HAZARD ANALYSIS AND CRITICAL CONTROL POINT ON THE MICROBIOLOGICAL STATUS OF SOME COMMERCIAL FISH SPECIES SMOKED AT VARIOUS LOCATIONS AROUND SHIRORO LAKE, NIGERIA

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ABSTRACT

Fish is a highly perishable commodity. Many processing and preservation methods have being employed to prolong its shelf life. Smoking is one of such method used in preserving fresh fish. After smoking, the microbial loads were categorized into Total Viable Count (TVC), Total Coliform Count (TCC) and Total Fungi Count (TFC), values obtained for Improved Traditonal Smoking Klin indicated that TVC had the highest occurrence of bacteria load ranging from $0.14X10^{2}$- $0.46X10^{8}$, for Clarias spp,Bagrus bayad, Lates niloticus and Heterotis niloticus, only Clarias spp had TCC value of $0.25X10^{7}$ and TFC value of $0.48X10^{8}$ S. nigrita, M.rume and Tilapia spp had no values were recorded. These values were significantly different (P< 0.05). TVC. The Values from Zumba studied indicated that TVC ranged between $1.81.x10^{8}$- $4.12x10^{8}$, TCC $0.53X10^{4}$-$1.35X10^{7}$ and TCF was between $0.14x10^{3}$ $0.82x10^{3}$ for all the seven fish species studied,Gwada site ranged between $0.73x10^{8}$- $2.63x10^{8}$ for TVC, only S.nigrita had TCC value of $0.51x10^{4}$ and TFC value of $0.11x10^{5}$, Kuta site had TVC values that ranged from $1.20x10^{8}$-$3.25x10^{8}$, only B. bayad and Clarias spp had TCC values of $0.40x10^{4}$ and $0.23x10^{4}$ respectively,(P>0.05).The Critical Control Limit (CCL), which according to International Commission on Microbiology Specification for foods (ICMSF) (1997) recommended bacteria limit values for dried fish to be $3.0x10^{3}$ and$3.0x10^{4}$, while fungi limit should not exceed $2.0x10^{2}$. Results obtained for smoke fish in this research fell above this recommendation except for ITSK that had the least microbial load forClarias specie, Additional bacteria isolates like Bacilius megaterium, Bacilius lichinforms, MicroccusRoseus and Microcusluteus were found present on fishes from Kuta and Zumba, suggesting poor handling, poor smoking technique and re-contamination after smoking. It is therefore recommended that, good processing practices like washing the fish thoroughly with clean water, gutting the fishes, bringing the fish before smoking ensure that microbial loads on fish are greatly reduced in other to prevent microorganisms attack and also prolong the shelf life of the fish.

Keywords: Fish species, Microorganisms, Locations and Hazard Analysis and Critical Control Point (HACCP).

INTRODUCTION

Fish quality deteriorates immediately it is out of water, and that if fish are not processed, preserved or stored it can lead to colossal post-harvest loss, in term of physical, economic and nutritional losses to the country and reduced the wellbeing of her citizen. (Oyero, 2018). Spacey (2017), documented that Quality Control (QC) is defined as the process of detecting mistake in operational outputs such as products and service. Quality, therefore, according to Eyo(2001), is the characteristic or rather the attributes that makes a fish acceptable to a consumer.

Oyediji (2010) therefore defined Hazard Analysis and Critical Control Point (HACCP) as a flow process, where if a significant action at a particular stage is omitted, it will result in either a physical, biological or chemical food hazards to the consumer. Such stages in the process are called the

Critical Control Points (CCPs) and the exact points are called the Critical Limit (CL). Hazard Analysis and Critical Control Point (HACCP) a method to identify process steps, where a loss or significant deviance from the required product quality and safety could occur if no targeted control is applied (CAC, 1997).

Ababouch*et al.* (2005) reported that an international drive towards reforming fish inspection system using Hazard Analysis Critical Control Point (HACCP) based on safety and quality came to limelight as early as 1980, however, Ababouch (2000) and Codex Alimentarius Commission (2001) traced the history of HACCP, though the original idea was first conceived by a private food company called Pillsbury in the late sixties, with the motive of guarantying the food safety intended for the U.S space program..

Gana*et al*. (2019 in press) reported that using the Improved Traditional Smoking Kiln-ITSK(steel drum) produced the best amino acid profile of 84.54% at a temperature of 60^0- 90^0C with GLUTAMIC acid ranked the highest when compared to Traditional Smoking Kiln that produced the least amino acid profile value of 68.55%.

