

Microbiological Flora and Proximate Composition of the Large African Cricket, *Brachytrupes Membranaceus*

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Received: September 19, 2016; **Published:** October 25, 2016

Abstract

The microbial flora and proximate composition of the large African cricket, *Brachytrupes membranaceus*, were examined. The heterotrophic bacterial, fungal and coliform counts of the external surface of the beetle were 1.71×10^7 cfu/g, 1.06×10^6 cfu/g and 4.40×10^4 cfu/g, respectively. The bacterial species isolated belonged to the genera, *Bacillus*, *Enterococcus*, *Proteus* and *Staphylococcus* while the fungi included the genera *Aspergillus* sp., *Cryptococcus* sp and *Fusarium* sp. These bacterial and fungal species are saprophytes and do not pose a health or sanitary problem. The proximate composition was similar to that of either lean meat or chicken at 20.22% while fat was considerably higher at 18.10% compared to lean meat (3.80%) or chicken (7.23%). Moisture content was 54.49%, about ten percentage points lower than that of either lean meat or chicken while carbohydrate was 1.22%. *Brachytrupes membranaceus* is a good source of protein.

Keywords: Microbiological flora; *Brachytrupes membranaceus*; cricket; *Bacillus*, *Enterococcus*, *Proteus* and *Staphylococcus*

Introduction

Entomophagy, the practice of eating insects and arachnids is becoming attractive globally. *Brachytrupes membranaceus* is a pest of crops (e.g. yam and tobacco) and a delicacy in many homes in Nigeria. Alamu., *et al.* [5] listed the species as one of the edible insects in Nigeria. It is not listed as an African edible insect by Kelemu., *et al.* [14] but listed as one of the World's edible insects by Jongema [13]. In Nigeria the practice of eating insects is common in the humid forest zone [5]. The genus occurs in the tropical and subtropical belt of Asia and Africa [9]. The insect lives entirely underground, is nocturnal, breeds once a year in February and March and adults die off between February and April [10]. In Nigeria *B. membranaceus* is known by different names such as apina (Eleme), pina (Ogoni), ediang (Ibibio) and gyare (Hausa). The insect is usually salted, seasoned and roasted before eating.

Amadi and Kinn-Kabari [3] gave a comprehensive review of the nutritional composition and microbiological flora of *B. membranaceus* and other insects commonly eaten in Africa and highlighted some inconsistencies in the results reported by various workers. For instance, considerable discrepancy exists in the proximate composition data for *B. membranaceus* as reported by Bukkens [8], Agbideye., *et al.* [2] and Adeyeye and Awokunmi [1]. Also there seems to be a dearth of information on the microbiological flora of *B. membranaceus*. This study, therefore, examined the microbial flora and proximate composition of this insect and compared the latter with those of other workers.

Materials and Methods

Materials

Live crickets were collected from a farmland in Mbiakpa village of Ini Local Government Area of Akwa Ibom State, Nigeria and transported to the laboratory in sterile perforated containers. Average weight of cricket was 4.54g. The crickets were either used immediately or within twenty-four hours after storage in a refrigerator.

The media used were Peptone water (Micro Master, India), Nutrient agar (Bio-Tech, UK), MacConkey agar (Titan Bio-Tech, India) and Sabourouds agar (Titan Bio-Tech, India). The carbohydrates used were glucose, maltose, mannitol, lactose, fructose and sucrose.

Lean meat and chicken were purchased from a daily local market in Port Harcourt, Rivers State, Nigeria.

Bacterial viable counts

The method used was the 10-fold serial dilution method of Harrigan and McCance [12]. Initial tenfold dilution of whole cricket was performed by adding an appropriate volume of physiological saline to a whole cricket, e.g., 6 ml was added to 4.0g cricket in a 50 ml conical flask and thoroughly shaken to dislodge bacteria. Subsequent 10-fold dilutions were carried out by adding 1.0 ml of the penultimate dilution to 9.0ml of fresh diluents. Finally, 0.1 ml of an appropriate dilution were placed on dry nutrient agar, evenly spread with a sterile glass spreader and incubated at 30°C for 24h. At the end of incubation, counts were performed for those dilutions which showed count between 30 and 300 colonies [4]. All counts were performed in duplicate and averages recorded.

