



Nutritional and microbial quality of fresh and dried *Clarias gariepinus* and *Oreochromis niloticus*

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ABSTRACT

Freshly harvested *Clarias gariepinus* and *Oreochromis niloticus* were purchased from vendors in Ogbomoso, Oyo State, Nigeria; and were divided into two parts. One part was used to determine the proximate composition and microbial analysis of the fresh fish while the other part was dried using a smoking kiln and was used for the same analysis. Fresh *O. niloticus* showed the highest total bacterial count of 1.8×10^7 cfu g⁻¹ and dried *C. gariepinus* showed the lowest total bacterial count of 2.0×10^4 cfu g⁻¹. Bacterial pathogens were also isolated from fresh and dried samples and the isolates were characterized and identified as *Pseudomonas fluorescens*, *Pseudomonas chlororaphis*, *Pseudomonas putida*, *Proteus vulgaris*, *Pseudomonas mirabilis* and *Enterobacter aerogenes*. The antibiotic susceptibility profile of the isolates was determined and 41.7% were sensitive to clinically relevant antibiotic disc while 58.3% were only resistant to antibiotics. The proximate analysis showed that dried *C. gariepinus* had higher fat content (11.02%) and also had the highest protein content (42.88%). Fresh *O. niloticus* had the highest moisture content (71.11%) whereas dried *O. niloticus* had the highest crude fiber and ash content (4.79 and 20.02%), respectively. The fresh fish samples had less percentage of crude fibre which is responsible for its faster digestibility when compared with that of the dried fish samples.

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INTRODUCTION

Fish is one of the sources of proteins, vitamins and minerals, and it has essential nutrients required for supplementing both infants and adults diet (Abdullahi et al., 2001). In Nigeria, fish is eaten fresh and smoked and form a much cherished delicacy that cut across socio-economic, age, religions and educational barriers (Adebayo et al., 2008).

Fish is an important source of protein to the large teeming population of Nigeria. Fish provides 40% of the dietary intake of animal protein to the average Nigerian (FDF, 1997). According to Adekoya and Miller (2004), fish and fish products constitute more than 60% of the

total protein intake in adults especially in rural areas. According to FAO (2006), to maintain the present per capita fish consumption level of 13 kg per year, 2.0 million metric tons of fish food would be required. It has been noted by some researchers that the only means of meeting up with this annual fish demand for the country would be through a pragmatic option of intensive fish farming (Ezeri et al., 2009).

There are various reasons for the merits of eating fish. One such reason is that fish is less tough and more digestible when compared with beef, mutton, chicken and bush meat. This is possible because of the greater ratio of muscles protein to connective tissues in fish in relation to other animals thus making fish acceptable by infant and adults. Because of its greater digestibility, fish is usually recommended to patients with digestive disorders

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such as ulcers (Eyo, 2001).

Fish product has a nutrient profile superior to all terrestrial meats of beef, pork and chicken etc., being an excellent source of high quality animal protein and highly digestible energy. It is a good source of sulphur and essential amino acids such as lysine, leucine, valine and arginine. It is therefore suitable for supplementary diets of high carbohydrate contents (Amiengheme, 2005). Attention has been focused recently on the relationship between fish consumption and reduced incidence of cardiovascular disease. The benefit has been attributed to the nature of the fats in fish. Unlike other fats in other food, it is the only type of fat that supplies omega-3 poly unsaturated fatty acids (PUFA) (Al-Jedah et al., 1999). PUFAs are essential in lowering blood cholesterol level and high blood pressure. It is able to migrate to alleviate platelet of (cholesterol) aggregation and various arteriosclerosis conditions in adult population. It helps in prevention of asthma, arthritis, psoriasis, and sonic type of cancer (Ward, 1995). It reduces the risk of sudden death from heart attack and reduced rheumatoid arthritis. Omega-3 fatty acid also lower the risk age related muscular degeneration and vision impairment, decrease the risk of bowel cancer, and reduce insulin resistance in skeletal muscle. Fish is abundant to some extent and occur free in nature. This may account for its relatively low cost compare with other meats. Fish is available in most market as fresh, smoked, dried, canned, chilled or frozen and as such the problem of scarcity is removed. The place of aquatic product in the food basket of the nation cannot be over-emphasized.

Fish drying is one of the key processes in fish meal production. The prime reason for fish drying is to reduce the moisture content of the non-aqueous material to such a level that insufficient water remains to support the growth of the micro-organisms which feed on it. This level is also sufficiently low to stop chemical degradation. It is necessary to dry the fish as quickly as possible from 45 to 60% moisture content to 10% moisture or less (Ruiter, 1995).

