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Assessment of the extent of microbial load of smoked dried African catfish (*Clarias gariepinus*) sold in Maiduguri markets

Garba AA, J Ochogwu, BI Usman and MO Adadu

Abstract
This study was carried out to determine the bacterial and fungal load of smoke-dried *Clarias gariepinus* sold in Maiduguri markets and to isolate and characterize the microbes. A total of forty five (45) Smoke-dried African catfish (*C. gariepinus*) were randomly purchased from the five different Markets (Muna, Gamboru, Bama road, Baga road and Abbaganaram) in Maiduguri, Borno State, Nigeria. Nine (9) samples were collected randomly between September 2020 and November, 2020 from each market on three sampling occasions and wrapped aseptically.
Samples were transported from Maiduguri to the microbiology Laboratory, Department of food Science Technology of the University of Agriculture, Makurdi in sterile polythene bags for microbial analysis. Portions of the skin surface, gills and tissue of all the samples were cut and 1g of each sample was weighed out and homogenized in sterile test tube containing 9ml of distilled water which became the stock solution (1:10 dilution). Serial dilutions were then made up to 10^{-9} and 10ml. The result of this study showed that pathogenic bacteria and fungi are present in smoked *C. gariepinus* sold in Maiduguri markets. The study also revealed that a total of eight (8) types of microbes [three (3) species of bacteria and five (5) species of fungi] were isolated from the samples of smoked *C. gariepinus.* The bacteria observed include, *Eschericia coli, Staphylococcus aureus* and *Salmonella spp.* The fungi present in the fish samples were *Fusarium spp., Mucor spp., Aspergillus fumigatus, Aspergillus niger* and *yeast*. Majority of the fungal isolates identified in this study produces mycotoxins. The presence of fungi particularly aflatoxigenic molds in these fish species is very significant as it was indicated by food safety standard that aflatoxigenic molds produce mycotoxins which have pathogenic effects on man. The result of this study shows that, Baga road market had highest microbial contamination ($1.567 \times 10^3 \pm 0.243 \times 10^3$ cfu/g), and Gamboru market recorded the lowest, $0.806 \times 10^3 \pm 0.131 \times 10^3$ cfu/g. However, there was no significant difference in terms of microbial load between Gamboru market and Abbaganaram market ($0.806 \times 10^3 \pm 0.131 \times 10^3$ cfu/g and $0.841 \times 10^3 \pm 0.105 \times 10^3$cfu/g respectively). Reverse was the case in Gamboru and Abbaganaram markets whose environments were at least the most hygienic of all the markets under study and this translates to their relatively low microbial load. In conclusion, microbial contamination or re-contamination of smoked catfish products has been seen to vary from one locality (market) to another and even within the same locality from one fish processor or seller to another. To enhance consumers' health, the use of mechanized smoking system that would completely dehydrate the fish in order to prevent contamination due to moisture is recommended. Also authorities such as National Agency for Food and Drugs Administration and Control (NAFDAC) should look into the environmental condition of our food handlers as it concerns the smoking factories, the markets and even the hawkers that carry the food from one place to another.

Keywords: microbial load, Maiduguri markets, *C. gariepinus,* smoked fish and processing

Introduction

Fish as an important dietary component of people around the world represents a relatively cheap and accessible source of high quality protein for poorer households (Ikutegbe and Sikoki, 2014) [23]. In West Africa, fish has been reported to provide 40-70% of the protein intake of the population (Bene and Heck, 2005; Ikutegbe and Sikoki, 2014) [12, 23] and is a crucial source of dietary protein that is not readily available in the carbohydrates based staple foods of the population. Depending on consumers' preference, there are several forms in which fish can be consumed. These can either be fresh, dried, fermented, brined etc.
Mafimibesi (2012) [24] reported that, majority of Nigerian people prefer fresh fish; however, limitations such as low keeping quality of the fish after harvest and distances between fishing

grounds and marketing outlets make this very difficult. This results in a higher reported consumption of smoked-dried fish, which has a longer shelf-life.

Smoked fish and shellfish products can be a source of microbial hazards including *Listeria monocytogenes, Salmonella* species, *Clostridium botulinum, Staphylococcus aureus,* and *Escherichia coli. According* Gram (2001) [20], these organisms have also been isolated from a variety of fish and shellfish products. Studies have shown that *L. monocytogenes* can significantly increase in numbers on smoked salmon during storage at 4ºC. Regulatory agencies in the United States have adopted a zero-tolerance policy toward this organism in ready-to-eat food products. *L. monocytogenes is* a non-spore forming, psychrotrophic bacterium that causes the disease, listeriosis. In humans, the primary manifestations of listeriosis are meningitis, abortion and pre-natal septicemia (Dillon *et al.,* 1994) [15]. Yusuf and Tengku Abdul Hamid (2012) [35] reported that the presence of *Salmonella spp.* indicates poor food preparation and handling practices and it is associated with food borne diseases. *E. Coli* is an enteric bacteria causing gastroenteritis and the contamination of fish or fish products with pathogenic *E. coli* is associated with improper handling of fish (Ayulo *et al.,* 1994; Asai *et al.,* 1999) [11, 8] and fecal contamination (Feng, 2002) [19]. *Staphylococci aureus* are capable of producing enterotoxins that cause gastroenteritis (Novotny *et. al.,* 2004) [26]. Thus, food poisoning and food borne diseases could occur as a result of intake of smoked fish.

Smoking reduced the total viable count significantly in all samples, but when the smoked products are constantly exposed to the effect of humid environment, the possibility for an increase in the moisture content of the smoke-dried product is inevitable thereby enhancing the activity/proliferation of these microorganisms. Eyo (2001) [16] stated that smoked fish samples may have a relatively high water activity level which is a prerequisite for microbial growth.

