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Determination of the Nutritive and Anti-Nutritive Values of *Pelophylax esculentus* (Edible Frog) Found in Hanyan Gwari, Minna Niger State, Nigeria

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Authors' contributions

This work was carried out as collaborative research among all the authors. Author JTM designed the study, wrote the protocol, wrote the first draft of the manuscript and carried out pretreatment of the sample. Author MMN managed the literature researches, analyses of the amino acid profile. Authors SSM and EYS managed the experimental processes. Authors ABS and YA identified the species of the amphibian, carried out the mineral and statistical analyses.

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ABSTRACT

The proximate, selected minerals, amino acid profile, functional properties and anti-nutrient composition of edible frog (*Pelophylax esculentus)* were determined using standard analytical methods. The crude protein was 31.17±1.36%, carbohydrate was found to be 29.02±1.16% while the crude fibre was 11.71±0.22%. The crude fat was 16.22±0.16%, ash content was 8.93±1.33% and moisture was 3.49±0.56%. The abundance of mineral elements found in the meat of *P.*

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esculentus was found to be in the order: sodium > phosphorus > potassium > calcium > zinc > magnesium > copper > iron > manganese. The calorific value was 506.17 kcal/100 g while the animal was also found to have reasonable amounts of essential amino acids: tryptophan (0.39), lysine (7.62), arginine (6.13), histidine (2.13), threonine (3.94), valine (4.82), methionine (2.89), leucine (7.22), isoleucine (3.83) and phenylalanine (4.14) all expressed as percentage of protein. Based on its anti-nutritional contents, *P. esculentus* meat could be considered as a good, low cost source of animal protein for man and his animals. It could also be a good source of calcium, potassium and sodium.

Keywords: Edible frog; functional properties; proximate analysis; amino acid profile.

1. INTRODUCTION

Meat is important to human beings and could be obtained from various sources. It is very good source of nutrients and vitamins to the body. Due to its high cost and some health problems associated with red meat, research is now focused on other alternatives especially the animals which would help to take care of these health challenges and would be cheaper and safer for consumption [1]. Since meats contain essential classes of food such as, carbohydrate, proteins, fat, vitamins and minerals, they provide the nutritional requirements of man in the appropriate quantities [2]. The provision of these nutritional entities becomes a major problem in most developing countries such as Nigeria leading to under- or malnutrition. In a view to reduce such menace in Nigeria some lesser known animals which can serve as food are studied for their nutritive and non-nutritive values for human consumption. One class of such known animals that could be considered for this purpose is the amphibian [3].

Pelophylax esculentus (edible frog), formally known as *Rana esculentus*is considered to be of good nutritional value [4]. It is a widespread natural hybrid that is produced as an offspring of the parent species *P. lessonae* and *P. ridibundus* [5]. This frog is the fertile hybrid of the Pool Frog (*Pelophylax lessonae*) and the Marsh Frog (*Pelophylax ridibundus*). It belongs to the kingdom: animalia, phylum: chordate, class: amphibian, order:anuran, family: ranida, genus: pelophylax and species: *P. lessonae* and *P. ridibundu* [5]. The aim of this study was to determine the proximate, mineral, functional properties, anti-nutritional factors and amino acid profile of *Pelophylax esculentus* in order to establish the safety or otherwise of its consumption by humans.

2. MATERIALS AND METHODS

The sample (*Pelophylax esculentus*) used in the course of this work was obtained on 24th May, 2013 from Hanya Gwari Bosso around F. U. T environment in Minna, Niger State. Samples were randomly collected and mixed to obtain a composite sample of the animals.

2.1 Sample Preparation and Treatment

The samples were cut opened (flesh**,** skin and bones) and dried in an air oven at 60°C for 10 hours for proper removal of moisture**.** The fleshy parts of the samples were scrapped using a clean laboratory stainless steel knife, dried again and milled. This was kept in an air tight polythene bag and stored in a desiccator prior to further analysis.

