



Pathogenic Organisms in Pre-Brined Fresh and Stored Hot Smoked *Clarias gariepinus* (Burchell, 1822)

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Abstract

Keywords:

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chloride

This study was carried out to assess the microbial qualities of smoked catfish (*Clarias gariepinus*) pretreated with sodium chloride, hot smoked and stored for a 2-weeks duration at ambient temperature. The average body weight of fish was 700g and the fresh catfish were treated with salt (sodium chloride) at a rate of 1/4w/v (700g of fish)(T1 and T2) and 1/8w/v (700g of fish)(T3 and T4) i.e soaking in salt solution at rate of 1/4 or 1/8 of the fish body weight each for either 30 minutes or 1hour prior to smoking. Fish samples were selected, washed and distributed into five groups including the control sample (T5). The four groups were subjected to soaking treatment for 30 minutes and 1hour respectively before drying. The non-treated (without salt T5) catfish served as the control. After soaking treatments, all the catfish belonging to both treatment and control groups were subjected to hot smoking and slowly cooked with wood heat at 70°C. The control sample was smoke dried without prior soaking in salt solution. The smoked samples were stored for one and two weeks before analyses. The fresh fish, control and the treated samples showed different types of microbes. Smoke dried fish had the lower microbial loads during the weeks of storage. The results from this study revealed the presence of eight bacterial species namely; *Staphylococcus aureus*, *Micrococcus luteus*, *Salmonella sonnel*, *Enterobacter aerogenes*, *Bacillus cereus*, *Salmonella typhimurium*, *Escherichia coli*, *Proteus mirabilis* and five fungi species namely; *Aspergillus flavus*, *Aspergillus fumigatus*, *Rhizopus stolonifer*, *Rhizopus oryzae*, and *Candida krusei*. *Escherichia coli* had the highest bacteria load of 3.5×10^5 cfu/ml immediately after smoking while *P. mirabilis* has the least bacterial count of 2.3×10^3 cfu/ml after one week of smoking the fish. *A. fumigates* has the highest fungi of 7.3×10^4 sfu/ml in fresh *C. gariepinus* while *R. oryzae* has the least fungi count of 1.1×10^2 sfu/ml after two weeks of smoking the fish. The result from this study revealed that common salt can extend the shelf life of hot smoked catfish (*C. gariepinus*) between one to two weeks under ambient storage condition, before the action and effects of spoilage microorganisms become significant.

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INTRODUCTION

Fish provides a high source of protein required in the diets of man as it contains essential nutrients such as vitamins, fats and minerals which help in the maintenance of life (Ashano and Ajayi, 2003). Apart from its food value, fish has been reported to possess medicinal values, such as the amelioration of asthma, arthritis, coronary heart diseases, goitre and cancer (Cobiac *et al.*, 1991). In spite of these valuable nutrients derivable from fish, however fish is an extremely perishable food commodity as it is highly susceptible to autolysis, rancidity as a result of oxidation of fat which creates unpleasant odors and flavors (FSA, 2004). Generally, fish have soft tissues and high amount of water and this enhances its susceptibility to microbial contamination (Olayemi *et al.*, 2012).

Smoke contributes greatly to fish preservation by acting as an effective antioxidant, bacteriostatic and bactericidal agent, as well as providing a protective film on the surface of the smoked fish (Eyo, 2001). Apart from giving the fish a desirable taste and odour, smoking provides a longer shelf-life through its anti-bacterial and oxidative effect, imparting desirable coloration as well as accelerating the drying process and preventing spoilage (Abolagba and Uwagbai, 2011). Other preservative measures are added as pre-treatments to smoking for improving the shelf life and the hygienic conditions.

LITERATURE REVIEW

Microbial studies have been reported by Abolagba and Igbinevbo (2010) for smoked fish (*Clarias spp*), Omojowo *et al.*, 2010 also work on microbial quality and safety of smoked Tilapia (*Oreochromis niloticus*) treated with Sodium benzoate; Jimoh *et al.*, 2012 and 2013; Akinwumi and Adegbehingbe, (2015) on *Clarias gariepinus* (African mud catfish), *Sardinella eba* (herring) and *Oreochromis niloticus* (Tilapia). It is generally accepted that fish with microbial load greater than 10^6 cfu/g is likely to be at the stage of being unacceptable for consumption (ICMSF, 1986). Akise *et al.*, (2013) reported that high microbial load and unfavourable composition from smoke-dried fish during shelf storage poses a serious health concern for consumers and public health workers.

This study, assessed the microbial qualities and safety of smoked catfish (*Clarias gariepinus*) pre-treated with sodium chloride (common salt) in respect to their body weight at $\frac{1}{4}$ w/v and $\frac{1}{8}$ w/v at different time variation.

MATERIALS AND METHODS

A total of four table fish size with average weight of 700g were used for the study. The fish samples (*Clarias gariepinus*) were obtained from Ministry of Agriculture, Department of Fisheries, Alagbaka ponds in Akure, Ondo State. They were transported live with a clean plastic bucket to the Department of Animal and Environmental Biology Laboratory for analyses.