Any of the processing methods used isto obtain a product called smoked fish. Smoked fish is a traditional part of the diet of a large section of the world's population especially where modern preservative methods like canning and freezing are not readily available (Eyo, 2001and Oyero, 2006). Smoked fish is known to have a slightly extended shelf-life, as compared to raw fish. Smoked fish is derived from traditional smoking kilns like the traditional smoke house, pit oven, traditional conical mud kiln, drum type kiln and earthen ware pot-type. Sadly, enough, this smoked fish from these means suffer a lot defects such as high moisture contents, heavy insect infestation, bacterial decomposition, and fungi attack (Oyero, 2006). These defects result in a short shelf life and remarkable inability to withstand handling and transportation practices by retailers. According to Agbolagba *et al*. (2011), microbial contamination or re- contamination of smoked fish is due to several factors such as poor smoking of fish products, inappropriate temperature control or application of poor personal hygiene of mongers, poor hygiene/ sanitary practices relating to smoked fish products, smoke/workhouse, packaging and storage as well as the use of inadequate and in efficient, traditional processing facilities, poor environmental sanitation and high human traffic are also implicated.Fish smoking techniques is based on temperature regime that is employed. There are three types of smoking techniques these are: cold smoking, hot smoking and smoke drying. In cold smoking, the fish are not cooked and the end products is similar in keeping qualities of fresh fish and the temperature at which this take place is between 30 - 60°C whereas, in hot smoked fish, the fish is cooked, a process which prevents spoilage for only a day or two, and is normally carried out between temperature of 60 - 90°C. In many traditional processing techniques, there is little distinction between hot smoking and smoke drying which can lead to cooked dried products which tend to break up on handling (Joules, 2011). Oyero (2006) and Eyo (2000 and 2001) all described the different types of fish smoking equipment and categorized them into traditional and mechanical. Among the traditional smoking kilns are: Traditional smoke house, pit oven, traditional conical mud kiln, drum type kiln, earthen ware pot type kiln. Potential hazards in fish that necessitate the use of HACCP can be categorized into three broad aspects. These are the biological, chemical and physical hazards. The biological food hazards are Microbes which include: Pathogenic bacteria (infectious toxin producing), fungi, nematodes/parasites protozoans and aquatic biotoxin (Price, 2000). Pathogenic bacteria cause illness in human. Shweihofor and Wells (2013) cited examples of these biological hazards to include *salmonella*, *E. coli*and *Clostridium botulinium.* Fish can be said to be a source of biological hazard because according to Austin and Austin (2007) fish are capable of harboring pathogenic bacteria and their toxins, parasites and biogenic amines that are sources of causal agent of food borne illness in humans. According to Gram and Huss (2000) pathogenic bacteria are defined as those bacteria that may cause illness inhuman. Some pathogenic bacteria are transmitted to human

via food. Food- borne bacteria are few. Bacteria food-borne pathogens may be grouped into those that cause food intoxication and those that result in food- borne bacterial infection. In case of bacteria food poisoning or intoxication, the causative organism multiplies in the food where it produces its toxins. A food poisoning is therefore characterized by rapid onset of the illness (typically symptoms are nausea, vomiting) as the toxins are already formed in the food before consumption.

Thus ingestion of viable bacteria is not a prerequisite for the induction of the - disease.
Most often intoxication requires that the toxin producing bacteria have grown to high numbers (10^5 - 10^8cfu/g) in the food before it is eaten (Gana*et al*.,2012). In contrast, the food merely acts as a carrier for the causative organism in food-borne infections. The infectious agent may or may not have multiplied in the food, but the ingested viable bacteria continue to grow within the host body to produce the typical symptoms (fever, diarrhea) the number of viable bacterial cells necessary to cause disease (the Minimum Infective Dose, MID) varies considerably between bacteria species thus the MID is known to be high (10^5 - 10^6 cells) for pathogenic Vibrio spp and very low for some *Salmonella typhi*and *Shigella*spp (Kothany and Babu, 2001). According to Gana*et al.* (2012), Seafood - borne pathogenic bacteria may conveniently be divided into three groups according to their ecology and origin as those who are indigenous to: The aquatic environment, the general environment and the animal/human reservoir.