2.2 Methods

2.2.1 Proximate analysis

The standard analytical procedures for food analysis were adopted for the determination of the moisture content, crude protein, crude fibre, percentage lipids, carbohydrate, ash and calorific value.

2.2.1.1 Moisture content

Two grammes of the sample were put into the crucibles, dried in an oven at 105°C overnight. The dried sample was cooled in a desiccator for 30 minutes and weighed to a constant weight. The percentage loss in weight was expressed as percentage moisture content on dry weight basis [6]. This was repeated three times to obtain triplicate values.

2.2.1.2 Ash content

From the dried and ground sample, 2.00 g was taken in triplicates and placed in pre-weighed crucibles and ashed in a muffle furnace at 600°C for 3 hours. The hot crucibles were cooled in a desiccator and weighed. The percentage residual weight was expressed as ash content [6].

2.2.1.3 Crude lipid content

From the pulverized sample, 2.00 g was used for determining the crude lipid by extracting the lipid from it for 5 hours with (60-80°C) petroleum ether in a soxhlet extractor [6]. Triplicate samples were extracted to obtain triplicate values that were later averaged.

2.2.1.4 Protein determination

Total protein was determined by the Kjedahl method. 0.5 g of the sample was weighed in triplicate into a filter paper and put into a Kjedahl flask, 8-10 cm^3 of concentrated H_2SO_4 were added and then digested in a fume cupboard until the solution became colourless. Distillation was carried out with about 10 cm^3 of 40% NaOH solution. The condenser tip was dipped into a conical flask containing 5 cm^3 of 4% boric acid in a mixed indicator till the boric acid solution turned green. Titration was done in the receiver flask with 0.01 M HCl until the solution turned red [6].

2.2.1.5 Crude fibre content

From the pounded sample, 2.00 g were used in triplicates for estimating the crude fibre by acid and alkaline digestion methods using 20% $H_2SO_4$and 20% NaOH solutions [6].

2.2.1.6 Carbohydrate determination

The carbohydrate content was calculated using the following formula:

Available carbohydrate (%), = 100 – [protein (%) + Moisture (%) + Ash (%) + Fibre (%) + Crude Fat (%)].

2.2.1.7 Caloric value

The caloric value was calculated in kilocalories per 100 g (kcal/100g) by multiplying the crude fat, protein and carbohydrate values by Atwater factors of 37, 17 and 17 respectively.

2.2.2 Minerals analysis

Sodium and potassium were determined using Gallenkamp Flame analyzer, while calcium, magnesium, iron, manganese, zinc and copper were determined using Buch Model 205 atomic absorption spectrophotometer. Phosphorus level was determined using the phosphovanadomolybdate colorimetric technique on JENWAY 6100 Spectrophotometer [7].

2.2.3 Amino acid contents

From the ground sample, 0.50 g was defatted with chloroform and methanol mixture in a ratio of 1:1. Then, 0.25 g of the defatted sample was put into a glass ampoule, 7 cm^3 of 6 M HCl prepared from 36% BDH stock solution was added and oxygen expelled by passing nitrogen into the ampoule. This was put in the oven at 105^0C for 22 h, allowed to cool and filtered. The filtrate was then evaporated to dryness at 40^0C under vacuum in a rotary evaporator. The residue was dissolved with 5cm^3 acetate buffer (pH 2.0) and loaded into the amino acid analyzer and the samples were determined by ion exchange chromatographic (IEC) method using the Technicon Sequential Multi-sample Amino acid Analyzer (Technicon Instruments Corporation, New York) [8].

2.2.4 Functional Properties

The standard analytical procedures for food analysis as described below were used.

2.2.4.1 Bulk density

Firstly, a dried and empty 10 cm^3 measuring cylinder was weighed. The sample was filled gently into the weighed 10 cm^3 measuring cylinder and then gently tapped at the bottom on a laboratory bench several times until there was no further diminution of the sample level after filling to the 10 cm^3mark. After this, the filled measuring cylinder was weighed and recorded. This process was repeated three times.