Pre-smoking of sample treatments/smoking technique

Fish samples were selected and randomly divided into five groups (T1, T2, T3, T4 and T5). Each group was subjected to soaking for 30min and 1hr in separate treatment before hot smoked drying. The four group samples were treated using $\frac{1}{8}$ %w/v (Sodium chloride) and $\frac{1}{4}$ % w/v (Sodium chloride) solution for 30mins and 1hour, the groups are $\frac{1}{4}$ %(NaCl)w/v for 30min (T1), $\frac{1}{4}$ %(NaCl) w/v for 1hour (T2) $\frac{1}{8}$ %(NaCl) w/v for 30min (T3), $\frac{1}{8}$ %(NaCl) w/v for 1hour (T4), and control (T5) hot smoke without Sodium chloride treatment. After soaking treatments, all the samples belonging to the same pretreatment and control groups were subjected to local hot smoking method and slowly cooked for about 8hours with wood heat at 60-70°C temperature. After smoking and cooling, each treatment group and the control were spread on a foil sheet at room temperature. Samples were subjected to microbial analyses at 0 to 2 weeks of storage. 10g of fish sample was macerated in a mortar and then dissolved in a test-tube containing 90ml of sterilized distilled water to obtain a solution of 1ml aliquots of 10^{-1} and 10^{-3} . Dilution was aseptically removed with a sterile pipette and transferred into labeled sterile petri dish and about 20ml of melted media were added by pour plate method. The plates were swirled gently to achieve homogeneity of the aliquot with the media used. The plates for bacteria isolation were allowed to set and then incubated at 37°C for 24hrs and room temperature for fungi isolation for 7days respectively based on the media used. After smoking, the samples were allowed to cooled at room temperature were then spread on a foil sheet and subjected to bacteriological and mycological sampling on 0 and 2 weeks of storage for viable count according to Olorok *et al.*, 2007. The colony identification was carried out using mycology atlases. (Willey *et al.*, 2008, Navi *et al.*, 1999 and Kidd *et al.*, 2016). Spots were counted and recorded as total viable counts using colony counter. The counts for each plate were expressed as colony forming unit (cfu/g) and spore forming unit (sfu/g) respectively. The indirect cell count method was used to measure the microbial load (viable counts) which involved the counting of numbers of pure culture arising from single cell that resulted after plating.

The result was recorded in cfu/ml (Olutiola et al., 2000 and Willey et al., 2008).

Culture media

The following media were used for determination of microbial load of fish samples, nutrient agar for the enumeration of total viable mesophilic bacteria count, MacConkey agar for coliform counts, Eosine-Methylene Blue agar for *Escherichia coli* counts, Mannitol Salt Agar for Staphylococcus species count and Salmonella Shigella agar for *Salmonella* and *Shigella* counts. Nutrient agar was used for subculturing the bacterial isolates, Potato-dextrose agar for subculturing fungi isolates. Each of the agar was prepared and sterilized according to the manufacturer's specification. The sterility and control of each batch of test medium was confirmed by incubating one or two uninoculated plates along with the inoculated plates. The un-inoculated plates were always examined to show no evidence of bacterial growth.

ENUMERATION OF MICROBIAL COLONIES

Colony counting was carried out visually by counting the number of visible colonies that appeared on the plates. Calculation of colony forming unit (CFU) per ml for the bacteria and the spore forming unit (SFU) per ml for the fungi was based on the formula:

$$\text{CFU/ML or SFU/ML} = \frac{\text{Number of colonies} \times \text{dilution factor}}{\text{ml of sample suspension}}$$

Identification of Bacteria and fungi Isolates

The identity of the organisms was confirmed by their morphological, cultural and biochemical characteristics according to John et al., (2013), while fungi colony identification was carried out by use of mycology atlases according to (Navi et al., 1999 and Kidd et al., 2016) and bacterial identification using Atlas Medical Bacteriology according to Halah, (2016).

Biochemical Test

Biochemical Characteristics test such as **Gram's staining**, (Fawole and Oso, 2001); **Coagulase Test** (Cappuccino and Sherman 1992); **Indole Test, Oxidase Test** (Cheesbrough, 2002); **Methyl-Red Test, Voges-Proskauer Test** (Olutiola et al., 2000); **Citrate Utilization, Citrate positive, Citrate negative** (Forbes et al., 2015); **Motility Test** (MacFaddin, 2000); **Sugar Fermentation test, Urease Test** (Fawole and Oso, 2001) were carried out on the isolated organisms

Data Analysis

The values obtained for triplicate samples were used for one-way analysis of variance (ANOVA) where $P < 0.05$ was applied to the different values obtained. Statistical Package for Social Science (SPSS) 20.0 software was used in the data analysis.

RESULTS AND DISCUSSIONS

This study, assessed the microbial load and safety of smoked catfish (*Clarias gariepinus*) pretreated with sodium chloride (common salt), in respect to their body weight at different salt concentration ($\frac{1}{4}$ w/v and $\frac{1}{8}$ w/v) respectively and time variation on pathogenic organisms and shelf life on the stored *Clarias gariepinus* from 0 to 2 weeks of storage at room temperature.

