

Original Research Article

Microbiological assessment of smoked *Clarias gariepinus* sold in open Yenagoa

Abstract

This study aimed to assess the microbial quality of smoked *Clarias gariepinus* sold in markets in Yenagoa, Bayelsa State, Nigeria. Fish samples processed using open drums, were purchased from retailers from Agudama, Akenfa, Kpansia Opolo and Tombia markets and analyzed for their microbial quality using viable plate count. Fish samples from Agudama market had total viable count (TVC) of 6.0×10^5 CFU/g and total fungi count (TFC) of 2.0×10^5 CFU/g; Akenfa market samples had TVC of 9×10^5 CFU/g and TFC of 7.0×10^5 CFU/g; Kpansia market samples had TVC of 3.5×10^6 CFU/g and TFC of 2.0×10^5 ; Opolo market samples had TVC of 3.0×10^6 CFU/g and TFC of 2.0×10^5 CFU/g while Tombia market samples had TVC of 2.0×10^5 CFU/g and TFC of 1.0×10^5 CFU/g. The bacterial isolates were identified as *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptobacillus* spp. while the fungal isolates were identified as *Aspergillus fumigatus*, *Aspergillus terreus*, *Candida* spp and *Mucor* spp. The fish samples, except those from Tombia market, had high microbial load and presence of pathogen, which is indicative of the poor conditions of processing, packaging, storage and display in the market. Improvement of processing practice and packaging method are recommended.

Keywords: *Clarias gariepinus*, microbial quality, processing practice and packaging method

1.0 Introduction

Fish is the principal animal protein consumed in the riverine and coastal states of Nigeria, especially in the rural areas harbouring plenteous person whose occupation is fishing or engage in fishing as a cultural practice [1,2]. In Nigeria, like in other dominions, the catches from natural water bodies are usually not enough to meet the huge demand for this cheap protein source, or in some cases, exotic protein, and is thus supplemented by domesticated fish from artificial ponds [3]. This has created a massive market for the common commercially farmed fish species in Nigeria, *Clarias gariepinus* [4]

The conditions under which fish are farmed or the environment from which they are harvested, will define their microbial flora. In fact, the slime that envelopes the surface of fish is a slurry of bacteria and fungi. Therefore, many dangers exist when fish having high microbial load is not fitly prepared before consumption. Spoilage of fish after harvest, is largely due to microbial spoilage [3]. Aside the loss of food value that can be brought about by spoilage microorganism, there is the risk of illness mediated by the presence of pathogenic microorganisms and or their toxins [5].

To ensure that fish retain its nutritive worth and serve food purpose when needed, preservation methods of several kinds are used [6]. Traditionally, smoking of fish impact on palate reception, flavour and ultimate acceptance of fish, aside being a practice passed down from generations from long past [3,7]. Chemicals in smoke depending on the wood type, also ensures that smoked fish stay long without spoilage, as they antagonize microorganism, while

Comment [RD1]: Microbiological Quality of Smoked *Clarias gariepinus* sold in Open Markets of Yenagoa Metropolis, Nigeria

Comment [RD2]: A total of xxxxx smoked fish processed by open drum method were purchased from retailers in Agudama, Akenfa, Kpansia Opolo and Tombia open markets. The samples collected were evaluated for microbiological quality following standard methods.

Comment [RD3]: The result showed that total viable bacteria count of fish ranged between . Besides, total fungi count of were observed on all the fish samples evaluated. Comparatively, the result showed or no statistically variation ($p < 0.05$) in the microbial contamination of the smoked fish in the sampled areas. Fish samples from xxxx was the most contaminated followed by yyyyyy, zzzz had least total viable bacteria count.

NOTE. My big worry is that total coliform count or Faecal coliform count that are indicators of food contamination, or hygiene practice were not determined. Therefore, conclusion based on these parameters is weak.

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Comment [RD5]: Bacteria species of *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptobacillus* sp. are dominant contaminants of the smoked fish. The fungi of *Aspergillus fumigatus*, *Aspergillus terreus*, *Candida* sp and *Mucor* sp. were also identified.

Comment [RD6]: The smoked fish are contaminated with high microbial load and dominant organisms that are indication of the poor hygiene, and potential human pathogens. Therefore, improvement in the hygiene practices during handling and processing is highly recommended to prevent microbial contamination of the smoked fish.

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the heat removes moisture from the fish to keep water activity low [8,9]. It is expected therefore, that smoking fish would kill off microorganisms present in fish so long as the heating is at high enough temperature (above 80°C) and processing was carried out under hygienic environment so also the packaging. This is augmented with adequate brining of not less than 5% sodium chloride [10].

