Original Research Article

2 DETERMINATION OF THE NUTRITIVE AND ANTI-NUTRITIVE VALUES OF 3 Pelophylaxesculentus (EDIBLE FROG) FOUND IN HANYAN GWARI, MINNA NIGER

- 4 STATE, NIGERIA
- 5

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6 Author's contributions

7 This work was carried out as collaborativeresearch among all the authors. Author JTM designed 8 the study, wrote the protocol, wrote the first draft of the manuscript and carried out pretreatment 9 of the sample. Author MMN managed the literature researches, analyses of the amino acid 10 profile. Authors SSM and EYS managed the experimental processes. AuthorsSAB and YA 11 identified the species of the amphibian, carried out the mineral and statistical analyses.

12

ABSTRACT

13 The proximate, selected minerals, amino acid profile, functional properties and anti-nutrient composition of edible frog (Pelophylaxesculentus) were determined using standard analytical 14 methods. The crude protein was 31.17±1.36%, carbohydrate was found to be 29.02±1.16% while 15 16 the crude fibre was $11.71\pm0.22\%$. The crude fat was $16.22\pm0.16\%$, ash content was $8.93\pm1.33\%$ 17 and moisture was $3.49\pm0.56\%$. The abundance of mineral elements found in the meat of P. esculentus was found to be in the order: sodium > phosphorus > potassium > calcium > zinc > 18 magnesium > copper > iron > manganese. The calorific value was 506.17 kcal/100g while the 19 animal was also found to have reasonable amounts of essential amino acids: tryptophan (0.39), 20 lysine (7.62), arginine (6.13), histidine (2.13), threonine (3.94), valine (4.82), methionine (2.89), 21 22 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 23 source of animal protein for man and his animals. It could also be a good source of calcium, 24 potassium and sodium. 25

26 Keywords: edible frog, functional properties, proximate analysis, amino acid profile

27

28 1.0 INTRODUCTION

Meat is important to human beings and could be obtained from various sources. It is very good 29 30 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 31 which would help to take care of these health challenges and would be cheaper and safer for 32 consumption [1]. Since meats contain essential classes of food such as, carbohydrate, proteins, 33 fat, vitamins and minerals, they provide the nutritional requirements of man in the appropriate 34 quantities^[2]. The provision of these nutritional entities becomes amajor problem in most 35 developing countries such as Nigeria leading to under- or malnutrition. In a view to reduce such 36 menace in Nigeria some lesser known animals which can serve as food are studied for their 37

- 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].
- 40 Pelophylaxesculentus (edible frog), formally known as Ranaesculentusis considered to be of
- 41 good nutritional value [4]. It is a widespread natural hybrid that is produced as an offspring of
- 42 the parent species *P. lessonae* and *P. ridibundus* [5]. This frog is the fertile hybrid of the Pool
- 43 Frog (Pelophylaxlessonae) and the Marsh Frog (Pelophylaxridibundus). It belongs to the
- 44 kingdom: animalia, phylum: chordate, class: amphibian, order:anuran, family: ranida, genus:
- 45 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
- 47 *Pelophylaxesculentus* in order to establish the safety or otherwise of its consumption by humans.

48 2.0 MATERIAL AND METHODS

- 49 The sample (*Pelophylaxescuslentus*) used in the course of this work was obtained on 24th May,
- 50 2013 from HanyaGwariBosso around F. U. T environment in Minna, Niger State. Samples were
- 51 randomly collected and mixed to obtain a composite sample of the animals.

52 **2.1Sample preparation and treatment**

53 Thesampleswerecutopened (flesh, skinandbones)anddried in an air oven at 60°C for 10 hours for 54 proper removal of moisture. The fleshy parts of the samples were scrapped using a clean 55 laboratory stainless steel knife, dried again and milled. This was kept in an air tight polythene 56 bag and stored in a desiccatorprior to further analysis.

57 **2.2Methhods**

58 **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.

