Original Research Article

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 is carried out in collaboration between all authors. Author JTM designed the study, wrote the protocol, wrote the first draft of the manuscript and he 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 process. Author UB and YA identified the species of insect, carried out mineral and statistical analysis.

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 of analysis. 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. 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), threosine (3.94), valine (4.82), methionine (2.89), leucine (7.22), isoleucine (3.83) and phyylalanine (4.14). Based on its anti-nutritional contents of *P. esculentus* meat could be considered as a good source of animal protein for man and his animals. From the result obtained *P.esculentus* could be a good low cost and easy source of animal protein, good of calcium, reasonable amount of potassium as well as 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 that would help to take care of this health challenges and which would be cheaper and safer for consumption especially the aquatic animals [1]. Since meats contain essential classes of food such as, carbohydrate, proteins, fat, vitamins and minerals, they provide the nutritional requirement 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 study 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 is to determine the proximate, minerals, functional properties, anti-nutritional factors and amino acid profile of *Pelophylax esculentus* in order to establish the safety or otherwise of the consumption of this amphibian by humans.

3.0 MATERIAL AND METHODS

The sample (*pelophylax escuslentus*) used in the course of this work were obtained on 24th May, 2013 from Hanya Gwari bosso around F. U. T environment in Minna, Niger State.

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, the small pieces were dry milled, kept in air tight polythene bag and stored in a dessicator (with dessicant) 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 moisture content, crude protein, crude fibre, percentage lipids, carbohydrate, acid insoluble ash and caloric value.

Moisture Content

Two grams of the sample was put into the crucible, dried in an oven at 105°C overnight. The dried samples were cooled in a dessicator 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.

Ash Content

2.00g of the ground sample was placed in a crucible and ashed in a muffle furnace at 600ºC for 3 hours. The hot crucibles were cooled in a dessicator and weighted. The percentage residual weight was expressed as ash content [6].

Crude Lipid Content

2.00g of the sample was used for determining crude lipid by extracting lipid from it for 5 hours with petroleum ether in a soxhlet extractor [6].

Protein Determination

Total protein was determined by the Kjedahl method. 0.5 g of the sample was weighed into a filter paper and put into a Kjedahl flask, 8-10 cm3 of concentrated H_2SO_4 were added and then digested in a fume cupboard until the solution becomes colourless. Distillation was carried out with about 10 cm^3 of 40% of NaOH. The condenser tip was dipped into a conical flash 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].

Crude Fibre Content

2.00 g of each sample were used for estimating crude fibre by acid and alkaline digestion methods with 20% H_2SO_4 and 20% NaOH solution [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 Kilojoules 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 techniques on JENWAY 6100 Spectrophotometer [7].

Amino acid contents

0.50 g of ground sample was defatted with chloroform and methanol mixture in a ratio 1:1, then, 0.25 g of the defatted sample was put into a glass ampoule, 7 ml of 6 M HCl was added and oxygen expelled by passing nitrogen into the ampoule was put in the oven at 105^{o}C for 22 h, allowed cool and filtered. The filtrate was then evaporated to dryness at 40^{o}C under vacuum in a rotary evaporator. The residue was dissolved with 5ml acetate buffer (pH 2.0) and loaded into the amino acid composition and the samples were determined by ion exchange chromatography (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 was carried out using the method of AOAC [6] while foam capacity and stability was determine using the method as described by Abbey and Ibeh [9]. The emulsification capacity was also determined by the method of Padmashree *et al.*, [10].

Anti-nutritional Properties

Oxalate: A modification of the titrimetric method of Day &Underwood [7]was used in the determination of oxalate in the Velvet bean samples. 75 ml of 3N H_2SO_4 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 ml of the filtrate was collected and titrated against hot (90^0C) 0.1 M $KMnO_4$ solution until a faint pink colour appeared that persisted for 30 seconds. The concentration of Oxalate in each sample was obtained from the calculation:

1ml of 0.1M $KMnO_4$ = 0.006303g Oxalate.

