

Bacteria and Fungi associated with Smoked Tilapia Fish sold in Kure Market Minna Niger State Nigeria

Abstract

Fish is a vital source of animal protein and a widely consumed food item in Nigeria due to its availability and nutritional value. This study assessed the microbial load and diversity associated with smoked tilapia fish sold in Kure Market Minna, Niger State, Nigeria. Samples were collected and analyzed using standard microbiological methods. The results revealed a mean bacterial colony count ranging from 120 ± 13.03 to 189 ± 11.03 CFU/ml across different parts of the fish. Seven bacterial species were isolated, including three Gram-positive bacteria (*Staphylococcus aureus*, *Micrococcus sp.*, and *Bacillus sp.*) and four Gram-negative bacteria (*Escherichia coli*, *Proteus sp.*, *Salmonella sp.*, and *Pseudomonas sp.*). Additionally, four fungal species were identified: *Aspergillus fumigatus*, *Aspergillus flavus*, *Penicillium sp.*, and *Aspergillus niger*. The presence of these microorganisms is of significant public health concern, as several of the identified species are known to cause food spoilage and poisoning. These findings underscore the need for improved handling, processing, and storage practices to ensure the safety of smoked fish and protect consumer health.

Keywords; Smoked fish; Tilapia, Bacteria, Fungi, Kure Market, Minna

Introduction

Fish constitutes an important source of protein intake of many people, particularly in the developing countries. In Nigeria, where about 41% of the total animal protein intake is obtained from fishery products, the total fish consumption rate has risen to 2.66 million metric tons annually [1, 2]. Fish is a major source of animal protein and an essential food item in the diet of Nigerians because it is relatively cheaper than meat [1,3]. Fish contains most of the important essential amino acids, particularly, lysine, methionine and tryptophan that are lacking in plant proteins [1]. It is also an important source of vitamins and minerals which are important for good living [4]. Fishes are perishable food which are prone to fast spoilage and its spoilage are caused by enzymatic and bacteria breakdown of the flesh, especially in the tropic region, which is characterized with high temperature [5]. There is a need to preserve fish after capturing both from wild and artificial environments to preserved loss due to spoilage and the major process of preservation employed in the tropics is smoking [6].

Technically smoking is the process in which meat or fish flesh are exposed to thermal combustion of wood and it penetrates their flesh [7]. The quality of preserved fish also depends on the smoking procedure employed as fish smoking using wood for its preservation purpose dates back to civilization [2,6,7]. Smoking of fish gives the fish and its product a desirable taste and it also extends its shelf-life using its anti-bacterial and oxidative effects, lowering of pH, the drying process and acting as an antagonist to spoilage agents [6]. Fish contamination caused by microorganisms often results in food spoilage causing life-threatening health implications like food poisoning [8]. Prevention thus helps in the preservation of food quality and public health enhancement Fish and other food sources are considered to be microbiologically unsafe owing to the presence of microorganisms such as bacteria and fungi which can invade the human system and cause harm by producing toxins [9].

In many developing nations Nigeria Inclusive, smoked fish are usually hawked without taking cognizance of the microbial contamination from the environment. In Nigeria, smoked fish products could be contaminated with microorganisms from the processing units and the market centers before reaching the consumers because many processors and hawkers usually display them openly in a manner that could be potential sources of microbial contamination. Thus, this study is designed to assess the microorganisms associated with

Smoked Tilapia Fish sold in Minna Niger State Nigeria with a view of assessing the level of their public health importance.

Material and methods

Sample collection

A total of forty (40) samples of smoked Tilapia were purchased from fish traders in New Market popularly called Kure Market. Minna is the capital city of Niger State which is located in North-Central Eco region of Nigeria, which is characterized with two distinct season i.e the wet and dry season [10]. The study area is The fish samples were randomly purchased from the fish sellers. The fish samples collected were packed and covered in a sterile new polyethylene bag. The collected samples were transported to the laboratory for further analysis.