Fish spoilage is a complex process involving both non-microbiological and microbiological processes. Non-microbiological deterioration is caused by endogenous proteolytic enzymes, which are concentrated in the head and viscera and attack these organs and surrounding tissues after death. Enzymatic spoilage is followed by the growth of microorganisms, which invade the fish flesh, causing breakdown of tissues and a general deterioration of the product. According to Nyarko, *et al.* (2011) [27], smoked sardine from marketing centers had higher microbial counts than those from smoking sites due to the better sanitary conditions of the latter. Adegunwa, *et al.* (2013) [5] reported that smoked fish collected at a camp location in Odeda, Ogun State, Nigeria had higher microbial load than other locations as a result of handling, frequent exposure, poor environmental and sanitary conditions. The disparities in contamination levels between locations have been observed to be influenced by one or more of the factors enumerated by Tatcher and Clark (1973) [32] as follows: Source of the raw fish; Temperature of food during storage and processing; Severity of freezing process in terms of lethality to microorganisms; Additional contamination introduced by handlers; Contamination after the fish had already been processed.

Smoked fish is relished food item in many dishes in Nigeria; therefore, there is need for corresponding concern for safety issues in fish consumption (Riches, 2012) [31].

Bacterial pathogens, which may be transferred from fish to human beings include: *A. hydrophila* (septicemia, diarrhea), *Campylobacter jejuni* (gastro-enteritis), *Clostridiumbotulinum* type E (botulism*), Eduardsiella tarda* (diarrhea), *Erysipelothrix rhusiopathiae* (fish rose), *Leptospira interrogans* (leptospirosis), *Mycobacterium fortuitom/ marinum* (mycobacteriosis), *Plesiomonas shigelloides* (gastroenteritis), *Pseudomonas aeruginosa* (wound infections), *P. fluorescens* (wound infections), *Salmonella spp.*. (food poisoning), and *Vibrio parahaemolyticus* (food poisoning) (Austin and Austin, 1989) [9]. The main pathogens associated with contaminated fish are *Clostridium botulinum*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella spp.*., and *Vibrio spp.*. (Faber, 1991) [17]. These enteric pathogens such as *E. coli* are capable of causing intestinal disease (Cheah *et al.,* 2015) [13]. Odu and Imaku (2013) [28] enumerated diseases caused by *E. coli* to include diarrhoeal illness, urinary tract infections, meningitis, sepsis, wound infections, nosoco-mial pneumonia and dysentery. *S. aureus* is resistant to heat, drying and radiation and produces toxin which are not destroyed by heat (Odu and Imaku, 2013) [28]. The toxins and enzymes produced by *S. aureus* could increase the severity of certain diseases such as food poisoning, septic shock, and toxic shock syndrome

Daniel *et al.* (2013) [14] reported microbial diversity of smoked fish sold in Benin City, Nigeria to include *S. aureus, Pseudomonas, Streptococcus, Bacillus,* (Bacteria) and Yeast*, Aspergillus niger* and *Penicillium* spp. (fungi). Adebayo-Tayo *et al.* (2008) [4] reported *Aspergillus flavus, Aspergillus tereus, Aspergillus fumigatus, Absidia* spp., *Rhizopus* spp., *A. niger, Mucor* spp., *Cladosporum* spp., *Penicillium italicum, Penicilium viridatus, Candida tropical* and *Fusarium moniliformis* as fungal isolates found in smoked dried fishes sold in different markets in Uyo, Nigeria. Akise *et al.* (2013) [7] reported predominant fungi species isolated from three different anatomical parts of smoke-dried fish under storage to include *P. italicum*, *Penicillium oxalicum*, *Mucor, Saccharomyces, Rhodotorula* spp. and *Aspergillus* spp. Majority of the fungi produce mycotoxins. Mycotoxins produced by *Aspegillus* species are known to produce many types of toxins such as aflatoxins, ochratoxins and sterigmato-cystine (Hashem, 2011) [21]. Acute aflatoxicosis in humans has been reported in different parts of the world and was characterized by symptoms like vomiting, abdominal pain, pulmonary oedema, convulsion, coma, and death with cerebral oedema and fatty involvement of the liver, kidney, and heart (Akinyemi *et al.,* 2011) [6].

Fafioye *et al.* (2002) [18] studied the fungal infestation of five traditionally smoked dried freshwater fish in Ago-Iwoye, Nigeria and isolated and identified eleven different fungal species of which *Aspergillus flavus* was the most frequently encountered fungus on the fish species. Adebayo-Tayo *et al.* (2008) [4] reported the presence of aflatoxins and other metabolites in smoked fish due to *A. flavus* in smoked fish sold in Uyo, Akwa Ibom state, Nigeria and confirmed that consumers could have been at risk of aflatoxin poison.

According to Aberoumand (2010) [1], *Escherichia coli* are a classic example of enteric bacterial causing gastroenteritis*. E. coli* including other coliforms and bacteria such as *Staphylococcus spp.* and sometimes *enteroccoci* are commonly used as indices of hazardous conditions during processing of fish. *E. coli and Staphylococcus aureus* were reported as the predominant microorganisms present in smoked fish in Asaba area of Delta state, Nigeria (Okonta and Ekelemu, 2005) [29]. Outbreak of listerosis in different parts of the world in the last three decades as a result of eating smoked

fish has been a major public health concern. Due to the fact that fish are smoked and retailed openly in the markets, it is important to assess the quality of the product in order to protect consumers' health. In view of this, this study was therefore embarked upon to assess the microbial load and to isolate and characterize the microbes associated with smoked *C. gariepinus in* Maiduguri markets.

