

Evaluation of Bacteriological Quality and Proximate Composition of African Giant Snail (*Archachatina Marginata*) Sold in Port Harcourt, Nigeria

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Abstract

Snails are highly perishable, and prone to vast variations in quality due to differences in species, as well as the environmental and feeding habits. This study therefore seeks to evaluate the bacteriological and proximate composition of Snail (*Archachatina marginata*) sold in markets around Port Harcourt. In this study, a total number of sixty (60) samples were collected from three markets: Creek Road, Mile One and Rumuokoro. The samples were collected using sterile bags properly labeled according to standard microbiological procedures. Standard analytical procedures were employed to determine the bacteriological characteristics and proximate composition of the various parts such as flesh, intestine, and fluid of the samples. Statistical analyses were carried out using ANOVA and All Pairs Turkey-kramer. Results obtained from the study showed that Total heterotrophic bacteria counts of the snail parts from the three sampled markets ranged from $3.7 \pm 0.52 \times 10^6$ cfu/ml (fluid) to $8.6 \pm 1.24 \times 10^6$ cfu/g (Intestine), total coliform count ranged from $0.6 \pm 0.31 \times 10^4$ cfu/ml (fluid) to $4.3 \pm 1.1 \times 10^4$ cfu/g (Intestine), *Listeria* count ranged from $0.6 \pm 0.34 \times 10^4$ cfu/ml to $1.9 \pm 0.55 \times 10^4$ cfu/g while *Salmonella* counts ranged from $0.4 \pm 0 \times 10^4$ cfu/ml to $3.6 \pm 1.58 \times 10^4$ cfu/g. The mean values for all the microbial counts were significantly different ($P < 0.05$) in the three samples across the sampled markets. Using genomic identification technique *Listeria* species isolated and identified in this study include; *L.monocytogenes*, *L.grayi*, *L. seeligeri*, *L.ivanovii*, and *L.welshmeri* while species of *Salmonella* include *S. enterica* and *bongori*. Other bacterial isolates were identified as *Vibrio spp*, *Bacillus spp*, *Staphylococcus spp*, *Shigella spp*, *Pseudomonas spp*, *Enterobacter spp*, *E. coli*, *Micrococcus spp*, *Acinetobacter spp*, *Klebsiella spp*. Results of proximate analyses revealed that the protein content of the snail was relatively good which makes it a good alternative source of protein. This study revealed that the meat of snails traditionally sold in the city of Port Harcourt were of poor microbiological quality, The presence of pathogenic organisms isolated in this study is of public health concern as these organisms are known causes of food-borne diseases. Therefore, awareness of public health implication of under cooked food samples such as snails should be emphasized via television, radio stations and schools.

Keywords: Proximate, Bacteriological, Snail, Evaluation, Consumption.

Introduction

Land snails are ubiquitous creatures found on farms, forests and in homes. In some Communities with poor sewage disposal systems, snails are often found at the sewage disposal sites. Snails are popular protein sources consumed by people worldwide, apart from being a protein source, snails are a good source of iron, calcium, and phosphorus, and is said to contain almost all the amino acids needed by humans (Akinrotimi *et al.*, 2011). The African giant snails are considered as a delicacy in Nigeria, and they are sold in many markets within and outside Port Harcourt. The diverse environments in which these snails are found make them susceptible host to a vast range of microorganisms. Thus, they could harbour parasites, Bacteria, viruses as well as fungi. Bacteriological studies (Akpavie *et al.*, 2000), indicate that various potentially pathogenic bacteria inhabit different organs and tissues including the haemolymph of the African giant snail. The presence of pathogenic bacteria in the snails is a health threat to consumers. Studies done by Efuntoye *et al.* (2011). The close contact of wild snails with soil and organic debris, and their uncontrolled feeding pattern make them susceptible to microbial contamination. Snails are regarded to have high populations of indigenous bacteria and coliforms, and also suspected to contain certain poisonous substances which they ingest from their environment (Nyoagbe *et al.*, 2016). The meat of snails can be easily contaminated by microbial pathogens thereby serving as a vehicle for transmission of infectious agents to consumers. Microorganisms that have been found in snails include *Escherichia coli*, *Pseudomonas* spp., *Shigella* spp., *Enterobacter* spp., *Salmonella* spp., and *Klebsiella* spp. (Nwuzo *et al.*, 2016). The presence of *Listeria monocytogenes* have also been reported in snails (Kirkan *et al.*, 2006).