Sample preparation

In the laboratory, the fish samples were crushed separately into a smaller fraction using a mortar and the crushed samples were homogenized with the aid of a blender. For the Microbial analysis, 1.0 g of the crushed fish part samples were poured and mixed thoroughly in 9 ml of sterile water. The ten-fold serial dilution method was employed using sterile distilled water and 1 ml of the desired dilution levels were plated in duplicates on a Petri dish containing prepared media using the pour plate method [11].

Media preparation and inoculation of sample

28g of Nutrient agar was dissolved in 1000ml of distilled water and mixed till the suspension was uniformed, the media was heat to boiling point to dissolve the medium completely, the medium was sterilized by autoclaving at 121 °C for 15minutes. The media was dispensed accordingly in a sterilized Petri-dish and the Petri dishes was inverted to prevent condensation, droppings from the lid of the plate onto the surface of the media was allowed to gel by cooling, all the Petri dishes were carefully labeled. 1 ml of the samples was taken with the aid of a pipette from the serial diluted samples and was place over a prepared solidify nutrient, agar in a Petri dishes. The Petri dishes was incubated at 37 °C for 24hrs [11]. Each bacteria colonies that appeared on the culture plates were counted with the aid of a colony counter and recorded as a colony-forming unit (cfu/g). All isolates were sub-cultured and transferred to a slant media to obtain a pure culture where a gram-staining and other biochemical test was, catalase test, coagulase test, methyl red test, indole test, citrate

utilization and Sugar fermentation test were conducted to identify the isolates based on the method described by Cheesbrough [11].

Whilst for fungi isolation, the water sample from each sampling station was placed on sterile Potato Dextrose Agar (PDA) (Accumix(R) -Tulip Diagnostics (P) Ltd). Amended with tetracycline (100mg/kg) to prevent incubation of bacteria and inoculated at $28.00 \pm 2.00^\circ\text{C}$ for 5 days before sub-culturing. Pure culture of the different fungal species isolated were morphologically characterized following established procedures of Willoughby [12]. The isolates were macroscopically studied when each colony was stained with 0.05% Trypan blue in Lacto-phenol

Data Analyses

The data obtained were presented in mean \pm standard deviation. Data were analysed using the descriptive statistic function of Paleontological statistic software (PAST Version 4.05)

Results

The mean bacteria colony count of sampled smoked Tilapia fish sold in Kure Market Minna Niger State Nigeria is presented in Table 1. Mean bacteria colony was highest (189 ± 11.03) in the muscle of the sampled Tilapia, followed by the head (154 ± 10.30) and lowest mean colony (120 ± 13.03) was observed in the tails of the sampled Tilapia fish. The biochemical characteristics of the bacteria isolated from smoked Tilapia fish sold in Kure Market Minna Niger State Nigeria is presented in table 2. A total of seven (7) bacteria species were isolated from the smoke Tilapia fish samples collected. Which are *Staphylococcus aureus*, *Escherichia coli*, *Proteus* sp., *Micrococcus* sp., *Bacillus* sp., *Salmonella* sp., and *Pseudomonas* sp. The bacteria species comprises of three (3) gram positive bacteria (*Staphylococcus aureus*, *Micrococcus* and *Bacillus* sp) and four gram negative bacteria (*Escherichia coli*, *Proteus* sp., *Salmonella* sp., and *Pseudomonas* sp.)

The fungi species isolated from smoked Tilapia fish sold in Kure Market Minna Niger State Nigeria is presented in Table 3. A total of four (4) fungi species were isolated which are *Aspergillus fumigatus*, *Aspergillus fumigatus* *Penicillium* sp. and *Aspergillus niger*

Table 1: Mean Bacteria Colony count of sampled Smoked Tilapia Fish sold in Kure Market Minna Niger State Nigeria

Fish part	Mean colony	Colony form per ml (CFU/g)
Head	154 ± 10.30	1.54 ± 10.30 × 10 ⁻⁴
Muscle	189 ± 11.03	1.89 ± 11.03 × 10 ⁻⁴
Tail/fin	120 ± 13.03	120 ± 13.03 × 10 ⁻⁴

Table 2: Biochemical characteristic of bacteria isolated from smoked Tilapia fish sold in Kure Market Minna Niger State Nigeria

