Vol. 3 No. 1

NIGERIAN JOURNAL OF POST-HARVEST RESEARCH

www.nspri.gov.ng

EDITORIAL BOARD

JOURNAL MANAGEMENT COMMITTEE

N I G E R I A N J O U R N A L O F P O S T - H A R V E S T R E S E A R C H

Aims: The Nigerian Journal of Post-Harvest Research has its focus on the dissemination of information on the discoveries of new technologies through Post-harvest Research by publishing the manuscript of research.

Scope: The manuscripts of research to be accepted for publication would be from those disciplines whose spheres or relevant has its tentacles wallowing found the subject or Post Harvest Technology.

ISSN: 2630 - 7022

MICROBIAL STUDIES OF DRIED CATFISH (CLARIAS GARIEPINUS*)* FROM SOME MARKETS IN ILORIN, KWARA STATE.

Ige*, I. M, Nwaehujor, I., Dada O. A., Adediji, A. Y., Adebayo, O. B, and Oyebanji, A. O.

Perishable Crop Research Department, Nigerian Stored Products Research Institute, Ilorin. Nigeria

**Corresponding author's Email; itohanige@ yahoo.com*

ABSTRACT

Microbiological qualities (bacteria counts, mould load, identification) and moisture contents of smoked catfish samples obtained from four markets in Ilorin, Nigeria were determined and compared with that of smoked fish using the Nigerian Stored Products Research Institute (NSPRI) smoking kiln. The study was to ascertain safety of the processed fish for consumption and storage. The markets include Idi-ape, Ipata, Ojatuntun and Unity markets in Ilorin Metropolis. The mean moisture content of smoked fish from the market samples was higher (8.07%) with the highest obtained at Ojatuntun market (9.51%) and the lowest at Ipata market(6.78%).Moisture content of NSPRI smoked fish was 5.16%. Average bacteria counts of smoked fish samples from the local markets was 4.9×10^4cfu/g with highest being obtained from Unity market(20.1×10^4cfu/g) and the lowest at Ipata market (8.55×10^4cfu/g). This was higher than for samples from NSPRI smoking kiln (0.1×10^4cfu/g). For fungi count, the highest counts was obtained at Idi-ape market with 3.17×10^3cfug and the lowest at Ojatuntun and Unity markets with 0.67×10^3cfu/g with average of 1.9×10^3cfu/g while samples from NSPRI had no fungi. The average coliform count for market samples was 3.2×10^3 cfu/g but NSPRI smoking kiln samples had no coliform. *Staphylococcus aureus*, *Aspergillus niger* and *Aspergillus flavus* were among organisms isolated. The number of microorganisms on the smoked fish from the open markets, as compared to samples from NSPRI improved smoking kiln is an indication of low microbiological quality and poor hygiene practices in the production of smoked fish at the markets. Proper cooking and hygienic handling are therefore recommended.

***Keywords*:** Bacteria count, market samples, moisture content, mould count, Smoked fish.

INTRODUCTION

Fish is one of the nutritionally valuable food available (Khalil, 2017). It is also highly perishable (Ashie *et al.,* 2009). It can deteriorate rapidly at atmospheric temperature (Mahmud, 2018). They need-proper handling and preservation if they are to have a long shelf life and retain a desirable quality and nutritional value (Brewer, 1989). A central concern of fish processing is to prevent it from deteriorating. This remains an underlying concern during fish processing operations. Climate change is a threat and risk to world fisheries. The ambient temperature of the tropics which Nigeria is one can cause rapid deterioration of fish. This is what is responsible for about 50% wastage of the total catches worldwide. An estimated 40% of total fish harvested in Nigeria is usually lost and amount to post harvest losses. Reduction of post-harvest losses of fish will go a long way to meeting fish demand and increasing the income of those involve in the business (Mungai, 2014). Fish processing can be subdivided into fish handling and the manufacture of fish products. Another natural subdivision is into primary and secondary processes. The primary process is into filleting and freezing of fresh

fish, the secondary process involves the chilling and production of frozen and canned products for the retail and catering trades (Bekker- Nielsen, 2005)

Preservation techniques are needed to prevent fish spoilage and lengthen shelf life. They are designed to inhibit the activity of spoilage bacteria and the metabolic changes that result in loss of fish quality. Spoilage bacteria and fungi are specific bacteria and fungi that produce unpleasant odour and flavours associated with spoilt fish. Fish normally host many bacteria that are not spoilage bacteria. Some of the bacteria present in spoilt fish played no role in the spoilage (Huss, 1988). To flourish, bacteria need the right temperature, sufficient water and oxygen and a surrounding that is not acidic.