Proximate composition of food is the term applied to the proportion of moisture, fat, carbohydrate, protein, fibre and ash present in foods (Raimi, 2009). proximate composition include five constituents Ash, Moisture, Proteins, Fat and Carbohydrates. Analytically, four of the five constituents are obtained via chemical reactions and experiments. The fifth constituent, (carbohydrates) is a calculation based on the determination of the four others. Proximate should nearly always add up to 100%, any deviation from 100% displays the resolution of the chemical test i.e. small variations in the way each test is performed chemist to chemist will accumulate or overlap the composition make-up (Amadi *et al.*, 2016). The need for nutritional information on any edible food product, ever known to man, cannot be over-emphasized. This information serves as a guide to consumers on the quality assurance for already existing food products and establishes acceptability for new ones. Provision of safe, wholesome and acceptable snail, through quality control against contamination is essential for consumer safety (Daminabo *et al.*, 2021). Contamination of snails for sale often occur as a result of the way they are handled and exposed in the markets (Ogbonna and Inana 2018), also the inherent microbial load in this food product increases during exposure as they are not showcased at low temperatures that will retard microbial growth not only that but also consumption of snails laden with microbial pathogens can lead to food borne diseases that can result to

death. Hence, this work is aim to evaluate the bacteriological quality and proximate composition of Snail (*Archachatina marginata*) sold in markets around Port Harcourt.

Statement of Problem

Snail can also function as carriers of several microbial and other health hazards (Yagoub, 2009). Maintenance of quality is of utmost importance in production and trade of snail products, Hence, this study will provide specific information as regards the proximate composition and microbial load of and snails sold in Port Harcourt.

Objectives of the Study

The objectives of this study were;

1. Enumerate and isolate total heterotrophic bacteria associated with the fishes and
2. Evaluate the heavy metal contents and proximate composition of the fish and snail samples.

Materials And Methods

Description of Study Area

Port Harcourt is located in the Niger Delta region, Southern Nigeria. The city is situated between latitudes 3°37' and 3°56' N. and longitude 11°10' and 11°45' E, approximately 50km from the Atlantic coast. Precipitation averages 3,030mm annual and a temperature average of 23°C. Port Harcourt is in the Niger Delta region, Southern Nigeria. One of the major occupations of the residents/inhabitants in Port Harcourt is fishing around the marine communities in spite of the various exploitation of oil by multinational companies. Figure 1 shows the map of Port Harcourt indicating the sampled markets.