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One common fish species that is sold and consumed in most market in Nigeria is *Clarias gariepinus*. Typically, the smoked fish is displayed by vendors in open trays either in market or as it is hawked along the streets. Smoked fish of various kind have been reported to harbour objectionable microorganisms including *Bacillus cereus*, *Clostridium botulinum*, *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa* and *Salmonella* spp., *Staphylococcus aureus* among others, owing to improper and unhygienic processing, packaging and storage [11-14]. Such food is considered to be microbiologically unsafe, and in many instances, outbreaks results from lack of understanding of and adherence to food hygiene.

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For reason of food safety, which cannot be discern from organoleptic perception, the microbiological properties of ready-to-eat smoked fish needs to be evaluated. Therefore, this study aimed to assess the microbial quality of smoked *Clarias gariepinus* sold in markets in Yenagoa, Bayelsa State, Nigeria

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2.0 Materials and Methods

2.1 Sample Collection

Fish samples sold in open markets at Agudama, Akenfa, Kpansia Opolo, and Tombia, were purchased at random and conveyed to the laboratory for analysis. From each market, 3 retailers were picked, from which 3 smoked catfish were purchased, and with 3 samples of personally smoked catfish which served as control, the total was 48.

Comment [RD31]: A total of 48 smoked fish samples were purchased from open markets Yenagoa, Bayelsa State, Nigeria. Three samples each of smoked catfish were randomly collected from in Agudama, Akenfa, Kpansia Opolo, and Tombia markets. Samples of smoked catfish prepared under aseptic conditions served as control. All fish samples were transported to the microbiology laboratory of xxxxxx for analysis.

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2.2 Isolation and enumeration of bacteria and fungi

One gram (1g) of fish sample transferred into a beaker containing 9 ml sterile distilled water, under aseptic condition, and allowed to stand for about 30 minutes, for the dislodgement of attached microorganism. Serial dilution of the suspension was made and spread plated onto sterile nutrient agar which were incubated at 37°C for 24 hours and onto sterile saboraud dextrose agar which were incubated at room temperature (27°C) for 72 hours, to isolate and quantify the bacteria and fungi present.

Comment [RD35]: The stock suspension was tenfold serially diluted and spread plated on sterile nutrient agar. It was followed by aerobic incubation at 37°C for 24 h and distinct colonies formed enumerated as total viable bacteria count. Similarly, the total fungal count was carried out on sterile Saboraud Dextrose agar, incubated at room temperature (27°C) for 72, and colonies were enumerated as colony-forming units per gram of samples (cfu/g).

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2.3 Identification of Bacterial Isolates

Bacterial isolates were identified by adopting Gram staining, motility, and biochemical tests (catalase, citrate utilization, indole, sugar fermentation, coagulase, oxidase, and methyl red/voges proskauer) according to the scheme of Cheesbrough [15].

2.4 Identification of fungi

Fungi identification was based on the colonial and microscopic characteristics following the schemes of Salvamani and Nawa [16] and Pepper and Gerba [17].

3.0 Results

Table 1 shows the microbial load of smoked fish sold in Yenagoa. Fish samples from Kpansia market had the highest TVC of 3.5×10^6 CFU/g while those from Tombia market had the least TVC of 2.0×10^5 CFU/g. Fish samples from Akenfa market had the highest TFC of 7.0×10^5 CFU/g while those from Agudama market had the least TFC of 1.0×10^5 CFU/g. The control had 0 CFU/g and TFC of 3.0×10^4 CFU/g.

Table 2 shows the bacteria and fungi isolated from the fish samples. The bacterial isolates were identified as *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptobacillus* spp. while the fungal isolates were identified as *Aspergillus fumigatus*, *Aspergillus terreus*, *Candida* spp. and *Mucor* spp.

Table 1: Microbial load of smoked *Clarias gariepinus* sold in Yenagoa

Market	TVC (CFU/g)	TFC (CFU/g)
Agudama	6.0×10^5	1.0×10^5
Akenfa	9.0×10^5	7.0×10^5
Kpansia	3.5×10^6	2.0×10^5
Opolo	3.0×10^6	2.0×10^5
Tombia	2.0×10^5	2.0×10^5
Control	-	3.0×10^4

Table 2: Microbial isolates from smoked *Clarias gariepinus* sold in Yenagoa

Market	Bacterial isolate	Fungal isolate
Agudama	<i>Bacillus cereus</i> <i>Staphylococcus aureus</i>	<i>Aspergillus fumigatus</i>
Akenfa	<i>Bacillus cereus</i> <i>Staphylococcus aureus</i> <i>Staphylococcus epidermidis</i>	<i>Aspergillus fumigatus</i>
Kpansia	<i>Escherichia coli</i> <i>Staphylococcus aureus</i> <i>Staphylococcus epidermidis</i> <i>Streptobacillus</i> spp	<i>Mucor</i> spp.
Opolo	<i>Bacillus cereus</i> <i>Escherichia coli</i>	<i>Aspergillus terreus</i>
Tombia	<i>Staphylococcus aureus</i> <i>Bacillus cereus</i> <i>Staphylococcus aureus</i> <i>Staphylococcus epidermidis</i>	<i>Candida</i> spp.
Control	-	<i>Aspergillus fumigatus</i>

