

Biochemical and Microbiological Assessment of Smoked Dried *Clarias gariepinus*

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Abstract

Microbiological and Biochemical characteristics of smoke-dried *Clarias gariepinus* was studied for four weeks to assess changes related to belated consumption. Five fishes were bought from 3 different sellers and then mixed together to make a composite sample of fifteen. Analytical methods as described by Bergey's manual of bacteriology and AOAC were used for weekly Microbiological and Biochemical analysis while Mineral was analyzed biweekly. Microbiological properties fluctuated through the 4 weeks but were within acceptable limits as prescribed by the Food and Agriculture Organization (FAO), microflora isolated study are *Penicillium* and *Aspergillus niger*, the bacterial Species isolated are *Staphylococcus aureus*, *Klebsiella spp* and *E. coli*. There was significant ($p < 0.05$) rise in ash and protein while moisture and lipid declined significantly ($p < 0.05$) through the period of the study. Mineral content for Na, K, Ca and Fe did change significantly ($p < 0.05$) through the study. The quality of the smoke-dried *C. gariepinus* was wholesome at the time of purchase but progressively declined through the course of the study thereby making it of reduced nutritional benefit and potentially harmful for consumption.

Keywords: *Clarias gariepinus*; *Penicillium*; *Aspergillus niger*; Fish

Introduction

Fish is a very important staple food and a source of protein for the diets of the poor and low-income earners of most developing countries of the world.

Food and Agriculture Organisation [1] stated that fish makes up about 60% of the world's protein supply and that 60% of the developing countries obtain more than 30% of their animal protein from fish. Fish are generally regarded as safe, nutritious and beneficial it allows for protein improved nutrition in that it has a high biological value in terms of high protein retention in the body, the presence of essential amino acids and ability to lower cholesterol level [2].

Populations with the highest consumption of fatty fish appear to have the lowest incidence of cardiovascular diseases. Fish consumption has also been linked to reduced hypertension, reduced blood clotting tendencies, and more favorable plasma lipid and lipoprotein levels [3].

Fish as a choice protein source in developing countries is prepared fresh or processed into other forms for the purpose of preservation or the basic preference of the consumer. Fish could be seasoned and prepared fresh, cooked, dried, grilled, fried, salted or smoked [4]. However, preference for smoked dried fish is quite obvious as it constitutes condiment used in many delicacies.

Owing to the high perishability of fish and its product, varying methods of processing, preserving and prolonging the shelf life of fish and fish products are employed. Fresh fish rapidly deteriorates unless it is preserved; therefore it is preserved through various methods such as drying, icing, salting, freezing, and smoking etc. Smoking is one of the oldest food preserving methods, during smoking; the heat from the fire dries the fish while chemicals from the smoke impregnate the flesh. Smoking not only increases the resistance of food to spoilage but changes appearance, taste, and smell of foods [5]. The obtained flavors depend both on the raw materials used and the length of time the fish are smoked.

Poor Handling, long storage time and the generally unhealthy display conditions of smoked dried fish by retail fish vendors in open markets pose enormous challenges to the consumer safety and

nutritional integrity of smoked dried fish. This commodity which is bought in large quantities are most times exposed to conditions that lead to nutritional deterioration and potentially hazardous microbiological infestation when kept in storage, these conditions are promoted by poor handling and makeshift preservation techniques employed by both retailers and consumers, this study, therefore, aims at examining the biochemical and microbiological status of smoked dried *Clarias gariepinus* obtained from the market and kept in storage for a given time.

Materials and Methods

Smoked dried fish selection

Fifteen smoke-dried samples of *Clarias gariepinus* (African catfish) were purchased from the mobile fish market in Minna, Niger State, Nigeria. The fishes have been in storage by their sellers for indeterminate periods of time prior to their procurement for the investigations.