Calculation:

$$\text{The bulk density } \left(\frac{\text{g}}{\text{cm}^3}\right) = \frac{\text{Weight of sample (g)}}{\text{Volume of sample (cm)}^3} \quad [6]$$

2.2.4.2 pH measurement

The pH values of the samples were determined by suspending 10% W/V of the sample in distilled water in each case. It was then thoroughly mixed in a 100 cm^3 beaker, stirred and the pH was taken using. This was repeated three times and the average calculated [9].

2.2.4.3 Water/oil absorption capacity

From the ground sample, 1.00 g was weighed into a conical graduated centrifuge tube and 10 cm^3 of water or oil was added to the weighed

sample. A warring whirl mixer was used to mix the sample for 30 seconds. The sample was allowed to stand at room temperature for 30 minutes and then centrifuged at 5000 rpm for 30 minutes. After then the mixed sample was transferred from the graduated centrifuge tube into a 10cm^3 measuring cylinder to know the volume of the free water or oil. The absorption capacity was expressed as grammes of oil or water absorbed per gramme of sample.

2.2.4.4 Calculation

The water/oil absorption capacity of the sample was calculated as: (Total oil/water absorbed – free oil/water) × Density of oil/water [10].

2.2.4.5 Foam capacity and stability

From the powdered sample, 2.00 g were weighed, blended with 100 cm^3 of distilled water using warring blender (Binatone BLG-555) and the suspension was whipped at 1600 rpm for 5 minutes. The mixture was then poured into a 100 cm^3 measuring cylinder and its volume was recorded after 30 seconds. Foam capacity was expressed as percent increase in volume using the formula of Abbey and Ibeh [11].

$$\text{Foam capacity} = \frac{\text{volume after whipping} - \text{volume before whipping}}{\text{Volume before whipping}} \times 100$$

The foam stability of the sample was recorded at 15, 30, 60 and 120 seconds after whipping to determine the foam stability (FS).

$$\text{Foam stability} = \frac{\text{Foam volume after time t}}{\text{Initial foam volume}} \times 100$$

2.2.4.6 Emulsification capacity (EC)

From the sample, 2.00 g of sample were blended with 25 cm^3 of distilled water at room temperature for 30 seconds in a warring blender at 1600 rpm. After complete dispersion, 25 cm^3 of vegetable oil was gradually added and the blending continued for another 30 seconds. Then the mixture was transferred into a centrifuge tube and centrifuged at 1600 rpm for 5 minutes. The volume of oil separated from the sample was read directly from the tube after centrifuging.

Calculation: The emulsion capacity was expressed as the amount of oil emulsified and held per gramme of sample [11].

$$\text{Emulsion capacity} = \frac{X}{Y} \times 100$$

Where X = height of emulsified layer and Y = height of the whole solution in the centrifuge tube.

2.2.4.7 Wettability

Triplicate samples were weighed and in each case, 1.00 g was introduced into a 25 cm^3 measuring cylinder with a diameter of 1cm and a finger was placed over the end of the cylinder. The mixture was inverted and clamped at a height of 10 cm from the surface of a 250 cm^3 beaker containing 100 cm^3 of distilled water. The finger was removed to allow the test material to be dumped. In this case, the wettability was taken as the time required for the sample to become completely wet [12].

2.2.4.8 Gelation capacity

In every case for triplicate samples, 5 cm^3 of 2-20% (w/v) suspended samples were in test tubes and heated for 1 hour in a boiling water bath followed by rapid cooling under running cold tap water. The test tubes were further cooled for 2 hours at 4°C and the gelation capacity was the least gelation concentration determined as the concentration when the sample from the inverted test tube did not fall or slip [13].

2.2.4.9 Gelatinization temperature

In triplicates, 10% samples were suspended in test tubes, heated in a boiling water bath with continuous stirring and 30 seconds after gelatinization was visually noticed, the temperature of the samples were taken as the gelatinization temperature [13].