Fish drying is the processes that most affect the protein quality. Fish proteins are affected when exposed to high temperature for long period. This affects the nutritional value of fish. When the fish temperature is kept below 70°C during this process, the drying time and heat affect very little the protein quality. Exposure to high temperature for very short time (1 to 10 min) will have little effect on protein quality (Keller, 1990). For Salmon and Mink feeds, special low temperature fish meals have been found to give higher feed intake and better growth (Pike, 1989).

Nutrient content varies with fish species. There are limited data on the nutritional composition of fish species which are commonly consumed by the poor in developing countries of Asia and sub-Saharan Africa. Therefore, the crust of this study was to evaluate the nutritional and

microbiological quality of fresh and dried *Clarias gariepinus* and *Oreochromis niloticus*.

MATERIALS AND METHODS

Raw material and sample preparation

The freshly harvested fish samples (*C. gariepinus* and *O. niloticus*) were bought from the fish vendor at Ogbomoso, Oyo State, Nigeria. Twenty (20) fishes of each sample were bought and divided into equal parts. One part of the sample was used to determine the proximate compositions of the fresh fish while the second part was eviscerated, beheaded and washed, smoked and dried in a smoking kiln at a temperature range of 60-70°C for 24 h and used for proximate composition analysis of the dried fishes.

Culture media

The media used in this study includes nutrient agar and nutrient broth (Himedia, India). The media were prepared according to the manufacturer's specification. These media were sterilized in an autoclave at 121° C for 15 min.

Total bacteria count

One gram (1 g) of each sample was dissolved in sterile deionized water and serially diluted. One milliliter (1 ml) of appropriate dilutions was seeded on plate count agar using spread plate method, and the medium was then incubated at 37°C for 24 h. The plate count agar was examined and colonies present were counted and recorded after incubation at 37°C for 24 h to get the total colony count in cfu g⁻¹.

Isolation of microorganism

One gram (1 g) of each fish sample was serially diluted, 1 ml of an appropriate dilution was inoculated on nutrient agar plates and the plates were incubated for 24 h at 30°C. After 24 h, sterile wire loop was used to pick the isolate from the plate and was streaked on a freshly prepared sterile nutrient agar plates, then it was incubated for 24 h at 30°C in order to get pure cultures. The pure cultures were then stored in a refrigerator at 4°C. The routine laboratory method of Cruickshank et al. (1975) was used to characterize the different isolates. The isolates were identified using their macroscopic, cultural, physiological and biochemical characteristics.

Physiological tests

Isolates were subjected to standard cultural, morphological and physiological techniques and were identified according to Bergey's Manual of Systemic Bacteriology. Isolates were subjected to different physiological tests such as Gram staining, catalase test, coagulase test, methyl red test, Voges-Proskauer test, indole test, oxidase test, sugar fermentation test, etc and were differentiated on this basis with reference to Bergey's Manual of Systemic Bacteriology (Sneath, 1986).

Antibiotic susceptibility test

Mueller-Hinton agar was evenly seeded throughout the plate with the isolate which had been previously diluted at a standard concentration. Commercially prepared disks each was pre-impregnated with a standard concentration of a particular antibiotic and were lightly pressed onto the agar surface. The plates were incubated for 24 h at 37°C. The antibiotics used included Amoxicillin, Ofloxacin, Streptomycin, Chloramphenicol, Ceftriazone, Gentamycin, Pefloxacin, Cotrimoxazole, Ciprofloxacin and Erythromycin. After an overnight incubation, the bacterial growth around each disc was observed.

Proximate analysis

Determination of crude protein by Kjeldahl method

In this method, the fish sample to be analyzed was digested with concentrated sulphuric acid in the presence of a small amount of copper sulphate, selenium and sufficient sodium or potassium sulphate with mercury (Hg) as a metal catalyst. Under these conditions, the organic matter was oxidized and the protein nitrogen was converted to ammonium sulphate $(\text{NH}_4)_2\text{SO}_4$. The digestion was followed by the addition of a strong base (NaOH) to liberate ammonia. The ammonia distilled, trapped in 0.5% boric acid indicator which was then titrated with 0.01 M HCl. Almost all organic forms of nitrogen were converted to ammonia by the conditions of the digestion. The result of Kjeldahl analysis is usually expressed as crude protein. The weight of nitrogen in a sample can be converted to protein using the appropriate factor based on the percentage of nitrogen in the protein sample. To convert gram of nitrogen to gram of protein, the common factors 6.25 was used. The nitrogen value was therefore multiplied by 6.25 to get the weight of protein (Oladipo and Jadesimi, 2013).

