Journal of Research in Forestry, Wildlife & Environment Vol. 13(2) June, 2021
E-mail: jrfwe2019@gmail.com; jfewr@yahoo.com
http://www.ajol.info/index.php/jrfwe

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ISBN: 2141 – 1778
Ricketts and Oyero, 2021

NUTRITIVE QUALITY AND MICROBIAL ASSESSMENT OF STORED SMOKED DRIED *GYMNARCHUS niloticus* Cuvier, 1829

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ABSTRACT

The Proximate composition and Microbial load of smoke-dried Gymnarchus niloticus from Mobil fish market Minna Niger state was assessed to study changes related to belated consumption over a period of 28 days. A total of 15 fish samples shared from different traders were assessed. Values of proximate composition were significantly different (p<0.05) through the study. The crude protein and ash increased from 56.19 (Day 1) to 85.96 (Day 28) and 9.94 (Day 1) to 17.12 (Day 28) respectively while the moisture and lipid declined from 15.14 (Day 1) to 5.43 (Day 28) and 24.09 (Day 1) to 9.82 (Day 28) respectively. Mineral contents: Na, K, Ca and Fe changed significantly (p<0.05). Microbiological properties fluctuated through the period of assessment but were within acceptable limits. The fungi isolated were Penicillium and Aspergillus niger, while the bacterial species identified were Staphylococcus aureus, Klebsiella spp and E. coli. The nutritive quality of the smoke-dried Gymnarchus niloticus was wholesome at the time of purchase however the decline in lipid and presence of potentially harmful microorganism makes it of relatively reduced quality and potentially harmful for direct consumption after 28 days of storage under ambient conditions.

Keywords: *Gymnarchus niloticus*, proximate composition, microbial, safety

INTRODUCTION

Fish is very nutritious, tasty, easily digested, *and* much sought after by a broad cross-section of the world's population, particularly in developing countries (Ekelemu, 2010; Nwabueze and Nwabueze, 2010). Fish accounts for 17% of the global consumption of animal protein in 2015 and provides about 20% of the average per capita intake of about 3.2 billion people (FAO, 2018). Fish is rich in macro-nutrients and micro-nutrients (Proteins, Fats, vitamins and minerals) thereby providing essential nourishment (WorldFish Center, 2011). Fish also contains significant amounts of all essential amino acids, particularly methionine and lysine which are relatively poor in cereals. Fish protein can be used to complement the amino acid and improve the overall protein quality of a mixed diet.

Fish is however susceptible to deterioration as soon as it is harvested, globally fish losses are estimated to be 10 - 12 million tons per year, accounting for around 10% of the total production of capture fisheries and

aquaculture (Ansen and Davide, 2017; Francisca *et al.,* 2010). Because of their high-water content fish and fish products are very perishable, therefore, various methods of processing are used to prolong the shelf life of fish and fish products, the processing methods includes drying, icing, salting, freezing and smoking. Smoking is one of the oldest food preservation methods. The process changes appearance, smell and taste of foods (Abowei and Tawari, 2011; Foline *et al.,* 2011; Ikutegbe and Sikoki, 2014; Varlet *et al.,* 2007).

The nutritional and food safety conditions of smoked dried fish are best not compromised. The major aim of commercial fisheries and aquaculture is to provide accessible, affordable and consumable protein that can meet the nutritional demands of the world's increasing population while contributing to food security and providing livelihood along the value chain. This forms the basis of advocacy for minimal post-harvest losses of any form physical or nutritional. The problem of long storage time and the generally unhealthy display conditions of smoke-dried *Gymnarchus niloticus* by retail fish vendors in Mobil fish market (Minna, Niger state, Nigeria) poses both economic and health concerns to the unsuspecting consumer. This study therefore aims at examining the nutritive quality and consumption safety status of smoked dried *G. niloticus* obtained from the market and kept in storage for a given time.

MATERIALS AND METHODS

A total of fifteen smoke-dried samples of *Gymnarchus niloticus* were purchased from different traders at Mobil Fish market Minna, Niger State, Nigeria. The fishes have been in storage by the traders for indeterminate period of time prior to their procurement for the investigations. 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 proximate compositions and microbial of the samples were analysed and recorded (Day 1), representing the biochemical composition and microbiological characteristics of samples at the time of purchase. Thereafter, the samples were stored in local fabricated metal wire basket under ambient temperature and humidity. The biochemical and microbial composition of the samples were analysed weekly over a period of 28 days. Biochemical and microbial analysis were 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 assessed using the prescribed methods by (AOAC, 2014). The mineral analysis was carried out using one gram (1g) of sample which was weighed into a beaker to which 15ml of concentrated nitric acid was added 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 50ml with distilled water and stored in a sample bottle. The sample was then analysed using Atomic Absorption Spectrophotometer (AAS).

