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Nutritional and antinutritional composition of seeds of ripe and unripe *Blighia sapida* (K. Koenig)

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ABSTRACT

The proximate, antinutrient, mineral and amino acid composition of ripe and unripe *Blighia sapida* (*ackee*) seed were quantified using standard analytical techniques. Fat, fibre and ash were higher in the unripe seed while protein and carbohydrate were more in the ripe seed. The protein content of the ripe and unripe *Ackee* seed were 11.67% and 9.63% respectively; values obtained for fat were 18.00% and 22.50%, ash 2.83 and 4.17%, fibre 9.33% and 14.33%, moisture values 9.17% and 7.00%, carbohydrate 49.00% and 41.19%. The mineral constituents (mg/kg) for sodium 12.85±0.45, phosphorus 7.14±0.32, Zinc 3.10±0.09, and potassium 16.55±0.05 were higher in the ripe *ackee* seed while iron 43.75±1.16, manganese 3.09±2.81, calcium 2.85±0.15 and copper 2.15±0.45 were higher in the unripe *ackee* seed respectively. Eighteen amino acids were found in the samples in this study; 9 essential amino acids with histidine and 9 non-essential amino acids. Glutamate, aspartate and leucine, were the most abundant amino acids in the samples. The ratios of essential amino acids (EAA) to non-essential amino acids (NEAA) in the ripe and unripe *ackee* seeds was 0.71. The values of the common antinutrients; cyanide, phytate and oxalate were similar in both seed samples; however, trypsin inhibitory activity was higher in the unripe *ackee* seed. This study shows that *ackee* seeds can be a potential alternative source of livestock feed.

Introduction

Seeds are essential part of a balance diet because of their nutritive and calorific values. The ultimate achievement in employing plant proteins as food ingredients largely depends upon the nutritive value they impact to foods, which conversely depend largely on their functional and nutritional properties [45]. *Blighia sapida*, commonly known as *Ackee* (English), *Gwanja Kusa* (Hausa; Northern Nigeria), *Isin* (Yoruba; Western Nigeria), *Okpu* (Igbo; Eastern Nigeria) and *Yila* (Nupe; North Central Nigeria) is a soap berry plant of the family *Sapindaceae*. It is a perennial herbaceous plant that is prominently found in Western Tropical Africa and was imported to Jamaica in the16th century mainly as food for residents. It gained scientific recognition in 1793 when Captain William Bligh in honour of whom it was named '*Blighia sapida*' introduced it to England [27]. It produces a good yield of 7.5 to 10 cm long fruits almost all the year round, with two peak fruiting seasons of January to March and June to August [27]. Upon ripening the fruits splits, exposing freshly cream-coloured arils attached to shiny black seeds [3]. The fresh aril is eaten after been made into sauce or fried in oil [9]. *Ackee* widely serves as part of the national dish in Jamaica. It is also popular among Jamaicans in the United States and Canada, countries where it was previously prohibited. The aril annually generates more than 13 million U. S dollars for Jamaica [38]. In order to preserve it over a long period, it is sundried in Côte d'Ivoire, and this makes it readily available for consumption. However, an apparent setback in its application is the toxicity which manifests as diarrhoea, hypoglycaemia, nausea and vomiting commonly known as Jamaican vomiting sickness (JVS) or toxic hypoglycemic syndrome [30]. The toxicity is due to the toxic amino acids hypoglycin A and B present in the unripe arils but have been reported to decrease by 13 and 7 folds respectively upon ripening [30], hence, all self-opened *ackee* fruits have been found to be quite safe for consumption. The LD_{50} of hypoglycin A is 90 mg kg^{-1} for adult rats [24] and 10-20 mg $kg^{-1}$for rabbits and monkeys. Formulation of feed for livestock in Africa involves the use of energy-giving ingredients such as grain by-products (maize bran, wheat bran, rice by-products) and proteinous ingredients like (cotton seed cake, sunflower seed cake) and leaf meals. The utilization of indigenous food commodities to formulate local and home-based complementary foods is being practiced in many developing countries. Likewise, sustainable livestock production is dependent on the availability of various sources of nutrients that are required for the formulation of animal feed. Principally among these protein and energy sources are groundnut, soybeans and maize which are also important foodstuff for humans [21b, 46]. Thus, there is competition for the limited common foodstuff and hence the high cost which is ultimately translated into high cost of animal protein. With increasing global demand for livestock products, research into locally available food with potential use as additional sources of protein and energy is imperative. Therefore, the aim of this study was to assess the nutritional composition of the seeds of ripe and unripe *Ackee* (*Blighia sapida)* that may be useful for application in the feed industries.