Materials and Methods

Description of study area

Maiduguri, the Borno State capital lies between latitude $11^{0}15'$ North and longitude $13^{o}15'$East. The state occupies a land area of 70,898 Km^2 making it the second largest state in Nigeria, next to Niger State (Adadu and Ochogwu, 2020) [3]. The sahelial climate is characterized with their distinct seasons: wet and dry. The dry hot season starts from March to June with attendant temperature ranging from $27^{0}C$ to $40^{0}C$ making it the driest period with intense heat (CBDA). It is an urban settlement with many markets within the metropolis where food items are sold such as smoked fish, meat, frozen fish etc. under different conditions, environment, packaging and quality control.
The major markets in Maiduguri include Mu na market, Gamboru market, Bama road market, Baga road market and Abbaganaram market among others located in strategic and different parts of the town, hence their selection for this study.

Sample collection and preparation

A total of forty five (45) Smoke-dried African catfish (*C. gariepinus*) were randomly purchased from the five different Markets (Muna, Gamboru, Bama road, Baga road and Abbaganaram) in Maiduguri. Nine (9) samples were collected randomly between September 2020 and November, 2020 from each market on three sampling occasions and wrapped aseptically.
Samples were transported from Maiduguri to the microbiology Laboratory, Department of food Science Technology of the University of Agriculture, Makurdi in sterile polythene bags for microbial analysis. Portions of the skin surface, gills and tissue of all the samples were cut and 1g of each sample was weighed out and homogenized in sterile test tube containing 9ml of distilled water which became the stock solution (1:10 dilution). Serial dilutions were then made up to 10^{-9} and 10ml.

Media used

The media used included: Nutrient Agar (NA), which is suitable for the growth of most bacteria and was used for routine work in culturing microorganisms; Manitol Salt Agar (MSA) was used for *Staphylococcus aureus;* Eosin Methylene Blue Agar (EMBA) was for *E. coli*; Potatoes Dextrose Agar (PDA) was used for Mycological analysis; Mackonkey Agar (MCA) was used to enhance the growth of Gram-negative organisms and as a multipurpose agar; *Salmonella Shiggella* Agar (SSA) was used for culturing *Salmonella* and *Shigella* species. All media were prepared according to manufacturer's specifications.

Preparation of Nutrient agar (NA)

The Nutrient Agar was prepared by dissolving 7g of NA in 250ml of distilled water. The medium was autoclaved at $121^{O}C$ for 15 minutes and the pour plate method was ascetically carried out. The plate was allowed to gel and packed in incubator for sterility control.

Preparation of Manitol salt agar (MSA)

Twenty eight grams (28g) of Manitol Salt Agar (MSA) was dissolved in 250ml of distilled water. It was autoclaved at 121 ^{0}C for 15 minutes and the pour plate method was aseptically carried out. The plate was allowed to gel and was packed in incubator for 24 hours for sterility control.

Eosin methylene blue agar (EMBA)

Nine grams (9g) of EMBA was dissolved in distilled water and autoclaved at $121^{0}C$ for 15 minutes, pour plates method was conducted aseptically and the gelled plates were incubated for 24 hours for sterility control.

Preparation of Potato dextrose agar (PDA)

Seventeen grams (17g) of PDA was measured and dissolved in 250ml of distilled water and autoclaved at 121^{0} C for 15 minutes, 40ml of streptomycin was added to suppress the growth of bacteria. It was then removed and allowed to cool before the pour plate method was conducted aseptically and the gelled plate was incubated at room temperature for 5 days.

Inoculation of media plates

Pour-plate method was adopted for inoculation of all media plates. A loopful of each stock solution was picked with a well flamed wire loop and inoculated on each of the media plates by streaking across the surface of the media plates. The inoculums were spread by swirling the plate gently. The plates were then subjected to 24 hour incubation at $37^{o}C$ after which all plates were read. Each discrete colony observed was picked and streaked on fresh media plates to obtain pure cultures. All plates and test tubes used were properly labeled according to dilution, agar used and Market from which the sample was obtained. Bacterial growths from the plates were subjected to Gram staining test, motility test and Biochemical (Coagulate, Catalase, Citrate utilization test and Indole) tests, to confirm their identity. The total viable bacteria count of each sample was estimated using the method described by Collin and Lyne (1970). The mould count method of Harrigan and Maccance (1976) was adopted in enumerating yeast and mould.

Microscopy/biochemical test

Gram staining and motility test of all bacteria isolated in pure culture were made. Then the preparations were viewed under microscope. Mucor and yeast were also viewed.
The biochemical tests were carried out on the isolated organisms based on their ability to produce enzymes or gas.

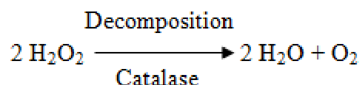
Coagulase test

Two drops of physiological saline solution were put on a clean grease-free microscope slide about 2cm apart; one colony was carefully emulsified in each drop of the saline.
A loopful of the attracted human plasma was added to the bacterial suspension on one slide and mixed, the slide was held and tilted for one minute. Clumping of cells under the coagulase positive in the bacterial suspension mixed with plasma, the other slide served as the control. This test was employed to differentiate *Staphylococcus aureus* from other members of the genus staphylococcus. If there is coagulation in both, then the organism is auto-coagulating, hence identity of the microbe cannot be made by coagulase test.