Fungi associated with smoked fish pose a great threat to the populace as the transfer of microorganism attack the immune system of the consumer, thereby giving room for invasion of disease (Adeyeye*et al.,* 2015). Furthermore, Agbolagba*et al.* (2011) stated that bacteria (*Staphylococcus aureus*), yeast (*Saccharomyces cerevisiae*) and mould (*Penicillium* and*Aspergillus)* are the commonest fungi associated with smoked fish capable of poising serious food borne illness to consumer. Salako and Anjorin (2012) reported that fungi implicated in biological food hazard are the genus *Aspergillus,* which are responsible for the disease known as Aspergillosis. Members of the genus are *Aspergillus Niger Aspergillus fumigatus* and *Aspergillus flavus.* The symptom of Aspergillosis includes: fever, cough, chest pain, breathlessness, cancer, high mortality and liver arrhosis, when ingested by man and animals. *A. fumigatus and A. Niger* have been observed to invade the human bronchus forming compact fungus balls. . In addition to this, Fafioye*et al.* (2002) reported high incidence of *Aspergillus flavus* in smoked fish in agogiwoye(Nigeria), While Adebayo-Tayo*et al.* (2008) was able to report aflatoxin in smoked fish.

Justification of the study
HACCP is a relatively new technology that is being employed in food safety management. Its use in Smoked fish (most especially in Niger State) is yet to be fully understood as well as undertaken. This study therefore is expected to widen the scope of research on HACCP as it relates to post harvest technology in smoked fish

Objective of the study
To use the HACCP principles to assess, the microbiological quality of smoked fishes from Shiroro Lake.

MATERIALS AND METHODS

Shiroro Lake
According to Kolo (2007) Shiroro dam Hydro-electric project was initiated by the defunct Northern Nigerian government and the former Electricity Corporation of Nigeria in 1957. Originally conceived to meet the electricity requirements of Kaduna, Zaria and Kano areas, work on the project commenced in 1987.It was impounded in 1984 and commissioned in 1990.The Lake is about 66km away from Minna, the capital of Niger state. River Sarkinpawa, Munya and Dinya are the major tributaries of the lake. It has a vast catchment area covering about 20,300km^2 in Niger state alone and draws about 27% of the total landmass of the state. It arises from the western slope of Jos plateau and has a grand total area of about 65, 580km^2 from its head water to the gauging

station at Wuya Bridge in Niger state. The dam itself has a surface area of 306km^2, an elevation of 382m and tremendous storage capacity of 605 billion metre cube, the second largest Hydro-electric dam in Nigeria with installed capacity of 600mw. WhereasKainji dam, being the largest has the installed capacity of 750mw (Fig.1). The climate of the lake is typical of Nigeria having distinct wet and dry season.