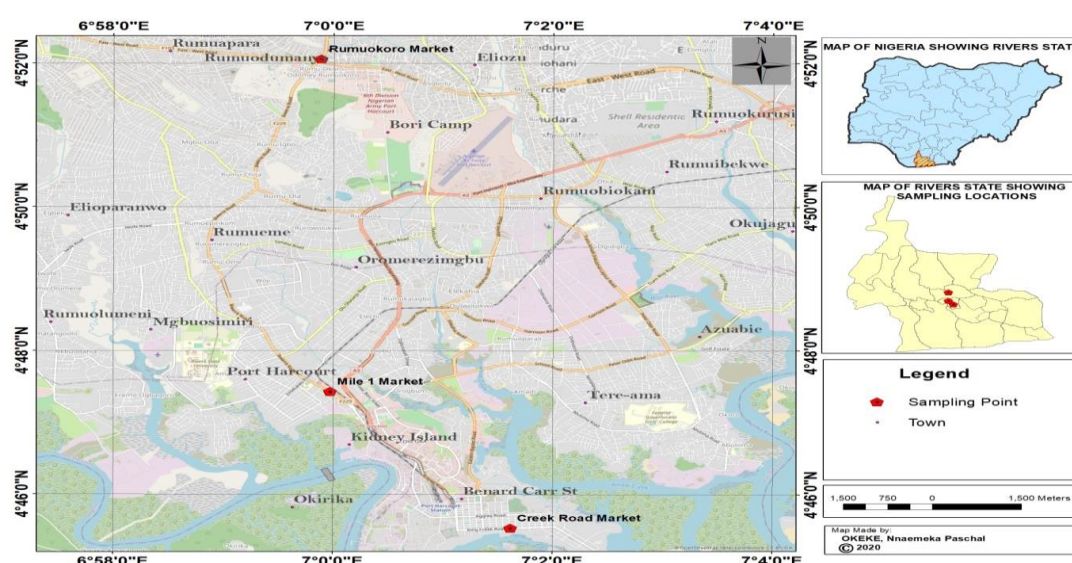


Figure 3.1: Map showing Study Area

Researcher's Field survey (2023)

Samples processing

Upon arrival at the laboratory, under sterile conditions, the snails were washed extensively with distilled water to remove contaminants present at the surface of their shells, rinsed with sterile distilled water, and disinfected with ethanol 70% (v/v). Then, the snails were removed from their shells, carefully eviscerated wash and rinsed thoroughly with sterile distilled water. The meat and intestine were then aseptically extracted from the shell and homogenized while the fluids were carefully collected in a sterile universal bottle.

Bacteriological Evaluation

Serial dilution

One gramme each of the respective samples was separately added to 9 ml of 0.1% peptone water diluent. After thorough shaking, further tenfold (v/v) serial dilutions were made by transferring 1 ml of the original solution to freshly prepared peptone water diluent to a range of 10^{-6} dilutions (Prescott *et al.*, 2005).

Inoculation and Incubation

Aliquots (0.1 ml) of various dilutions were inoculated to surface dried Plate Count Agar (PCA) in triplicates for enumeration of total heterotrophic bacterial population, McConkey agar for total coliform population, *Salmonella-Shigella* agar (SSA) for *Salmonella* and *Shigella* populations, and *Listeria* selective agar base supplemented with *Listeria* Selective Supplement II (FDo63) for *Listeria* population in triplicates and spread evenly with flamed bent glass spreader. The plates were incubated at 37°C for 24 hours.

Enumeration and Isolation of Pure Culture:

Bacteria were enumerated as described by Prescott *et al* (2005). Bacterial colonies that appeared on the respective plate were counted and the mean expressed as cfu/g for the respective samples (Odokuma and Nrior, 2015). The colony forming unit per gramme of the sample was calculated using the formula below;

$$CFU/G = \frac{\text{number of colonies}}{\text{Dilution} \times \text{volume plated}}$$

Maintenance of pure culture

Discrete bacterial colonies that grew on the respective media were sub cultured using streak plate method onto freshly prepared nutrient agar and incubated at 37°C for 24 hours in order to obtain pure culture. The pure bacteria cultures were then maintained according to the method adopted by Amadi *et al.* (2014) using ten percent (v/v) glycerol suspension at -4°C.

Identification of the Bacterial Isolates

Pure bacterial isolates were identified by the method described by Collins *et al.* (1989), and Cheesbrough (2002). Pure bacterial isolates were subjected to Biochemical tests which

include oxidase test, Catalase test, Indole test, methyl red test, Voges Proskauer test, Starch hydrolysis test, Urease test, Citrate test, Sugars fermentation test and Triple sugar iron agar test. And further identification using molecular technique was also carried on the isolates.