Catalase test

A single colony was picked with a sterile wire loop and emulsified in a drop of normal saline on a clean microscope slide and a drop of hydrogen peroxide was added to the smear. Catalase production test on the mixture with 0.5ml of 3% hydrogen peroxide (H_2O_2) shows an immediate bubbling

and frothing. Absence of bubble shows the culture is catalase negative.



This test was used to differentiate members of the genus *Streptococcus* from that of the genus *Staphylococcus*.

Citrate utilization test
Simon's citrate agar slant was inoculated in a bijou bottle and incubated for 24 hours at 37 ºC. The development of a deep yellow colour indicates a positive reaction. This test was used to differentiate the members of the genus *klebsiellae* from members of the genus *Escherichia*, which are distinctly negative.

Indole test
Each bacteria isolate was grown (sub-cultured) in 5ml of peptone water for 24 hours at 35 ºC and the mixture was shaken gently, a red color development above the reagent layer within 1minute indicated a positive result.

Selective isolation of *Salmonella* and *Shiggella* spp.
Doxycholate citrate agar (DCA) hynes modification as recommended by Collins and lyne (1970) was utilized for isolation of *Salmonella* species and *Shiggella* species by means of transfer loop, a portion of the mixed bacteria colonies on nutrient agar plates was inoculated on DCA by streaking across the surface. Plates were incubated at 35ºC for 24 hours. Salmonella colonies present on DCA medium appeared creamy brown with black centre while *Shiggella* appeared slightly pink and do not have black centre.

Formulae used for calculation of counts
The method described by Collins and Lyne (1970) for estimation of microbial number was used to enumerate the total viable count of the samples.
Countable plates showing 20-100 colonies were selected and counted. The mean of the count of the duplicate on each given dilution was used to estimate the total viable count for the sample in the colony forming units per gram (cfu/g).

Calculation of colony forming unit per gram
Let the dilution factor be (10^{-a}-10^{-9})
Let the average number of colonies per dilution be = C. Total viable count per gram = dilution factor X colonies counted = 1/D X C = C/D (Cfu/g^{-1}).

Calculation of average sum of means (cfu/g^{-1})
Let the arithmetic mean of the set of Cfu/g value N be denoted by X and it is defined as X = ∑×/N where ∑× = Sum of the mean of the total (Cfu/g^{-1}) of the entire individual sample, N = Number of sample tested.
Total bacteria count was reported (visible counts and total counts), colony forming units per gram (cfu/g^{-1}) and calculated as total microbial counts (V) = N/VXD where V = viable count (cfu/g^{-1}), N = Number of colonies, V = Volume transferred into petri dishes, D = Dilution factor.

Statistical analyses
The data collected were subjected to statistical analyses (ANOVA).

Results
Results of analysis of bacteria isolates in samples from the five markets in Maiduguri are as shown in Table 1. This study shows that pathogenic bacteria and fungi are present in smoke-dried *Clarias gariepinus* sold in Maiduguri markets. Samples from Baga road market recorded the highest mean total viable count (1.567 x 10^3 ± 0.243 x 10^3 cfug-1), while samples from Gamboru market recorded the least (0.806 x 10^3 ± 0.131 x 10^3 cfug-1), this was closely followed by samples from Abbaganaram market (0.841 X 10^3 ± 0.105 x 10^3 cfug-1) although, the difference was not statistically significant. A total of three species of bacteria (*Staphylococcus aureaus, Salmonella* spp. and *Escherichia coli)* were isolated. There was no significant difference (p>0.05) in the plate count of *S. aureus* and *E. coli* between locations. However, a significant difference existed in plate count of *Salmonella* spp., with Muna market recording the highest (1.021 x 10^3 ± 0.215 x 10^3) and Abbaganaram market recording the lowest (0.249 x 10^3 ± 0.150 x 10^3).

Table 1: Analysis of bacteria isolates of smoke-dried *Clarias gariepinus* from the five markets

Isolates/Location	*S. aureus* (Cfu/g)	*Salmonella* spp. (Cfu/g)	*E. coli* (Cfu/g)	Mean total viable count (cfu/g)
Gamboru	0.881 x 103 ± 0.090 x 103b	0.865 x 103 ± 0.578 x 103a	0.681 x 103 ± 0.195 x 103c	0.806 x 103 ± 0.131x 103
Abbaganaram	1.115 x 103 ± 0.134 x 103b	0.249 x 103 ± 0.150 x 103b	0.701 x 103 ± 0.167 x 103c	0.841 x 103 ± 0.105 x 103
Bama Road	0.861 x 103 ± 0.220 x 103b	0.701 x 103 ± 0.177 x 103a	1.379 x 103 ± 0.177 x 103b	1.001 x 103 ± 0.131x 103
Baga Road	1.813 x 103 ± 0.354 x 103a	0.876 x 103 ± 0.225 x 103a	1.774 x 103 ± 0.576 x 103a	1.567 x 103 ± 0.243 x 103
Muna	1.161 X 103 ± 0.331 x 103c	1.021 X 103 ± 0.215 x 103a	1.530 X 103 ± 0.220 x 103a	1.269 X 103 ± 0.148 x 103

Mean values in the same column with the same superscript are not significantly different (p>0.05)

Results of the Biochemical Characteristics of the bacterial Isolates are presented in Table 2. All the bacteria species isolated were catalase positive and none of the isolates was oxidase positive. Coagulase test was only positive for *Staphylococcus aureus,* while only *E. coli* indicated indole positive.