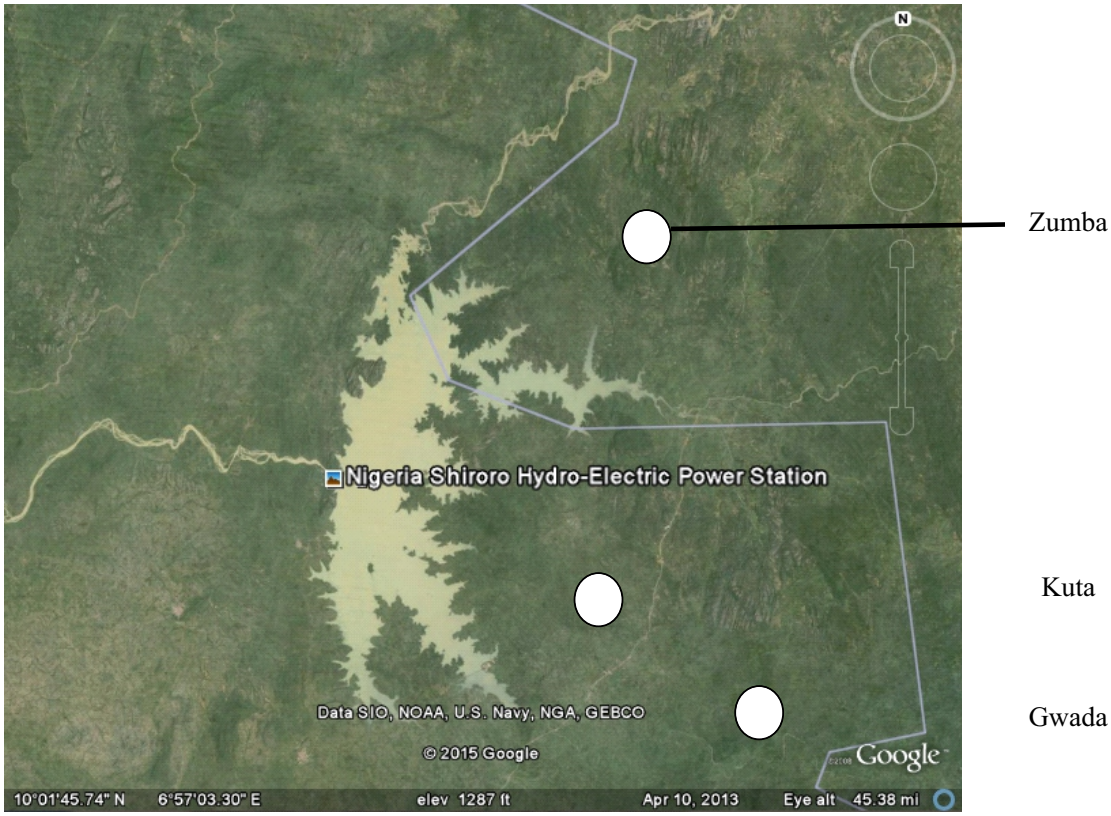

Figure 1: Aerial view of Shiroro Dam showing Gwada, Zumba and Kuta as dotted spots on the map

(Source: Ibrahim, 2015)

A total of six hundred and seventy-two fish samples used in this research were sourced from three markets, namely Gwada, Zumba and Kuta fish market. Fresh samples of fish species (that were thoroughly inspected to ascertain their quality) were purchased and then transported to each location where smoking was carried out. The smoking activity was carried on a biweekly basis for a period of sixteen weeks (4 months).Each of the seven fish species were replicated three times to give a total of twenty-one samples per week. Three Traditional Smoking Kilns stationed in each of the locations (Zumba, Gwada and Kuta) were used.Hardwoods were used as the source of heat. The smoking activity lasted for duration of fifteen (15) hours. A fabricated Improved Traditional Smoking Kiln was used, which serve as the control. A sensitive thermometer was attached to kiln. Smoking was carried out for duration of eight (8) hours in the control.

Determination of Biological (status) Hazard.

The microbial analysis was carried out by the method as described by Oyeleke and Manga (2008)

and Chessbrough (2000) to identify bacteria isolates. Ayanwale*et al*. (2009) described the procedure of identifying fungi. The two groups of microorganisms studied were the bacteria and fungi. The procedure for their determination entails the preparation of culture media which involves the weighing of 12.6gms of salmonella shigella agar and 10.4gms of mac- conkey agar which were later autoclaved and poured into sterilized plates.

In the fungi analysis, the procedure involves slicing 300gram of Irish potato which was added with 500millilitre of distilled water and allowed to simmer for 30 to60 minutes, after which it was filtered through layers of cheese cloth. 20gram of agar and 20gm of glucose was added to the filtrate and autoclaved at 121°C for 15minutes to prevent the growth of bacteria in the primary culture as much as possible. 0.5gm of chloramphenicol was added to the autoclaved medium aseptically. Smearing of thefish sample were carefully rubbed with distilled water in a test-tube and was covered with cotton wool plugs. Swabs were cultured in varied media based on concentration in 3 replicates test tubes containing distilled water were labeled A-D and with separate sterile pipettes serial dilution of the fungi culture were prepared. With separate sterile pipette 1ml of smear suspension was poured into 3 test tubes head/gill and head (10^1,10^2 and 10^3) respectively

Description of Improved Traditional Smoking Kiln (ITSK) used for the experiment.

The improved smoking kiln was designed and fashioned after the traditional drum kiln with slight modification to conserve fuel and improve the quality of smoked fish.

The kiln was fabricated from a 44-gallon drum (plates 3.7 and 3.8), the height of which is 0.92m. The drum is cut vertically to create door of 0.67m x 0.48m. The improved kiln is equipped with steel rods drilled into the inner compartment and wire mesh placed on them to form three fish trays that are 0.18m apart, increasing its carrying capacity.

At the bottom of the drum is located a stokehole which is 0.33m x 0.17m. A perforated metal sheet is incorporated at 0.1m above the stokehole. This act as a damper to prevent direct contact of fire with the fish loaded on the bottom trays to prevent them from being charred (FAO, 2011).

The back top of the drum was drilled to provide a chimney where excess smoke and heat escape from. During smoking, the door of the kiln must be kept closed to further conserve heat.