Preservation techniques work by interrupting one or more of the following factors. The factors include the control of temperature which can be achieved by refrigerating and freezing. Another is the control of water which can be achieved by salting, drying and smoking Also the use of heat or ionizing radiation can be used to control microbial load to preserve the fish. Controlling the oxygen around the fish can also increase the shelf life of the fish. This is done by reducing the oxygen potential. Another method of fish preservation is chemical control of microbial load. This can be achieved by Bio preservation and fermentation of fish. (Kauffeid, 2005)

Lastly combined technique can be employed in which case two or more of the aforementioned methods can be employed to improve preservation. These combined techniques also reduce unwanted side effects such as denaturation of nutrients by severe heat treatment. Common combination techniques include salting/drying, salting/marinating, salting/smoking, pasteurization/refrigeration and controlled atmosphere/refrigeration. For the purpose of this study, the salting and smoking method was employed. Preservation technique can also be classified as follows salting, freezing, canning, smoking and pickling.

Smoking preserves the fish by drying. Fish smoking is a very popular and old method of processing fish in Europe, Africa and the Far East (Adeyeye, 2019). When properly processed, the smoked can be of high quality which supports the marketing of dried fish as a health food that is thriving in the traditional markets. The possible source of contamination during the processing of fish includes the use of contaminated fresh fish and washing the fish with contaminated water. The use of contaminated utensils and spices can also be sources of contamination. Furthermore, the handlers hand can also be a source of contamination. It is also important to note that the smoking process of fish may destroy vegetative bacteria cells but may not destroy some spores (Edema, 2010)

This study aimed at investigating bacteria counts, mould counts and moisture content of smoked fish from markets in Ilorin metropolis and compared with fish samples processed with NSPRI Smoking Kiln.

MATERIALS AND METHOD

Sample collection

Twenty kilograms (20kg) of fresh catfish (*Clarias gariepinus*) was purchased from fish farmers from Asa-dam fish farmers' settlement in Ilorin. The fish was processed for drying using the Nigerian Stored Products Research Institute's smoking kiln following the flow chart shown in Figure 1.

Figure 1: Steps for processing of the fish

Also already processed dry catfish was purchased from four different markets in Ilorin metropolis. The markets include Unity market, Idi-ape market, Ojatuntun and Ipata market. Three samples were purchased from each sample site

Determination of moisture content

Ten grams of dried fish sample from each sample site was weighed and dried in the oven at 80°C to a constant weight. The moisture content was determined as the difference between the weight of the fresh sample and that of the sample after drying to a constant weight. This was expressed as the percentage of the total weight of the sample (AOAC, 2019).

$$M_c = \frac{L_{ws}}{W_{fs}}\ x\ 100\%$$

(Where Mc = Moisture constant %, Lws = Loss in weight of sample, Wfs = Weight of fresh sample)

Microbiological Analyses of Samples

Sample Preparations

All the samples were properly labeled. Ten grams (10g) of each fish sample were weighed and homogenized into 90 ml of sterile distilled water using a sterile warring blender. Tenfold dilution of the homogenates was made using sterile pipettes as described by the methods of Fawole and Oso (2001). All the chemicals and reagents used were of analytical grade, obtained from Sigma chemical Co. Ltd, England. Media used in this study included: Plate count Agar (PCA), Potato Dextrose agar (PDA) and Eosin Methylene Blue (EMB). All media were prepared according to the manufacturer's specification and sterilized at 121^0Cfor 15 min. Aliquots (0.1ml) of each dilution were transferred in replicate into corresponding media. Plate count agar for bacteria growth, Eosin Methylene Blue agar as selective media for coliform while Potato Dextrose Agar as enrichment media for moulds. Serial dilutions were prepared up to 10^{-4} dilutions. 0.1ml of each dilution was spread uniformly on the petri dish using a hockey stick. The different agar was poured on the petri dish containing the diluted sample. The plates were then incubated aerobically at 37°C for 18 to 24hrs (for bacteria) and at 25°C for 72hrs (for fungi). Discrete colonies were sub-cultured into fresh agar plates aseptically to obtain pure cultures of the isolates. Pure isolates of resulting growth were then stored for further identification. Eosin Methylene blue agar was used for coliform enumeration while Mannitol salt agar was used for the isolation of *Staphylococcus aureus*. Total viable aerobic bacteria count was performed on Plate Count Agar. At end of the incubation periods, colonies (30-300) that appeared on each plate for the different dilution were counted using the illuminated colony counter (Gallenkamp, England). The counts for each plate were expressed as colony forming unit of the suspension (cfu/g).