2.2.4.10 Viscosity

In each case, 10% suspended sample in distilled water was taken and mechanically stirred for 2hours at room temperature. Thereafter, the viscosities of the samples were measured using Oswald type viscometer [12].

2.2.5 Anti-nutritional Properties

2.2.5.1 Oxalate

A modification of the titrimetric method of Day and Underwood [14] was used in the determination of oxalate in the frog meat

samples. In this method, 75 cm^3 of 1.5 M H_2SO_4 (made from 99% BDH AnalaR grade) was added to 1 g of the ground samples and the solution was carefully stirred intermittently with a magnetic stirrer for 60 minutes and filtered using Whatman No 1 filter paper after which 25 cm^3 of the filtrate was collected and titrated against hot (90°C) 0.1M $KMnO_4$ (BDH AnalaR grade) solution until a faint pink colour that persisted for 30 seconds appeared. This was repeated twice more and the concentration of oxalate in each sample was obtained from the calculation:

1 cm^3 of 0.1M $KMnO_4$ = 0.006303g Oxalate.

2.2.5.2 Alkaloids

The quantitative determination of alkaloids was carried out by the alkaline precipitation through gravimetric method described by Day and Underwood [14]. Two grammes (2.00 g) of the sample was soaked in 20 cm^3 of 10% ethanolic acetic acid (BDH AnalaR grade). The mixture was allowed to stand for 4 hr at room temperature. Thereafter, the mixture was filtered through Whatman filter paper no. 40. The filtrate (extract) was concentrated by evaporation over a steam bath to ¼th of its original volume. For the alkaloids to be precipitated, concentrated ammonia solution was added in drops to the extract until it was in excess. The resulting alkaloid precipitate was recovered by filtration using a previously weighed filter paper. After filtration, the precipitate was washed with 1% ammonia solution and dried in the oven at 60°C for 30 min, cooled in a desiccator and reweighed. The experiment was repeated two more times and the average was taken. The weight of alkaloids was determined by difference and expressed as a percentage of the weight of the sample analysed as shown.

$$\%\ \text{Alkaloids} = \frac{w_2 - w_1}{\text{wt of sample}} \times 100$$

Where; w_1 = weight of filter paper and w_2 = weight of paper + alkaloid precipitated

2.2.5.3 Tannins

0.2 g of sample was measured into a 50 cm^3 beaker. 20 cm^3 of 50% methanol was added and covered with paraffin and placed in a water bath at 77-80°C for 1 hr. It was shaken thoroughly to ensure a uniform mixture. The extract was quantitavely filtered into a 100 cm^3 volumetric flask using a double layered Whatman No.41 filter paper. 20 cm^3 of water was added followed by 2.5 $cm^3$of Folin-Denis reagent and 10 cm^3 of Na_2CO_3 (prepared from Kem-light product) This was then thoroughly mixed and the mixture was made up to mark with distilled water and allowed to stand for 20 minutes for the development of a bluish-green colour. The absorbances of the tannic acid standard solutions as well as samples were read after colour development on a UV-spectrophotometer model 752 at a wavelength of 760 nm [15].

2.2.5.4 Saponin

0.5 g of the sample was added to 20 cm^3 of 1MHCl and was boiled for 4h. After cooling it was filtered and 50 cm^3 of petroleum ether was added to the filtrate and the ether layer evaporated to dryness. 5 cm^3 of acetone/ethanol mixture was added to the residue. 0.4 cm^3 of each was taken into 3 different test tubes. 6 cm^3 of ferrous sulphate reagent was added into them followed by 2 cm^3 of concentrated H_2SO_4. It was thoroughly mixed and after 10 min the absorbance was taken at 490 nm. Standard saponin was used to establish the calibration curve [15].

2.2.5.5 Flavonoids

1 g of the sample was weighed and repeatedly extracted with 100 cm^3 of80% methanol at room temperature. The mixture was then filtered through filter paper into a 250 cm^3 beaker and the filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed. The % flavonoid was calculated using the formula:

$$x = \frac{w_2 - w_1}{w_3} \times 100$$

Where x = percentage flavonods, w_1 = weight of empty beaker, w_2 =weight of empty beaker+ flavonoid and w_3 = weight of sample

2.3 Statistical Analysis

All determinations were performed in triplicates. The results obtained were subjected to statistical analysis using means and standard deviations.