Proximate Analysis

The proximate composition of the different parts of the snail were evaluated using standard analytical procedures. The moisture was determined using the gravimetric method AOAC (1995). The ash content was assessed following the method AOAC (1990) while the protein content was estimated by the method of Devani *et al.* (1989), lipids by the method of Bourelly (1982) and total sugars (Carbohydrate) by the method of Dubois *et al.* (1956).

Statistical Analysis

All experiments were repeated at least three times. The data obtained were expressed as mean standard deviation. Analysis of variance (ANOVA) and Tukey's test were used to compare means at $p < 0.05$. These analyses were performed using Minitab 16 software (Minitab Ltd., Coventry, UK).

Results

Mean bacteriological counts of the various snail parts analyzed in the study is presented in Table 1-4 respectively. Total heterotrophic bacteria count of the snail parts from the three sampled markets ranged from $3.7 \pm 0.52 \times 10^6$ cfu/ml (fluid) to $8.6 \pm 1.24 \times 10^6$ cfu/g (Intestine). the results showed that intestine have the highest bacteriological counts across the three markets.

Total coliform counts of the various sample parts from the three sampled markets ranged from $0.6 \pm 0.31 \times 10^4$ cfu/ml (fluid) to $4.3 \pm 1.1 \times 10^4$ cfu/g (Intestine). These shows that at $P < 0.05$ there were significant differences between the count obtained from the fluid across the three sampled markets (Table 2).

Listeria count ranged from $0.6 \pm 0.34 \times 10^4$ cfu/ml to $1.9 \pm 0.55 \times 10^4$ cfu/g while *Salmonella* counts ranged from $0.4 \pm 0 \times 10^4$ cfu/ml to $3.6 \pm 1.58 \times 10^4$ cfu/g of the various sample parts from the three sampled markets (Table 3 and 4). The results obtained in this study agree with the observation of Adebayo *et al.* (2012) who obtained similar bacterial count in their research on microbial quality of different snail sold in Uyo metropolis except that the values obtained in this study were significantly high. These could be attributed to environmental factors and the feeding habit (Arannilewa *et al.*, (2006).

These results revealed that total heterotrophic bacteria, total coliform, *Salmonella* and *Listeria* species counts followed similar trend of having the highest in the intestine and lowest in the fluid for the snail samples this may be because of the high nutritional values stored in the intestines and the low composition in the fluid and also the present of certain antimicrobial property such as slime contained in the fluid of snails. (Ajao *et al.*, 2009). Similar trends were observed across the three markets though there were significant

differences between the bacterial count among the various sample parts analysed in this study but no significant difference at $P < 0.05$ within samples parts when compared to the various markets Okonko *et al.*, (2009)

The values of bacteriological counts obtained in this study were above the permissible limit as recommended by regulatory bodies. According to the World Health Organization (WHO) directives on microbial limits, total bacterial count should not exceed 5×10^5 colonies per gramme of sample. The result of the conventional and genomics identification shows that five *Listeria* species which include, *L.monocytogenes*, *L.graji*, *L. seeligeri*, *L.ivanovii*, and *L.welshmeri* were isolated and identified in this study. While three species of *Salmonella*: *S. arizonae*, *S. gallinarum*, and *S. typhi* were isolated. Other bacterial isolates were identified as *Vibrio* spp, *Bacillus* spp *Staphylococcus* spp *Shigella* spp *Pseudomonas* spp. *Enterobacter* spp. *E.coli* *Micrococcus* spp. *Acinetobacter* spp. *Klebsiella* spp. The presence of contaminating bacteria obtained in this study could be attributed to cross- contamination from environment, source, and handling by the sellers. These results are in line with reports of other studies in Nigeria by various authors (Okonko *et al.*, 2008, Chukwuka *et al.*, 2010; Akintobi *et al.*, 2011; Al-Hindi *et al.*, 2011). It showed that the organism isolated in this study suggest the high level of contamination of environment where the samples were obtained, meaning that water body is not free from microorganisms. Apart from the *Listeria*, *Salmonella* and other enteric organisms which serve as indicator of pathogens, *Staphylococcus* species isolated in this study are known enterotoxin producing agent. This is in agreement with the previous reports of some other workers (Adebayo *et al.*, 2012, Okonko *et al.*, 2009). Similar bacteria isolated in this study were also reported for fish and fish products by other researchers (Koutsoumanis and Nychas 2000, Herrera *et al.* 2006, and Yagoub 2009).