Microbiological Analysis

Nutrient agar (NA), MacConkey agar (MAC), *Salmonella shigella* agar (SSA) and Manitol Salt agar (MSA) were the culture media used in the bacteriological investigation. Sabouraud Dextrose agar (SDA) was used in the culture, isolation and identification of fungal colonies present on the fish samples. All apparatus and culture media (petri dishes, beakers, test tubes etc.) used in this study was sterilized by autoclaving at 121^0C for 15 minutes in an electrically powered autoclave (Ikutegbe and Sikoki, 2014).

Identification and Classification
The identification of micro-organisms as well as biochemical tests such as motility, indole, catalase, oxidase etc. were based on characteristics as described by (Buchanan and Gibbons, 1974).

Statistical Analysis
Data was analysed at 5% level of significance with SPSS Version 22 (IBM Corporation, 2013). Mean change in values between the initial and subsequent readings were compared using one-way analysis of variance. Microbial counts were log transformed for data analysis and then reversed.

RESULTS
Mean percentage moisture started at 15.14 ± 3.56% (Day 1) and declined significantly (P<0.05) to 5.43 ± 0.71% (Day 28) (*Table 1)* the difference between base and final mean percentage moisture was -13.55 ± 3.58. The mean moisture content recorded for Day 1 and 7 was significantly (P<0.05) different from Days 14, 21 and 28. The atmospheric humidity ranged between 35 to 76% and averaged at 55% through the period of the study.

The initial (Day 1) mean percentage protein was 56.19 ± 2.12%, the protein content increased through the study period to a final value of 85.96 ± 0.79% (Day 28) (Table 1) the difference between base and final mean percentage protein was 29.77 ± 2.91. Apart from Day 14 and 21 which were not significantly (P>0.05) different the mean percentage protein was significantly (P<0.05) different

Mean percentage lipid progressively declined from 24.09±2.14% (Day 1) to 9.82±1.42% (Day 28) (*Table 1*) the difference between base and final mean percentage Lipid was -13.45 ± 2.22. The mean Lipid on days 1, and 7 were significantly (P<0.05) higher than Days 14 and 21wich were significantly (P<0.05) higher than the final (Day28).

The initial mean percentage ash for *G. niloticus* was 9.94±0.11%, the ash content increased through the study period to a final value of 17.12±1.95% (Day 28) (*Table 1*) the difference between base and final mean percentage Ash was 7.99 ± 2.32. The final (Day 28) mean ash content was significantly (P<0.05) different from Day 1, 7 14 and 21.

Table 1: Change in proximate composition of smoke dried *Gymnarchus niloticus*

Proximate Composition	Length of time in Storage (days)					Difference (Final-Initial)
	1	7	14	21	28	
Protein	56.19 ± 2.12^{d}	70.96 ± 0.98^{c}	81.50 ± 1.48^{b}	81.95 ± 1.97^{b}	85.96 ± 0.79^{a}	29.77 ± 2.91
Moisture	15.14 ± 3.56^{a}	14.20 ± 0.90^{a}	8.35 ± 0.37^{b}	7.45 ± 1.30^{b}	5.43 ± 0.71^{b}	-13.55 ± 3.58
Lipid	24.09 ± 2.14^{a}	22.65 ± 1.40^{a}	16.32 ± 0.97^{b}	17.87 ± 0.69^{b}	9.82 ± 1.42^{c}	-13.45 ± 2.22
Ash	9.94 ± 0.11^{b}	10.08 ± 0.95^{b}	11.57 ± 1.08^{b}	10.90 ± 0.83^{b}	17.12 ± 1.95^{a}	7.99 ± 2.32

**Values in the same row with different superscript letters are significantly different (p<0.05)*

Mineral Assessment
Table 2 shows the initial (Day 1) mean Sodium (Na) (mg/kg) recorded was 38.65±1.06, this declined to 25.5 ± 1.14. The mean Sodium (Na) (mg/kg) recorded was significantly (P<0.05) different throughout the study. Mean Calcium ranged from 3.50 ± 1.41 to 5.53 ± 0.59 and did not differ significantly (P>0.05) through the study. The Potassium (K) of 91.40 ± 1.56 (Day 1) was significantly lower than Days 14 and 28. Mean Iron did not differ significantly (P>0.05) however it ranged from 3.09 ± 0.77 (Day 14) to 5.26 ± 2.25 (Day 28).