Description of the Traditional Smoking Kiln

The traditional smoking kiln used was a 44-metalic drum also having an equal height of 0.92m like the ITSK. The top of the drum was completely opened, while half of the base was cut out to serve as the stokehole for the use of firewood. On the top of the drum an appropriate wire mesh was placed for spreading of the intending fish to be smoked**.**

Experimental Design

The experimental design is a completely randomizeddesign.

Data Analysis

Statistical Package for Social Science (SPSS version 16) was used to analyze the data. The data was subjected to one-way Analysis of Variance (ANOVA) and means between the treatments were separated using Duncan multiple range test (Cite author)

Results

Tables 1 to 4 represent values of microbial load analyzed after smoking processes had been carried out on the seven fish species studied. Table 1, showed the values of the microbial load of seven different fish species, obtained for the ITSK, after undergoing smoking processes. The values showed three groups of microbes identifiable. These were the Total Viable Count (TVC), Total Coli form Count (TCC) and Total Fungi Count (TFC). Among the seven fish species studied, *Clarias spp*had the highest occurrence of microbes (bacteria and fungi

i.e.,*Bacillus substilis, Klebisellapneuemonea, Streptococcus feacalis, Aspergillus Niger, Aspergillus flavus* and *Aspergillus mucor*). These values were significantly different (P>0.05)
*Bacillus substilis,Klebisellapneuemonea*and *Streptococcus feacalis* belong to the group TVC, while coli forming bacteria like*Klebisellapneuemonea* belong to the group TCC and lastly, *A. Niger, A.flavus*and *A. mucor* belong to group TFC. However, *Bagrusbayad, Latesniloticus and Heterotisniloticus*only had one type of microbe (bacteria i.e.,*Bacillus substilis*). There were no occurrences of microbe presence for the remaining three species of Fish (*Synodontisnigrita, Tilapia spp and Mormyrusrume*). This implies that *Clarias spp*is a type of fish that is capable of abhorring high prevalence of microbes.

Table 2 represents the values of the microbial load from Zumba-TSK. The values were for six different fish species. *Heterotisniloticus*had the highest value of $4.12x10^{8}$, while the lowest value was from *Tilapia spp* which had the lowest $1.8x10^{8}$for TVC, while TCC for *Clarias and mormyrusrume* both had the highest value of $1.35x10^{7c}$and*Bagrusbayad*had the least value of $0.53x\ 10^{4,}$ for the TFC value, *Bagrusbayad* had the highest value of $0.82x10^{3}$the least value came from *Heterotisniloticus*with a value $0.14x10^{3}$

Table 3 represents the values of microbial load from Gwada -TSK. Interestingly, Gwada site performed better off than the two other sites. In the sense, that it had the least values of microbial incidence among the fish samples studied. It could therefore be said that Gwada site performed next to the ITSK. A critical look at this table show that it was only *Synodontisnigrita*had occurrence of microbial load for TVC, TCC and TCF. However, the TVC value show that the highest value of $2.63x10^{8}$, while the lowest value was from *Tilapia spp*with a value of $0.73x10^{8}$. These values were significantly different from each other. These values were significantly different (P>0.05)

Table 4 showed the value of microbial load for the Kuta site. The value for TVC show that *Latesniloticus* had the highest value of $3.25x10^{8}$ while the least value was for *Tilapia spp* with a value of $1.20x10^{8}$. Value of TCC show that *Bagrusbayad*had value of $0.40x10^{4}$, while there was no values recorded for the other fishes except for *Clarias spp*that had $0.23x10^{4}$. Values for TFC show that *Latesniloticus*had the highest value of $0.25x10^{5}$ while *Clarias spp*had value of $0.23x10^{5}$, while the remaining fishes had no values. Finally, it was observed that there was an initial reduction inthe population of microbial load during and immediately after smoking; however there was subsequentlyincrease in population growth of microbes as the duration of storage of smoked fish increases. Table 5 show the characteristization of the bacteria isolates. This table revealed the family, species and genus that the bacteria isolate belong to.