Five samples of each species were obtained from three different sellers and then mixed together to make composite samples of fifteen. The samples were hand-picked with sterilized gloved hands and taken to the laboratory in sterilized polythene bags to avoid contamination from handling. On commencement of the study, the initial microbial and proximate compositions of the samples were analyzed and recorded (Week 0), representing the biochemical composition and microbiological characteristics of samples at the time of purchase. Thereafter, the samples were stored under ambient temperature and humidity. The microbial and biochemical composition of the

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samples were analyzed weekly over a period of 4 weeks. Microbial and biochemical analysis carried out at the Department of Water Resources, Aquaculture and Fisheries Technology and Department of Animal Production laboratories respectively.

Biochemical analysis

The initial and weekly proximate composition of the samples were taken using the prescribed methods for Protein, Lipid, Ash and moisture content analyses as prescribed by the Association of Official Analytical Chemists [6].

Mineral analysis

One gram (1 g) of the sample was weighed into a beaker to which 15 ml of concentrated nitric acid was added to the sample and was allowed to digest into a clear solution on a hot plate. Distilled water was added to the digest and then the digest was filtered, the filtrate was then made up to 50 ml with distilled water and then stored in a sample bottle. The sample was then analyzed using Atomic Absorption Spectrophotometer (AAS) machine.

Microbiological analysis

The culture media used in the bacteriological analysis is Nutrient agar (NA), MacConkey agar (MAC), Salmonella-Shigella agar (SSA) and Mannitol Salt Agar (MSA). Sabouraud Dextrose Agar (SDA) was used in the culture, isolation, and identification of fungal colonies present on the fish samples. In an electrically-operated autoclave, All growth media and glassware (Petri dishes, beakers, test tubes etc.) used in this study was sterilized by autoclaving at 121°C for 15 minutes.

Identification

The identification of micro-organisms as well as biochemical tests such as motility, indole, catalase, oxidase etc would be based on the characteristics described in Bergey's Manual of Determinative Bacteriology [7].

Gram staining was used to provide a broad classification of bacteria either as gram-positive or gram-negative. A smear was made for 18-hour old isolates on clean, dry and grease-free slides. The smear would then be fixed by passing through an open flame and flooded with 0.5% crystal violet for 60 seconds and then rinsed off with water. A mordant Gram's iodine solution was then applied and left for another 60 seconds before it was drained off and decolorized by holding the slide in a slant position while applying absolute alcohol. It was then rinsed off with water and counterstained with carbon fuchsin for 30 seconds and then rinsed off again. Finally, the stained smear would be air dried and examined under a microscope using oil immersion objective

Statistical analysis

Data was analyzed at 5% level of significance with SPSS Version 22 [8]. Mean change in values between the initial and subsequent Readings were compared using independent samples t-test while the test of homoscedasticity (equality of variance) was carried out with Levene's test of equality of variances.

Results

Biochemical assessment

Proximate composition for *Clarias gariepinus*: The initial mean percentage ash for *C. gariepinus* was $9.35 \pm 0.69\%$, the ash content increased through the study period to a final value of $16.34 \pm 0.22\%$ (Week 4), the difference between the base and final mean percentage

Ash was 6.99 ± 0.47 . The mean change in week 1,2,3 and 4 were not significantly ($p>0.05$) different from the initial (week 0) (Figure 1).

Mean percentage lipid was $35.63 \pm 1.14\%$ at Week 0 and progressively declined to $16.06 \pm 2.09\%$ (week 4), the difference between the base and final mean percentage Lipid was -19.57 ± 1.63 . The mean change in week 1,2,3 and 4 were not significantly ($p>0.05$) different from the initial (week 0).

Mean percentage Moisture was $20.49 \pm 7.30\%$ at week 0 and declined to $5.55 \pm 0.40\%$ (week 4), the difference between the base and final mean percentage moisture was -14.94 ± 7.62 . The mean change in week 1 and 2 was not significantly ($p>0.05$) different from the initial (week 0), while the mean change in week 3 and 4 differed significantly ($p<0.05$) to the initial (week 0).

The initial mean percentage protein was $59.22 \pm 3.14\%$, the ash content increased through the study period to a final value of $76.83 \pm 2.08\%$ (Week 4), the difference between the base and final mean percentage protein was 17.61 ± 2.84 . The mean change in week 1, 2, 3 and 4 were not significantly ($p>0.05$) different from the initial (week 0).