3. RESULTS AND DISCUSSION

The nutritional value of a given food depends on its nutritional and anti-nutritional constituents [16]. Table 1 shows that the selected mineral elements in the sample were in the order: sodium > phosphorus > potassium > calcium > zinc >

magnesium > copper > iron > manganese. The ratio of Na/K in the body is of great importance in the control of high blood pressure and the Na/K ratio of less than one is recommended [17]. Hence *Pelophylax esculentus* meat may not be a good protein source for a diabetic patient since it had a Na/K ratio of 3.76.

Table 1. The selected mineral contents (mg/100g) of the edible frog (*Pelophylax esculentus*) meat

Parameter	Content
Iron	35.93±0.67
Zinc	219.45±0.71
Copper	54.55±0.86
Sodium	2,550.00±2.17
Calcium	477.50±0.36
Potassium	679.00±1.01
Phosphorus	1,220.54±1.57
Manganese	2.75±0.35
Magnesium	87.56±0.04

Values are means of triplicate determination ± standard deviation

McDonald [18] reported that calcium in conjunction with magnesium, phosphorus, manganese, vitamins A, C and D, chlorine and protein is involved in bone formation. From the results obtained *Pelophylax esculentus* will serve as a good source of some minerals involved in bone formation since it contains large and considerable amounts of calcium and magnesium respectively. It however, had little amount of manganese. Ozkan, [19] considered a food source to be good if its Ca/P ratio is above one and poorif the ratio is less than 0.5. The Ca/P ratio of *Pelophylax esculentus* was 0.39 and based on this, the meat may have to be augmented with a higher calcium source in order to meet up the calcium requirement of the body. However, the 477.50±0.36mg/1000g calcium value obtained in this work was higher than the 126.55±0.53, 46.50±1.64, 19.04±0.28, 16.11±0.83, 7.83±1.31 and 11.71±0.63 mg/kg reported in literature for quail, beef, lamb, turkey, broiler and ostrich respectively [20]. This thus placed this meat at a higher advantage as a source of calcium in animal nutrition over these animal meats mentioned above. Furthermore, the 31.17±1.36% crude protein content of *Pelophylax esculentus obtained in this work* was higher than the 29.05% crude protein content of duckweed [21] and the 22.80% crude protein value of chicken [22]. This was however lower than the 53.74±0.98% reported as the crude protein content of *Rana galamensis* [22].

Tannins and oxalates affect the bioavailability of composite nutrients, complexing with the bivalent ions: Ca^{2+}, Mg^{2+}, Fe^{2+} and Zn^{2+}. This makes them unavailable especially in monogastric animals [23]. From Table 2, all the anti-nutrient contents of *Pelophylax esculentus* were very low compared with the values reported for other meat sources [24].

Table 2. Some anti-nutritional factors (mg/100g) of the edible frog (*Pelophylax esculentus*) meat

Anti-nutritional factors	Content
Saponin	1.75±0.35
Tannin	5.37±0.53
Flavonoid	1.75±0.35
Alkaloid	2.80±0.00
Oxalate	2.78±0.00