Discussion

This research work was able to identify the bacteria isolates to be *Bacillus substilis, Streptococcus feacalis, klebsiella pnenumonea,* for the ITSK. It was observed that these bacteria were found on four species of fish, these were *Clarias spp, Bagrus bayad, Lates niloticus* and *Heterotis niloticus.* The predominate isolate was *B.substilis*. *A. Niger, A.flavus* and *A. mucor* fungi found was only on *Clarias spp*. Values of $0.46x10^{8}$ TVC, $0.25x10^{7}$ TCC, $0.48x10^{8}$ TFC was obtained for *Clarias spp*. whereas only values of 0.14- $0.17x10^{2}$ TVC were obtained for *B.bayad. L.niloticus* and *H. niloticus.*

Zumba site showed additional bacteria isolates of *B. megaterium, B.lichinformis, Micrococcus roseus* and *M. leteus* with values ranging from values of $1.81x10^{8}$- $4.12x10^{8}$ TVC, $0.53x10^{4}$- $1.35x10^{7}$ TCC and $0.14x10^{3}$- $0.29x10^{5}$TFC. Gwada site had similar bacteria isolates when compared with that of ITSK. The values obtained here ranged from $0.73x10^{8}$- $2.63x10^{8}$for TVC, while only *Synodontis nigrita* has values of both TCC and TFC as $0.5x10^{4}$and $0.11x10^{5}$ respectively. The Kuta site had microorganisms similar to that of Zumba site with

values ranging from $1.20x10^8$- $3.25x10^8$ TVC, $0.23x10^4$- $0.40x10^4$TCC and $0.23x10^5$ TFC.

This result is similar to the findings of Gram and Huss, (2000), that reported that the common spoilage bacteria of fish includes species of mesophilic gram-positive, micro flora consisting of Micrococcus, Bacillus and Coryneforms found in fresh water fish. Agbolagba *et al. (*2011) stated that bacteria *Staphylococcus aureus*, yeast *Saccharomyces cerevisiae* are the commonest microorganism associated with smoked fish capable of posing food borne illness to consumers. Fafioye *et al*. (2002) reported high incidence of *Aspergillus flavus,* while Adebayo-Tayo *et al.* (2008) was able to report aflotoxin poison in smoked fish. The micro-organism associated with smoked fish pose a great threat to the populace as the transfer of micro-organisms attack the immune system of the consumer. ICMSF (1996) recommended bacteria limit values for smoke - dried fish to be 10^3 and 10^4, while fungi limit should not exceed 10^2. Judging from this result, values obtained from this research work show that ITSK had values of $0.46x10^8$ TVC, $0.25x10^7$ TCC, and $0.48x10^8$TFC for *Clarias spp*only. Whereas only values of 0.14- $0.17x10^2$ TVC were obtained for *B.bayad.* Thisresult show that values obtained for *Clarias spp*were above the recommended limit, while values obtained. Values of $1.81x10^8$- $4.12x10^8$ TVC, $0.53x10^4$- $1.35x10^7$ TCC and $0.14x10^3$- $0.29x10^5$ TFC obtained for all the fishes from Zumba site were above the recommended limit. Gwada site values ranged from $0.73x10^8$- $2.63x10^8$ for TVC, while only *Synodontisnigrita*had values of both TCC and TFC as $0.5x10^4$ and $0.11x10^5$respectively; again these values were above the recommended limits.TheKuta site had values ranging from $1.20x10^8$- $3.25x10^8$ TVC, $0.23x10^4$- $0.40x10^4$ TCC and $0.23x10^5$ TFC. These values were above the recommended limit set by ICMSF. It could be possible re- contamination of microbes did occur during storage as a result of poor storage method.

Conclusion

Seven different species were subjected to smoking, using two different methods. That is the Traditional Smoking Kilns (TSK) and Improved Traditional Smoking Kilns (ITSK). Results obtained from the ITSK perform better than that of TSK (wherein fishes from three locations were used) in terms of microbial load, representing biological hazards.1. ITSK had the least microbial load except for *Clarias specie*. 2. It was observed that only four species of fish had bacteria isolates (Gwada and ITSK). 3.Additional bacteria isolates like B. *megaterium B. lichinforms, MicroccusRoseus*and *Microcusueteus*were found present on fishes from Kuta and Zumba. suggesting poor handling, poor smoking technique and re-contamination after smoking.

