

Original Article**Quality Assessment of Edible Fish Species at Flood Basin of Omambala River, South East Nigeria**

Uwakwe Simon Onoja¹, Nwachukwu Romanus Ekere^{*2}, Joy Uzoamaka Ezugwu¹, Remigius Ibe Onoja³

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ABSTRACT

Background: Food poisoning resulting from microbial and heavy metals pollution is commonplace. These contaminants do not only reduce the nutritional value of the food but are in many cases toxic. In this study, the proximate composition, bacterial quality and some heavy metals (Cd, Pb, Hg) concentration in three common fish species (*Claria gariepinus*, *Heterobranchus bidorsalis* and *Channa obscura*) found in flood basin of Omambala River in Anambra, Nigeria were evaluated from 2016 to 2017.

Methods: Wet digestion was used for the samples preparation and AOAC methods were adopted for the proximate analysis. Standard aerobic pour-plate techniques were used for bacterial enumeration while heavy metal concentrations were determined using Atomic Absorption Spectrophotometer (AAS).

Results: All the fish species had high protein, moisture, lipid, ash and minerals contents. Mean bacteria count of 3.36×10^6 cfu/g was observed in *C. gariepinus* while *C. obscura* had mean bacterial count of 3.23×10^6 cfu/g and *H. bidorsalis* had mean bacterial count of 3.40×10^6 cfu/g. *C. gariepinus* had a cadmium and lead concentration of 0.012 ± 0.012 mg/kg, and 0.0043 ± 0.0012 mg/kg respectively while *C. obscura* had 0.010 ± 0.005 mg/kg and 0.037 ± 0.013 mg/kg of cadmium and lead. *H. bidorsalis* had 0.011 ± 0.002 mg/kg, and 0.048 ± 0.005 mg/kg of cadmium and lead respectively.

Conclusion: All the fish species are good sources of nutrient. The microbial and heavy metals concentrations were within the acceptable international limits for heavy metals in foods. However, the presence of these toxicants in the samples is a source of concern in relation to the health of the consumers.

Keywords: Bacteria, Fish, Flood Basin, Heavy Metal, Nutrients, Proximate.

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INTRODUCTION

One major public health problem in developing countries is malnutrition. Fish makes a very significant contribution to nutrition because it is a source of vital nutrients to many people worldwide. Fish is a highly proteinous food consumed by many people especially in the developing countries due to their availability and palatability [1, 2]. Fish is consumed more than meat in Nigeria because it is cheaper and relatively abundant [3]. The increased awareness of the diseases of modern civilization notably, non-communicable diseases make fish the better choice as it contains long-chain polyunsaturated omega-3 fatty acid. This compound is known to improve lipid profile, reduce cholesterol

levels, the risk of coronary heart diseases, stroke and preterm diseases [4-6]. Fish has hypolipidemic/or antiatherogenic effects and is also a source of vitamins and minerals [6]. It can also decrease the risk of prostate cancer, reduce occurrence of renal-cell carcinoma in women and reduce risk of dementia and Alzheimer disease [5]. Fish is consumed fresh or smoked and form by all socio-economic, socio-cultural, age and religious groups in Nigeria [7, 8]. In many areas, fishermen hunt fish in natural water bodies or in artificial ponds as a means of income [9].

Regular fish intake is known as a potential source of human exposure to toxic chemicals [4, 6]. Some heavy metals found in fish samples toxic and may enter water bodies from natural and anthropogenic

1. Department of Home Science, Nutrition, and Dietetics, University of Nigeria, Nsukka, Nigeria.

2. Department of Pure and Industrial Chemistry, University of Nigeria, Nsukka, Nigeria.

3. Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka, Nigeria.

* Corresponding Author: E-mail: nwachukwuekere64@gmail.com

sources [10]. Fish can also be polluted by microbes. These microorganisms especially bacteria enter water through animal excreta, agricultural runoff, industrial and domestic wastes [11-13].

In Nigeria, many fish species are found in flood basin where they are hunted for food. The nutrient composition of these species may be different when compared to fish bred in captivity. Scanty information exists on the nutritional and contaminants status of fish species commonly found in flood basins in developing countries like Nigeria [14]. Hence, the nutritional status and the risk associated with eating such fish makes it imperative to evaluate the proximate composition, microbial quality and heavy metals content of the fish species.

The objective of this study was to assess the proximate composition, bacterial and some heavy metal contents of predominantly consumed fish species (*C. gariepinus*, *H. bidorsalis* and *C. obscura*), found in Omambala River flood basin in Anambra, South eastern Nigeria.

MATERIALS AND METHODS

Study Area

The study was conducted from 2016 to 2017 in Omambala River in Anambra State which flows into the River Niger and is found in Anambra State, Nigeria. The river is the most important feeder of the River Niger South of Lokoja. Anambra is a southeastern state of Nigeria and its name was from Oma Mbala, the native name of the Anambra River. The location of Anambra is in the coordinates 6°21'N 7°E with area of 4844km².