Mineral assessment

Mineral assessment for *Clarias gariepinus*: Mean Sodium (Na) (mg/kg) for *C. gariepinus* at week 0 (initial) was 23.00 ± 6.18 , At week 2 the mean values was 30.83 ± 6.17 , by the end of the study the mean mineral value was 28.23 ± 5.39 . The mean change in week 2 and 4 were not significantly ($p>0.05$) different from the initial (week 0).

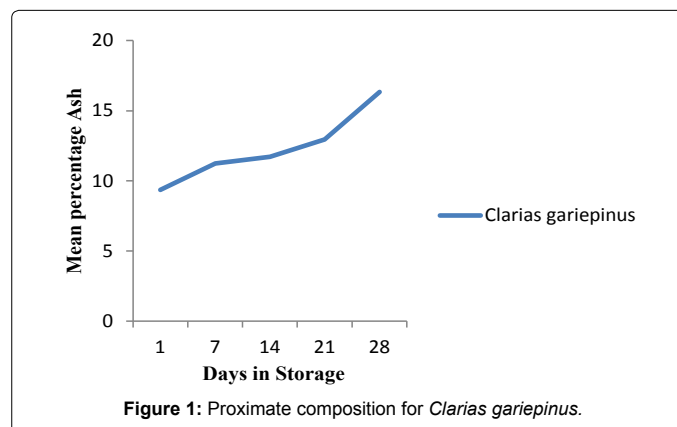
Mean Potassium (K) at week 0, was 93.5 ± 3.35 , at week 2 the mean values were 95.07 ± 2.47 . Finally, at week 4 Mean K was 93.70 ± 3.14 . The mean change in week 2 and 4 were not significantly ($p>0.05$) different from the initial (week 0).

Mean values of Calcium (Ca) at week 0 was 14.00 ± 0.98 , 14.00 ± 0.92 by week 2 and declined to 8.50 ± 1.20 by the end of the study (week 4). The mean change in week 2 was not significantly ($p>0.05$) different from the initial (week 0), while week 4 differed significantly ($p<0.05$) from week 0.

The initial (week 0), Mean mineral values for Iron (Fe) was 4.08 ± 1.00 by week 2 the mean value for Fe increased to 5.27 ± 1.04 , finally at week 4 the mean value of Fe declined to 4.60 ± 0.10 . The mean change in week 2 and 4 were not significantly ($p>0.05$) different from the initial (week 0).

Microbiological assessment

Microflora and bacterial species identified: The bacterial Species isolated are *Staphylococcus aureus*, *Klebsiella spp.* and *Escherichia coli*, the microflora isolated from the fish species sampled were *Penicillium*



and *Aspergillus niger*, these fungal species were present on the fish sample.

Microbiological assessment for *Clarias gariepinus*: Mean Total Viable Count (TVC) on Nutrient Agar (NA) for *C. gariepinus* at the initial (week 0) was $1.2 \times 10^4 \pm 0.119$. The values of NA at Week 1 increased to $2.9 \times 10^5 \pm 0.085$, The TVC declined at week 2 and then plateaued to week 3. At week 4 the TVC on NA for *C. gariepinus* increased to $2.7 \times 10^5 \pm 0.098$. The mean change in week 1 and 4 differed significantly ($p < 0.05$) to the initial (week 0), while week 2 and 3 were not significantly ($p > 0.05$) from week 0 (Table 1).

Mean viable count values for Mannitol Salt Agar (MSA) *C. gariepinus* had non analyzable data (one plate growth out of triplicate). Mean viable count values for MSA At week 1 was $2.0 \times 10^5 \pm 0.216$, declining to $4.5 \times 10^4 \pm 0.210$. At week 2, by week 3 the MSA value for *C. gariepinus* further declined to $2.6 \times 10^4 \pm 0.174$. The final values (week 4) for *C. gariepinus* rose to $6.9 \times 10^4 \pm 0.207$ the mean change in week 2,3 and 4 differed significantly ($p < 0.05$) to week 1.