The total heterotrophic count was done using method adapted from the Laboratory manual for Tropical Countries for general microbiology (Monica Cheesbrough). The plates which contained between 30 and 300 colonies after 18 to 24 hours of incubation were counted. The number of colonies was multiplied by the reciprocal of the dilution factor and the volume plated. Colony forming unit/gram = number of colony x 1/dilution x 1/volume plated.

Colonies identifiable as discrete on the Plate count Agar were carefully examined macroscopically for cultural characteristics such as the shape, color, size, and consistency. Bacterial isolates were characterized based on microscopic appearance, colonial morphology, and Gram staining reactions as well as appropriate biochemical tests for example Kligler's Iron Agar (KIA) test, Indole production test, Methyl Red (MR) test, Voges-Proscauer (VP) test, Citrate utilization test, Motility Indole Urea (MIU). Mannitol Salt Agar was used for the selective cultivation of S. aureus. Also, Catalase and coagulase tests were used for the confirmation of S. aureus.

Statistical analysis

Statistical analysis was carried out using one-way ANOVA and a p value of ≥ 0.05 was considered statistically significant

RESULTS AND DISCUSSION

Variations in moisture content of fish samples

The moisture content of the NSPRI samples and that of market samples are as shown in Table 1. The range for the NSPRI samples was 5.15% to 5.17% with an average of 5.16%. The samples from Idi-ape market had moisture content ranging between 7.35-9.31 % with an average of 8.22%. Ipata samples had moisture content of 6.17-7.47% with an average moisture content of 6, 78%. The samples obtained from Oja tuntun market had moisture content ranging from 6.36-14.57% with an average of 9.51%. Lastly the moisture content of samples from the Unity market had moisture content ranging from 6.89-7.81% and had average moisture of 7.79%.

Table 1. Moisture content of fish samples

Sample site	**Moisture content**	**Average moisture content**	**SD**	
NSPRI 1	5.15%			
NSPRI 2	5.17%	5.16%	0.01	----
NSPRI 3	5.16%			
IDI-APE MARKET 1	9.31%	8.22%	1.00	NS
IDI-APE MARKET 2	7.99%			
IDI-APE MARKET 3	7.35%			
IPATA 1	7.47%			
IPATA 2	6.17%	6.78%	0.65	NS
IPATA 3	6.71%			
OJA TUNTUN 1	14.57%			
OJA TUNTUN 2	6.36%	9.51%	4.42	NS
OJA TUNTUN 3	7.61%			
UNITY MARKET 1	8.66%			
UNITY MARKET 2	7.81%	7.79%	0.89	NS
UNITY MARKET 3	6.89%			

There were marked difference in the moisture content of NSPRI processed fish and that of market samples. The results showed higher moisture content for market samples. The fish sample with the highest moisture content was from Ojatuntun Market (14.57%) while the lowest from the market samples was from Ipata market (6.17%). The mean moisture content of the market samples was 8.07%. The samples processed using NSPRI Smoking kiln had an average moisture content of 5.16%. For all location sampled, NSPRI had the lowest moisture content (Table 1). Though there was difference in the moisture content for the four sample sites the difference compared to NSPRI samples was not significant. The reduced moisture content of the NSPRI processed fish might explain the reduced number of microorganisms found in the fish samples. Adequate drying creates unfavorable conditions for growth of microorganisms (Islam, 2013). This should in turn lead to prolonged shelf life of the fish

Microbial load

The result for the Bacteria count for this study is seen in Table 2. There were also marked difference between bacteria counts in the varying sampling sites. It was only for the third location for Ipata market ($p=0.025$) and third location for Oja tuntun market ($p=0.021$) that the difference was significant

Table 2. Bacterial count on fish samples from the markets and NSPRI-processed samples