Materials and methods

Materials

Chemicals: All chemicals used were of analytical grade and were products of BDH Chemicals Ltd, Poole, England unless otherwise stated.

Collection of Samples:

Blighia sapida (ackee) seeds used for this study were obtained from Garatu village Niger State, Nigeria and were

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identified in the Department of Crop Science, Federal University of Technology Minna Niger State, Nigeria. Seeds were screened to remove bad ones, washed, sundried to constant weight, pulverized using pestle and mortar, sieved, put in an air-tight container and stored until further analysis.

Methods

Proximate Analysis:

The proximate analysis of the samples for moisture, total ash, crude fibre and fat were carried out in triplicate using methods described by [37]. The nitrogen was determined by micro Kjeldahl method described by [37] and the nitrogen content was converted to protein by multiplying by a factor of 6.25. Total carbohydrate content was estimated by 'difference'. All the proximate values were reported in percentage.

Moisture

Moisture was determined by oven drying method. Two grams of well-mixed sample was accurately weighed into clean, dried crucible (W_1). The crucible was allowed in an oven at 105 ^{0}C for 12 hours until a constant weight was obtained. Then the crucible was placed in the desiccator for 30 minutes to cool. After cooling it was weighed again (W_2). The percentage moisture was calculated using the expression:

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

Where

W_1 = Initial weight of crucible + Sample

W_2 = Final weight of crucible + Sample

Ash:

For the determination of ash, clean empty crucible was placed in a muffle furnace at 550 ^{0}C for an hour, cooled in desiccator and the weight of empty crucible was noted (W_1). Two gram of each of sample was placed in the crucible (W_2) and pre-ashed over a burner, until it charred. Then the crucible was placed in a Muffle furnace for ashing at 550^{0}C for 2-4 hours. The appearance of gray white ash indicates complete oxidation of all organic matter in the sample. After ashing, the crucible was cooled and weighed (W_3). Percentage ash was calculated by the following formula:

$$\% \text{ Ash } = \frac{\text{Difference in Weight of Ash x 100}}{\text{Weight of sample}}$$

Difference in weight of Ash = $W_3 - W_1$

Crude Protein

Protein in the sample was determined by Kjeldahl method. 0.25 gram of dried samples was placed in digestion flask, with 6 ml of concentrated Tetraoxosulphate (vi) acid (H_2SO_4) and a speck of Kjeldahl catalyst (mixture of 1g Na_2SO_4 + 0.05g selenium). The flask was swirled in order to mix the contents thoroughly and digested on the digestion block till the mixtures become clear (colourless or greenish in color). The digest was cooled and transferred into 100 ml volumetric flask and made up to marked level with distilled water. Distillation of the digest was performed in Markham Distillation Apparatus. Ten millilitres of digest was introduced in the distillation tube after which 10 ml of 40 % NaOH was gradually added through the same way. Distillation was continued for at least 10 minutes and NH_3 produced was collected as NH_4OH in a conical flask containing 5 ml of 4 % Boric acid solution with few drops of methyl red indicator. During distillation, yellowish colour appears due to NH_4OH. The distillate was then titrated against standard 0.1 N HCl solution till the appearance of pink colour. A blank was also run through all steps as above. Percentage crude protein content of the sample was calculated using the following formula:

% Crude Protein = 6.25 x %N (Correction factor)

$$\%\text{N} = \frac{(S - B) \text{ x N x } 0.014 \text{ x D x } 100}{\textit{Weight of the sample} \text{ x V}}$$