Table 2: Biweekly Evaluation of some Mineral compositions of *Gymnarchus niloticus*

Minerals	Length of time in Storage (days)		
	1	14	28
Calcium (Ca)	5.53 ± 0.59^{a}	3.50 ± 1.41^{a}	4.40 ± 0.80^{a}
Potassium (K)	91.40 ± 1.56^{b}	97.95 ± 0.35^{a}	97.40 ± 2.69^{a}
Sodium (Na)	38.65 ± 1.06^{a}	33.45 ± 1.63^{b}	25.50 ± 1.41^{c}
Iron (Fe)	3.95 ± 0.54^{a}	3.09 ± 0.77^{a}	5.26 ± 2.25^{a}

Values in the same row with different superscript letters are significantly different ($p<0.05$)

Microbiological Assessment

Table 3 shows the mean total viable count (TVC) on Nutrient Agar (NA) for smoked dried G. *niloticus* fluctuated through the study. It ranged from $2.91 \times 10^4 \pm 0.43$ (Day 28) to $7.42 \times 10^4 \pm 0.12$ (Day 7) although it fluctuated through the study means were not significantly different ($P>0.05$). This trend was also observed on MAC growth medium however there were no growth on the medium for Day 1 and 7. Ranging from $8.04 \times 10^3 \pm 0.27$ (Day1) to $1.02 \times 10^5 \pm 0.17$ (Day 7) Growth on MSA fluctuated and varied significantly ($P<0.05$) through the study. There was no growth recorded on SSA growth medium throughout the study. Day 1 of the SDA growth medium also had no growth but ranged from $3.04 \times 10^6 \pm 0.44$(Day 28) to $4.00 \times 10^7 \pm 0.28$ (Day 7) with means being significantly different ($p<0.05$). The bacterial species isolated were *Staphylococcus aureus*, *Klebsilla spp* and *Escherichia coli*, while the mycoflora isolated from the smoked fish sampled were *Penicillium* and *Aspergillus niger*.

Table 3: Total heterotrophic bacterial and fungal counts (cfu/g) observed on the *Gymnarchus niloticus*

Medium	Length of time in Storage (Days)				
	1	7	14	21	28
NA	$3.02 \times 10^4 \pm 0.14^{a}$	$7.42 \times 10^4 \pm 0.12^{a}$	$3.55 \times 10^4 \pm 0.20^{a}$	$3.58 \times 10^4 \pm 0.22^{a}$	$2.91 \times 10^4 \pm 0.43^{a}$
MAC	NG	$9.12 \times 10^3 \pm 0.27^{a}$	$1.55 \times 10^4 \pm 0.36^{a}$	$2.77 \times 10^4 \pm 0.25^{a}$	NG
MSA	$8.04 \times 10^3 \pm 0.27^{c}$	$1.02 \times 10^5 \pm 0.17^{a}$	$1.64 \times 10^4 \pm 0.07^{bc}$	$2.23 \times 10^4 \pm 0.13^{c}$	$1.93 \times 10^4 \pm 0.19^{c}$
SSA	NG	NG	NG	NG	NG
SDA	NG	$4.00 \times 10^7 \pm 0.28^{a}$	$3.83 \times 10^6 \pm 0.27^{b}$	$2.92 \times 10^7 \pm 0.14^{a}$	$3.04 \times 10^6 \pm 0.44^{b}$

**Values in the same row with different superscript letters are significantly different ($p<0.05$)*

**NG (No growth)*

DISCUSSION

Protein

The protein value range between 56.19 and 85.96% through the study, this is similar to the range of values reported by Adeyeye and Adamu, (2005) and Alfa *et al.* (2014). This highlights the fact that smoked *Gymnarchus niloticus* is a good source of protein that can help cub malnutrition if food safety protocols are strictly followed. Being inversely proportional to moisture the protein increase through the study is in line with the findings of Oyero *et al.*, (2007) and Ikutegbe and Sikoki, (2014) which stated an observed increase in protein with a corresponding decrease in moisture content of smoked dried *Oreochromis niloticus* and decrease in protein concurrent to increase in moisture of stored *Chrysichthys nigrodigitatus* and *Pseudotolithus typus*, (Chukwu and Shaba, 2009; Fapohunda and Ogunkoya, 2006).

Moisture

In this study the moisture content of the fish progressively reduced through the period of the study. Atmospheric humidity during the

study elicited the continued decrease in the moisture content of the fish to 4.43% representing a respective 13.55% drop from the initial moisture content of the fish by the end of the study. The low moisture implies a reduced microbial activity on the fish samples (Oyero *et al.,* 2007), this also explains the observed brittleness and insect infestation of the fish samples concurring with the reports of Oparaku *et al.* (2003) and Francisca et al. (2010) which stated that at moisture content below 15 % the product is fragile and prone to crumbling and attack of insects.