Table 2: The biochemical characteristics of the bacterial isolates of smoke-dried African catfish *(Clarias gariepinus)*

S/No.	Isolates	Oxidase test	Catalase test	Coagulase test	Indole test
1	*S. aureus*	-	+	+	-
2	*Escherichia coli*	-	+	-	-
3	*Salmonella* Spp.	-	+	-	+

+ = Positive, - = Negative

Results of Morphological and Cultural Characteristics of the bacterial Isolates are presented in Table 3. Two gram negative rod shaped bacteria species and one gram positive *Coccus* bacteria species were isolated. The gram positive *Coccus* bacteria isolated was non-motile while all the gram negative bacteria were motile. Each bacteria species showed different growth characteristics.

Table 3: Morphological and chemical characteristics of the bacteria isolates of smoke-dried African catfish *(Clarias gariepinus)*

S No.	Isolate	Gram reaction	Shape	Culture medium	Description
1	*Escherichia coli*	-	Straight rod	EMBA	It showed blue-black by transmitting light and having metallic sheen incident light.
2	*Staphylococcus*		Clustered	MSA	Deep yellow about 0.75 mm aureus + cocci diameter. Colonies of having uniform colouration
3	*Salmonella* spp.	-	Straight rod	SSA	Creamy, 2-3 mm brown in diameter at 24 hours, with black or brown center

- = Gram negative, + = Gram positive

Table 4 Shows percentage frequency of bacteria isolated from the five (5) markets sampled. A total of three bacteria in seventy nine (79) occurrences were recorded.

Table 4: Percentage frequency of bacteria isolates of smoked-dried African folk-tailed catfish *(Chrysichthys nigrodigitatus)* from the five (5) markets

S. No.	Isolates	Frequency	Percentage occurrence
1	*S. aureus*	29	36.71
2	*E. coli*	27	34.18
3	*Salmonella* spp.	23	29.11
∑F = 79			

∑F = Sum of frequency

Results of mycological analysis are presented in Table 5. This Shows Mycological Analysis using Potatoes Dextrose Agar (PDA). A total of five (5) species of fungi were isolated. All the fungi isolated from the various markets had different growth characteristics and shapes. *Mucor* was isolated from Gamboru market, *Aspergillus fumigatus* was isolated from Abbaganaram market, *Fusarium* spp. was isolated from Bama road market, *Aspergillus niger* was isolated from Baga road market, yeast was found in samples from Muna market.

Table 5: Mycological analysis using potato dextrose agar (PDA)

Sampling sites	Pathogen isolates	Growth rate	Morphological appearance	Microscopic appearance in lacto phenol cotton blue preparation
Gamboru Market	*Mucor*	3-4 days (Slow)	Yellow-white fluffy with brown reverse sides	Hyphae without rhizoids, large globose sporaniphore
Abbaganaram Market	*Aspergillus fumigatus*	2-5 days (Slow)	Creamy yellow filamentous colonies	Large/globose conidiphore, loose columna with biserited hypha
Bama road Market	*Fusarium* Spp.	2-3 days (Fast)	Whitish cotton aerial	Enlongated ovoid curved microconidia
Baga road Market	*Aspergillus niger*	1-2 days (Fast)	White to yellow but later turns distinct black as colony develops	Large conidiosphore with 2 series of sterigmata over its entire surface, Brown to black conidia and rough walled spores, black and green
Muna Market	Yeast	3-5 days (Slow)	Creamy white	B-polar budding cells with lemon-shaped mother tips

Results of mean water activity are presented in Table 6. This shows the Mean water activity (aw) of all samples used, ranging from 43.076 ± 0.292 (Baga road market) to 40.487 ± 0.294 (Abbaganaram market). The difference between mean water activity of the samples were not statistically significant.

Table 6: Mean water activity (a_w) of fish from the five markets

Market location	Water activity
Gamboru	41.310 ± 0.497
Abbaganaram	40.487 ± 0.294
Bama road	41.310 ± 0.547
Baga road	43.076 ± 0.292
Muna	42.112 ± 0.350

Values were not significantly different (p>0.05)