Fish Part	Gram reaction	Shape	Catalase	Oxidase	Urease	Citrate	Methyl red	Indole	Sucrose	Glucose	Lactose	Probable organisms
Head	+	Cocci	+	-	+	+	+	-	+	+	+	<i>Staphylococcus aureus</i>
	-	Rod	+	-	-	-	+	-	+	+	+	<i>Escherichia coli</i>
	-	Rod	+	-	+	-	+	-	-	+	-	<i>Proteus</i> sp.
	+	Cocci	+	-	+	-	-	-	-	-	-	<i>Micrococcus</i> sp.
Muscle	+	Cocci	+	-	+	+	+	-	+	+	+	<i>Staphylococcus aureus</i>
	-	Rod	+	-	-	-	+	-	+	+	+	<i>Escherichia coli</i>
	-	Rod	+	-	+	-	+	-	-	+	-	<i>Proteus</i> sp.
	+	Cocci	+	-	+	-	-	-	-	-	-	<i>Micrococcus</i> sp.
	+	Rod	+	+	+	+	+	-	+	+	+	<i>Bacillus</i> sp.
	-	Rod	+	-	-	-	+	-	-	+	-	<i>Salmonella</i> sp.
	-	Rod	+	-	+	-	+	-	+	+	+	<i>Pseudomonas</i> sp.
Tail	+	Rod	+	+	+	+	+	-	+	+	+	<i>Bacillus</i> sp.
	-	Rod	+	-	-	-	+	-	-	+	-	<i>Salmonella</i> sp.
	-	Rod	+	-	+	-	+	-	+	+	+	<i>Pseudomonas</i> sp.
	+	Cocci	+	-	+	+	+	-	+	+	+	<i>Staphylococcus aureus</i>
	-	Rod	+	-	-	-	+	-	+	+	+	<i>Escherichia coli</i>

Table 3: Fungi species isolated from smoked Tilapia fish sold in Kure Market Minna Niger State Nigeria

Fish part	Macroscopic Appearance	Microscopic Appearance	Probable Isolate
Head	Green in color with light green pigmentation	Septae hyphae, divided by cross wall called septa, Conidiospores	<i>Aspergillus fumigatus</i>
	Black in colors with white pigmentation	Conidial heads are black and radiated conidia are globule to subglobose brown with irregular ridges	<i>Aspergillus niger</i>
Muscle	Blue ash in colour with pigmentation	Chains of single celled conida are produced in basipetal succession from a specialized conidiogenous cell called a phialide	<i>Penicillium</i> sp
	Green in color with light green pigmentation	Septae hyphae, divided by cross wall called septa, Conidiospores	<i>Aspergillus fumigatus</i>
	Black in colors with white pigmentation	Conidial heads are black and radiated conidia are globule to subglobose brown with irregular ridges	<i>Aspergillus niger</i>
Tail/Fin	Black in colors with white pigmentation	Conidial heads are black and radiated conidia are globule to subglobose brown with irregular ridges	<i>Aspergillus niger</i>

Yello-green in colour with light green pigmentation	Septate hyphae, divided by cross wall called septa conidiospores	<i>Aspergillus flavus</i>
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Discussion

This study revealed seven bacterial species and four fungal species in smoked fish samples, with considerable changes in microbial loads throughout different regions of the fish. The bacterial isolates included *Staphylococcus aureus*, *Escherichia coli*, *Proteus sp.*, *Salmonella sp.*, *Pseudomonas sp.*, *Micrococcus sp.*, and *Bacillus sp.*, with the highest colony count observed in the fish muscle (189 ± 11.03 CFU/ml), followed by the head (154 ± 10.30 CFU/ml) and the tail (120 ± 13.03 CFU/ml). This pattern fits with previous studies, of Ayelaja et al. [9], who attributed increased microbial loads in muscle tissue to its high moisture content, creating a favorable environment for bacterial multiplication.