Where

S = Sample titration reading

B = Blank titration reading

N = Normality of HCl

D = Dilution of sample after digestion

V = Volume taken for distillation

0.014 = Milli equivalent weight of Nitrogen

Crude Fat

Crude fat was determined by ether extract method using Soxhlet apparatus. Approximately 2 gram of moisture free sample was wrapped in filter paper, placed in fat free thimble and then introduced in the extraction tube. A weighed, cleaned and dried receiving flask was filled with petroleum ether and fitted into the apparatus. The Soxhlet apparatus was assembled and allowed to reflux for 6hours; extract was transfered into clean glass dish with ether washing which was evaporated on water bath. Then the dish was placed in an oven at 105^{0}C for 1hour and cooled it in a desiccator. The percentage crude fat was determined by using the following formula:

$$\% \text{ Crude Fat} = \frac{\text{Weight of ether extract x 100}}{\text{Weight of sample}}$$

Crude Fibre:

2gram of sample was defatted with petroleum ether; 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 or several layers of cheese cloth on fluted funnel, washed with boiling water until the washings are no longer acidic then the residue was transferred into a beaker and boiled for 30 minutes with 200 ml of solution containing 1.25 g of carbonate free NaOH per 100 ml. The final residue was filtered through a thin but close pad of washed and ignited asbestos in a Gooch crucible, dried in an electric oven, weighed, incinerated, cooled and reweighed. The loss in weight after incineration × 100 is the percentage crude fibre.

Trypsin Inhibitory Activity

A modified method of [47] was employed in the determination of trypsin inhibitory activity. 2 g of the sample was dispensed into 20 ml of 0.50 % (w/v) $NaHCO_3$ buffer, pH 8.3 at 37^{0}C and shaken for 30minutes. The mixture was filtered and the filtrate heated for 10minutes at 60^{0}C. Assay for trypsin activity involved mixing a portion (1ml) of the extract with 2.5 ml of 2.50 % trypsin substrate (albumin) in a test tube containing 1ml of trypsin enzyme solution (10 mg solid / ml). After swirling, the mixture was incubated at 37^{0}C for exactly 30minutes. 1ml of aliquot was removed after incubation and mixed with 4 ml of trichloroacetic acid (TCA), swirled and centrifuged for 5mins at 3000 rpm, the supernatant was collected and the absorbance read at 440 nm in a spectrophotometer. A control which consisted of 1ml enzyme solution in 2.5 ml of trypsin substrate (albumin) but no extract was set up as described above and its absorbance was read. Trypsin inhibitor activity was extrapolated from a standard tyrosin curve.

Phytic Acid

The Phytic acid content was determined using a modified indirect colorimetric method of [49]. The method depends on an iron to phosphorus ratio of 4:6 and is based on the ability of standard ferric chloride to precipitate phytate in dilute HCl extract of the sample. 5 g of the sample was extracted with 20 ml of 3 % trichloroacetic acid and filtered. 5 ml of the filtrate

was used for the analysis; the phytate was precipitated as ferric phytate and converted to ferric hydroxide and soluble sodium phytate by adding 5 ml of 1M NaOH. The precipitate was dissolved with hot 3.2 M HNO_3 and the absorbance read immediately at 480 nm. Preparation of standard curve for phytic acid was done as follows: standard curve of different $Fe(NO_3)_3$ concentrations was plotted against the corresponding absorbance of Spectrophotometer to calculate the ferric iron concentration. The phytate phosphorus was calculated from the concentration of ferric iron assuming 4:6 iron: phosphorus molar ratio.

Cyanide

Cyanide content was determination by alkaline picrate method according to Wang and Filled method as described by [37]. 5 g of powdered sample was dissolved in 50 ml of distilled water in a corked conical flask and the extraction was left undisturbed over-night and filtered. 1ml of the filtrate was mixed with 4 ml of alkaline picrate in a corked test tube and incubated in a water bath for 5minutes. After colour development (reddish brown colour), the absorbance was read at 490 nm. The absorbance of the blank containing 1ml distilled water and 4ml alkaline picrate solution was also recorded. The cyanide content was extrapolated from a cyanide standard curve prepared from different concentrations of KCN solution containing 5-50µg cyanide in a 500l conical flask followed by addition of 25 ml of 1NHCl.