Isolation and identification of isolates

Representative colonies from the initial 10-fold dilution were picked and sub-cultured several times onto nutrient agar until pure isolates/cultures were obtained. Such pure cultures were stored as frozen glycerol suspensions at -35°C [16]. These frozen glycerol suspensions served as a means for long term storage and as a source for working cultures.

Isolates were identified following the scheme of Cowan and Steel [11] and Buchanam and Gibbons [7]. The tests employed included catalase test, oxidase test, indole production, methyl-red test, motility and oxidation/fermentation tests for fructose, glucose, lactose, maltose, mannitol and sucrose.

Fungal isolates were identified using macro- and micro-morphology.

Chemical analyses for proximate composition

Proximate analyses were carried out on wet weight basis for moisture, protein (N x 6.25), ash, fibre and fat according to AOAC [6]. Total available carbohydrate was analysed using the difference method of Raghuramulu, *et al.* [15]. All analyses were performed in duplicate and averages taken.

Moisture content

The moisture content in the sample was determined using an air circulating oven (Gallenkamp, UK). Aluminium moisture cans were weighed and dried in the oven for 25 minutes and transferred to the desiccator to cool. Two grams (2g) of sample were weighed into the cans and the weight of moisture cans plus content noted. The cans were then transferred into the oven and heated to a temperature of 105°C for 4 hours. The cans and contents were then removed, placed in the desiccator, allowed to cool and re-weighed. The moisture content was calculated using the formula:

$$\text{Moisture (\%)} = \frac{\text{weight loss after drying (g)}}{\text{weight of sample (g)}} \times 100$$

Crude protein determination

The micro-Kjeldahl method was used for the determination of total nitrogen. Thus 0.5g of sample was weighed into 300ml digestion flask. One tablet of Kjeldahl catalyst was added followed by the addition of 10 ml concentrated sulphuric acid. The digest was cooled and transferred into 100 ml volumetric flask and made up to 100 ml mark with distilled water. Ten millilitres of the digest was placed into the micro- Kjeldahl distillation apparatus and neutralized with 10 ml of 40% sodium hydroxide. The content was then distilled into 25 ml of boric acid/indicator. Finally, the ammonium borate complex produced was titrated against 0.05M sulphuric acid. A blank determination without sample was also carried out. The amount of total nitrogen in the sample was calculated using the formula:

1 ml 0.05 sulphuric acid = 1.4 mg nitrogen.

$$\text{Nitrogen (\%)} = \frac{\text{titre (ml)} - \text{blank (ml)} \times 1.4}{\text{weight of sample (g)}} \times 100$$

$$\text{Crude protein (\%)} = \text{nitrogen (\%)} \times 6.25$$

Ash

The ash content of the sample was determined using the muffle furnace (Model SK, China). Porcelain crucibles were washed and dried in the oven after which 1g of sample in a crucible was placed in a muffle furnace at 550°C for 3 hours. At the end the crucibles were placed in a desiccator to cool and the re-weighed with its contents. The ash content was calculated as follows:

$$\text{Ash (\%)} = \frac{\text{weight of ash (g)}}{\text{weight of sample}} \times 100$$

Determination of crude fibre

Half a gram of moisture-free sample was extracted for three hours with petroleum ether (bp 60 – 80°C) using a soxhlet apparatus. The fat-free material was placed in a 200 ml beaker and 50 ml of 1.25% (v/v) sulphuric acid was added and covered with a watch glass. The content of the beaker was heated gently on a hot plate for 30 minutes (acid hydrolysis). At the end of the acid hydrolysis, the content of the beaker was filtered under vacuum through a buchner funnel fitted with filter paper (Whatman No. 4) and washed with boiling water until the washing was no longer acid to litmus. The residue was washed back into the beaker with 1.25% sodium hydroxide, covered with a watch glass and boiled for 30 minutes. The resultant insoluble material was transferred to a dried pre-weighed ash-less filter paper and washed thoroughly, first with hot water until it is no longer alkaline to litmus and then with 95% (v/v) ethanol and finally dried at 105°C to a constant weight for one hour. The filter paper and its contents were incinerated to ash at 500°C for one hour. The ash was then cooled

and weighed. The weight of the ash was subtracted from the increase of weight on the filter paper due to the insoluble material and the difference reported as fibre.

$$\text{Crude fibre (\%)} = \frac{\text{wt. of fibre}}{\text{wt. of sample}} \times 100$$