Sample site	First/Second samples	t-value	p value	
IDI-APE				
First location	20 / 200	1.08292	0.196017	NS
Second location	20 / 25	2.82843	0.052786	NS
Third location	25 / 250	1.06497	0.199222	NS
IPATA				
First location	35 / 350	1.14271	0.185754	NS
Second location	25 / 48	2.03933	0.089128	NS
Third location	25 /30	4.24264	0.025658	NS
OJA TUNTUN				
First location	300 / 32	1.14532	0.185321	NS
Second location	320 / 15	1.01626	0.208222	NS
Third location	24 /26	4.64238	0.021701	NS
UNITY				
First location	20 /35	1.89737	0.099108	NS
Second location	50 / 500	1.1666	0.18183	NS
Third location	550 /55	1.17166	0.18101	NS

Bacterial counts were widely dispersed in all the markets surveyed with mean and standard deviations of 90(105.78), 85.5(129.86), 119.5(147.79) and 201.7(251.02) at Idi-ape, Ipata, Oja tuntun and Unity markets respectively. Unity market had the highest number of bacteria counts while Ipata market had the lowest among the market samples. Though this was not statistically significant at $p<0.05$.(Table 2). Of note are the bacteria Staphylococcus aureus isolated from the market samples. This is also in line with results obtained by other workers (Bujjamma, 2015). They also isolated S. aureus from processed fish samples. Staphylococcus species are part of the important food borne opportunistic bacteria in fishes. When the load of the bacteria is reasonably high, it can result in food spoilage. (Albuquerque, 2007). The presence of Staphylococcus specie is an indication of poor handling and has been indicted in food poisoning (Gupte 2006)

Coliform Counts

The result for coliform count for this study is seen in Table 3. There is difference in the coliform counts for the different sampling sites. Though for most of them this difference was not statistically different. The difference in second location in Oja tuntun market ($p=0.013$) and the second location in Unity market ($p=0.024$) were statistically significant (Table 3).

Table 3. Coliform count on fish samples from the markets and NSPRI-processed samples

Sample site	First/Second samples	t-value	p value	
IDI-APE				
First location	0 / 8	1	0.211325	NS
Second location	0 / 2	1	0.21135	NS
Third location	3 / 8	2.2	0.079404	NS

IPATA				
First location	0 / 2	1	0.21135	NS
Second location	0 / 3	1	0.211325	NS
Third location	0 / 6	1	0.211325	NS
OJA TUNTUN				
First location	6 / 0	1	0.211325	NS
Second location	7 / 5	6	0.013336	NS
Third location	0 /2	1	0.21135	NS
UNITY				
First location	0 / 4	1	0.211325	NS
Second location	5 / 8	4.33333	0.024673	NS
Third location	2 / 7	1.8	0.106833	NS

The counts for coliform bacteria were not widely dispersed in the markets surveyed. The means and standard deviations are 3.5(3.67), 1.83(2.40), 3.3(3.077) and4.3 (3.01) for Idi-ape, Ipata, Ojatuntun and Unity markets respectively. Though Idi-ape market had the highest number of coliform organism, the other sampled locations had varying numbers of coliform though this result was not statistically significant (Table 3). Similar result was obtained in a study carried out in Benin Republic where they also isolated coliform organisms from processed fish samples (Anihouvi, 2019). The presence of coliform organism on samples from market is an indication of fecal contamination

Fungi Counts

The fungi count for this study are seen in Table 4. For all the sampling sites in Idi-ape market, the difference as compared to NSPRI samples was statistically significant ($p>0.05$). One location in Ipata market, Oja tuntun market and Unity market had statistically different results as compared to NSPRI samples.

Table 4-Fungi Count on fish samples from the markets and NSPRI-processed samples

Sample site	**First/Second samples**	**t-value**	**p value**	
IDI-APE				
First location	5 / 4	9	0.006061	S
Second location	1 / 2	3	0.047733	S
Third location	4 / 3	7	0.009902	S
IPATA				

First location	0 / 1	1	0.211325	NS
Second location	5 / 2	2.33333	0.072407	NS
Third location	1 / 2	3	0.047733	S
OJATUNTUN				
First location	0 / 0	0	0.5	NS
Second location	2 / 1	3	0.047733	NS
Third location	0 / 1	1	0.211325	S
UNITY				
First location	6 / 2	2	0.091752	NS
Second location	2 / 1	3	0.047733	NS
Third location	0 / 1	1	0.211325	S