The results from this study revealed the presence of eight bacterial species namely; *Staphylococcus aureus*, *Micrococcus luteus*, *Salmonella sonnel*, *Enterobacter aerogenes*, *Bacillus cereus*, *Salmonella typhimurium*, *Escherichia coli* and *Proteus mirabilis*. And five fungal species namely; *Aspergillus flavus*, *Aspergillus fumigatus*, *Rhizopus stolonifer*, *Rhizopus oryzae* and *Candida krusei*. From table 1, bacteria microbial loads for fresh *Clarias gariepinus* without salt was higher than the smoked salted *Clarias gariepinus* ($p > 0.05$). This shows that treatment with salt does not have significant impact on microbial contamination of fresh fish.

However, there was significant difference ($P < 0.05$) between $\frac{1}{4}$ w/v(T1) 30minutes and $\frac{1}{4}$ w/v(T2) 1hour immediately after smoking with the bacteria count of 4.2×10^5 cfu/ml and 2.5×10^5 cfu/ml and fungi count of 4.5×10^3 sfu/ml and 2.0×10^4 sfu/ml. There was significant difference ($p > 0.05$) between $\frac{1}{8}$ w/v(T3) 30minutes and $\frac{1}{8}$ w/v(T4) 1hour after one week of smoking with the bacteria count of 6.5×10^4 cfu/ml and 7.9×10^4 cfu/ml and fungi count of 9.7×10^3 sfu/ml and 1.1×10^4 sfu/ml as shows in table 5.

Similarly, bacteria loads for *Clarias gariepinus* for fresh, immediately after smoking, one week after smoking and two weeks after smoking were higher than the smoked *Clarias gariepinus* (700g) for $\frac{1}{4}$ w/v (175g) of salt per hour in triplicates. There was also insignificant difference ($p > 0.05$) between bacteria loads and fungi loads for fresh *Clarias gariepinus* from two weeks after smoking. (table 5) while there was significant difference ($p < 0.05$) between bacteria loads such as *Bacillus cereus*, *Escherichia coli*, *Salmonella typhimurium*, *Salmonella sonnel*, and *Enterobacter aerogenes* and fungi loads for *Clarias gariepinus* such as *Aspergillus flavus*, *Candida krusei* and *Rhizopus* sp. immediately after smoking and one week after smoking *Clarias gariepinus* (table 2) (table 6) for all the periods

involved. The natural microflora of catfish and the manner in which the fish was handled determine the number and the types of microorganisms in catfish during fillets harvesting, processing and storage (Nickelson and Finne, 1992).

Likewise the bacteria loads for *Clarias gariepinus* for fresh, immediately after smoking, one week after smoking and two weeks after smoking were higher than fungi loads for 1/8w/v (87.5g) of salt per 30 minutes in triplicate. There was also insignificant difference ($p>0.05$) between bacteria loads such as *Bacillus cereus*, *Escherichia coli*, *Salmonella typhimurium*, *Salmonella sonnel*, and *Enterobacter aerogenes* and fungi loads such as *Aspergillus flavus*, *Candida krusei*, *Rhizopus stolonifer* and *Rhizopus oryzae* (table 2) for fresh *Clarias gariepinus* immediately after smoking, one week after smoking and two weeks after smoking (table 6).

Furthermore, bacteria loads for *Clarias gariepinus* for fresh, immediately after smoking, one week after smoking and two weeks after smoking were higher than fungi loads for 1/4w/v(175g) of salt per hour in triplicates. There was also insignificant difference ($p>0.05$) between bacteria loads such as *Bacillus cereus*, *Escherichia coli*, *Salmonella typhimurium*, *Salmonella sonnel*, and *Enterobacter aerogenes* and fungi loads *Aspergillus flavus*, *Candida krusei*, *Rhizopus stolonifer* and *Rhizopus oryzae* (table 3) for fresh *Clarias gariepinus* one week after smoking and two weeks after smoking (table 9) while there was significant difference ($p<0.05$) between bacteria loads and fungi loads for *Clarias gariepinus* immediately after smoking (table 6) for all the periods involved.

The result also showed that bacteria loads for *Clarias gariepinus* for fresh, immediately after smoking, one week after smoking and two weeks after smoking were higher than fungi loads for 1/8w/v (87.5g) of salt per 30 minutes in triplicates. There was also insignificant difference ($p>0.05$) between bacteria loads and fungi loads for fresh *Clarias gariepinus*, immediately after smoking and one week after smoking (table 6), while there was significant difference ($p<0.05$) between bacteria loads for *Bacillus cereus*, *Escherichia coli*, *Salmonella typhimurium*, *Salmonella sonnel*, and *Enterobacter aerogenes* and fungi loads for *Aspergillus flavus*, *Candida krusei*, *Rhizopus stolonifer* and *Rhizopus oryzae* for *Clarias gariepinus* two weeks after smoking as shown in tables 4 and 6 for all the periods involved.