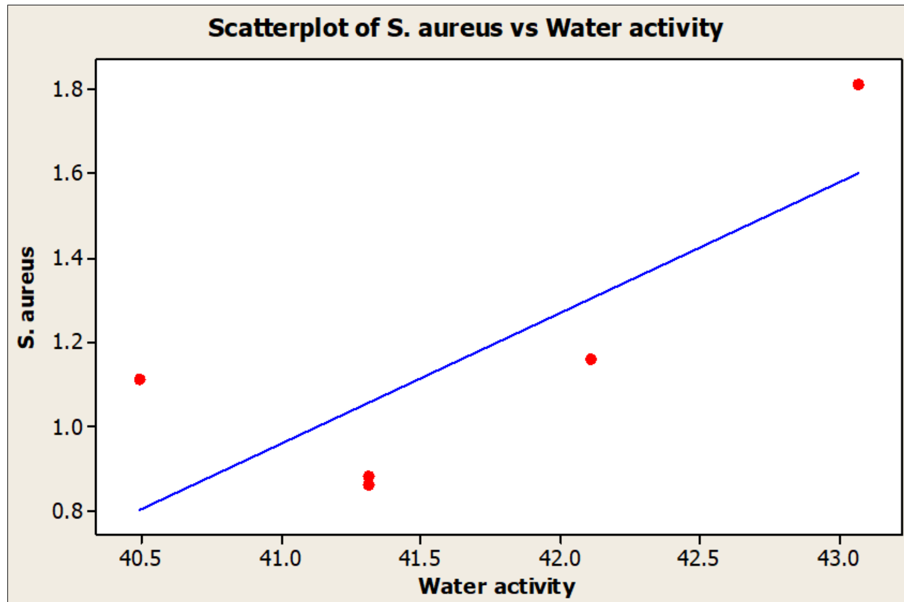


Fig 1: Linear relationship between *S. aureus* and water activity

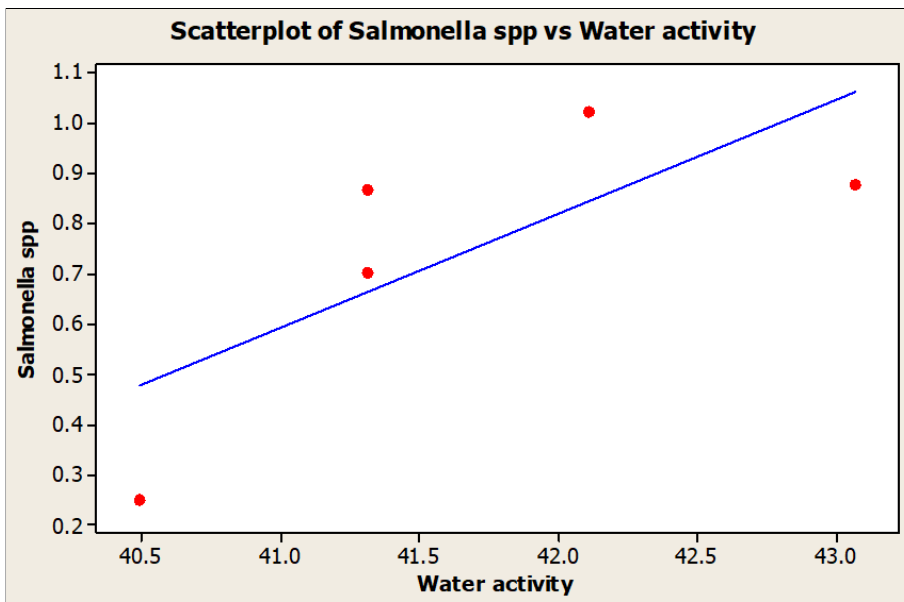


Fig 2: Linear relationship between *Salmonella* spp. and water activity

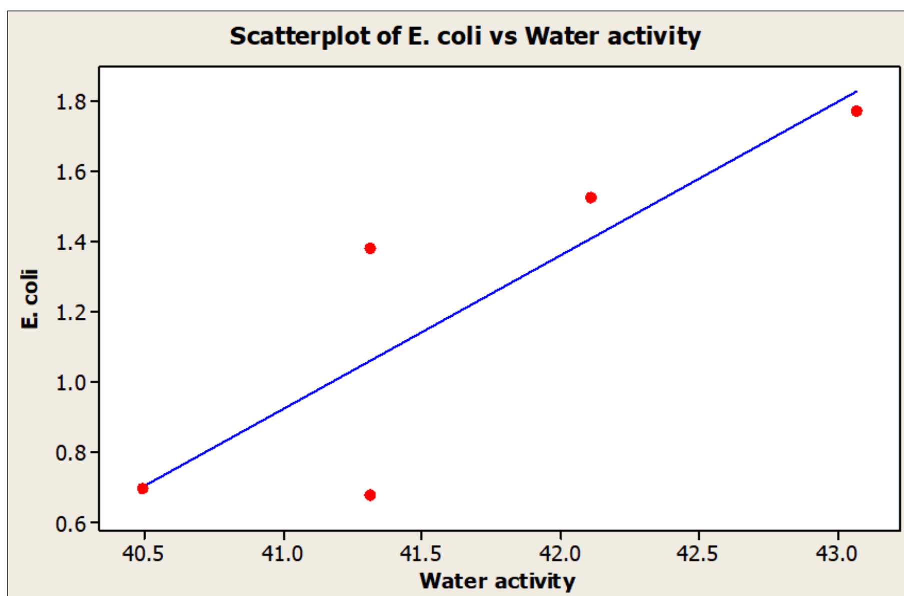


Fig 3: Linear relationship between *E. coli* and water activity

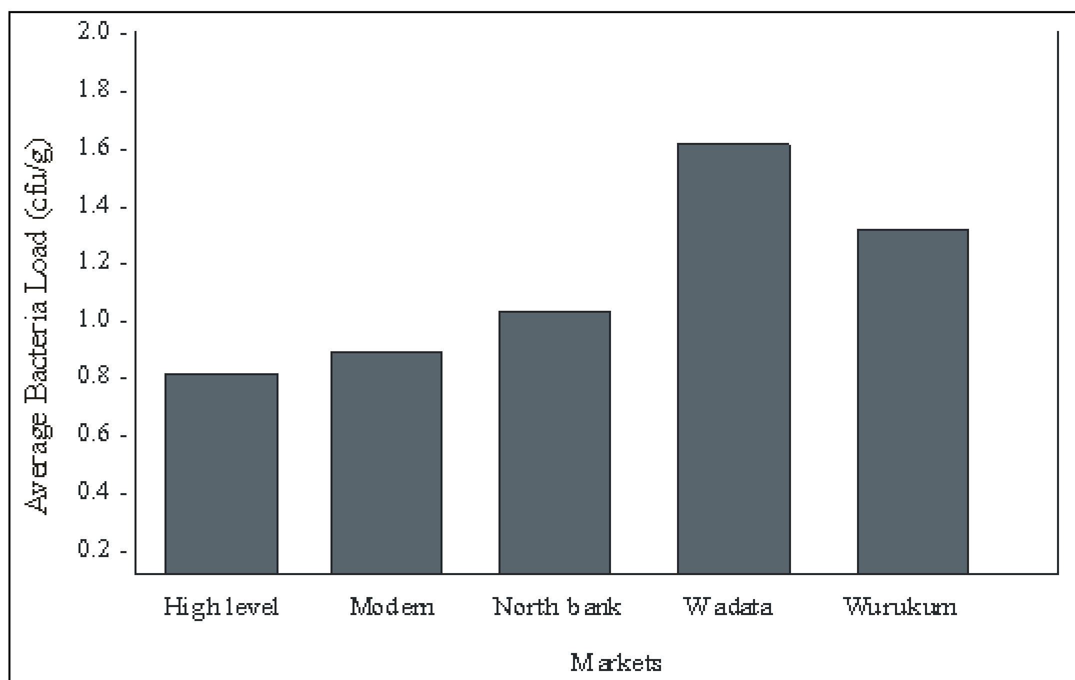


Fig 4: Average bacteria load of smoked dried *C. gariepinus* sold in five markets in Maiduguri