Oxalate

Oxalate in the sample was determined by permanganate titrimetric method by [33] and [37]. 2 g of the sample was suspended in 190 ml of distilled water in 250 ml volumetric flask, 10 ml of 6 M HCl was added and the suspension digested at 100^0C for 1hr, cooled, then made to the mark before filtration. Duplicate portion of 125ml of the filtrate were measured into beakers and 4 drops of methyl red indicator added. This was followed by the addition of conc. NH_4OH solution drop wise until the test solution changes from salmon pink colour to a faint yellow colour (pH 4-4.5). Each portion was then heated to 90^0C, cooled and filtered to remove precipitate containing ferrous ion. The filtrate was again heated to 90^0C and 10 ml of 5 % $CaCl_2$ solution added while being stirred constantly. After heating, it was cooled and left overnight at 5^0C. The solution was then centrifuged at 2500 rpm for 5minutes, the supernatant decanted and the precipitate completely dissolved in 10ml of 20 % (v/v) H_2SO_4 solution. The total filtrate resulting from the digestion was made up to 300ml. 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 colour which persisted for 30seconds. The calcium oxalate content (mg/100g) was calculated using the formular:

$$\frac{T \times (Vme)\,(Df) \times}{(\mathrm{ME}) \times \mathrm{Mf}} 10^5$$

Where T is the titre of $KMnO_4$ (ml), Vme is the volume-mass equivalent ($1cm^3$ of 0.05 M $KMnO_4$ solution is equivalent to 0.00225 g anhydrous oxalic acid), Df is the dilution factor V_T/A (2.4 where V_T is the total volume of titrate (300 ml) and A is the aliquot used (125 ml), ME is the molar equivalent of $KMnO_4$ in oxalate ($KMnO_4$ redox reaction) and Mf is the mass of flour used).

Amino acid analysis

The amino acid profile of the sample was determined using methods described by [16,43], with the Technicon sequential multisample amino acid analyzer (TSM), Model DNA 0209 Technicon instrument company limited Hamilton close Basingstoke RG21 2YE United Kingdom. A known weight of the sample was defatted with chloroform/methanol mixture (2:1) using Soxhlet extraction apparatus for 15 hours as described by AOAC (2006). A known weight of defatted sample was weighed into glass ampoule. 7 ml of 6 N HCl (10ml of 4.2 M NaOH in the case of tryptophan) was added and oxygen expelled by passing nitrogen into the ampoule. The sealed ampoule was put in an oven at about 105°C ± 5°C for 22 hours. The ampoule was allowed to cool before opened at the tip and the content was filtered to remove humins. The filtrate was then evaporated to dryness at 40°C under vacuum in a rotary evaporator. The residue was dissolved with 5ml of acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in the freezer; from this about 5-10 microlitre was loaded into the cartridge of the analyzer for analysis.

Mineral analysis

The mineral composition of the sample was analyzed on aliquots of dry-ashing. 1g of the test portion, was weighed into glazed, high-form porcelain crucible, ashed for 2 hours at 500^0C, and allowed to cool. 10 drops of distilled water was added to the ash, and carefully 3-4 ml of HNO_3 was added. Excess HNO_3 was evaporated on hot plate set at 120^0C. The crucible was returned to the furnace for reashing for additional 1hour at 500^0C after which the crucible was cooled, and the ash dissolved in 10 ml HCl. This was transferred quantitatively into 50 ml volumetric flask. The mineral elements were determined by Atomic Absorption Spectrophotometer (Model Accusy 211 Bulk Scientific USA), sodium and potassium by Flame photometer (Model FP6410 Harris Medical Essex, England), phosphorus was determined by colorimetric means using the vanadomolybdate (yellow) method of [8].