The microorganisms isolated in this study have been reported in some fish species. Adelaja et al., (2013) isolated *Staphylococcus* sp, *E. coli* and *A. fumigatus* in smoked *Chysichthys nigrodigatatus* which is in agreement with this study. *E.coli* and *Salomonella* sp. are fecal borne pathogens and they could occur as a result of contamination from the handlers.

The bacteria isolated in this study are also similar to those reported by Adeparusi et al., 2007, Okonta and Ekelemu, 2005; Abolagba and Igbinevbo, 2010; Jimoh et al., 2014; Akinwumi and Adegbehingbe, (2015) on their findings on both fresh and smoked African catfish and other fish species .

The occurrence of *Staphylococcus aureus* and *E.coli* in the smoked dried fish samples were similar to the report of Martin, (1994) where he stated that these organisms were the commonest micro-organisms associated with smoked fish. The presence of *Staphylococcus aureus* in fish samples according to Okonko et al., (2008) might have been through contamination by handling. Okareh and Erhahon (2015) reported that *Staphylococcus* sp. has pathogenic strains which could cause food poisoning due to the heat stable *Staphylococcus* enterotoxin which is resistant to gastrointestinal enzymes. *Staphylococcus aureus* can rapidly develop resistance to many antimicrobial agents and pose therapeutic problems (Thrower, 2000). *Staphylococcus aureus* shows the highest percentage prevalence in smoked *Clarias gariepinus*. This confirmed the work of Okonta and Ekelemu, (2005) who reported *Staphylococcus* as one of the predominant microorganisms affecting smoked fish and causing their spoilage. This study shows that *E. coli* has the highest bacteria load of 3.5×10^5 cfu/ml immediately after smoking the fish and 3.7×10^4 cfu/ml after one week of smoking. This also confirmed the work of Novotny et al., (2004) who reported that the contamination of food of fish origin with pathogenic *E. coli* probably occurs during handling of fish and during the production process.

According to Aberoumand, (2010), *Escherichia coli* is a classic example of enteric bacteria causing gastroenteritis. *E. coli* including other coliforms and bacteria as *Staphylococcus aureus* and sometimes *Enterococci* are commonly used as indices of hazardous conditions during processing of fish. *Escherichia coli* was the dominant bacteria found in *Clarias gariepinus* immediately after smoking with percentage prevalence of 62.2% (Table 2) and one week after smoking with percentage prevalence of 38% (Table 3) which is in agreement with Aberoumand, (2010) finding .

Fish harvested from contaminated waters can harbor *Salmonella* sp. (Alexander and Austin, 1986). Miller et al., (1973) observed that not all bacteria present on fresh fish are spoilers but there are certain active spoilers which are the major pathogens on fish spoilage. These bacteria have the ability to reduce trimethylamine and produce Hydrogen sulphide from Sodium thiosulphate as a secondary metabolite that constitute fresh fish spoilage. The microorganisms isolated and identified from the fresh fish samples can be said to be normal flora of the fish e.g. *Bacillus* sp. (Ola and Oladipo, 2004) which is in line with this present study as *Bacillus cereus* has the highest bacteria load of 2.7×10^5 cfu/ml in fresh *Clarias gariepinus*. The normal microbial flora of the fish are not initially harmful, as they even help in preventing the invasion of the fish flesh by other microorganisms but they become pathogenic when there is an enabling environment that promotes their growth due to bad handling which can lead to bruises, poor hygiene and delayed processing and preservation of the fish after harvest.

Bacillus was present in virtually all fish samples tested (smoked and fresh) as it was the dominant bacteria found in fresh *Clarias gariepinus* with percentage prevalence of 24%. It is a gram-positive, obligate aerobic rod shaped, endospore forming bacteria (Todar, 2008). Two *Bacillus* sp. are considered to be medically significant; *B. anthracis* which causes anthrax and *B. coagulase* which causes food spoilage. Colonially, they are large, spreading and irregularly shaped. When viewed under microscope, they appear as rods with a bulge which contains the endospore (Martinko, 2005).

The study also showed the presence of *Micrococcus luteus*, *Proteus mirabilis*, *Salmonella sonnel*, *Enterobacter aerogenes* in the already smoked fish. *Micrococcus luteus* was the dominant bacteria found in *Clarias gariepinus* two weeks after smoking with the load of 2.1×10^4 cfu/ml and percentage prevalence of 27.8%. The presence of *Enterobacter aerogenes* could be indicative of fecal contamination. All the pathogens isolated are of food and public health implication and hence hazardous and injurious to human health if consumed above the maximum recommended bacteria count for good product. The maximum recommended bacteria count for good quality product is 5.0×10^5 (5.7Log cfu/g) according to International Commission on Microbiological Specification for Food (ICMSF, 1986). The total bacterial count on fish rarely indicate the quality of the fish but it gives an indication of the risk of spoilage induced since each of these organisms had different ways of affecting health

conditions of consumers of such contaminated fish as reported by (Gram and Huss, 2000).