Discussion

This study showed that pathogenic bacteria and fungi are present in smoked *C. gariepinus* sold in Maiduguri markets. The study also revealed that a total of eight (8) types of microbes [three (3) species of bacteria and five (5) species of fungi] were isolated from the forty five (45) samples of smoked *C. gariepinus*. The bacteria present in the fish samples include, *Eschericia coli, Staphylococcus aureus* and *Salmonella spp.* This is in agreement with the findings of Ayuba *et al.* (2013) [10] who reported the presence of *Escherishia coli, Salmonella* spp. and *Staphylococcus aureus* in smoke-dried sardine in makurdi markets. The bacteria: *Staphylococcus aureus and Eschericia coli* in the smoked-dried fish samples are the commonest bacteria found in the smoked fish samples. This is in agreement with Martin (1994) [25] who also stated that these organisms were the commonest micro-organisms associated with smoked fish. The bacteria group of *Staphylococcus aureus* isolated from this study is one of the most common causes of human disease and they constitute the normal flora of the human skin and mucous membrane without resulting in a diseased condition. This is in agreement with Ayuba *et al.* (2013) [10] who stated that, *Staphylococcus aureus* was one of the most common causes of human diseases. This bacteria class may also cause superficial and systemic infections such as boils, impetigo and folliculitis while more serious and more common infections could be pneumonia, bacteremia and other infections of the bones and wounds. Odu and Imaku, (2013) [28] also reported that, *S. aureus* is resistant to heat, drying and radiation and produces toxin which could not be destroyed by heat. The toxins and enzymes produced by *S. aureus* could increase the severity of certain diseases such as food poisoning, septic shock, and toxic shock syndrome. Also, the intensity of the disease symptoms produced may depend on the amount of contaminated food ingested and susceptibility of the individuals to the toxin. *Escherichia coli* also, usually cause diarrhea and kidney damage as well as uncomplicated community acquired urinary tract infections (Odu and Imaku, 2013) [28].

The fungi present in the fish samples were *Fusarium spp., Mucor spp., Aspergillus fumigatus, Aspergillus niger* and *yeast*. Majority of the fungal isolates identified in this study produce mycotoxins. Mycotoxins by *Aspegillus* species are known to produce many types of toxins such as aflatoxins, ochratoxins and sterigmato-cystine. This agrees with the report of Hashem (2011) [21]. Acute aflatoxicosis in humans has been reported in different part of the world and is characterized by symptoms like vomiting, abdominal pain, pulmonary oedema, convulsions, coma, and death with cerebral oedema and fatty involvement of the liver, kidney, and heart (Akinyemi *et al.*, 2011) [6]. Most of the fungal species isolated such as, *Fusarium* and *Aspergillus* spp. are known to produce mycotoxins in food products especially carbohydrate. Mycotoxins are secondary metabolites produced by microfungi that could cause disease condition. Aflatoxin can occur in diversity of protein sources including plants and animals (Adebayo-Tayo *et al.*, 2008) [4]. *A. flavus* produces aflatoxin that contaminate not just smoked fish but also plants such as cereal grains and legumes such as peanuts, corn, and rice. Aflatoxin in food affects its nutritional quality. This conforms to the findings of Pratiwi *et al.* (2015) [30], who reported that aflatoxins in food materials affects the nutritional quality as well as its safety. In line with this, a study on the mycoflora of smoked fish species of *Ethmalosafimbriata* (Bonga fish), *Tilapia* sp. (Banda mangala), *Gadus morhua* (stock fish), *Pseudotolithus typhus* (croak*er), Ariushendeloti* (cat fish) and *Drepane Africana* (spade fish) revealed the presence *of Aspergillus niger, A. flavus, Penicillium* sp, *Fusarium* sp. *Rhizopus* sp. and *Trichoderma* sp. in their order of decreasing frequency in all the fish samples (Wogu and Iyayi, 2011) [34].

It was observed in this study that the presence of fungi particularly aflatoxigenic molds in these fish species is very significant as it was indicated by food safety standard that aflatoxigenic molds produce mycotoxins which have pathogenic effects on man; it destroys the liver and kidney resulting to death. The presence of the organisms could be as a result of poor handling during smoking and also cross contamination during storage (after smoking) and handling during sales of smoked fish. These reports from various

authors indicate that these microbial contaminants are not peculiar only to this fish species under study, as contamination by bacteria and fungi cuts across various smoked fish species.