Sample Collection

Thirty-three fresh fish samples made up of eleven samples each of different species (*C. gariepinus*, *H. bidorsalis*, and *C. obscura*) were purchased from fishermen at Omambala river bank immediately they were caught from the river basin. The samples were collected from flood basin using equipment like baited foul-hook, lift net, flattened hook, gill net, traps, spring-loaded set lines, cages of mesh sizes of 50mm, 75mm, and 100mm each kept inside open container containing water.

The identities of the samples were authenticated at the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka.

Sample Preparation

The samples thoroughly washed with deionized water to remove any adhering contaminants, followed by draining under folds of filter paper. They

were dissected with a stainless knife to remove the gut and bones. The muscles were homogenized with an electric food blender and stored at -20°C prior to analysis [8].

Proximate Analysis

The determinations of moisture, ash, crude protein, fat and carbohydrate content were done according to the method of AOAC [15]. The percentage of protein content was calculated according to the following equation:

$$\% \text{ Nitrogen} = \frac{0.014 \times \text{VD} \times \text{N} \times 100 \times \text{TV}}{\text{Weight of sample} \times \text{AD}}$$

$$\% \text{ Protein} = \% \text{N} \times \text{F}$$

where, VD = volume of digest; N = normality of acid; TV = titre value; AD is the aliquot of digest and F = conversion factor for nitrogen to protein (6.25). Crude fat got gravimetrically from complete extraction from 2.0 gr of each sample in a Soxhlet apparatus using petroleum ether as solvent. Carbohydrate content was calculated by based on difference calculation:

$$\text{Carbohydrate} = 100\% - (\% \text{ moisture} + \% \text{ ash} + \% \text{ crude protein} + \% \text{ fat}).$$

Microbial Examination

The microbial (bacterial) examinations of the samples were done by standard plate count technique. A mass of 1.0 gr of each of the samples was homogenized in 9ml saline to make up the volume of the homogenate to 10ml suspension. The suspension was further diluted in 10 folds and 0.1ml of the dilution evenly spread on MacConkey and *Salmonella shigella* agar plates. The plates were incubated at 37°C for 24h. The growth was observed and colonies were counted. Microscopic examination was carried out to identify the different bacteria present.

Heavy Metals Determinations

The edible portions of the fish samples (muscles and gills) were removed and homogenized. Each sample (2.0 g) was subjected to wet digestion using 14 ml of nitric acid - perchloric acid (HNO₃:HClO₄) mixture in 5:2 ratio overnight at room temperature. The digest was, allowed to cool to room temperature, filtered with glass wool and made up to 50ml with deionized water. The filtrates were analyzed in triplicate, using Buck 2000 Atomic Absorption Spectrophotometer (AAS). Cd and Pb were analyzed using FAAS while Hg was assayed with cold vapour technique.

Statistical Analysis

Data obtained were subjected to analysis using SPSS statistical package version 15 for windows. Significant differences were accepted at $P < 0.05$ probability level.

Quality Assurance

The apparatuses used were washed thoroughly, rinsed and soaked overnight in 5% v/v HNO_3 . Blanks were taken through same digestion procedure. Limit of detection (LOD) and limit of quantitation (LOQ) were estimated using the method [16]. Recovery Analysis was carried using six randomly selected samples (two each of the species) to determine equipment working conditions and method accuracy. The fish samples were spiked with three different known quantities of the pure metals salts. Calibrations were done with 1000mg/L stock solutions of the respective salts supplied by BDH, UK. The stock solutions were serially diluted to get

the required concentration levels for calibrations. R^2 values ranging from 94 to 99% were obtained. The LOD and LOQ of 0.003 and 0.009 for Cd and 0.005 and 0.016 for Pb were respectively obtained. Recoveries between 97% and 104% were obtained from the calculations.

There is no conflict of interest in this work and all the authors contributed equally in all aspects of it. Human or animal study/usage was not part of the work.

RESULTS

The results of the analysis conducted on the samples were presented in this section. These include proximate composition, microbial study and heavy metals contamination. The result of proximate compositional analysis of the fish species showed in Table 1. Table 2 and 3 present the microbial content while Table 4 is the metal toxicants in the samples.

Table 1. Means \pm standard deviations of the proximate composition of the fish species (g/100 g wet weight).

Fish species	Fat	Ash	Protein	Carbohydrate	Moisture
<i>C. gariiepinus</i>	12.1 \pm 0.14	3.38 \pm 0.46	22.14 \pm 0.12	5.48 \pm 0.13	57.22 \pm 1.80
<i>C. obscura</i>	12.06 \pm 0.11	4.11 \pm 0.13	21.82 \pm 0.01	3.80 \pm 0.51	58.14 \pm 0.36
<i>H. bidorsalis</i>	11.49 \pm 0.51	3.00 \pm 0.49	21.12 \pm 0.45	4.01 \pm 0.49	60.34 \pm 0.10

H. bidorsalis had the highest moisture content (60.34%) while the lowest was found in *C. gariiepinus* (57.22%). *C. gariiepinus* had the highest protein content value of 22.14% while the lowest value of 21.12% was found in *H. bidorsalis*. *C. obscura* had the highest ash content of 4.11% among the three fish species while *H. bidorsalis* recorded the lowest value of 3.00%. *C. gariiepinus* had the highest value of 12.1% while *H. bidorsalis* recorded the lowest value of 11.49%. The carbohydrate content of *C. gariiepinus* was the highest and *C. obscura* had the lowest value.