For fungi, the counts were not widely dispersed as these were the means and standard deviations for Idi-ape 3.167(1.472) while for Ipata, Ojatuntun and Unity markets were 1.67(1.751), 0.67(0.816) and 2.167(2.041) respectively. Idi-ape had the highest fungi counts and this was statistically significant ($p<0.05$) .The fungi organism identified in this study include *Aspergillus niger* and *Aspergillus flavus* which were isolated from the market samples. Fatima et al (2016) also isolated *Aspergillus niger* from smoked fish samples in Maiduguri, Nigeria. *Aspergillus flavus* was also isolated from smoked fish samples in Cotonou (Benin Republic) in a study carried out by Yann (2019). *Aspergilus niger* and *Aspergilus flavus* identified in this study have been incriminated in food spoilage. The isolation of the aforementioned microorganisms from the smoked fish samples from the market shows partial drying during the processing of the fish. A study by Schewan (1977) also isolated these fungi and attributed their presence to partial dehydration during processing.

In all the locations sampled, NSPRI had the lowest counts for bacteria, coliform, fungi, and yeast. Unity market has the highest average bacterial count (201.67) when compared with Ojatuntun, Idi-ape and Ipata with 119.5, 90 and 85.5 respectively.

The average counts of organisms is shown in Figure 2.

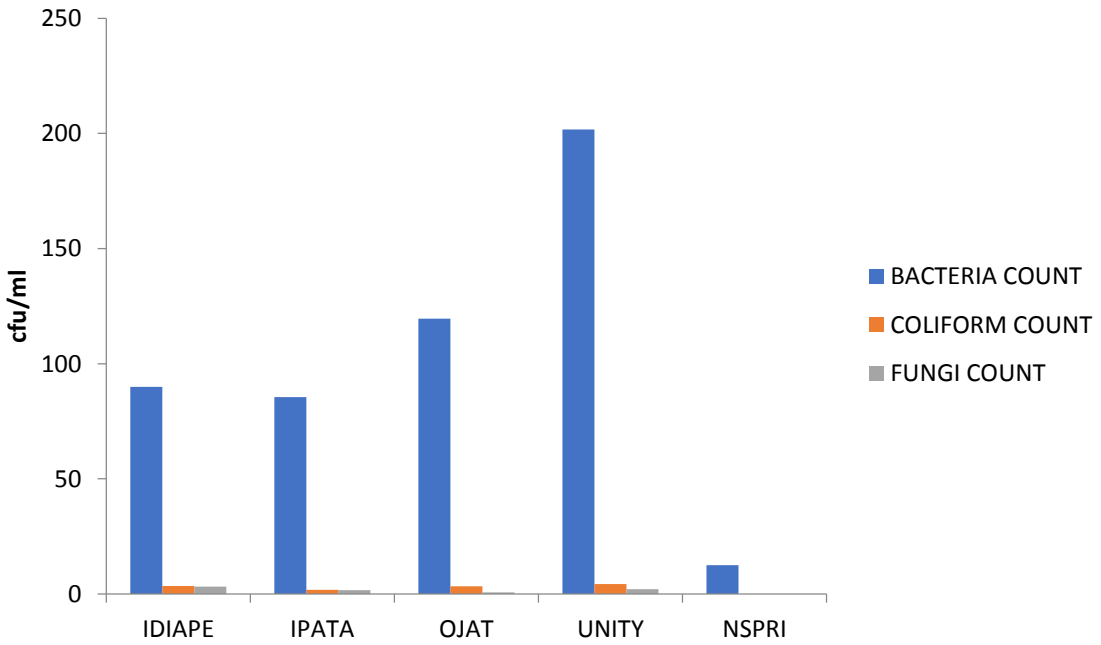

Figure 2. Average microorganisms counts on fish samples from the different sample sites

CONCLUSION

The result of this study shows that using a more improved mechanized smoking system that will completely dry the fish is a preferred method of drying for improved shelf life extension. This will prevent contamination due to high moisture content. There should be regular monitoring of food handlers dealing with the processing and marketing of smoked fish. Their hygienic condition should be examined regularly before allowing them to handle food for public consumption.
Lastly it is recommended that food for consumption should be properly cooked before eating.

ACKNOWLEDGEMENT

Authors acknowledge NSPRI's sponsorship of this project and appreciate support and assistance of all research and technical staff in cooperation and collaboration.

REFERENCES

Adeyeye, S. A. O. (2019). Smoking of fish: a critical review. *Journal of Culinary Science and Technology,* 17(6):559-575.

