], which showed *C. esculenta* to have 69.2%. However, this value differs slightly with some previous report which showed *C. esculenta* to have about 73-78% moisture. So many factors including time of harvest of samples, age and

environmental conditions contributed to the variance in the moisture contents reported by some other author.

Of the solid nutrient content in roots and tuber crops, carbohydrates predominate [19] and as such is proven by the result of this analysis which shows the carbohydrate of *C. esculenta* flour peeled with the bark (75.85%) to be higher than the flour peeled without the bark (70.25%) and in agreement with the value of 84.1%in the work of [38].

Furthermore, the anti-nutrient results showed oxalate content of the flour peeled with the bark (0.027 mg/g) to be the same as the flour peeled without the bark (0.027 mg/g). This result agrees with the work of Famurewa et al. [39] which shows oxalate to be low in *C. esculenta* flours. On the other hand, the result of this study is not comparable with that of the works of Eddy et al, 2007 [19] and Adane et al., 2013 [30] whose results of oxalate are relatively on the high side with 5.01 mg/g and 243.06 mg/g respectively.

The tannin content of the *C. esculenta* flour processed without the bark (2.5%) is slightly higher than the flour processed with the bark (2.1%). The values in this study is in line with the work of Famurewa et al. [39] with the value of 2.7% but different with the finding of Adane et al [30] whose value is on a high side with 47.39%.

The phytate content of the *C. esculenta* flour processed with the bark (0.58%) is same value with the flour processed without the bark (0.58%). The values of this analysis shows phytate to be higher in concentration than the work of Famurewa et al. [39] with the value of 0.24% but varies with the works of Eddy et al. [19] and Adane et al, 2013 [30] with the values of 115.43 mg/g and 88.21 mg/g respectively.

The saponin content of the *C. esculenta* flour processed with the bark (15.34%) is much higher than the flour processed without the bark (5.14%). The work Famurewa et al. [39] falls into the range of (5.14%-15.34%) of the present study.

The cyanide content of the *C. esculenta* flour processed without the bark (0.864 mg) is slightly higher than the flour processed with the bark (0.756 mg). The lethal dose of cyanide was reported by Eddy et al [19] to be 35 mg which is relatively higher than the values of the present study, while Famurewa et al. [39] did not detect any at all in his work. Beinroth et al. [40] reported

that cyanogenic glycoside exerts inhibitory effect at the cytochrome oxidase level in the electron transport chain.

The phenol content of this present study for the *C. esculenta* flour processed with the bark (5.99 mg/kg) is higher than the flour processed without the bark (1.874 mg/kg). These values is higher than the phenol content of the red and white *C. esculenta* leaves with the values of (0.02 mg/kg and 0.27 mg/kg) respectively in the work of Adeniyi [41].

The cardiac glycoside content of this analysis for the *C. esculenta* flour processed with the bark (5.06%) is higher than the flour processed without the bark (4.74%).

Among the minerals analyzed, the composition of Fe is the least with values of 1.613 ppm for the flour processed with the bark and 1.852 ppm for the flour processed without the bark and when compared to the value in the work of Adane et al. [30] it is lower. The values for the Zn of the *C. esculenta* flour processed with and without the bark of this study are 6.124 ppm and 3.036 ppm and the values are low as in the findings of Eddy et al. [19] but not in line with the work of Adane et al. [30] whose value is relatively on the high side with the value of 43.08 mg/g.

The calcium content of *C. esculenta* in the present analysis with the values of 65.673 ppm and 47.843 ppm of the processed with the bark and without the bark respectively is within the range reported by Huang et al. [42] and higher than the value 13.953 mg/g in the work of Eddy et al. [19]. The value for Na in the work of Adane et al. [30] is less than the values previously reported by Eddy et al. [19], Njoku and Ohia [43], including the present study with the values of 29.497 ppm and 103.303 ppm for the *C. esculenta* flour processed with and without the bark respectively.

The potassium content of the *C. esculenta* flour processed with the bark (14.637 ppm) is lower than the flour processed without the bark (18.537 ppm). Both values does not agree with the work of Eddy et al. [19] whose value (500.510 mg/g) is high.

5. CONCLUSION

The aim of this work was to investigate the proximate, anti-nutrients and elemental constituents of *C. esculenta*. This was done to ascertain the degree of variance present in both

samples. It is important to note that *C. esculenta* can be a good source of dietary energy and essential minerals. On the other hand, it contains some anti-nutritional factors such as oxalates and phytates which can limit the utilization of *C. esculenta* nutrients for human consumption and animal feed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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