Determination of crude fibre by trichloroacetic acid

The organic residue left after sequential extraction of sample with ether can be used to determine the crude

fibre; however if a fresh sample was used, the fat in it could be extracted by adding petroleum ether, stirred and allowed to settle and decanted. This was done three times. The fat-free material was then transferred into a flask/beaker and 200 ml of pre-heated 1.25% H_2SO_4 was added and the solution was gently boiled for about 30 min, maintaining constant volume of acid by the addition of hot water. The Buckner flask funnel fitted with Whatman filter was pre-heated by pouring hot water into the funnel. The boiled acid sample mixture was then filtered hot through the funnel under sufficient suction. The residue was then washed several times with boiling water (until the residue was neutral to litmus paper) and transferred back into the beaker. Then 200 ml of pre-heated 1.25% Na_2SO_4 was added and boiled for another 30 min, filtered under suction and washed thoroughly with hot water and twice with ethanol. The residue was dried at 65°C for about 24 h and weighed; and then transferred into a crucible and was placed in muffle furnace (400-600°C) and ashed for 4 h. It was then allowed to cool in a desiccator and was weighed (Oladipo and Jadesimi, 2013).

$$\% \text{ crude fibre} = \frac{\text{dry wt. of residue before ashing} - \text{weight of residue after ashing}}{\text{weight of sample}} \times 100$$

Determination of moisture

Moisture was determined by the reduction in weight when the sample was dried to a constant weight in an oven. About 2 g of fish sample was weighed into a silica dish which was previously dried and weighed; the sample was then dried again in an oven at 65°C for 36 h, cooled in a desiccator and weighed. This process was continued until a constant weight was achieved (Oladipo and Jadesimi, 2013).

$$\% \text{ moisture} = \frac{\text{weight of sample + dish before drying} - \text{weight of sample + dish after drying}}{\text{weight of sample taken}} \times 100$$

Determination of crude fat

The ether extract of a feed represent the fat and oil in the feed. Soxhlet apparatus is the equipment used for the determination of ether extract. It consists of 3 major components; an extractor: comprising the thimble which holds the sample, a condenser: for cooling and condensing the ether vapor and 250 ml flask.

About 150 ml of an anhydrous diethyl ether (petroleum ether) of boiling point of 40-60°C was placed in the flask. 2-5 g of the sample was weighed into a thimble and the thimble was plugged with cotton wool. The thimble with content was placed into the extractor; the ether in the flask was then heated. As the ether vapor reached the condenser through the side arm of the extractor, it

condensed to liquid form and dropped back into the sample in the thimble; the other soluble substances were dissolved and carried into solution through the siphon tube back into the flask.

Extraction continued for at least 4 h. The thimble was removed and most of the solvent was distilled from the flask into the extractor. The flask was then disconnected and placed in an oven at 65°C for 4 h, cooled in a desiccator and weighed (Oladipo and Jadesimi, 2013).

$$\% \text{ of fat} = \frac{\text{weight of flask + extract} - \text{tare wt of flask}}{\text{weight of sample}} \times 100$$

Determination of crude ash

Ash is the inorganic residue obtained by burning off the organic matter of the samples at 400 – 600°C in a muffle furnace for 4 h. 2 g of the sample was weighed into a pre-heated crucible. The crucible was placed in muffle furnace at 400 – 600°C for 4 h or until a whitish-grey ash was obtained; and then was placed in the desiccators and weighed (Oladipo and Jadesimi, 2013).

$$\% \text{ ash} = \frac{\text{weight of crucible + ash} - \text{wt of crucible}}{\text{weight of sample}} \times 100$$

RESULTS

A total of 13 organisms were isolated from fresh and dried fishes of *C. gariepinus* and *O. niloticus*. The 13 isolates were subjected to biochemical, macroscopical, microscopical and physiological test. And it was discovered that all the organisms were negative to Gram's reaction and positive to catalase reaction, coagulase reaction and motility reaction. The isolates were identified to be *Proteus vulgaris* (4), *Pseudomonas mirabilis* (2), *Pseudomonas fluorescens* (4), *Pseudomonas putida* (1), *Pseudomonas chlororaphis* (1) and *Enterobacter aerogenes* (1).

The values for total colony count for bacterial isolates is shown in Table 1; fresh *O. niloticus* showed the highest total bacteria count of 1.8×10^7 cfu g⁻¹ and dried *C. gariepinus* showed the lowest total bacteria count of 2.0×10^4 cfu g⁻¹.