Recommendations

1. Micro organisms capable of causing serious health risk have been identified in this research work, this is attributed to the poor handling processing used during smoking procedures. If fish mongers can improve on these unhygienic methods of handling fish it will go a long way to reduce the presence of micro-organisms.
2. The primitive kiln used in smoking fish need to be improve upon as this result in the generation of too much soot and less moisture which give room for the presence of micro-organisms as compare to the improve traditional smoking kiln used in this experiment

Table 1. Microbial load values of fish smoked with ITSK.1

Fish Species	TVC (cfu/g)	SEM	TCC (cfu/g)	SEM	TFC	OrganismsPresent
Clarias spp	$0.46x10^{8a}$	±0.23	$0.25x10^{7c}$	±0.19	$0.48x10^{8a}$	*Bacillus substilis, klebisella,pneuemonea,streptococcus Feacalis,Aspergillusniger, Aspergillusflavus, Aspergillusmucor*
Bagrusbayad	$0.17x10^{2b}$	±0.18				*Bacillus substilis*
Latesniloticus	$0.14x10^{2b}$	±0.44				*Bacillus substilis*
Heterotis niloticus	$0.14x10^{2b}$	±0.33				*Bacillus substilis*
Synodontis nigrita						
Tilapia spp						
Mormyrus rume						

Key=
TVC= Total viable count
TCC= Total coli form count
TFC= Total fungal count
Sem= standard error of the Mean
x= fell below recommended ICMSF 1996 value for bacteria (10^3 and 10^4) and fungi (10^2)
xx= fell above the recommended ICMSF 1996 value for bacteria (10^3 and 10^4) and fungi (10^2)

Means in the same column carrying different superscripts are significantly different (p< 0.05)

Table 2: Microbial load values of fishes from Zumba location using TSK2

Fish Species	TVC (cfu/g)	SEM	TCC (cfu/g)	SEM	TFC	SEM	Organisms Present
Clarias spp	$3.89x10^{8c}$	±0.52	$1.35x10^{7c}$	±0.60	$0.29x10^{5c}$	±0.19	*Bacillussubstilis,Klebsiell pneumonia, E.coli, Micrococcus roseus, Bacillus lichniformis Aspergillusniger α Aitermiaspp*
Bagrus bayad	$2.56x10^{8b}$	±0.66	$0.53x10^{4a}$	±0.53	$0.82x10^{3b}$	±0.37	*Bacillus substilis, Bacillus lichinformis, Micrococcus roseus Streptococcus feacalisAspergillus flavus*
Lates niloticus substilis, Bacillus	$2.46x10^{8b}$	±0.67	_____	_____	_____	_____	*Bacillus Megaterium, Microccusroseus*
Heterotis niloticus	$4.12x10^{8d}$	±0.41	$1.2x10^{4b}$	±0.63	$0.14x10^{3a}$	±0.14	*Bacillus substilis,Klebsiella pneumonia, E.coli, Aspergillusniger, Aspergillus flavus,Staphyloccusaureus*
Tilapia spp substilis, Streptococcus,	$1.81x10^{8a}$	±0.60	_____	_____	_____	_____	*Bacillus feacalis, Micrococcus leteus*
Mormyrus rume	$2.62x10^{8b}$	±0.81	$1.35x10^{7c}$	±0.60	$0.29x10^{5c}$	±0.19	*Bacillus substilis,E.coli, Streptococcus feacalis, Staphylococcusaureus, Klebisella Pnuemonea A. flavus, A.niger*

Means in the same column carrying different superscripts are significantly different (p< 0.05)
x= fell below recommended ICMSF 1996 value for bacteria (10^3 and 10^4) and fungi (10^2), xx= fell above the recommended ICMSF 1996 value for bacteria (10^3 and 10^4) and fungi (10^2). TSK means Traditional Smoking Kiln

Table 3: Microbial load values of fishes smoked from Gwada location using TSK3

Fish Species Present	TVC (cfu/g)	SEM	TCC (cfu/g)	SEM	TFC (cfu/g)	SEM	Organisms
Clarias Spp	$2.44x10^{8a}$	±0.54	_____	_____	_____	_____	*Bacillus substilis, &Streptococcus feacalis.*
Bagrusbayad	$2.54x10^{8b}$	±0.50	_____	_____	_____	_____	*Bacillus substilis*
Lates niloticus	$1.75x10^{8c}$	±0.31	_____	_____	_____	_____	*Bacillus substilis*
Heterotis niloticus	$1.57x10^{8ab}$	±0.48	_____	_____	_____	_____	*Bacillus substilis,*
Synodontis nigrita	$2.63x10^{8ab}$	±0.22	$0.51x10^{4a}$	±0.40	$0.11x10^{5a}$	±0.1	*Bacillus substilis, Bacillus lichinformis, Staphylococcus aureaus, Aspergillus niger& Aspergillus flavus*
Tilapia Spp	$0.73x10^{8d}$	±0.18	_____	_____	_____	_____	*Bacillus substilis*
Mormyrus rume	$0.95x10^{8d}$	±0.47	_____	_____	_____	_____	*Bacillus substilis& Streptococcus feacalis*