62 **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.
- 67

68 Ash Content

From the dried and ground sample, 2.00g was taken in triplicates and placed in pre-weighedcrucibles and ashed in a muffle furnace at 600°C for 3 hours. The hot crucibles were cooled in a

- 71 desiccator and weighed. The percentage residual weight was expressed as ash content [6].
- 72

73 Crude Lipid Content

From the pulverized sample, 2.00g was used for determining thecrude lipid by extracting thelipid
from it for 5 hours with (60-80⁰C)petroleum ether in a soxhlet extractor [6]. Triplicate samples
were extracted to obtain triplicate values that were later averaged.

77

78 **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 cm³ 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 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].

86

87 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% H₂SO₄and 20% NaOH solutions [6].

90

91 Carbohydrate Determination

- 92 The carbohydrate content was calculated using the following formula:
- 93 Available carbohydrate (%), = 100 [protein (%) + Moisture (%) + Ash (%) + Fibre (%) +
- 94 Crude Fat (%)].

95

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

100 2.2.2 Minerals analysis

Sodium and potassium were determined using GallenkampFlame analyzer, while calcium,
 magnesium, iron, manganese,zinc and copper were determined using Buch Model 205 atomic
 absorptionspectrophotometer. Phosphorus level wasdetermined using the
 phosphovanadomolybdate colorimetrictechnique on JENWAY 6100 Spectrophotometer [7].

106 2.2.3 Amino acid contents

107 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 HClprepared 108 109 from 36% BDH stock solution was added and oxygen expelled by passing nitrogen into the ampoule. This was put in the oven at 105^oC for 22 h, allowed to cool and filtered. The filtrate 110 was then evaporated to dryness at 40° C under vacuum in a rotary evaporator. The residue was 111 dissolved with 5cm³ acetate buffer (pH 2.0) and loaded into the amino acid analyzer and the 112 113 samples were determined by ion exchange chromatographic (IEC) method using the Technicon 114 Sequential Multi-sample Amino acid Analyzer (Technicon Instruments Corporation, New York) [8]. 115

116

117 2.2.4 Functional Properties

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

119 Bulk density

Firstly, a dried andempty 10cm³ measuring cylinder was weighed. The sample was filled gently into the weighed 10cm³ 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 10cm³mark. After this, the filled measuring cylinder was weighed and recorded. This process was repeated three times.

125 Calculation:

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

127 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 100cm³ beaker, stirred and the pH was takenusing. This was repeated three times and the average calculated [9].

131 Water/oil absorption capacity

From the ground sample, 1.00g was weighed into a conical graduated centrifuge tube and 10cm³ 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 10cm³ 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

139 Calculation:

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

142 Foam capacity and stability

From the powdered sample, 2.00g were weighed, blended with 100cm³ of distilled water using warring blender(Binatone BLG-555) and the suspension was whipped at 1600rpm for 5 minutes. The mixture was then poured into a 100cm³ 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].

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 todetermine the foam stability (FS).

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

151

152 Emulsification capacity (EC)

From the sample, 2.00g of sample were blended with 25cm³ of distilled water at room temperature for 30 seconds in a warring blender at 1600rpm. After complete dispersion, 25cm³ 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 1600rpm for 5 minutes. The volume of oil separated from the sample was read directly from the tube after centrifuging.

158 Calculation: The emulsion capacity was expressed as the amount of oil emulsified and held per159 gramme of sample [11].

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

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

161 Wettability

162 Triplicate samples were weighed and in each case, 1.00g was introduced into a 25cm³ measuring 163 cylinder with a diameter of 1cm and a finger was placed over the end of the cylinder. The 164 mixture was inverted and clamped at a height of 10cm from the surface of a 250cm³ beaker 165 containing 100cm³ of distilled water. The finger was removed to allow the test material to be 166 dumped. In this case, the wettability was taken as the time required for the sample to become 167 completely wet [12].

168 Gelation capacity

169 In every case for triplicate samples, 5cm^3 of 2-20% (w/v) suspended samples were in test tubes 170 and heated for 1hour in a boiling water bath followed by rapid cooling under running cold tap water. The test tubes were further cooled for 2hours at 4^{0} 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].

174 Gelatinization temperature

175 In triplicates, 10% sampleswere suspended in test tubes, heated in a boiling water bath with 176 continuous stirring and 30 seconds after gelatinization was visually noticed, the temperature of 177 the samples were taken as the gelatinization temperature [13].

178 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].