The Mean viable count value on MacConkey Agar (MAC) for *C. gariepinus* at week 0 was not statistically analyzable (no growth on medium) at week 1 the value for *C. gariepinus* was $4.2 \times 10^4 \pm 0.991$ and increased to $9.1 \times 10^4 \pm 0.32$ in week 2, by week 3 *C. gariepinus* declined to $3.2 \times 10^4 \pm 0.53$ and then declined further to $1.7 \times 10^4 \pm 1.00$ by the end of week 4. The mean change in week 2, 3 and 4 were not significantly ($p > 0.05$) different from week 1. No growth on Salmonella Shigella Agar (SSA) for *C. gariepinus* through the period of the study (Table 2).

The Mean viable count values for Sabouraud Dextrose Agar (SDA) at week 0 were not statistically analyzable (one out of the triplicate on *C. gariepinus*), Mean viable count values for SDA At week 1 was $5.8 \times 10^7 \pm 0.217$, declining to $1.1 \times 10^7 \pm 0.578$ At week 2, by week 3 the SDA value for *C. gariepinus* increased to $3.8^7 \pm 0.239$. The final values (week 4) for *C. gariepinus* declined to $5.4 \times 10^6 \pm 0.796$. The mean change in week 2, 3 and 4 was not significantly ($p > 0.05$) different from week 1.

Discussion

Protein

The initial protein value of 59.2% concurs with the range of values contained in literature of Daramola, et al., [9] buttressing the fact that protein forms a chunk of fish dry matter as reported by Peter [10], Oyero [11], Adeyeye and Adamu [12], Orire and Ricketts [13] and Pannevis [14] (Figure 2).

The increase in protein of the fish sample was proportional to the decrease in moisture through the study, showing that the dryer the fish sample got the higher its corresponding protein concentration this aligned with the findings reported by Oyero [11] which stated an observed increase in protein with a corresponding decrease in moisture content of smoked dried *Oreochromis niloticus*. In contrast to the report of Victoria and Francis [15] the result of this study shows an increase in protein with respect to storage time, this may be the effect of the different climatic condition of the site of this study which is the northern part of Nigeria which is a lot dryer than the southwestern part of the country.

When compared with the initial protein values, the results from week 2 through to week 4 showed a significant increase in protein attributed to a loss in moisture as a result of a decline in atmospheric humidity caused by the onset of the harmattan season.

Moisture

Oyero [11] stated that the factors which influence the stability of foods can be related to water activity (a_w), being a measure of available water in a food that is able to react chemically or, in spoilage to support the growth of microorganism such as bacteria and molds, water activity is directly proportional to the moisture content of the food samples therefore it can be lowered by drying (Figure 3).

In this study, the fish sampled showed acceptably low moisture contents. The initial moisture content of 20.49% is low enough to retard bacterial spoilage and greatly reduce autolytic activity but still sufficient to sustain mold growth which was observed on the fish samples in the first seven days in storage Trim and Curran [16]. The moisture content of the fish reduced by 14.94% through the period of the study this change was facilitated by the onset of the harmattan season. The atmospheric humidity ranged between 35% to 76% and averaged at 55% through the period of the study, the low atmospheric humidity would permit evaporation of moisture from the fish sample thereby lowering the water activity hence reducing microbial activity on the fish sample. The dryness of the sample below 15% explains the observed brittleness and insect infestation of the fish sample [17,18].

Lipid

The initial percentage lipid of both fish sample recorded in this study was 35.63% reaffirming the fact smoked dried fish is a very good source of nutritional beneficial oil to the consumer [19] (Figure 4).

Alias and Linden [20] as well as Oyero [11] stated that there is a relationship between the extent of lipid oxidation and water activity

Mineral	Week 0	Week 2	Week 4
Na	23.0 ± 6.18	30.83 ± 6.17	28.23 ± 5.39
K	93.5 ± 3.35	95.07 ± 2.47	93.7 ± 3.14
Ca	14 ± 0.98	14.1 ± 0.98	8.5 ± 1.2
Fe	4.08 ± 1.00	5.27 ± 1.40	4.6 ± 0.10

Table 1: Biweekly evaluation of some mineral compositions of *Clarias gariepinus*.