The distribution of the bacteria species present in all samples is shown in Table 2. *P. putida*, *P. fluorescens* and *E. aerogenes* were present in fresh *O. niloticus* while *P. vulgaris* and *P. mirabilis* were present in fresh *C. gariepinus*. Also, *P. fluorescens*, *P. chlororaphis* and *E. aerogenes* were present in dried *O. niloticus* while *P. vulgaris* and *P. fluorescens* were present in dried *C. gariepinus*.

Antibiotics susceptibility profile of the isolated organisms was evaluated and it was noted that *P. vulgaris* was resistant to all the antibiotics such as Amoxicillin, Ofloxacin, Streptomycin, Chloramphenicol, Ceftriazone, Gentamycin, Pefloxacin, Ciprofloxacin, Cotrimoxazole, and Erythromycin. *P. mirabilis* was resistant to Amoxicillin, Ceftriazone, and Gentamycin but sensitive to Ofloxacin, Streptomycin, Chloramphenicol, Pefloxacin, Cotrimoxazole, Ciprofloxacin and Erythromycin with zones of inhibition of diameter 17.0, 17.0, 13.5, 17.5, 13.0, 17.5 and 12.5 mm, respectively. *P. putida* was resistant to Amoxicillin, Ceftriazone, Cotrimoxazole and Erythromycin but sensitive to Ofloxacin, Streptomycin, Chloramphenicol, Gentamycin, Pefloxacin and Ciprofloxacin with zones of inhibition of diameter 13.5, 15.0, 11.0, 11.5, 14.5 and 13.0 mm, respectively. *P. fluorescens* was resistant to Amoxicillin, Streptomycin, Chloramphenicol, Gentamycin, Cotrimoxazole and Erythromycin but sensitive to Ofloxacin, Ceftriazone, Ciprofloxacin and Pefloxacin, with zone of inhibition of diameter 12.5, 12.5, 17.0 and 14.5 mm, respectively. *P. chlororaphis* was resistant to Amoxicillin, Streptomycin, Chloramphenicol, Ceftriazone, Gentamycin and Cotrimoxazole but sensitive to Ofloxacin, Pefloxacin, Ciprofloxacin and Erythromycin with zone of inhibition of diameter 12.0, 13.0, 17.5 and 14.0 mm, respectively; and *E. aerogenes* was resistant to Amoxicillin, Chloramphenicol, Ceftriazone, Gentamycin, Cotrimoxazole and Erythromycin but sensitive to Ofloxacin, Streptomycin, Pefloxacin and Ciprofloxacin with zone of inhibition diameter 1 of 3.0, 14.0, 13.0 and 13.0 mm, respectively (Table 3).

The proximate analysis was done for the fish samples which included fresh and dried *C. gariepinus*, and *O. niloticus*. The percentages of the crude protein for the four samples were 17.50, 42.88, 16.63 and 42.13%, respectively. The percentage of crude fiber were 1.02, 4.56, 2.05 and 4.79%, respectively, the percentage of fat for the four samples were 6.55, 11.02, 4.46 and 10.13%, respectively; the percentage of ash included 3.85, 17.69, 7.28 and 20.02% and the percentage of moisture were 70.35, 18.32, 71.11 and 8.22%, respectively (Table 4).

DISCUSSION AND CONCLUSION

All the isolates of the present study were found to be positive for catalase, citrate, and motility and were all negative for Gram's reaction. The isolated bacteria include species of the genera *Proteus*, *Pseudomonas* and *Enterobacter*, this supports the previous work of ICMSF (1998) who reported that bacteria present in fishes are normally associated with those found in their natural environment and influenced by the season and the harvesting conditions. The proportion of the initial population can easily be changed after harvesting but this

Table 1. Total bacteria count for samples.

Sample description	Total colony count for bacteria (cfu g ⁻¹)
Fresh <i>C. gariepinus</i>	1.4 X 10 ⁶
Fresh <i>O. niloticus</i>	1.8 X 10 ⁷
Dried <i>C. gariepinus</i>	2.0 X 10 ⁴
Dried <i>O. niloticus</i>	2.9 X 10 ⁴

Table 2. Distribution of bacteria isolates in different samples.

Isolate	DON	FON	DCG	FCG
<i>P. vulgaris</i>	-	-	+	+
<i>P. mirabilis</i>	-	-	-	+
<i>P. putida</i>	-	+	-	-
<i>P. fluorescens</i>	+	+	+	-
<i>P. chlororaphis</i>	+	-	-	-
<i>E. aerogenes</i>	+	+	-	-

DON, Dried *O. niloticus*; FON, fresh *O. niloticus*; DCG, dried *C. gariepinus*; FCG, fresh *C. gariepinus*.