Results and discussion

The ripe *ackee* seed has a higher percentage of carbohydrate (49.00±3.73) while the unripe *ackee* seed yielded a significant ($p<0.05$) percentage of crude fat (22.50±1.80) as shown in Table 1. These values indicate that both samples can serve as good sources of energy, as carbohydrate and fat are known to be the main sources of energy for organisms [29, 42]. The percentage carbohydrate obtained in this study is higher than those reported for papaya, apple, water melon, guava, orange, prickly pear, apricot and paprika seeds which were in the range of (8.54 – 34.74 %) while the percentage fat in this study compared with those reported for guava, prickly pear and paprika seeds (16.20 – 26.88%) but lower than those for papaya, apple, water melon, orange, and apricot seeds (30.73 – 54.2%) [44]. Dietary fats are essential for the structure and biological functions of cells and also increase the palatability of food by absorbing and retaining flavours [7]. A diet providing 12% of its calorie of energy as fat is said to be sufficient for human beings as excess fat consumption is implicated in atherosclerosis, cancer and aging [7].

The percentage crude protein reported in this study is higher in the ripe *ackee* seed (11.67±0.58) than in the unripe *ackee* seed (Table 1), this may be as a result of the utilization of most of the amino acids in the ripe *ackee* seed for protein synthesis. This value is lower than those of protein rich foods such as soybean, cowpea, pigeon pea and pumpkin with protein content ranging between 23.1 and 33.0% [36] and those reported for papaya, apple, water melon, prickly pear, apricot and paprika in the range of 16.60 – 33.79%, but higher than those for orange and guava (3.06 and 7.90%) respectively [44]. The protein content of *ackee* seeds in this study agrees with the percentage recommended by the Food and Agriculture Organisation (FAO, 2003) which is in the range of 12-15%.

Table 1. Proximate composition of ripe and unripe *Blighia sapida* seeds

Parameters (%)	Ripe *Blighia sapida* seeds	Unripe *Blighia sapida* seeds
Moisture content	9.17 ± 0.33^{a}	7.00 ± 0.29^{b}
Total Ash	2.83 ± 0.44^{a}	4.17 ± 0.17^{b}
Crude Fat	18.00 ± 0.29^{a}	22.50 ± 1.80^{b}
Crude Fibre	9.33 ± 3.18^{a}	14.33 ± 6.17^{b}
Crude Protein	11.67 ± 0.58^{a}	9.63 ± 2.20^{b}
Carbohydrate	49.00 ± 3.73^{a}	42.37 ± 3.36^{b}

Values are means of three different determination ± SEM.
Rows with different superscripts are significantly different

Table 2: Quantitative Antinutrient constituents of ripe and unripe *Blighia sapida* seeds

Parameters(mg100g^{1})	**Ripe *Blighia sapida* seeds**	**Unripe *Blighia sapida* seeds**
Cyanide	215.80 ± 0.92^{a}	217.53 ± 0.66^{b}
Phytate	24.80 ± 0.81^{a}	35.27 ± 0.24^{b}
Oxalate	151.14 ± 0.01^{a}	144.32 ± 0.01^{a}
TIA (%)	17.26 ± 2.06^{a}	37.50 ± 0.69^{b}

Values are means of triplicate determination ± SEM.
Rows with different superscripts are significantly different TIA: Trypsin inhibitory activity

Table 3: Mineral composition of ripe and unripe *Blighia sapida* seeds

Parameters (mg/kg)	**Ripe *Blighia sapida* seeds**	**Unripe *Blighia sapida* seeds**
Na^{+}	12.85 ± 0.45^{a}	6.55 ± 0.05^{b}
P	7.14 ± 0.32^{a}	5.09 ± 0.62^{b}
K^{+}	16.55 ± 0.05^{a}	15.7 ± 0.00^{b}
Ca^{2+}	0.35 ± 0.05^{a}	2.85 ± 0.15^{b}
Zn^{2+}	3.10 ± 0.09^{a}	0.31 ± 0.05^{b}
Cu^{2+}	0.34 ± 0.02^{a}	2.15 ± 0.45^{b}
Fe^{2+}	25.90 ± 1.90^{a}	43.75 ± 1.16^{b}
Mn^{2+}	0.58 ± 0.34^{a}	3.09 ± 2.81^{b}
Pb^{2+}	0.10 ± 0.00^{a}	0.40 ± 0.10^{b}