The result of this study shows that, Baga road market had highest microbial contamination ($1.567 \times 10^3 \pm 0.243 \times 10^3$ cfu/g), while Gamboru market recorded the lowest, $0.806 \times 10^3 \pm 0.131 \times 10^3$ cfu/g. However, it was observed that there was no significant difference in terms of microbial load between Gamboru market and Abbaganaram market ($0.806 \times 10^3 \pm 0.131 \times 10^3$ cfu/g and $0.841 \times 10^3 \pm 0.105 \times 10^3$cfu/g respectively). The high microbial load recorded in Baga road market could be associated with the location of the market which was observed to be highly polluted due to anthropogenic activities, such as lack of proper drainage system, refuse dump and lack of proper smoking on the side of the fish processors or/and improper hygienic and handling procedures adopted by the smoked fish sellers. This is in agreement with the findings of Abolagba and Iyeru (1998) [2] who reported that lack of proper smoking and proper hygienic handling of smoked fish products would result in a very high microbial load. Vincent (1989) [33] similarly reported higher microbial load on *Capsicum annum* procured from New Benin market in contrast to lower numbers obtained from Oba market. These differentials were linked with the higher human traffic and poor environmental sanitation of the New Benin market. Baga road market is congested and with poor sanitation as the New Benin market explaining the higher microbial activities and densities. Baga road market was closely followed by Muna market ($1.269 \times 10^3 \pm 0.148 \times 10^3$cfu/g-1), this may be due to exposure and improper handling of the commodity in Muna market as the commodity was observed to be displayed in open trays, exposing it to insects, dust particles and even consumers who were observed to be making direct contact with the commodity with bare hands while bargaining. Adebayo-Tayo, *et al.* (2008) [4] observed a similar scenario in Uyo, Nigeria, where retailers displayed smoke-dried fish samples in open trays beside gutters or refuse heaps.

The reverse was the case in Gamboru and Abbaganaram markets whose environments were at least the most hygienic of all the markets under study and this translates to their relatively low microbial load. The result of this study conforms to the findings of Adegunwa, *et al.* (2013) [5] who reported that smoked fish collected at a camp location in Odeda, Ogun State, Nigeria had higher microbial load than other locations as a result of handling, frequent exposure, poor environmental and sanitary conditions.

The disparities in contamination levels between locations have been observed to be influenced by one or more of the factors enumerated by Tatcher and Clark (1973) [32] as follows: Source of the raw fish; Additional contamination introduced by handlers; Temperature of food during storage and processing; Severity of freezing process in terms of lethality to microorganisms; Contamination after the fish had already been processed. For instance, apart from the mentioned reasons, the regular power fluctuation within Maiduguri metropolis may make the freezing process of fish inefficient and that may result in high contamination even before the fish are smoked.

It could also be deduced that *S. aureus* was more predominant in all the locations (Markets) except in Muna which had more occurrances of *E.coli*. This is still attributable to insanitary conditions of Muna Market. Although the average total bacterial count of all locations was within the acceptable range of Aerobic Plate count of 5.0×10^5 cfu/g as stipulated by International Commission on Microbiological Specification for Food (ICMSF, 1986) [22], the count of *Salmonella* spp., *E. Coli* and *Staphylococci aureus* for all the locations had exceeded the recommended level of 0, 11 and 10^3 cfu/g respectively. The presence of *Salmonella* spp. indicates poor food preparation and handling practices. This is supported by Yusuf and Tengku Abdul Hamid (2012) [35] and it is associated with food borne diseases. *E. Coli* is anenteric bacteria causing gastroenteritis and the contamination of fish or fish products with pathogenic. *E. coli* is associated with improper handling of fish and fecal contamination (Feng, 2002) [19]. *Staphylococci aureus* are capable of producing enterotoxins that cause gastroenteritis (Novotny, *et al.,* 2004) [26]. Thus, food poisoning and food borne diseases could occur as a result of intake of this commodity. Similarly, the mycoflora contaminants (*Aspergillus niger, Aspergillus fumigates, Mucor* spp. and *Yeast* spp.) isolated in this study are associated with the production of toxins which are hazardous to human health. This research is therefore instructive as consumption of contaminated smoked fish could pose serious health problems to the consuming masses.

The result of this study shows a strong correlation between water activity and Bacterial load, indicating that a high water activity connotes a high bacterial load. This corroborate with Eyo (2001) [16] who stated that smoked fish samples may have a relatively high water activity level which is a prerequisite for microbial growth.

Conclusion

In conclusion, microbial contamination or re-contamination of smoked catfish products has been seen to vary from one locality (market) to another and even within the same locality from one fish processor or seller to another. Thus, microbial contamination of smoked fish has been found to be due to several factors such as poor smoking of fish products (i.e. inappropriate temperature control or application), poor personal hygiene of processors/seller, poor hygiene/sanitary practices relating to smoked fish products, smoke/workhouse, packaging and storage as well as the use of inadequate and inefficient traditional processing facilities. Poor environmental sanitation and high human traffic are also implicated.

From the above, it can be deduced that smoked fish sold in Maiduguri markets are contaminated right from the factory point/processing area as well as improper handling by sellers. This implies that smoking is not an effective means of preservation and prevention of microbial proliferation in fish and that bacteria and fungi are responsible for the microbial contamination of smoked fish.

Recommendation

For consumers' health and safety, the following recommendations are drawn:

The use of mechanized smoking system that would completely dehydrate the fish in order to prevent contamination due to moisture is highly recommended. At the same time, fish should be properly smoked with hard wood unvarnished and untreated.

Authorities such as National Agency for Food and Drugs Administration and Control (NAFDAC) should look into the environmental condition of our food handlers as it concerns the smoking factories, the markets and even the hawkers that

carry the food from one place to another. Their hygienic condition must be ascertained before authorizing them to handle public food. People should cook their fish properly before eating even when it is smoked to avert food poisoning.
Finally, fish marketers should showcase the commodity in closed transparent containers instead of displaying in open trays to minimize contamination. In addition, consumers bargaining for the commodity should avoid making direct contact with the commodity using bare hands to curtail cross contamination.

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