Means in the same column carrying different superscripts are significantly different (p< 0.05)

x= fell below recommended ICMSF 1996 value for bacteria (10^3 and 10^4) and fungi (10^2), xx= fell above the recommended ICMSF 1996 value for bacteria (10^3 and 10^4) and fungi (10^2

Table 4. Microbial load values for fish smoked from Kuta location using TSK4

Fish Species	TVC (cfu/g)	SEM	TCC (cfu/g)	SEM	TFC (cfu/g)	SEM	Organisms Present
ClariasSpp	$2.70x10^{8e}$	±0.56	$0.23x10^{4a}$	±0.23	$0.23x10^{5a}$	±0.23	*Bacillus substilis, Bacillus lichinformis, Micrococcus roseus, Aspergillus flavus, klebsiella pneumonia*
Bagrus bayad	$2.55x10^{8e}$	±0.27	$0.40x10^{4b}$	±0.33	_____	_____	*Bacillus substilis, Bacillus lichinformis, Micrococcus roseus, Bacillus megaterium*
Lates niloticus	$3.25x10^{8f}$	±0.75	_____	_____	$0.25x10^{5a}$	±0.25	*Bacillus substilis, Streptococcus feacalis Aspergillus flavus*
Heterotis niloticus	$2.05x10^{8d}$	±0.20	_____	_____	_____	___	*Bacillus substilis,*
Synodontis nigrita	*Streptococcus feacalis& micrococcus roseus* $1.83x10^{8c}$	±0.56	_____	_____	_____	_____	*Bacillus substilis,*
Tilapia spp	$1.20x10^{8a}$	±0.33	_____	_____	_____	___	*Bacillus substilis, Streptococcus feacalis, Micrococcus leteus*
Mormyrus rume	$1.55x10^{8b}$	_____	_____	_____	_____	_____	*Bacillus substilis, E. coli, &Streptococcus,feacalis.*

Means in the same column carrying the different superscript are significantly different (p< 0.05)

x= fell below recommended ICMSF 1996 value for bacteria (10^3 and 10^4) and fungi (10^2), xx= fell above the recommended ICMSF 1996 value for bacteria (10^3 and 10^4) and fungi (10^2

Table 5: Morphology and Biochemical characteristics of Bacteria Isolates from commercially smoked fish species of Shiroro Lake5

Bacteria Conolonies	MAC ONNA	G.Stain	Cat. Test	Coag.test	Indo. Test	H_2S	MF	VP CIT
Bacillus substilis	Dull White mucoid rod in color incluster	+ve +ve	-ve	-ve -ve	-ve -ve +ve			
Bacillus megaterium	Whitish like beadsin long non mucoid chain	+Rod	+ve -ve	-ve	-ve -ve +ve	-ve		
Bacillus Lichiniformis	Whitish *big*nonsome in mucoid cluster Coloniessome in chain	+ve rod	+ve -ve	-ve -ve -ve	-ve +ve			
Streptococci faecalis	Whitish tiny discrete Colonies pattern	+ve	-ve -ve	-ve	-ve -ve -ve			
Staphylococcus auerus	whitish tiny discretecocci Colonies in long chain	+ve	+ve +ve	____	____			
Micrococcus Roseus	yellowish ting non cocci Mucoid in pair Discrete colonies	+ve	-ve -ve	-ve	-ve -ve -ve	-ve		
Micrococcus luteus	pinkish tiny discrete in cluster nonmucoid colonies	-ve Rod	-ve -ve	-ve	+ve -ve -ve			

Key =+ve = Positive (there is reaction) -ve = Negative (no reaction) G.stain = Gram stain Cat. Test = Catalyst Test
Coag. Test=Coagulate Test H_2s =Hydrogen sulphide production Mac on NA = macro culture on Nutrient Agar (i.e. colonial morphology characteristic) MR = Methyl Red Test VP = Vogasprausker Test CIT = Citrate Utilization Test
Indo. Test= Indole Test +ve rod = Gram positive rod -ve rod = Gram negative rod +cocci = Gram positive cocci
Clusters = scattered Chain = join together Pair = two join together

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