Values are means of duplicate determination ± SEM.
Rows with different superscripts are significantly different

Table 4: Amino acid composition (g/100g crude protein) of *Blighia sapida* seeds

Parameters	Ripe *Blighia sapida* seeds	Unripe *Blighia sapida* seeds
Lysine*	3.21	3.75
Histidine*	2.05	2.20
Arginine	4.83	5.35
Aspartate	8.06	8.50
Threonine *	2.95	3.09
Serine	2.17	2.28
Glutamate	9.09	10.00
Proline	2.24	2.65
Glycine	3.31	3.70
Alanine	3.46	4.10
Cystine	0.62	0.85
Valine *	3.16	3.47
Methionine *	0.89	1.22
Isoleucine*	2.44	2.82
Leucine *	6.94	7.45
Tyrosine	2.70	3.17
Phenylalanine*	3.97	4.22
Tryptophan*	0.44	0.62

*Essential amino acid

Table 5: Classification of amino acid composition (g/100g crude protein) of *B. Sapida* Seeds

Classification	RAS	RAS (%)	UAS	UAS (%)
TAA	62.53	---	69.44	---
TEAA: TNEAA	0.71	---	0.71	---
TSAA: TNSAA	0.02	---	0.03	---
TArAA: TNArAA	0.13	---	0.13	---
TEAA without Histidine	24.00	38.38	26.64	38.36
TEAA with Histidine	26.05	41.66	28.84	41.53
TNEAA	36.48	58.34	40.60	58.47
TSAA	1.51	2.41	2.07	2.98
TNSAA	61.02	97.59	67.37	97.02
TArAA	7.11	11.37	8.01	11.54
TNArAA	55.42	88.63	61.43	88.46
TAAA	17.15	27.42	18.50	26.64
TBAA	10.09	16.14	11.30	16.27

TAA – total amino acid, TEAA – total essential amino acid,
TNEAA – total non-essential amino acid, TSAA – total sulphur amino acid
TNSAA – total non-sulphur amino acid, TArAA – total aromatic amino acid
TNArAA – total non-aromatic amino acid, TBAA – total basic amino acid
TAAA – total acidic amino acid, RAS – ripe *ackee* seed, UAS - unripe *ackee* seed

However, a diet base on *ackee* seed may be supplemented with a complementary protein source to make it more nutritionally efficient.

The ash content is an indication of the degree of the inorganic matter of the samples. The percentage ash content is higher in the unripe *ackee* seed (4.17±0.17), this can be attributed to the mineral composition of the soil. The ash content of both samples in this study compare with the 3.66% obtained for *B. Sapida* root [1], 4.7% for soy bean, 2.7% for roasted peanut [18] and 3.7% obtained for melon seed and also for apple, water melon, orange, prickly pear, apricot and paprika seeds which is in the range (2.32 – 5.42%), and lower than that of papaya (8.89%) but more than that of guava seed (0.96%) [44] and little above the range of 1.5-2.5% recommended for seeds and tubers for animal feed formulation by [40]. If ash content is high, there is a very good chance the forage is contaminated with soil which is not desirable [25]. The normal ash content of legume grass forage is near 9.0% (dry matter basis). Those with more than 10 – 18% ash are likely contaminated with increasing amount of soil. For example in cattle, excess ash can have negative effect on lactation thereby increasing the amount of non- fermentable inorganic matter in some dairy cattle diets [25], on this basis, *ackee* could be considered suitable for animal feeds.

The percentage crude fibre was significantly ($P<0.05$) higher in the unripe *ackee* seed (14.33±6.17). Crude fibre content is commonly used as a measure of the nutritive value of poultry and livestock feeds and also in the analysis of various foods and food products to detect adulteration, quality, and quantity [25]. The value obtained for the samples in this study are higher than those reported for legumes (5-6%) [12], papaya, apple, water melon, orange and apricot ranging between (3.43 – 8.32%) but lower than that of paprika, prickly pear and guava in the of (33.83 – 64.67%) [44].