The isolated fungi in this study also similar to the microorganisms reported by Abolagba and Uwagbai, (2011) in smoke-dried Fishes (*Ethmalosa Fimbriata* and *Pseudotolithus Elongatus*); Jimoh et al., 2012 and 2014 in smoked African catfish (*Clarias gariepinus*) and *Tilapia* fish; Edema and Agbon, (2010) in smoke-cured *Ethmalosa fimbriata* and *Clarias gariepinus*

The presence of *A. flavus* and *A. fumigates* in the studied fish samples is of great health importance because of their mycotoxigenic potentials. Essiem et al., (2005) reported that *A. flavus* and *A. fumigates* produced aflatoxins, which destroyed the liver and kidney in man resulting to death. The presence of these organisms in the fish could be as a result of handling process during smoking and cross contamination during storage. *Aspergillus fumigates* was the dominant fungi found in fresh *Clarias gariepinus* with the load of 7.3×10^4 sfu/ml and percentage prevalence of 40.6% and also two week after smoking with the load of 5.6×10^3 sfu/ml and percentage prevalence of 45.2%. *Aspergillus flavus* was the dominant fungi found in *Clarias gariepinus* immediately after smoking with load of 4.5×10^4 sfu/ml and percentage prevalence of 100%. *Candida krusei* has the highest load of 1.0×10^4 sfu/ml after one week of smoking while *Rhizopus oryzae* was the dominant fungi found in *Clarias gariepinus* after one week with percentage prevalence of 49.2%. The presence of *Aspergillus fumigates*, *Aspergillus flavus*, *Rhizopus stolonifer* and *Rhizopus oryzae* in the smoked fish species could be due to the fact that during storage, the fish samples reabsorbed moisture from the environment which then supported the growth of the microorganisms in addition to the contamination during processing which is in consistent with Christianah et al., (2010) findings.

In this study microbial loads of *Clarias gariepinus* immediately after smoking has significant difference using $\frac{1}{4}$ w/v (30 minutes and 1hour but there was no significant difference between the microorganisms isolated in fresh *Clarias gariepinus*. Microbial loads of *Clarias gariepinus* one week after smoking has significant difference using $\frac{1}{8}$ w/v T1 and T2 (30 minutes and 1 hour). The microbial loads of *Clarias gariepinus* immediately after smoking was reduced when compared to the loads of fresh *Clarias gariepinus*. This may occur as a result of the fact that some microbial agents may likely to die in the course of smoking. The microbial loads of *Clarias gariepinus* after one week of smoking was higher when compared to immediately after smoking and the microbial loads of *Clarias gariepinus* after two weeks smoking was higher when compared to immediately after smoking and one

week smoking. Hood *et al.*, (1983) reported that microbial load increases with duration of storage and temperature which is in an agreement with this present study.

In this study, the variations observed in microbial analysis of the samples from the ponds may be due to smoking methods, different salt concentration and time variation. This is because traditional fish smoking devices are poorly constructed; the technology employed by local fishermen in smoking is not standardized and lack mechanisms for the control of smoke and heat production, so this most parameters remain uncontrolled. This is in agreement with Olopade *et al.*, 2013 who reported that, essential drying parameters such as duration, air humidity and temperature which affect the efficiency of smoking and the quality of the final products are not precisely determined and mastered.

The study showed that though smoking helps in inhibiting the activities of microorganisms, different salt concentrations as pre-treatment and smoking time variation also have a significant effect on the inhibition of microorganisms. When smoking is not properly carried out, microbial growth and activities still continue, leading to the deterioration of the fish. It can be concluded that some of the microbial species associated with the water environment of the fish are of food and public health implication and hence hazardous and injurious to human health. Hazards related to contamination, recontamination during processing cause infections in man. It is recommended that good handling and processing practices be carried out under hygienic condition. These will reduce such health hazard and promote good health on the part of the consumers.

CONCLUSION

Table 1: Analysis of different types of Bacteria (cfu/ml) and Fungi (sfu/ml) present in fresh *Clarias gariepinus* at different salt concentration and time variation.

	Control Fresh unsalted (cfu/ml)	Control for freshly smoked unsalted (cfu/ml) T5	¼(30min) 25% (cfu/ml) T1	¼(1hr) 25% (cfu/ml) T2	¼(30min) 12.5% (cfu/ml) T3	¼ (1hr) 12.5% (cfu/ml) T4	Percentage prevalence (%)
Bacteria							
<i>Micrococcus luteus</i>	-	1.8× 10 ⁵	-	-	-	-	8.5
<i>Proteus mirabilis</i>	-	-	-	9.7× 10 ⁴	-	-	4.5
<i>Bacillus cereus</i>	7.6× 10 ⁴	1.6× 10 ⁵	-	-	2.7× 10 ⁵	-	24
<i>Escherichia coli</i>	3.5× 10 ⁴	2.1× 10 ⁵	1.3× 10 ⁴	4.1× 10 ⁴	-	1.7× 10 ⁵	22.1
<i>Salmonella sonnel</i>	9.7× 10 ⁴	-	4.1× 10 ⁴	5.1× 10 ⁴	8.3× 10 ⁴	-	12.6
<i>Salmonella typhimurium</i>	5.4× 10 ⁴	-	-	4.4× 10 ⁴	-	-	4.5
<i>Enterobacter aerogenes</i>	-	-	7.6× 10 ⁴	-	1.3× 10 ⁵	1.4× 10 ⁵	16.5
<i>Staphylococcus aureus</i>	8.3× 10 ⁴	-	-	7.6× 10 ⁴	-	-	7.3