Table 2. Total bacteria count TBC ($\times 10^6$ cfu/g) in the different fish species.

Fish species	$\times 10^6$ cfu/gml
<i>C. gariiepinus</i>	3.36
<i>C. obscura</i>	3.23
<i>H. bidorsalis</i>	3.40

H. bidorsalis had the highest bacterial count of 3.40×10^6 cfu/g while *C. obscura* had the lowest bacterial count of 3.23×10^6 cfu/gml. All the fish species had the same total coli form count values of 2.6×10^6 cfu/gml.

Table 3. Isolated bacteria from fish species collected from the two.

Fish species	Bacteria isolated
<i>C. gariiepinus</i>	<i>Peadiococcus</i>
	<i>Alkaligenase</i>
	<i>Pseudomonas</i>
<i>C. obscura</i>	<i>Micrococcus</i>
	<i>Enterobacter</i>
	<i>Staphylococcus</i>
<i>H. bidorsalis</i>	<i>E. coli</i>
	<i>Alkaligenese</i>
	<i>Staphylococcus</i>

Table 4. Heavy metal Analysis concentrations in mg/kg of fish species.

Fish species	Cd	Pb	Hg
<i>C. gariiepinus</i>	0.012 \pm 0.012	0.043 \pm 0.0012	0.012 \pm 0.01
<i>C. obscura</i>	0.010 \pm 0.005	0.037 \pm 0.013	0.014 \pm 0.00
<i>H. bidorsalis</i>	0.011 \pm 0.002	0.048 \pm 0.005	0.012 \pm 0.002

C. gariiepinus had the highest Cd concentration of 0.012 \pm 0.012mg/kg while *C. obscura* had the lowest value of 0.010 \pm 0.005mg/kg. Pb concentration of *H. bidorsalis* was highest (0.048 \pm 0.005 mg/kg) and the lowest was in *C. obscura*. The highest concentration of Hg was 0.014 \pm 0.00mg in *C. obscura*. *C. gariiepinus* and *H. bidorsalis* had Hg concentrations of

0.012±0.01mg/kg and 0.012 ± 0.002mg/kg respectively. The order of heavy metal content in all the fish species is Pb > Cd > Hg

DISCUSSION

The proximate composition the three fish species indicated that they are nutritious. The moisture levels in two of the species were below the acceptable range (60% to 80%). Low moisture content of fish could be an advantage in fresh fish storage as the microbial spoilage and oxidative degradation is reduced [17]. The observed protein levels of fish species indicated that they belonged to the high-protein (18%-23%) category [18]. High crude protein in fish could be due to feed consumption and conversion efficiency of these fish species [19]. Their protein contents were not significantly different suggesting that they are all under the same health condition [20]. The highest protein content of *C. gariepinus* may be attributed to its carnivorous nature. The ash content levels of the fishes (3.00%-4.11%) showed that the species are good source of minerals such as calcium, potassium, zinc, iron and magnesium [8]. The fat content in this study which ranged from (11.49%-12.86%) places these fish species under the high-fat content category (>8%) [21, 22]. The high values of carbohydrate obtained in this study were within the permissible limit of 2%-5% [8, 23] and higher than that reported for the wild tilapia [24].

The isolation of bacteria from the fishes is an indication of contamination of the water body where they were caught. *E. coli* is found in gastrointestinal tracts of mammals and reach water with other microorganisms through animal excreta, agricultural runoff, industrial and human wastes [7]. *E. coli* is the cause of diarrhoea, dysentery, hemolytic uremic syndrome, bladder and kidney infection, septicemia, pneumonia and meningitis [11 - 13].

All the heavy metals analyzed in this study were found to be below the National Environmental Standard and Regulation Enforcement Agency (NESREA) [25] and WHO [26] standards. This may be due to less industrial activity within the study area. These metals can bioaccumulate and biomagnify after exposure to low levels for a long period of time [5]. Muscles are reputed to have high metals accumulating potential [27-30] and such have been reported in some fish muscles [31, 32]. In low concentrations, these metals are toxic [33-35]. A fish contaminated with these metals can enter man's food chain and biomagnifications of such heavy metal may be harmful to man's health [8].

CONCLUSION

All the three fish species in Omambala River flood basin in Anambra State, *C. gariepinus*, *H. bidorsalis* and *C. obscura* are good sources of protein, lipid, ash and minerals. The study has also revealed unacceptable level of bacteria and levels of some heavy metals within their permissible safe levels for human consumption. However, there is need for constant monitoring due to the current climate change which usually results in over flooding of rivers. Safe and efficient disposal of domestic wastes and industrial effluents are recommended to avoid these heavy metals and bacteria from gaining access to the aquatic environment.

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