The moisture content was higher in the ripe *ackee* seed (9.17 ± 0.33) than in the unripe *ackee* seed (Table 1). The moisture content obtained in this study agrees with those reported for legumes by [13] ranging between 7-10%, and higher than that reported for melon seed (4.60%) by [23] and [26] for pumpkin seeds. The low moisture contents obtained for both samples is a good attribute for storage. In addition, the lower moisture contents of the unripe *ackee* seed shows that it may have a longer storage live than that of the ripe *ackee* seed if well packaged.

There was no significant ($P>0.05$) difference in the oxalate content of both samples in this study. However, the values obtained for both samples are much higher than those reported for papaya, apple, water melon, guava, orange, prickly pear, apricot and paprika which are in the range of (0.41 – 3.74mg100g^{-1}) [44]. The concentration of oxalates obtained in this study were quite below the 15-30 g reported to be the lethal dose for this antinutrient hence revealing a safe margin for the *ackee* seeds analysed.

A higher concentration of phytate (35.27±0.24) and cyanide (217.53±0.66) were obtained for the unripe *ackee* seed. The concentration of phytate obtained for the ripe *ackee* seed in this study compares favourably with that of papaya and apple (23.25 and 29.19 mg100g^{-1}), but the concentration of phytate for both samples were higher than that of water melon, guava, orange, prickly pear, apricot and paprika in the range of (1.28 – 13.22 mg100g^{-1}) [44]. However, the values obtained in this study were below reported lethal dose (250-500 mg100g^{-1}) [19].

The values obtained for cyanide in this study were well above the reported lethal dose of 35mg100g^{-1} body weight [21a] or between 50-300 mgKg-1 body weights [17, 4]. High concentrations of antinutrients such as phytate, oxalate, tannins and cyanide have been found to exert substantial effects on mineral bioavailability in foods [48]. Oxalate salts are poorly soluble at intestinal pH (7.8-8) and oxalic acid is known to decrease calcium absorption in monogastric animals [6]. These antinutrients form complexes with nutritionally important minerals such Ca^{2+}, Mg^{2+}, Co^{2+}, Fe^{2+}, Mn^{2+} and Zn^{2+}, thereby limiting the absorption maxima of these minerals by the body systems [5].

The antitryptic activity was significantly ($P<0.05$) higher in the unripe *ackee* seed (37.50±0.69). This result reflects the presence of strong protease inhibitors in the unripe *ackee* seeds. Protease inhibitors, such as trypsin inhibitor in diets lead to the formation of irreversible trypsin enzyme - trypsin inhibitor complexes. This causes a decrease in trypsin in the intestine and subsequently in the digestibility of dietary protein, thus leading to slower animal growth [41].

Iron and potassium were significantly ($P<0.05$) high in both the ripe and unripe *ackee* seed. A higher value was obtained for sodium (12.85±0.45), phosphorus (7.14±0.32), zinc (3.10±0.09) and potassium (16.55±0.05) from the ripe *ackee* seed. The values obtained for calcium (2.85±0.15), copper (2.15±0.45), iron (43.75±1.16), manganese (3.09±2.81), and lead (0.40±0.10)

was higher in the unripe *ackee* seed. Iron was found to be the most predominant mineral in *ackee* seed. This contrast with the observation of [35, 11] that reported potassium as the most abundant mineral in Nigerian agricultural products. However, potassium is second in concentration to iron in *ackee* seed in this study. The Na^+ / K^+ ratio of *ackee* seed is less than one. Thus, on the basis of the recommendation of [32], suggests that *ackee* seed would be suitable for reducing high blood pressure. Conversely, the Ca^{2+} / P ratio of *ackee* seed is far less than one, thus its consumption is likely to reduce the intestinal absorption of calcium. An excess of phosphorus (Ca^{2+} / P ratio lower than 1) has been shown in several species of animals to lower the blood calcium level and to cause secondary hyperparathyroidism with resorption and loss of bone [14]. Also, the elevation of dietary Ca^{2+} / P ratio may inhibit bone loss and increase intestinal calcium absorption in ovariectomized rats [31].