At 0.05 level of Significance

	Control smoked unsalted (sfu/ml)	Control for fresh smoked unsalted (sfu/ml) T5	¼(30min) 25% T (sfu/ml) T1	¼(1hr) 25% T (sfu/ml) T2	1/8(30min) 12.5% (sfu/ml) T3	1/8(1hr) 12.5% (sfu/ml) T4	Percentage prevalence (%)
Fungi							
<i>Aspergillus flavus</i>	3.4× 10 ³	3.0× 10 ³	-	2.8× 10 ⁴	4.5× 10 ²	2.1× 10 ³	18.9
<i>Aspergillus fumigates</i>	-	-	7.2× 10 ³	7.3× 10 ⁴	-	-	40.6
<i>Candida krusei</i>	1.9× 10 ³	5.3× 10 ³	9.2× 10 ³	-	7.0× 10 ²	3.2× 10 ³	10.3
<i>Rhizopus stolonifer</i>	2.8× 10 ³	7.2× 10 ³	8.6× 10 ³	3.7× 10 ⁴	7.5× 10 ²	3.2× 10 ³	30.2

At 0.05 level of Significance

Table 2: Analysis of different types of Bacteria (cfu/ml) and Fungi (sfu/ml) present in *Clarias gariepinus* immediately after smoking at different salt concentration and time variation.

	Control smoked unsalted (cfu/ml) T5	¼w/v(30min) 25% (cfu/ml) T1	¼w/v(1hr) 25% (cfu/ml) T2	½w/v(30min) 12.5% (cfu/ml) T3	½w/v(1hr) 12.5% (cfu/ml) T4	Percentage prevalence (%)
Bacteria						
<i>Escherichia coli</i>	2.1× 10 ⁴	3.5× 10 ⁵	1.6× 10 ⁵	4.8× 10 ⁴	2.8× 10 ⁵	62.2
<i>Staphylococcus aureus</i>	3.1× 10 ⁴	7.0× 10 ⁴	9.1× 10 ⁴	1.3× 10 ⁵	1.9× 10 ⁵	37.8

At 0.05 level of Significance

	Control smoked unsalted (sfu/ml) T5	¼(30min) 25% (sfu/ml) T1	¼(1hr) 25% (sfu/ml) T2	1/8(30min) 12.5% (sfu/ml) T3	1/8(1hr) 12.5% (sfu/ml) T4	Percentage prevalence (%)
Fungi						
<i>Aspergillus flavus</i>	1.9× 10 ⁴	4.5× 10 ³	2.0× 10 ⁴	4.0× 10 ⁴	4.5× 10 ⁴	100

At 0.05 level of Significance

Table 3: A Bacteria (cfu/ml) and Fungi (sfu/ml) present in *Clarias gariepinus* one week after smoking of different salt concentration pre-treatment

	Control smoked unsalted (cfu/ml) T5	¼w/v(30min) 25% (cfu/ml) T1	¼w/v(1hr) 25% (cfu/ml) T2	½w/v(30min) 12.5% (cfu/ml) T3	½w/v(1hr) 12.5% (cfu/ml) T4	Percentage prevalence (%)
Bacteria						
<i>Micrococcus luteus</i>	-	-	2.7× 10 ⁴	-	-	7.8
<i>Proteus mirabilis</i>	2.3× 10 ³	3.2× 10 ⁴	-	1.7× 10 ⁴	2.7× 10 ⁴	22.5
<i>Escherichia coli</i>	2.5× 10 ⁴	2.7× 10 ⁴	2.4× 10 ⁴	1.9× 10 ⁴	3.7× 10 ⁴	38
<i>Enterobacter aerogenes</i>	-	-	2.1× 10 ⁴	-	-	6.1
<i>Staphylococcus aureus</i>	2.2× 10 ⁴	2.5× 10 ⁴	-	2.8× 10 ⁴	1.4× 10 ⁴	25.6

At 0.05 level of Significance

	Control smoked unsalted (sfu/ml) T5	¼w/v(30min) 25% (sfu/ml) T1	¼w/v(1hr) 25% (sfu/ml) T2	½w/v(30min) 12.5% (sfu/ml) T3	½w/v(1hr) 12.5% (sfu/ml) T4	Percentage prevalence (%)
Fungi						
<i>Rhizopus oryzae</i>	5.7× 10 ³	9.1× 10 ³	3.9× 10 ³	5.5× 10 ³	4.2× 10 ³	49.2
<i>Aspergillus fumigates</i>	5.3× 10 ³	1.9× 10 ³	-	4.2× 10 ³	7.4× 10 ³	32.5
<i>Candida krusei</i>	-	-	1.0× 10 ⁴	-	-	18.3

At 0.05 level of significance

Table 4: Bacteria (cfu/ml) and Fungi (sfu/ml) present in *Clarias gariepinus* two weeks after smoking at different salt concentration and time variation.

