

DETERMINATION OF THE NUTRITIVE AND ANTI-NUTRITIVE VALUES OF

***Pelophylax esculentus* (EDIBLE FROG) FOUND IN HANYAN GWARI, MINNA NIGER STATE, NIGERIA**

Author's 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 UB and YA identified the species of the amphibian, carried out the mineral and statistical analyses.

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 $31.17 \pm 1.36\%$, carbohydrate was found to be $29.02 \pm 1.16\%$ while the crude fibre was $11.71 \pm 0.22\%$. The crude fat was $16.22 \pm 0.16\%$, ash content was $8.93 \pm 1.33\%$ and moisture was $3.49 \pm 0.56\%$. The abundance of mineral elements found in the meat of *P. esculentus* was found to be in the order: sodium > phosphorus > potassium > calcium > zinc > magnesium > copper > iron > manganese. The calorific value was 506.17 kcal/100g 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

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: ranidae, 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.

3.0 MATERIAL 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.

3.4 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.

3.5 METHODS

3.5.1 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.

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.

Ash Content

From the dried and ground sample, 2.00g 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].

Crude Lipid Content

From the pulverized sample, 2.00g 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.

Protein Determination

Total protein was determined by the Kjeldahl method. 0.5 g of the sample was weighed in triplicate into a filter paper and put into a Kjeldahl flask, 8-10 cm³ of concentrated H₂SO₄ were added and then digested in a fume cupboard until the solution became colourless. Distillation was carried out with about 10 cm³ of 40% NaOH solution. The condenser tip was dipped into a conical flask containing 5 cm³ 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].

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% H₂SO₄ and 20% NaOH solutions [6].

Carbohydrate Determination

The carbohydrate content was calculated using the following formula:

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

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.

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 phosphovanado molybdate colorimetric technique on JENWAY 6100 Spectrophotometer [7].

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 cm³ of 6 M HCl was added and oxygen expelled by passing nitrogen into the ampoule. This was put in the oven at 105⁰C for 22 h, allowed to cool and filtered. The filtrate was then evaporated to dryness at 40⁰C under vacuum in a rotary evaporator. The residue was dissolved with 5cm³ 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].

Functional Properties

The standard analytical procedures for food analysis were used for the determination of bulk density, gelation capacity, water/oil absorption capacity, wettability, gelatinization temperature, viscosity and pH determination were carried out using the methods of AOAC [6] while foam capacity and stability were determined using the methods as described by Abbey and Ibeh [9]. The emulsification capacity was determined by the method of Padmashree *et al.*, [10].

Anti-nutritional Properties

Oxalate: A modification of the titrimetric method of Day and Underwood [7] was used in the determination of oxalate in the frog meat samples. In this method, 75 cm³ of 1.5M H₂SO₄ 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 cm³ of the filtrate was collected and titrated against hot (90⁰C) 0.1M KMnO₄ 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:

1cm³ of 0.1M KMnO₄ = 0.006303g Oxalate.

Alkaloids

The quantitative determination of alkaloids was carried out by the alkaline precipitation through gravimetric method described by Day and Underwood [7]. Two grammes (2.00g) of the sample was soaked in 20cm³ of 10% ethanolic acetic acid. 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 60⁰C for 30min, 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; w₁ = weight of filter paper and w₂ = weight of paper + alkaloid precipitated

Tannins

0.2 g of sample was measured into a 50cm³ beaker. 20 cm³ of 50% methanol was added and covered with paraffin and placed in a water bath at 77-80⁰C for 1 hr. It was shaken thoroughly to ensure a uniform mixture. The extract was quantitatively filtered into a 100 cm³ volumetric flask using a double layered Whatman No.41 filter paper. 20 cm³ of water was added followed by 2.5 cm³ of Folin-Denis reagent and 10 cm³ of Na₂CO₃. 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 [8].

Saponin

0.5 g of the sample was added to 20 cm³ of 1MHCl and was boiled for 4h. After cooling it was filtered and 50 cm³ of petroleum ether was added to the filtrate and the ether layer evaporated to dryness. 5 cm³ of acetone/ethanol mixture was added to the residue. 0.4 cm³ of each was taken into 3 different test tubes. 6 cm³ of ferrous sulphate reagent was added into them followed by 2 cm³ of concentrated H₂SO₄. It was thoroughly mixed and after 10min the absorbance was taken at 490 nm. Standard saponin was used to establish the calibration curve [8].

Flavonoids

1 g of the sample was weighed and repeatedly extracted with 100 cm³ of 80% methanol at room temperature. The mixture was then filtered through filter paper into a 250 cm³ 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 flavonoids, w₁ = weight of empty beaker, w₂ = weight of empty beaker + flavonoid and w₃ = weight of sample

Statistical Analysis

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

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177 **4.0 RESULTS AND DISCUSSION**

Table 1: The selected mineral contents (mg/1000g) 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

178 Values are means of triplicate determination ± standard deviation

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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

180 Values are means of triplicate determinations ± standard deviations

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

Parameter	Content
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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(°C)	69.00±0.71
Wettability (s)	60.04±0.66
Viscosity (s)	23.27±1.66
pH	8.60±0.00

181 Values are means of triplicate determinations ± standard deviations

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

Parameter	Percentage
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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/100 g)	506.17

182 Values are means of triplicate determinations ± standard deviations

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
Aspartic acid	9.16
*Threonine	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.

4.1 DISCUSSION OF RESULT

The nutritional value of a given food depends on its nutritional and anti-nutritional constituents [13]. 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 [14]. 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. McDonald [15] 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, [16] considered a food source to be good if its Ca/P ratio is above one and poor if 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 $477.50 \pm 0.36 \text{ mg/1000g}$ 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 $11.71 \pm 0.63 \text{ mg/kg}$ reported in literature for quail, beef, lamb, turkey, broiler and ostrich respectively [17]. 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 $31.17 \pm 1.36\%$ crude protein content of *Pelophylax esculentus* obtained in this work was higher than the 29.05% crude protein content of duckweed [18] and the 22.80% crude protein value of chicken [19]. This was however lower than the $53.74 \pm 0.98\%$ reported as the crude protein content of *Rana galamensis* [20].

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 [21]. From Table 2, all the anti-nutrient contents of *Pelophylax esculentus* were very low compared with the values reported for other meat sources [22].

From Table 4, the meat of *Pelophylax esculentus* had low moisture value (3.39%) which means that it might have a good shelf value [23]. 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 [24]. 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 $29.04 \pm 0.01\%$ reported for *Rana galamensis* [20]. The crude fat value of 16.22% obtained in this study was however, higher than the $9.52 \pm 0.31\%$ reported for *Rana galamensis* [20]. Since crude fat is an important part of diet which increases serum cholesterol level thus increasing the risk of coronary heart disease, hypertension, diabetes and breast cancer [25], 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 fibre although this fell short of the respective ranges of 19-25%, 21-30% and 29% required for children, adult, pregnant and lactating mothers [25]. 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 ($56.70 \pm 0.00\%$) was higher than the 40-50% range reported for some oil seeds [22] and the 34.00% reported for kersting's groundnut flour in NaNO_2 [23]. The low gelation capacity ($2.00 \pm 0.41\%$) 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 [26].

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 *Hoplobat rachus occipitalis* [3]. Since these 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.

4.2 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.

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