The moisture content of the fish samples were inversely proportional to the values of protein and ash, the dryer the samples were the higher their respective protein and ash content, this is consistent with the reports of Idah and Nwankwo, (2013), Fapohunda and Ogunkoya, (2006) and Obande et al. (2012) which generally reported increase in protein with respect to decreased moisture content of fish samples.

Lipid

The initial percentage lipid of *Gymnarchus niloticus* recorded in this study was 22.06%, this indicate that the fish is rich in oil which is beneficial to the consumer because fish has been reported to contain poly-unsaturated fatty acids (PUFA) which is important in lowering cholesterol and preventing or reducing certain premature heart disease (Petenuci *et al.,* 2008; Dhanapal *et al.,* 2010; Güler and Yildiz, 2011)

The downward stride of lipid in the smoked dried fish sample from 24.09% to 9.82% (13.45% decline) can be credited to oxidation of poly-unsaturated fatty acids (PUFA) present in the tissue of fish samples(Abolagba *et al.,* 2011; Varlet *et al.,* 2007). The PUFA rich lipid present in fish is highly susceptible to oxidation because of its numerous double bonds which make them very unstable when exposed to atmospheric oxygen. Lipid oxidation is a major contributor to deterioration in food quality leading to off odors and flavors (Fapohunda and Ogunkoya, 2006; Ghaly *et al.,* 2010; Skałecki *et al.,* 2016; Varlet *et al.,* 2007).

Ash

At 9.94% ash content the initial result of this study is similar to the findings of Alfa *et al.,* (2014) who reported 6.51% in *G.niloticus*. The ash content of the fish samples can be credited to numerous factors such as environment, age, size, diet and species as well as the variation in the amount and quality of food it eats and in the amount of movement it makes (Adeniyi *et al.,* 2012; Adewumi *et al.,* 2014; Emurotu *et al.,* 2014; Hei and Sarojnalini, 2012).

Minerals

The results of this study proved *G. niloticus* to be a good source of both micro and macro minerals, this is similar to the findings of Olagbemide, (2015) which stated that fish remained a good source of micro and macro minerals in spite of the processing effects of smoking. According to Hei and Sarojnalini, (2012) the changes in mineral value that occurred during storage of the smoked dried fish can be a function of availability of these elements in the environment diet and the ability of fish to absorb them.

Microbiological Assessment

Generally, the microbial load of the samples over time did not follow a linear pattern. This suggests that the initial and final values of microbial count of smoked fish may not represent a clear picture of all possible microbiological changes or fluctuations that may be obtainable while the commodity is in storage but it serves as an indicator to the wholesomeness of the smoked fish for consumption (Centre For Food Safety, 2014).

Through the study the TVC of the fish sample was at satisfactory levels (FASI, 2015; Food Standards Australia New Zealand (FSANZ), 2018; Centre For Food Safety, 2014), the

increased dryness reduced the available moisture necessary for metabolic activities of the microorganism present on the samples there by leading to their decline in selective growth media (Ikutegbe and Sikoki, 2014; Oyero *et al.,* 2007).

The opportunity for microbial contamination is facilitated by the unhygienic environment in which the smoked dried fish is processed, stored, distributed and displayed for sale after smoking (Abowei and Tawari, 2011; FAO, 2011; Ugwueze and Igbegu, 2013). Freshly smoked dried fish are generally of acceptable microbial standard due to the thermal intolerance of most microorganism that can grow on the fish (Oyero *et al.,* 2007), the low microbial load on the fish sample bought from the market for this study indicates that the fish may have been freshly smoked or recently re-smoked prior to purchase, however as the smoked fish cools down they become susceptible to microbial infestation depending on the relative humidity and hygienic condition of processing, handling and display environment the smoked fish can be re-contaminated (Akinwumi and Adegbehingbe, 2015;Ikutegbe and Sikoki, 2014).

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

CONCLUSION

Considering the drastic loss in fish lipid and presence of potentially harmful microorganisms that can infect consumers with gastrointestinal infections, smoked dried fish should not be kept for too long in ambient conditions, 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. The result of this study shows that there is a significant difference between the initial and final values of nutritive quality and microbial load of smoked dried G. *niloticus* stored for 28 days.

Author Contributions: The authors shared the conceptualization, data collection, statistical analysis and writing of manuscript.

Funding: This research received no external funding

Acknowledgments: The authors acknowledge Reverend and Mrs Angus Ricketts and the entire laboratory staff of the Department of Water Resources Aquaculture and Fisheries Technology for their support

Conflicts of Interest: The authors declare no conflict of interest.

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