Medium (agar)	Length of storage (Days)				
	1	7	14	21	28
NA	$1.2 \times 10^4 \pm 0.12$	$2.9 \times 10^5 \pm 0.09$	$1.0 \times 10^5 \pm 0.52$	$1.0 \times 10^5 \pm 0.47$	$2.7 \times 10^5 \pm 0.10$
MSA	0	$2.0 \times 10^5 \pm 0.22$	$4.5 \times 10^4 \pm 0.21$	$2.6 \times 10^4 \pm 0.17$	$6.9 \times 10^4 \pm 0.21$
MAC	0	$4.2 \times 10^4 \pm 0.99$	$9.1 \times 10^4 \pm 0.32$	$3.2 \times 10^4 \pm 0.53$	$1.7 \times 10^4 \pm 1.00$
SSA	NG	NG	NG	NG	NG
SDA	0	$5.8 \times 10^7 \pm 0.22$	$1.1 \times 10^7 \pm 0.58$	$3.8 \times 10^7 \pm 0.24$	$5.4 \times 10^6 \pm 0.80$

Table 2: Total heterotrophic bacterial and fungal counts (cfu/g) observed on the *Clarias gariepinus*.

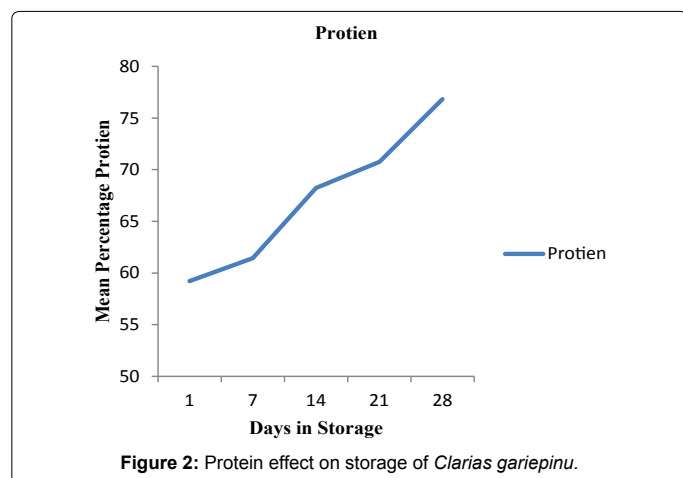


Figure 2: Protein effect on storage of *Clarias gariepinus*.

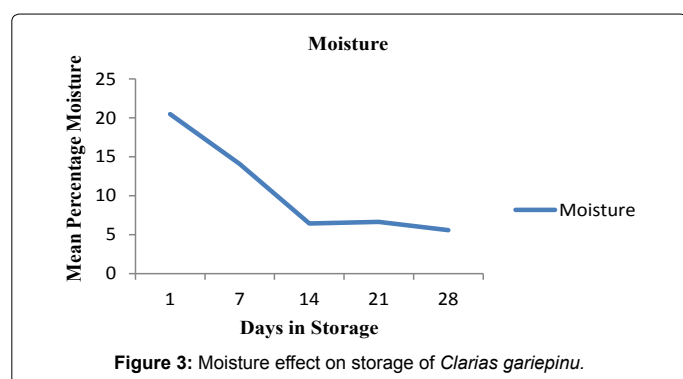


Figure 3: Moisture effect on storage of *Clarias gariepinus*.

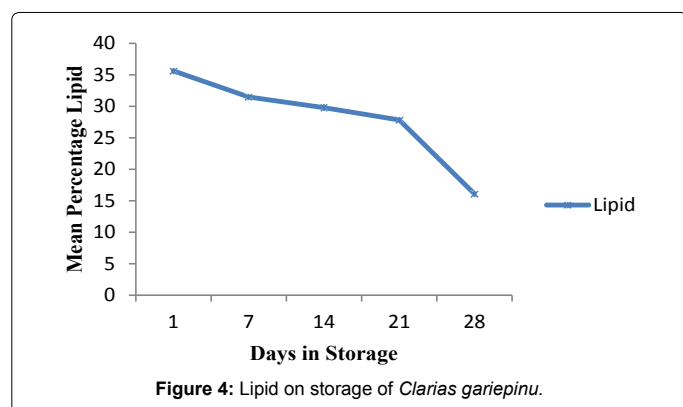


Figure 4: Lipid on storage of *Clarias gariepinus*.