182 2.2.5 Anti-nutritional Properties

- 184 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 cm³ of 1.5M H₂SO₄(made
- 186 from 99% BDH AnalaR grade) was added to 1 g of the ground samples and the solution was
- 187 carefully stirred intermittently with a magnetic stirrer for 60 minutes and filtered using Whatman
- 188 No 1 filter paper after which 25 cm³ of the filtrate was collected and titrated against hot $(90^{\circ}C)$
- 189 0.1M KMnO₄(BDH AnalaR grade) solution until a faint pink colour that persisted for 30
- 190 secondsappeared. This was repeated twice more and the concentration of oxalate in each sample
- 191 was obtained from the calculation:
- 192 $1 \text{ cm}^3 \text{ of } 0.1 \text{ M KMnO}_4 = 0.006303 \text{ g Oxalate.}$
- 193

- 194 Alkaloids
- 195 The quantitative determination of alkaloids was carried out by the alkaline precipitation through
- 196 gravimetric method described by Day and Underwood [14]. Two grammes(2.00g) of the sample

was soaked in 20cm³ of 10% ethanolic acetic acid (BDH AnalaR grade). The mixture was 197 198 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 199 200 bath to ¹/4th 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 201 was recovered by filtration using a previously weighed filter paper. After filtration, the 202 precipitate was washed with 1% ammonia solution and dried in the oven at 60° C for 30min, 203 cooled in a desiccator and reweighed. The experiment was repeated two more times and the 204 average was taken. The weight of alkaloids was determined by difference and expressed as a 205 percentage of the weight of the sample analysed as shown. 206

% Alkaloids
$$= \frac{W_2 - W_1}{Wt \text{ of sample}} \times 100$$

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

- Tannins 208 0.2g of sample was measured into a 50cm³ beaker. 20cm³ of 50% methanol was added and 209 covered with paraffin and placed in a water bath at 77-80⁰C for 1 hr. It was shaken thoroughly to 210 ensure a uniform mixture. The extract was quantitavely filtered into a 100 cm³ volumetric flask 211 using a double layered Whatman No.41 filter paper. 20 cm³ of water was added followed by 2.5 212 cm^{3} of Folin-Denis reagent and 10 cm³ of Na₂CO₃(prepared from Kem-light product)This was 213 then thoroughlymixed and the mixture was made up to mark with distilled water and allowed to 214 stand for 20 minutes for the development of a bluish-green colour. The absorbances of the tannic 215 acid standard solutions as well as samples were read after colour development on a UV-216 spectrophotometer model 752 at a wavelength of 760nm [15]. 217
- 218 Saponin

- 219 0.5 g of the sample was added to 20 cm³ of 1MHCl and was boiled for 4h. After cooling it was
- 220 filtered and 50 cm³ of petroleum ether was added to the filtrate and theether 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 cm^3 of ferrous sulphate reagent was added into them followed by 2
- 223 cm^3 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 [15].

- 226 Flavonoids
- 1 g of the sample was weighed and repeatedly extracted with 100 cm^3 of 80% methanol at room
- temperature. The mixture was then filtered through filter paper into a 250 cm^3 beaker and the
- 229 filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed. The
- 230 % flavonoid was calculated using the formula:

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

- 231 Where x = percentage flavonods, w_1 = weight of empty beaker, w_2 =weight of empty beaker+
- flavonoid and w_3 = weight of sample
- 234 Statistical Analysis
- All determinations were performed in triplicates. The results obtained were subjected to
- 236 statistical analysis using means and standard deviations.
- 237

233

238 3.0 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 >
- $\label{eq:phosphorus} 241 \qquad phosphorus > potassium > calcium > zinc > magnesium > copper > iron > manganese. The ratio$
- 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 *Pelophylaxesculentus*meat may not be a good
- 244 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(Pelophylaxesculentus) meat

Parameter	Content
Iron	35.93±0.67
Zinc	219.45±0.71

Copper	54.55±0.86
Sodium	$2,550.00\pm2.17$
Calcium	477.50±0.36
Potassium	679.00±1.01
Phosphorus	$1,220.54{\pm}1.57$
Manganese	2.75±0.35
Magnesium	87.56±0.04