Table 1: Total Heterotrophic Bacteria

Snail Parts	Sample Markets			Mean of MEAN	
THB	Mile 1 (n=20)	Creek road (n=20)	Rumuokoro (n=20)	P value	Significant Difference
Snail Fluid (cfu/ml)	4.9 ±0.96x10 ⁶ b	4.4 ±0.87x10 ⁶ d	3.7 ±0.52x10 ⁶ c	0.109	No
Snail Intestine (cfu/g)	8.6 ±1.24x10 ⁶ a(x)	8.2 ±1.23x10 ⁶ a(x)	6.7±0.99x10 ⁶ a(y)	0.0497*	Yes
Snail Meat (cfu/g)	5.4 ±3.01x10 ⁶ b	5.0 ±2.56x10 ⁶ cd	4.3 ±2.32vbc	0.792	No

Table 2: Total Coliform

Snail Parts	Sample Markets			Mean of MEAN	
TC	Mile 1 (n=20)	Creek road (n=20)	Rumuokoro (n=20)	P value	Significant Difference
Snail Fluid	1.7 $\pm 0.67 \times 10^4 f(x)$	0.6 $\pm 0.21 \times 10^4 e(y)$	0.6 $\pm 0.31 \times 10^4 e(y)$	0.006*	Yes
Snail Intestine	4.3 $\pm 1.1 \times 10^4 de$	3.7 $\pm 1.53 \times 10^4 cd$	3.8 $\pm 0.88 \times 10^4 c$	0.714	No
Snail Meat	3.5 $\pm 0.84 \times 10^4 e$	2.9 $\pm 0.92 \times 10^4 d$	2.6 $\pm 0.73 \times 10^4 d$	0.264	No

Table 3: Listeria count

Snail Parts	Sample Markets			Mean of MEAN	
LISTERIA	Mile 1 (n=20)	Creek road (n=20)	Rumuokoro (n=20)	P value	Significant Difference
Snail Fluid	1.1 $\pm 0.18 \times 10^4 c(x)$	0.6 $\pm 0.34 \times 10^4 d(y)$	0.7 $\pm 0.29 \times 10^4 d(y)$	0.025*	Yes
Snail Intestine	1.9 $\pm 0.55 \times 10^4 c$	1.6 $\pm 0.44 \times 10^4 c$	1.9 $\pm 0.57 \times 10^4 c$	0.672	No
Snail Meat	1.2 $\pm 0.16 \times 10^4 c$	0.8 $\pm 0.44 \times 10^4 cd$	1.1 $\pm 0.18 \times 10^4 d$	0.235	No

Table 4: Salmonella count

Snail Parts	Sample Markets			Mean of MEAN	
SALMONELLA	Mile 1 (n=20)	Creek road (n=20)	Rumuokoro (n=20)	P value	Significant Difference
Snail Intestine	3.6 $\pm 1.58 \times 10^4 b$	1.7 $\pm 0.96 \times 10^4 b$	1.5 $\pm 1.15 \times 10^4 b$	0.341	No
Snail Meat	1.7 $\pm 0.96 \times 10^4 b$	1.5 $\pm 1.0 \times 10^4 b$	1.0 $\pm 1.0 \times 10^4 b$	0.19	No
Snail Fluid	1.0 $\pm 0 \times 10^4 b$	0.4 $\pm 0.58 \times 10^4 b$	0.4 $\pm 0 \times 10^4 b$	0.823	No