Values are means of triplicate determinations ± standard deviations

From Table 4, the meat of *Pelophylax esculentus* had low moisture value (3.39%) which means that it might have a good shelf value [25]. The ash content of this sample was slightly high (8.71%) and this was expected because the sample was prepared by crushing both the meat and bones together. This value was far higher than the respective 0.60, 1.20, 0.80, 1.00 and 1.20% ash contents of pork carcass, beef (lean), beef carcass, pork (lean) and chicken [26]. The carbohydrate value of 29.02% showed that *Pelophylax esculentus*, though being an animal, could be a fairly good source of carbohydrate and this value was similar to the 29.04±0.01 % reported for *Rana galamensis* [22]. The crude fat value of 16.22% obtained in this study was however, higher than the 9.52±0.31% reported for *Ranagalamensis* [22]. Since crude fat is animportant part of diet which increases serum cholesterol level thus increasing the risk of coronary heart disease, hypertension, diabetes and breast cancer [27], this could not be a good diet to these groups of people. The crude fibre contents of the meat was 11.71%, which meant that *Pelophylax esculentus* could be a fairly rich source of fibreal though this fell short of the respective rangesof 19-25%, 21-30% and 29% required for children, adult, pregnant and lactating mothers [27]. The crude protein of *Pelophylax esculentus* was 31.17% which could be used to qualify it as a good source of low cost animal protein.

From Table 3, the foaming capacity of *Pelophylax esculentus* meat obtained in this

study (56.70±0.00%) was higher than the 40-50% range reported for some oil seeds [24] and the 34.00% reported for kersting's groundnut flour in $NaNO_2$ [25]. The low gelation capacity (2.00±0.41%) of the sample in this study suggested that it might not be a good gel forming agent however, its high emulsification capacity indicated the significant role it might play on many food systems where its protein might conveniently bind many fats [28].

Table 3. The functional properties of the edible frog (*Pelophylax esculentus*) meat

Parameter	Content
Bulk density (g/cm^3)	0.60±0.01
Oil absorption capacity (%)	2.01±0.23
Water absorption capacity (%)	4.55±0.11
Foaming Stability (%)	56.70±0.00
Emulsification capacity (%)	50.08±1.96
Gelation capacity (%)	2.00±0.41
Gelatinization temperature(^{0}C)	69.00±0.71
Wettability (s)	60.04±0.66
Viscosity (s)	23.27±1.66
pH	8.60±0.00

Values are means of triplicate determinations ± standard deviations

Table 4. Proximate composition (%) of the meat of edible frog (*Pelophylax esculentus*)

Parameter	Percentage
Moisture content	3.49±0.56
Ash content	8.93±1.33
Crude fat	16.22±0.16
Crude fibre	11.71±0.22
Crude protein	31.17±1.36
Carbohydrate	29.02±1.16
Calorific value (kcal/100g)	506.17

Values are means of triplicate determinations ± standard deviations

The result of essential and non essential amino acid profiles of the *Pelophylax esculentus* was as presented in Table 5. This showed that non-essential amino acids had higher percentage (52.40%) while the essential amino acid contents amounted to 47.60%. Similar amino acid composition was reported for *Hoplobatrachus occipitalis* [3]. Since tthese essential and non-essential amino acids complement one another when present in foods and *Pelophylax esculentus* meat contained these acids in reasonable amounts, it could be a good source of these amino acids.

Table 5. The amino acid contents (%) of edible frog (*Pelophylax esculentus*) meat

Parameter	Concentration in g/100 g
*Lysine	7.62
*Histidine	2.13
*Arginine	6.13
Asparti acid	9.16
*Threosine	3.94
Serine	4.24
Glutamic acid	13.86
Proline	4.04
Glycine	7.24
Alanine	5.60
Cysteine	0.93
*Valine	4.82
*Methionine	2.89
*Isoleucine	3.83
*Leucine	7.22
Tyrosine	3.06
*Phenylalanine	4.14
*Tryptophan	0.93
EAA (%)	47.60
NEAA(%)	52.40

** = essential amino acid, EAA = essential amino acid, NEAA = non-essential amino acid*

5. CONCLUSION

From the results obtained in this study, it could be inferred that meat of *Pelophylax esculentus* has high nutrient composition and calorific value. It also indicated that it has high content of mineral elements although given that the Na/K ratio is above 1, it may not be too good for a diabetic patient. *Pelophylax esculentus* also showed higher nutritional values than some meat most especially considering its crude protein value. Thus, this probably makes *Pelophylax esculentus* meat a better source of animal protein than some animal sources.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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