	Control smoked unsalted (cfu/ml) T5	¼w/v(30min) 25% (cfu/ml) T1	¼w/v(1hr) 25% (cfu/ml) T2	½w/v(30min) 12.5% (cfu/ml) T3	½w/v(1hr) 12.5% (cfu/ml) T4	Percentage prevalence (%)
Bacteria						
<i>Micrococcus luteus</i>	2.1 × 10 ⁴	9.5 × 10 ³	1.5 × 10 ⁴	6.5 × 10 ³	1.1 × 10 ⁴	27.8
<i>Proteus mirabilis</i>	1.3 × 10 ⁴	1.3 × 10 ⁴	7.5 × 10 ³	1.1 × 10 ⁴	9.4 × 10 ³	23.6
<i>Escherichia coli</i>	1.5 × 10 ⁴	-	9.5 × 10 ³	1.7 × 10 ⁴	1.9 × 10 ⁴	26.7
<i>Staphylococcus aureus</i>	7.5 × 10 ³	1.9 × 10 ⁴	7.6 × 10 ³	5.1 × 10 ³	1.1 × 10 ⁴	22

At 0.05 level of Significance

	Control smoked unsalted (sfu/ml) T5	¼w/v(30min) 25% (sfu/ml) T1	¼w/v(1hr) 25% (sfu/ml) T2	½w/v(30min) 12.5% (sfu/ml) T3	½w/v(1hr) 12.5% (sfu/ml) T4	Percentage prevalence (%)
Fungi						
<i>Rhizopus oryzae</i>	3.5 × 10 ³	3.1 × 10 ³	1.0 × 10 ³	1.1 × 10 ²	1.2 × 10 ³	26.7
<i>Aspergillus fumigates</i>	4.4 × 10 ³	2.1 × 10 ³	1.3 × 10 ³	1.5 × 10 ³	5.6 × 10 ³	45.2
<i>Rhizopus stolonifer</i>	3.1 × 10 ³	1.0 × 10 ³	5.6 × 10 ²	4.5 × 10 ³	1.2 × 10 ²	28.1

At 0.05 level of Significance

Table 5: Analysis of time and salt variation for ¼w/v (175g) of salt for 30 minutes and 1 hour (T1 and T2) and ½w/v (87.5g) of salt for 30 minutes and 1 hour (T3 and T4) for 700g of fresh smoked and stored hot smoked *Clarias gariepinus*

		Fresh washed unsalted fish Microbial loads				Fresh washed unsalted fish Microbial loads		
Organisms	¼w/v 30mins 25% T1	¼w/v 1hour 25% T2	T-test	Decision	½w/v 30mins 12.5% T3	½w/v 1hour 25% T4	T-test	
Bacteria (cfu/ml)	1.3 × 10 ⁵	2.9 × 10 ⁵			4.9 × 10 ⁵	3.2 × 10 ⁵		
			1.465	Insignificance			1.564	Insignificance
Fungi (sfu/ml)	2.5 × 10 ⁴	1.3 × 10 ⁵			1.9 × 10 ³	8.6 × 10 ³		
		Microbial loads of Immediately smoked fish (day 1)				Microbial loads of Immediately smoked fish (day 1)		
Organisms	¼ 30mins 25% T1	¼ 1hour 25% T2	T-test		1/8 30mins 12.5% T3	1/8 1hour 25% T4	T-test	
Bacteria (cfu/ml)	4.2 × 10 ⁵	2.5 × 10 ⁵		Significance	1.8 × 10 ⁵	4.7 × 10 ⁵		

			2.671				0.198	Insignificance
Fungi (sfu/ml)	4.5×10^3	2.0×10^4			4.0×10^4	4.5×10^4		
		Microbial loads of smoked fish stored after One Week Smoking				Microbial loads of smoked fish stored after One Week Smoking		
Organisms	$\frac{1}{4}$ 30mins 25%	$\frac{1}{4}$ 1hour 25%	<i>T-test</i>		$\frac{1}{8}$ 30mins 12.5%	$\frac{1}{8}$ 1hour 25%	<i>T-test</i>	
Bacteria (cfu/ml)	8.5×10^4	7.3×10^4		Insignificance	6.5×10^4	7.9×10^4		
			1.772				2.165	Significance
Fungi (sfu/ml)	1.1×10^4	1.4×10^4			9.7×10^3	1.1×10^4		
		Microbial loads of smoked fish stored after Two Week Smoking				Microbial loads of smoked fish stored after Two Week Smoking		
Organisms	$\frac{1}{4}$ w/v 30mins 25% T1	$\frac{1}{4}$ w/v 1hour 25% T2	<i>T-test</i>		$\frac{1}{8}$ w/v 30mins 12.5% T3	$\frac{1}{8}$ w/v 1hour 25% T4	<i>T-test</i>	
Bacteria (cfu/ml)	5.7×10^4	4.0×10^4		Insignificance	4.0×10^4	5.1×10^4		
			1.653				1.673	Insignificance
Fungi (sfu/ml)	6.3×10^3	2.9×10^3			4.9×10^3	7.7×10^3		

Table 6: Microbial Loads of *Clarias gariepinus* :Fresh, After Smoking, One week and Two weeks of storage.