Alkaloids

The quantitative determination of alkaloids was carried out by the alkaline precipitation through Gravimetric method described by Day &Underwood [7]. Two grams 2g of the sample was soaked in 20ml 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 ¼ 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.

$$\% \text{ Alkaloids} = \frac{W2 - W1 \times 100}{\text{Wt of sample}}$$

Where; W1 = Weight of Filter paper and W2 = Weight of paper + alkaloid precipitate

Tannins

0.2 g of sample was measured into a $50cm^3$ beaker. 20 cm^3 of 50 % methanol was added and covered with para film and placed in a water bath at 77-80^OC for 1 hr. It was shaken thoroughly

to ensure a uniform mixture. The extract was quantitavely filtered using a double layered whatman No.41 filter paper into a 100 cm^3 volumetric flask, 20 cm^3water added; 2.5 cm^3 Folin-Denis reagent and 10 cm^3 of Na_2CO_3 were added and mixed properly. The mixture was made up to mark with water mixed well and allowed to stand for 20 min for the development of a bluish-green colour. The absorbencies 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 cm^3 of 1NHCl and was boiled for 4h. After cooling it was filtered and 50 cm^3 of petroleum ether was added to the filtrate and ether layer evaporated to dryness. 5 cm^3 of acetone ethanol 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 after 10min and 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 cm^3 of 80% aqueous 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:

$$X = \frac{w_2 - w_1}{W_3} \times 100$$

W_1 = weight of empty beaker, w_2 =weight of empty beaker + flavonoid and w_3 = weight of sample

Statistical Analysis

All experiments were performed in triplicate. The results obtained were subjected to statistical analysis using mean standard deviation and analysis of variance (ANOVA)

4.0 RESULTS AND DISCUSSION

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

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

180 Values are means of triplicate determination ± standard deviation

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

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

182 Values are means of triplicate determination ± standard deviation

Table 3: Functional properties of the edible frog (*Pelophylax esculentus*)

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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 (cm^3)	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

183 Values are means of triplicate determination ± standard deviation

Table 4: Proximate composition (%) of the 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/100 g)	506.17

184 Values are means of triplicate determination ± standard deviation

Table 5: Result of amino acids contents in edible frog (*Pelophylax esculentus*)

Parameter	**Concentration in mg/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.

4.1 DISCUSSION OF RESULT

The nutritional value of a given food depends on the nutrient and anti-nutritional constituents of the food [13]. Table 1 shows that the presence of the selected mineral elements in the sample was in order: sodium > phosphorus > potassium > calcium > zinc > magnesium > copper > iron > manganese. The calculated ratio of Na/K in the body is of great importance in the control of high blood pressure. 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, vitamin 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 minerals involved in bone formation since it contains large amounts of calcium and considerable amounts of magnesium but 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 augumented with a higher calcium source in order to meet up the calcium requirement of the body.

Tannins and oxalate affect the bioavailability of composite nutrients, complexing with bivalent ions Ca^{2+}, Mg^{2+}, Fe^{2+} and Zn^{2+}. This makes them unavailable especially in monogastric animals [17]. From Table 2, all the anti-nutrient contents of *Pelophylax esculentus* were very low compared with the values reported for other meat sources [18].

From Table 4 it indicates that, the meat of *Pelophylax esculentus* contains lower moisture value (3.39%) which means that it might have a good shelf value [20]. 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. The carbohydrate value of 29.02% showed that *Pelophylax esculentus*, being an animal, is not a good source of carbohydrate. The crude fat value in the meat was much 16.22%, since crude fat is important part of diet, which decreases serum cholesterol levels risk of coronary heart disease, hypertension, diabetes and breast cancer [21]. The crude fibre contents of the meat was 11.71%, which meant that *Pelophylax esculentus* could not be a rich source of crude fibre because since this value fell short of the respective ranges of 19-25%, 21-30% and 29% required for children, adult, pregnant and lactating mothers as reported by Ishida *et al.*, [21]. 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 and relatively high biological value.

The result of essential and non essential amino acid profile of the *Pelophylax esculentus* was presented in Table 5. The result showed that non-essential amino acids content had higher percentage - 52.40% while essential amino acid contents amount to 47.60%. Similar amino acid compositions was recorded for *Hoplobat rachus occipitalis* reported by Onadeko *et al.*, [3]. The percentage present in both essential and non-essential amino acid were to complement each other when present in food; though they were desire in a certain quantity.

4.2 CONCLUSION

From the results obtained in this study, it shows that the meat of *Pelophylax esculentus* have reasonable amount of nutritional contents. It's indicates high content of mineral composition. The Na/K ratio is above 1 which may not be good for a diabetic patient. *Pelophylax esculentus* also showed higher content of amino acids which may be good for body buiding.

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