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Discussion

In this study, the microbial qualities of smoked catfish was carried out to ascertain the microbial load and type, with regards to objectionable pathogens. The plate count results for both bacteria and fungi revealed that fish samples from Agudama, Akenfa, Kpansia, Opolo and Tombia which were TVC of 6.0×10^5 CFU/g and TFC of 2.0×10^5 CFU/g; 9×10^5 CFU/g and 7.0×10^5 CFU/g; 3.5×10^6 CFU/g and 2.0×10^5 , 3.0×10^6 CFU/g and 2.0×10^5 CFU/g, and 2.0×10^5 CFU/g and 1.0×10^5 CFU/g, respectively, surpassed the 5×10^5 CFU/g maximum load for good quality for all but Tombia marker samples, but within maximum range (10^7) for marginal satisfactoriness, [18,19]. Ariyo and Omokaro [12] sampled smoked *Clarias gariepinus* from other markets in Yenagoa, and similarly found the microbial load to be high, ranging from 4.0×10^5 CFU/g - 5.4×10^6 CFU/g (TVC) and 1.1×10^3 CFU/g - 3.5×10^3 CFU/g (TFC). Studies on the microbial quality of farmed and fish harvested from the wild have shown marked differences in count, depending on the location, the aquaculture condition, and other factors that influence microbial proliferation, which preponderance of the studies substantiating that the microbial loads were margin of good quality [20,21]. Dillon et al [9] established that smoking reduced the microbial load of fish, but the number eventually increased in the course of conveyance and stowage.

The bacteria isolated from the fish samples were identified as *B. cereus*, *E. coli*, *Staphylococcus aureus*, *S. epidermidis* and *Streptobacillus* spp. The presence of these bacteria can be attributed mainly to unhygienic processing, as the use of open drums in available outdoor spaces suggest; poor handling by processors as well as vendors who display the smoked fish in open trays under equally unsatisfactory sanitary conditions, since the control samples processed in open kiln firewood smoke, and properly wrapped, had none of the bacteria detected in the other samples. Olayemi et al [4] demonstrated that by using special kilns fabricated by Nigerian Stored Products Research Institute, smoked *Clarias gariepinus* met acceptable standard, by the absence of potential and common pathogens associated with fish. According to Lyhs [22] bacteria such as *B. cereus*, *E. coli*, *S. aureus* and *S. epidermidis* in fish are not likely of endogenous origin but from the environment fish are exposed to.

S. aureus and *E. coli* are commonly isolated pathogens from smoked *Clarias gariepinus*, claimed Daramola et al. [20]. Both bacteria are linked to food-borne diseases of varying severities [15]. *E. coli* is an enteric pathogen and its presence in the fish samples suggest very poor hygiene, with likelihood to cause gastrointestinal disease [23,24]. Dalsgaard et al. [25] averred that the presence of *E. coli* in fish and fishery product should straightforwardly be seen as an indication of unacceptable hygiene and sanitation during processing. *Streptobacillus* spp is rare food-borne pathogen well known to cause the rat bite fever in human [26]. *S. aureus* cause food poisoning by its toxins which are resistant to heat, and could cause discomfort when ingested in large number [19,23]. *Bacillus cereus* are spore formers that also produces toxins resistant to heat [23] and can causes two distinct food poisoning syndromes such as emetic and diarrhoea syndrome.

The fungal isolated from the fish samples were identified as *Aspergillus fumigatus*, *Aspergillus terreus*, *Candida* spp and *Mucor* spp. Several studies to ascertain the microbial composition of smoked fish sold in Nigeria, have equally reported the presence *Aspergillus fumigatus*, *Aspergillus terreus*, *Candida* spp and *Mucor* spp among spoilage fungi [11-14,27]. *Candida* spp and *Mucor* spp are common food spoilage fungi [12-14,27]. *A. fumigatus* and *A. terreus* are common environmental moulds. Though *A. fumigatus* and *A. terreus* are not common cause of aspergillosis, they are opportunistic pathogen of concern for

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immunocompromised persons [28]. *A. fumigatus* is a thermotolerant mold [29] and this could explain its presence in smoked fish beside possible contamination during storage and marketing, since its conidia usually high in the environment. Both *A. fumigatus* and *A. terreus* identified in this study do not produces mycotoxins, so the concern for their presence in food is mainly owing to their opportunistic pathogenicity.

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Conclusion

This study provided evidence that fish samples sold in most of the markets in Yenagoa had poor microbiological quality in terms of microbial load and the presence of pathogens. Consumption of the processed fish will put consumers at risk of staphylococcal and mycotoxin food poisoning and diarrheic infection by *E. coil*.

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