Determination of fat

Fat was extracted using the micro Soxhlet extraction unit. Thus, 1g of sample was weighed into an extraction thimble, placed in the extractor and fitted to the unit. Fat was extracted with 100 ml petroleum ether (boiling point, 60 - 80°C) for 5 hours using a reflux condenser. The extracted fat was transferred to an air circulating oven at 80°C for 20 minutes to evaporate the solvent. The flask was then cooled in a desiccator and re-weighed. Crude fat was calculated from the equation below:

$$\text{Crude fat (\%)} = \frac{\text{weight of extract (g)}}{\text{weight of sample (g)}} \times 100$$

Sample	Proximate composition (%)					
	Moisture	Ash	Fat	Protein	Fibre	Carbohydrate
<i>B. membranaceus</i>	54.49	0.97	18.10	20.22	5.0	1.22
Chicken	65.20	2.61	7.23	19.80	2.86	2.30
Lean meat	66.40	1.10	3.80	21.71	5.57	1.42

Table1: Comparison of Proximate compositions (on wet wt. basis) of *Brachytrupes membranaceus*, chicken and lean meat.

Results and Discussion

The Bacterial populations for the cricket were 1.71×10^7 cfu/g (total heterotrophs), 1.06×10^6 cfu/g (coliforms) and 4.40×10^4 cfu/g (fungi). Although the heterotrophic bacterial load or count is high, the roasting process will considerably reduce it. The bacterial species isolated belonged to the genera, *Bacillus*, *Enterococcus*, *Proteus* and *Staphylococcus* while the fungi included the genera *Aspergillus* sp., *Cryptococcus* sp and *Fusarium* sp. These are normal soil micro-biota and not pose a sanitary or health problem. Table 1 shows the proximate composition of *B. membranaceus* compared with that of chicken and lean meat. Thus, protein content of *B. membranaceus* was similar to that of either chicken or lean meat while the fat content was considerable higher for the insect at 18.10% as compared to 7.23% for chicken or 3.80% for lean meat. Moisture content was higher in both chicken and lean meat (65.20% and 66.40%) than *B. membranaceus* which was 54.49%. Total carbohydrate was low for all three at 1.22%, 2.30% and 1.42% for *B. membranaceus*, chicken and lean meat, respectively. Our values for the proximate composition were much lower for the same parameters except for moisture when compared with those of Agbidye, *et al.* [2]. For those parameters where our values were lower, they vary between 1 and 35 percentage points and ca 43 percentage points for moisture (Table 2). Our results were also lower than those of Adeyeye and Awokunmi [1] except for moisture and fat (Table 2). Thus, with the exception of moisture and fat our values vary from those of Adeyeye and Awokunmi [1] between 3 and 14 percentage points. Our values were on wet weight basis while those of Agbidye and co. and Adeyeye and Awokunmi [1] were on dry weight basis. Considerable variations were also evident when the data of Agbidye and co. and Adeyeye and Awokunmi [1] were compared (Table 2), both data obtained on dry weight basis. Amadi and Kinn-Kabari [3] had recently called attention to the disparity observed in proximate composition by various workers and called for standardisation. We attribute this disparity to the formula used in the conver-

sion of wet weight values to dry weight or vice versa. What is clear or evident from this comparison is that whether dry weight result is compared with dry weight or wet weight results, disparity or differences exists. What is needed, therefore, is a method that would lend itself to comparison and produce comparable and reliable data. Finally, based on this result and those of other workers, *B. membranaceus* is a good source of protein.

Parameter	Author			Differences in Percentage point (B - A)	Differences in Percentage point (C - A)	Differences in Percentage point (B - C)
	This work A	Agbidye., et al. (2) B	Adeyeye and Awokunmi (1) C			
Moisture	54.49	11.60	0.90	- 42.89	- 53.59	10.70
Ash	0.97	3.25	5.70	2.28	4.73	- 2.45
Fat	18.10	53.05	4.20	34.95	- 13.90	48.85
Protein	20.22	35.06	29.10	14.84	8.80	5.96
Fibre	5.0	6.30	8.20	1.30	3.20	- 1.90
Carbohydrate	1.22	2.33	51.90	1.11	50.68	- 49.57

Table2: Comparison of wet wt. values (this work), versus dry wt. values (Agbidye., et al. (2) and Adeyeye and Awokunmi (1).

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Volume 5 Issue 3 October 2016

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