Albuquerque, W.F., Macrae, A., Sousa, O.V., Vierra, G H.F, Vieira, R.H.S. 2007. Multiple drug resistant Staphylococcus aureus strain isolated from fish market and from fish handlers. *Braz. J. Microbiol*., 38:131-134.

Ashie, I. N. A., Smith J. P, Simpson B. K and Norman, F. H. (2009). Spoilage and shelf-life extension of fresh fish and shellfish. Critical Reviews in Food Science and Nutrition.

Bekker- Nielsen, T. 2005 Ancient fishing and fish processing in the Black Sea region. Volume 2 of Black Sea studies, Aarhus University Press ISBN 978-87-7934-096-1

Bene C, Macfayden G, Allison, E H. (2007). Increasing the contribution of small-scale fisheries to poverty alleviation and food security. Italy; FAO Fisheries Technical guidelines for responsible fisheries

Brewer, D. J. and Freidman, R. F. (1989). Fish and Fishing in ancient Egypt. Cairo press: The American University in Cairo ISBN 978-977-424-224-3.

Bujjamma P. and Padmavathi, P. (2015). Prevalence of Staphylococcus aureus in Fish samples of Local Domestic Fish Market. *International Journal of Current Microbiology and Applied Sciences*, 5: 427-433.

Cheesbrough M. Medical Laboratory Manual. Tropical Health Technology, Low priced Edition. Doddington, Cambridgeshire, England, 2003; pp. 20-35

Anihouvi, D. G. H., Kpoclou, Y. E., Abdel, Massih, M., Iko Afé, O. H., Assogba, M. F., Covo, M., Scippo, M. L., Hounhouigan, D. J., Anihouvi, V., Mahillon, J. (2019). Microbiological characteristics of smoked and smoked-dried fish processed in Benin. F*ood Sci Nutr,* 7(5):1821-1827.

Edema, M. O., Agbon, A. O. (2010). Significance of fungi associated with smoke- cured ethmalosa fimbriata and *Clarias Gariepinus*. *Journal of food processing and preservation,* 34(s1):355-363.

Fatima M. S., Idris A. N and Gloria T. (2016). Mycological Evaluation of Smoked Dried Fish sold at Maiduguri metropolis, Nigeria: Preliminary Findings and Potential Health Implications. *European Journal of Health Sciences*, 2(1):5-10.

Fawole MO, Oso BA. Laboratory manual of Microbiology: Revised edition spectrum books Ltd, Ibadan, 2001; p. 127.

Gupte, S. (2006). The short Textbook of Medical Microbiology (9th Edition). Jayppee Brothers Medical Publishers (P) Ltd. New Delhi India, 164 & 209.

Huss, H. H. (1988). Fresh fish quality and quality changes. FAO Fisheries technical paper 348, Rome. ISBN 92-5-103507-5

Islam, T. M, Ahmed, S., Sultana, A., Tumpa, S. A. and Flowra, F. A, 2013. Nutritional and food quality assessment of dried fishes in Singra Upazila under Natore district of Bangladesh. *Trends in Fisheries Research*, 2(1):2319-4758

Kauffeld, M. K. and Egoi, P. W. (Eds) 2005. Handbook on ice slurries: fundamentals and engineering, International Institute of refrigeration 978-2-913149-42-7

Mahmud, A., Abraha, B., Samuel, M., Hamada, M., Winta, A. and Elham, M. (2018). Fish Preservation: a multi- dimensional approach, *MOJ Food Process Technol*,6(3); 303-310

Mungai, D. M. (2014) Biology assessment of post-harvest losses of Nile Perch (Lates Nicoticus) incurred by fishermen from Lake Victoria, Kenya.

Sarvenaz, K. T. and Sabine, S. (2018). Nutritional Value of Fish; Lipids, Proteins, Vitamins and Minerals. *Reviews in Fisheries Science and Aquaculture*, 26:243-253.

Schewan, J. M. (1977). Microbiology of Fish and Fish Production. Progress Report. *Journal of Applied bacteriology*, 34: 299-315.

Yann, C.S.A., Gerard T., Benoit, G. G. and Ambaliou, S. (2019). Morphological and Molecular characterization of Aspergillus flavus Isolated from smoked, Fermented and Dried Fishes Sold in Main Markets of Cotonou (Benin). *Journal of Food and Industrial Microbiology*. 5(1):1-6.