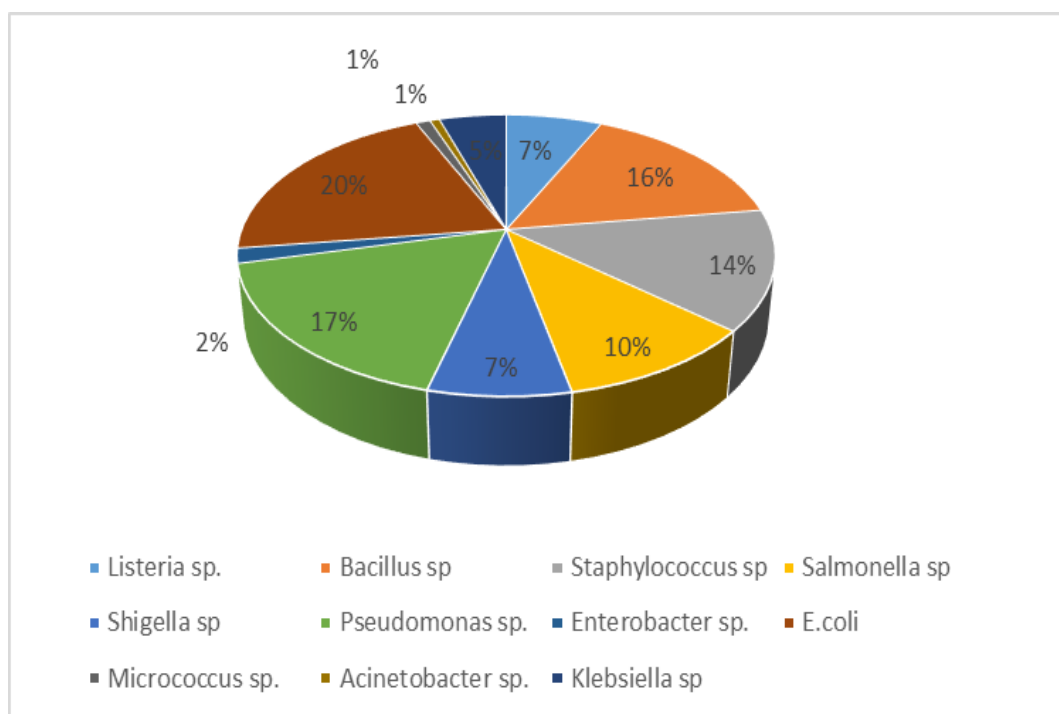


Figure 1: Percentage Occurrence of the Bacteria Isolated

Source: Researcher's Field survey (2023)

The presence of *Listeria* spp in food which colonizes the gastrointestinal tract, especially *Listeria monocytogenes* being the pathogenic specie has the unique ability to cause mammalian cells to absorb the organism into their cytoplasm. Using this ability, the pathogen penetrates the intestinal mucosa and can disseminate by cell-to-cell spread or hematogenously (Omorodion *et al.*, (2016). *L. monocytogenes* has the ability to cross three key barriers; the intestinal barrier, the blood–brain barrier and the feto placental barrier, so that it can infect organs such as the brain or uterus, and cause severe life-threatening infections such as meningitis, encephalitis, spontaneous abortion, or miscarriage (Adebayo *eta el.*, 2012). According to Håstein *et al.*, (2002) *L. monocytogenes* can survive for long periods of time in a seemingly hostile environment such as a food processing facility partially due to its ability to endure various stresses, such as sanitizers, pH and temperature and its ability to form biofilm leading to persistence.

Proximate Analysis of Different Parts of (Snail) *A. Marginata*

The result of proximate composition of snail revealed the following ranges: Moisture content 61 to 95 %, Ash 0.59 -2.16%, Fat 0.04 –12.3%, crude protein 0.16– 19.69%, Crude fibre 0 – 3.7% and Carbohydrate 4.03 – 11.08% as presented in the Figure 2 below.