(a_w). in this study it is seen that the moisture content of the fish sample declined drastically thereby lowering the water activity which in turn limited microbial activity but aided lipid oxidation on the smoked dried fish samples hence the reduction of percentage lipid in the smoked dried fish sample from 35.63% to 16.06% (19.59% decline) thereby decreasing the nutritive value of the fish in storage.

Furthermore, the high initial lipid content of 35.63% may have been a direct impact of the practice of fish processors to apply palm oil to improve the appearance of smoked dried fish after smoking or re-smoking as observed in the market during the purchase of fish samples.

Ash

The total mineral content of fish samples is expressed by the percentage ash content [21]. The initial ash content of the fish samples

was 9.35% this result is similar to the findings of Daramola et al. [7], who reported 8.54% for *C. gariepinus*.

At the end of the study the percentage ash content of the samples had increased by 6.99%, this can be attributed to the loss of moisture in the fish samples as seen in the report of Oparaku and Mgbenka [18], which stated that the ash content of fresh *C. gariepinus* increased from 1.79% (fresh) to 4.85% (solar dried) and 3.08% (electric oven dried) emphasizing that decrease in moisture of fish samples results in a corresponding increase in ash content of the sample.

Minerals

From the results of this study *C. gariepinus* proved to be a good source of both micro and macro minerals, this is similar to the findings of Peter [10] who asserted that *Clarias gariepinus* in spite of the processing effects of smoking remained a good source of micro and macro minerals which can contribute to the health, growth, and development of human beings.

In this study it is observed that the mineral value followed this order K>Na>Ca>Fe, Fe was the smallest most likely because it is a trace element, K and Ca were also abundant because they are constituent of the fish skeleton, the abundance of Na may be as a result of application of table salt during processing and after processing in order to ward off insect in storage before sale [21,22].

Microbiological assessment

The microflora isolated this studies are *Penicillium* and *Aspergillus niger* while the bacterial Species isolated were *Staphylococcus aureus*, *Klebsiella spp.* and *Escherichia coli* [23]. The presence of these microorganisms on the fish samples pose a potent threat to the health of the consumer, as they have been implicated in many foods borne gastrointestinal diseases.

The result of this study shows a general increase in microbial loads of smoked dried fish for the first week when compared to the findings of other research [24,25]. However, from the second week, the observed decline and fluctuation could be a result of ambient conditions and decrease in moisture content of the smoked dried fish [15]. Through the study the TVC of the smoked fish samples where at satisfactory levels [26-29], the increased dryness reduced the available moisture necessary for metabolic activities of the microorganism present on the samples thereby leading to their decline in selective growth media [11,15]. Due to increased air movement occasioned by the harmattan season, other microorganisms would have been transported and deposited on the samples by the wind thereby leading to fluctuations in TVC particularly in fungi colonies [30].

The fluctuations in microflora can be attributed to microbiological activity on any substrate which is characterized by rapid growth of organism due to a favorable substrate and ambient conditions [31], the organisms then compete for food and release metabolite on the samples thereby actively furnishing their decline. Decaying dead microorganisms provide an impetus for surviving colonies to thrive and increase exponentially until decline sets in for the same reasons mentioned above [32,33].

Conclusion and Recommendation

The initial (week 0) result of this study shows that smoked dried fish sold in Mobil fish market Minna Niger State Nigeria are of good and wholesome Microbial and Biochemical quality for immediate consumption, however, the quality of the smoked fish in storage

progressively declined thereby making it of reduced nutritional benefit and potentially harmful for consumption.

It is therefore recommended that smoked fish be bought in quantities that can be exhausted as quickly as possible in order to avoid any form of contamination or deterioration that may occur as a result of prolonged storage in ambient conditions also smoked dried fish can be sealed and refrigerated or kept in freezers pending time of consumption because reduced temperature is a proven way to slow down the rate of microbial activity furthermore proper packing would help curtail gain or loss of moisture, oxidation, and insect infestation.

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