²⁴⁵ Values are means of triplicate determination \pm standard deviation

McDonald[18] reported that calcium in conjunction with magnesium, phosphorus, manganese, 246 247 vitamins A, C and D, chlorine and protein is involved in bone formation. From the results obtained *Pelophylaxesculentus* will serve as a good source of some minerals involved in bone 248 249 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 250 be good if its Ca/P ratio is above one and poorif the ratio is less than 0.5. The Ca/P ratio of 251 *Pelophylaxesculentus* was 0.39 and based on this, the meat may have to be augmented with a 252 higher calcium source in order to meet up the calcium requirement of the body. However, the 253 254 477.50±0.36mg/1000g calcium value obtained in this work was higher than the 126.55±0.53, 255 46.50±1.64, 19.04±0.28, 16.11±0.83, 7.83±1.31 and 11.71±0.63mg/kg reported in literature for quail, beef, lamb, turkey, broiler and ostrich respectively [20]. This thus placed this meat at a 256 higher advantage as a source of calcium in animal nutrition over these animal meats mentioned 257 above. Furthermore, the 31.17±1.36% crude protein content of Pelophylaxesculentus obtained in 258 this work was higher than the 29.05% crude protein content of duckweed [21] and the 22.80% 259 crude protein value of chicken [22]. This was howeverlower than the 53.74±0.98% reported as 260 the crude protein content of *Ranagalamensis* [22]. 261

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	

Table 2: Some anti-nutritional factors (mg/100g) of the edible frog (Pelophylaxesculentus) meat

262 Values are means of triplicate determinations ± standard deviations

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 *Pelophylaxesculentus* were very low compared with the values reported for other meat sources [24].

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	

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

268 Values are means of triplicate determinations \pm standard deviations

269

270 From Table 4, the meat of *Pelophylaxesculentus*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 271 272 (8.71%) and this wasexpected 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% 273 ash contents of pork carcass, beef (lean), beef carcass, pork (lean) and chicken [26]. The 274 275 carbohydrate value of 29.02% showed that *Pelophylaxesculentus*, though being an animal, could be a fairlygood source of carbohydrate and this value was similar to the 29.04±0.01 % reported 276 for Ranagalamensis [22]. The crude fat value of 16.22% obtained in this study was however, 277 higher than the 9.52±0.31% reported for *Ranagalamensis* [22]. Since crude fat is an important part 278 of diet which increases serum cholesterol level thus increasing the risk of coronary heart disease, 279 hypertension, diabetes and breast cancer [27], this could not be a good diet to these groups of 280 people. The crude fibre contents of the meat was 11.71%, which meant that 281 282 Pelophylaxesculentus could be a fairly rich source of fibrealthough this fell short of the respective ranges of 19-25%, 21-30% and 29% required forchildren, adult, pregnant and lactating 283 mothers[27]. The crude protein of Pelophylaxesculentus was 31.17% which could be used to 284 qualify it as a good source of low cost animal protein. 285

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	
рН	8.60 ± 0.00	

Table 3: The functional properties of the edible frog (Pelophylaxesculentus) meat

286 Values are means of triplicate determinations \pm standard deviations

From Table 3, the foaming capacity of *Pelophylaxesculentus* meat obtained in this study ($56.70\pm0.00\%$) was higher than the 40-50% range reported for some oil seeds [24] and the 34.00% reported for kersting's groundnut flour in NaNO₂ [25]. The low gelation capacity ($2.00\pm0.41\%$) 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 5: The amino acid contents (%) of edible frog (*Pelophylaxesculentus*) 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
Senne	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.

293

The result of essential and non essential amino acid profiles of the *Pelophylaxesculentus* 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 *Hoplobatrachusoccipitalis*[3]. Since these essential and nonessential amino acidscomplement one another when present in foods and *Pelophylaxesculentus* meat contained these acids in reasonable amounts, it could be a good source of these amino acids.

301

302 5.0 CONCLUSION

303

From the results obtained in this study, it could be inferred that meat of *Pelophylaxesculentus* 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. *Pelophylaxesculentus* also showed higher nutritional values than some meat most especially considering its crude protein value. Thus, this probably makes *Pelophylaxesculentus* meat a better source of animal protein than some animal sources.

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