Periods	Microbial Loads	Control Fresh without salt	Control for fresh smoked without salt T5	$\frac{1}{4}$ w/v 30mins 25% T1	$\frac{1}{4}$ w/v 1hour 25% T2	$\frac{1}{8}$ w/v 30mins 12.5% T3	$\frac{1}{8}$ w/v 1hour 12.5% T4
	Bacteria (cfu/ml)	3.2×10^5	5.7×10^5	1.3×10^5	2.9×10^5	4.9×10^5	3.2×10^5
For Fresh	Fungi (sfu/ml)	8.2×10^3	1.5×10^4	2.5×10^4	1.3×10^5	1.9×10^3	8.6×10^3
	P-value	0.256	0.176	0.180	0.172	0.149	0.248
	t-test	1.283	1.574	1.557	1.593	1.705	1.307
	Decision	Insig.	Insig.	Insig.	Insig.	Insig.	Insig.
Immediately	Bacteria (cfu/ml)	5.2×10^4	-	4.2×10^5	2.5×10^5	1.8×10^5	4.7×10^5
After	Fungi (sfu/ml)	1.9×10^4	-	4.5×10^3	2.0×10^4	4.0×10^4	4.5×10^4
Smoking	P-value	0.201	-	0.068	0.171	0.234	0.192

	t-test	1.472	-	2.322	1.596	1.353	1.506
	Decision	Insig.	Insig.	Insig.	Insig.	Insig.	Insig.
One Week	Bacteria (cfu/ml)	5.7×10^4	-	5.7×10^4	4.0×10^4	4.0×10^4	5.1×10^4
After	Fungi (sfu/ml)	1.1×10^4	-	6.3×10^3	2.9×10^3	4.9×10^3	7.7×10^3
Smoking	P-value	0.151	-	0.140	0.145	0.131	0.131
	t-test	1.693	-	1.755	1.724	1.804	1.804
	Decision	Insig.	Insig.	Insig.	Insig.	Insig.	Insig.
Two weeks	Bacteria (cfu/ml)	7.0×10^4	-	8.5×10^4	7.3×10^4	6.5×10^4	7.9×10^4
After	Fungi (sfu/ml)	1.1×10^4	-	1.1×10^4	1.4×10^4	9.7×10^3	1.1×10^4
Smoking	P-value	0.167	-	0.164	0.160	0.169	0.128
	t-test	1.614	-	1.630	1.649	1.609	1.819
	Decision	Insig.	Insig.	Insig.	Insig.	Insig.	Insig.

At 0.05 level of Significance

Table 7: Minimum and maximum microbial loads in fresh and stored hot smoked *Clarias gariepinus*.

	Bacterial(cfu/ml)		Fungi (sfu/ml)	
	Minimum	Maximum	Minimum	Maximum
FRESH	0	2.3×10^5	0	0
	0	1.3×10^4	0	7.3×10^4
	0	0	0	4.5×10^2
AFTER SMOKING	0	3.5×10^5	0	0
	0	2.1×10^4	0	4.5×10^4
	0	0	0	4.5×10^3
AFTER ONE WEEK	0	3.7×10^4	0	1.0×10^4
	0	2.3	0	1.9×10^3
	0	2.1×10^4	0	0
AFTER TWO WEEKS	0	2.1×10^4	0	0
	0	5.1×10^3	0	5.6×10^3
	0	0	0	1.1×10^2

Table 8: Prevalence of Bacteria and Fungi in Fresh and Stored Hot Smoked *Clarias gariepinus* In Percentage (%)

	For fresh (%)	Immediately after smoking (%)	One week after smoking (%)	Two weeks after smoking (%)
Bacteria				
<i>Micrococcus luteus</i>	12.5	0	12.5	62.5
<i>Proteus mirabilis</i>	12.5	0	50	62.5
<i>Bacillus cereus</i>	37.5	0	0	0
<i>Escherichia coli</i>	62.5	62.5	62.5	50
<i>Salmonella sonnel</i>	50	0	0	0
<i>Salmonella typhimurium</i>	25	0	0	0
<i>Enterobacter Aerogenes</i>	37.5	0	12.5	0
<i>Staphylococcus aureus</i>	25	62.5	62.5	62.5
Fungi				
<i>Rhizopus oryzae</i>	0	0	62.5	62.5
<i>Aspergillus flavus</i>	62.5	62.5	0	0
<i>Aspergillus fumigates</i>	25	0	50	62.5
<i>Candida krusei</i>	62.5	0	12.5	0
<i>Rhizopus stolonifer</i>	75	0	0	62.5

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