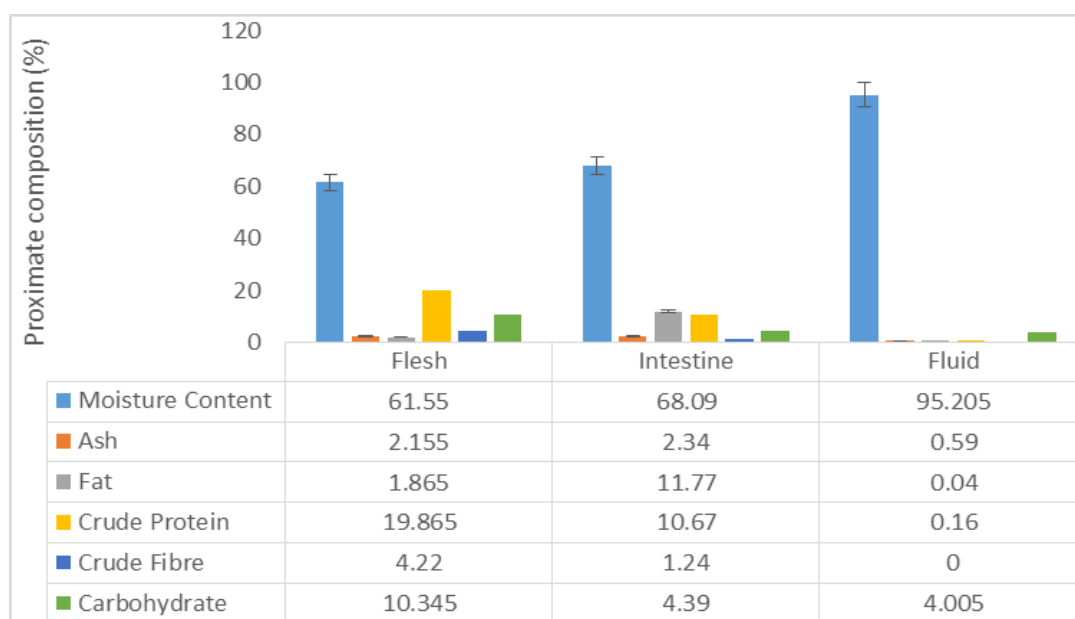


Figure 2: Proximate analysis of different parts of (snail)

Source: Researcher's Field survey (2023)

The moisture content recorded for snail meat is less than the 84.91% reported by Babalola and Akinsoyinu (2009) indicating some levels of disparity. Raimim (2019) however reported a moisture content of 66.27% similar to the record obtained in this study. Likewise, Babalola and Akinsoyinu (2009) reported a very low crude protein content (5.86%) in their research compared to the 20.04% this study established in snail meat Raima, (2019), However reported a 26.12% crude protein content in snail meat analysed.

Conclusion

This study revealed that the meat of snails traditionally sold in the city of Port Harcourt were of poor microbiological quality. The presence of pathogenic organisms isolated in this study is of public health concern as these organisms are known causes of food-borne diseases. They contained several pathogens which rendered questionable the safety issue of these meat products. The fact that snails' meat is consumed either cooked or roasted might considerably reduce these contaminations and make the product suitable for human consumption. However, the presence of microorganisms with spores forming and toxins-producing abilities which are heat stable suggests that particular attention should be given to these products in order to prevent high level of public health risk to consumers. The proximate analysis revealed relatively good amount of protein in the snail flesh which makes it a good alternative source of protein.

Recommendations

1. Awareness of public health implication of under cooked food samples such as snails should be emphasized via television, radio stations and schools.

2. It is important that all hazard analysis critical control point be adhered to for good production processes.
3. Proper blanching and heating methods should be employed during preparations of snail.

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