Table 3. Antibiotics sensitivity pattern of isolates.

Isolate	AMX 25 µg	OFL 5 µg	STR 10 µg	CHL 30 µg	CRO 30 µg	GEN 10 µg	PFX 5 µg	COT 25 µg	CPX 10 µg	ERY 5 µg
<i>E. aerogenes</i>	R	13.0	14.0	R	R	R	13.0	R	13.0	R
<i>P. vulgaris</i>	R	R	R	R	R	R	R	R	R	R
<i>P. putida</i>	R	13.5	15.0	11.0	R	11.5	14.5	R	13.0	R
<i>P. fluorescens</i>	R	12.5	R	R	12.5	R	14.5	R	17.0	R
<i>P. mirabilis</i>	R	17.0	17.0	13.5	R	R	17.5	13.0	17.5	12.5
<i>P. chlororaphis</i>	R	12.0	R	R	R	R	13.0	R	17.5	14.0

R, Resistant; AMX, amoxicillin; OFL, ofloxacin; STR, streptomycin; CHL, chloramphenicol; CRO, ceftriazone; GEN, gentamycin; PFX, pefloxacin; COT, cotrimoxazole; CPX, ciprofloxacin; ERY, erythromycin.

Table 4. Proximate analysis on dried and fresh fish sample.

Sample description	% Crude protein	% Crude fibre	% Crude fat	% Crude ash	% Moisture
Fresh <i>C. gariepinus</i>	17.50	1.02	6.55	3.85	70.35
Fresh <i>O. niloticus</i>	16.63	2.05	4.46	7.28	71.11
Dried <i>C. gariepinus</i>	42.88	4.56	11.02	17.69	18.32
Dried <i>O. niloticus</i>	42.13	4.79	10.13	20.02	8.22

depends on the ability of those bacteria to adapt to the new conditions.

Comparison of the total bacterial count of the fresh and dried fish samples showed that fresh *O. niloticus* had the highest colony count of 1.8 x 10⁷ and dried *C. gariepinus* had the lowest total colony count of 2.0 x 10⁴, which

indicates that drying reduced the microbial load of the samples.

The level of resistance and sensitivity of these bacteria to clinically relevant antibiotics differs; the isolates showed 58.3% resistance to clinically relevant antibiotics and 41.7% susceptibility. *P. vulgaris* was found to be

resistant to all the commercially available antibiotic discs used while *E. aerogenes*, *P. putida* and *P. fluorescens* were resistant to six of the antibiotics used. Levy (1992) reported that antimicrobial resistance in bacterial pathogens is a major impediment to successful therapy, and in several instances, bacterial strains have arisen that are resistant to most available antimicrobial treatments. The public health consequences of antimicrobial resistance to many antibiotics have been debated. However until recently, clear evidence of health risk was not available. The multiple nature of drug resistance of these bacteria create an extremely serious public health problem and it has always been associated with the outbreak of major epidemic throughout the world (Prescott et al., 2002).

Dried fish had higher protein than the fresh fish. Increase of protein may be due to the dehydration of water molecule present between the proteins thereby, causing aggregation of protein and thus resulting in the increase in protein content of dried fishes (Ninawe and Rathnakumar, 2008). Ogbonnaya and Shaba (2009) reported that protein nitrogen was not lost during drying, so that protein content increased with the reduced moisture content in the fish samples.

Ash content of dried fish was higher than that of fresh fish. Clucas and Ward (1996) reported that the inorganic content remain as ash after the organic matter is removed by incineration. Dried fish had higher fat content than the fresh fish. After drying, there was an increase in fat content and this variation could be the result of evaporation of moisture contents which is in agreement with the previous work of Ogbonnaya and Shaba (2009). Moisture content of fresh fish was higher than that of dried fish as a result of dehydration of water molecule present in dry fish.

In conclusion, fresh fish consumption is recommended for children because of less percentage of crude fiber which is responsible for its faster digestibility compared to that of dry fish. This work supports the work of Eyo (2001) who reported that fish has a great digestibility. Dried fish also contain higher percentage of fats which is responsible for the supplies of omega-3 PUFAS for lowering blood cholesterol level and high blood pressure.

In general, there were great influences of drying on proximate compositions of both *C. gariepinus* and *O. niloticus*. These result showed that different nutritional components of fish undergo different changes at elevated temperatures.

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