The result presented in Table 4 shows that the unripe *ackee* seeds contained a higher concentration of all the amino acids in contrast to the concentration obtained for the ripe *ackee* seeds. These variations may be attributed to the utilization of the amino acids in the ripe *ackee* seeds for protein synthesis as the fruit ripens. The result shows that glutamate, aspartate and leucine are the three most abundant amino acids in *ackee* seed. This observation closely agrees with that reported for melon seed by [36, 34, 2, 12, 23], except that in this study, in place of arginine that was reported by these authors, leucine was predominant. In addition, this result shows that *ackee* seeds contain essential amino acids such as leucine, isoleucine, lysine, phenylalanine, valine, arginine and threonine. Among the essential amino acid obtained in this study, leucine was present in the highest amount in both the ripe *ackee* seed (6.94 g/100 g protein) and the unripe *ackee* seed (7.45 g/100 g protein). These values obtained for leucine compared very well with the values reported for papaya, apple, water melon, guava, prickly pear, and paprika seeds which are in the range of ($5.61 – 7.76 g100g^{-1}$), but higher than that of apricot and orange seeds in the range (3.45 – 3.94 g/100 g protein) [44]. Sulphur containing amino acids have been reported to be the limiting amino acids in legumes [28, 15]. *Ackee* seed contained moderate amounts of methionine (0.71) which is lower in comparison with that of cashew nut (1.7 g/100 g protein) [10] and cystine, and also lower than the reported value for papaya, apple, water melon, guava, orange, apricot and paprika in the range (0.92 – 4.09 g/100 g protein) [44].

The nutritive value of a protein depends primarily on the capacity to satisfy the needs for nitrogen and essential amino acids. The total essential amino acids (with histidine) of ripe *ackee* seed protein (26.05 g/100 g protein) and unripe *ackee* seed protein (28.84g/100 g protein) shown in Table 5, compares favourably with that reported for papaya, apple, water melon, guava, prickly pear and apricot seeds in the range of (26.17 – 29.49 g/100 g protein) and is greater than that of orange (18.3 g/100 g protein) but lower than that of paprika (34.14 g/100 g protein) [44]. The total acidic amino acid (TAAA) for ripe *ackee* seed protein (17.15 g/100 protein) and unripe *ackee* seed protein (18.50 g/100 protein) was greater than the total basic amino acid (TBAA) for ripe *ackee* seed protein (16.14 g/100 g protein) and unripe *ackee* seed protein (16.27 g/100 g protein) respectively, implying that *ackee* seed protein is probably acidic in nature.

The total sulphur amino acid (TSAA) of the ripe *ackee* seed protein and unripe *ackee* seed protein were (1.51 g/100 g protein) and (2.07 g/100 g protein) respectively, which is about one-third the value (5.8 g/100 g protein) recommended for infants [22]. The aromatic amino acid (ArAA) for the current report (Table 5) are within the range suggested for ideal infant protein (6.8-11.8 g/100 g protein) [22] indicating that if the antinutrients present in *ackee* is removed, *ackee* seed could be used to prepare gruel as weaning food, and should be supplemented with protein rich foods. The percentage ratio of EAA to TAA in the ripe *ackee* seed and the unripe *ackee* seed was 41.66 and 41.53 respectively. These values are well above the 39% considered to be adequate for ideal protein food for infants, 26% for children and 11% for adults [22]. The percentage of EAA/TAA for *ackee* seed could be favourably compared with that of pigeon pea (43.6%) [39], beach pea protein isolates (43.8-44.8%) [20]. Hence, the amino acid profile of *ackee* seed suggests that its protein has nutritive value.

Conclusion

The findings in this study confirm that *ackee* seeds are nutritionally valuable seeds with potentials that could be explored in the industry for livestock feed formulation if the antinutrient composition can be sufficiently eliminated.

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