The presence of *Escherichia coli* and *Salmonella sp.* is particularly problematic due to their link with foodborne diseases. *E. coli*, especially *enterotoxigenic* (ETEC) and *enterohemorrhagic* (EHEC) strains, can cause serious gastrointestinal illnesses through toxin production. For instance, EHEC strains, such as O157:H7, release Shiga toxin, leading to bloody diarrhea and potentially life-threatening consequences such as hemolytic uremic syndrome [13, 14, 15]. *Salmonella sp.*, including *S. Enteritidis* and *S. Typhi*, offers considerable dangers, with *S. Typhi* producing typhoid fever and non-typhoidal *Salmonella* contributing to gastroenteritis [14]. These findings underscore the necessity for safe food handling, adequate cooking, and enhanced sanitation to decrease contamination risks [16, 17]. The presence of these pathogens in smoked tilapia fish indicates probable fecal contamination, possibly introduced during handling or processing. Akinwumi & Adegbehingbe [2] similarly observed *E. coli* and *Salmonella sp.* in smoked fish from Ondo State, Nigeria, attributing contamination to unsanitary methods and environmental exposure.

The investigation also discovered *Staphylococcus aureus* and *Pseudomonas sp.*, which are significant pollutants influencing food safety and quality. *S. aureus* is particularly harmful because of its ability to create heat-stable enterotoxins that stay active even after cooking, causing staphylococcal food poisoning characterized by nausea, vomiting, and diarrhea [18]. *Pseudomonas sp.*, though not normally pathogenic, is a prominent spoiling bacteria in perishable foods, resulting in sensory and economic losses through slime

generation, discolouration, and off-odors [19]. The presence of these bacteria suggests post-processing contamination, likely due to inappropriate handling and inadequate storage conditions [20, 21]. These findings are consistent with Ibrahim et al. [6], who identified comparable bacterial profiles in smoked catfish marketed in Bida, Nigeria, attributing contamination to inadequate market cleanliness.

The fungal isolates, including *Aspergillus fumigatus*, *A. niger*, *Penicillium sp.*, and *A. flavus*, further underline the hazards associated with smoked smoked tilapia fish. The prevalence of *Aspergillus species* is similar with Nwachukwu & Madubuko [5], who observed substantial fungal contamination in smoked fish stored under suboptimal conditions. Of particular concern is *A. flavus*, which produces aflatoxins carcinogenic chemicals associated to liver cancer, especially in locations with heavy fish consumption [22]. The discovery of *Penicillium sp.* also implies poor storage conditions, as these fungi flourish in wet environments [23]. This fungal species have also been reported in water sample subjected to different anthropogenic activities [24]

The measured microbial load (120–189 CFU/g) in this study exceeds the values reported by Yusuf & Hamid [25] in Bauchi Metropolis, demonstrating regional disparities in hygiene practices and environmental factors. Contamination likely happens at numerous locations along the supply chain, including processing, storage, and market display. Traditional smoking procedures, using open fires and exposure to ambient contaminants, may introduce germs and fungi into the fish. Additionally, the practice of exposing fish publicly at marketplaces exposes them to dust, insects, and other sources of infection. Addressing these concerns requires a holistic strategy, including improved sanitary methods, increased storage conditions, and consumer education on food safety [6].

The ramifications of microbial contamination in smoked fish are extensive, offering serious public health and economic issues. Pathogenic bacteria such as *E. coli* and *Salmonella sp.* can cause illnesses ranging from mild gastroenteritis to life-threatening infections, while aflatoxins produced by *A. flavus* entail long-term health hazards [26]. Economically, deterioration induced by microbial contamination affects the shelf life and marketability of smoked fish, leading to financial losses for vendors and processors. Persistent contamination may decrease customer faith in smoked salmon as a safe protein source, further harming livelihoods.

Conclusion and recommendation

The smoked tilapia fish sold in Kure Market in Minna, Niger State, Nigeria, had a high level of microbiological contamination, according to this study. Four fungal species (*Aspergillus fumigatus*, *Aspergillus flavus*, *Penicillium sp.*, and *Aspergillus niger*) and seven bacterial species (*Staphylococcus aureus*, *Escherichia coli*, *Proteus sp.*, *Micrococcus sp.*, *Bacillus sp.*, *Salmonella sp.*, and *Pseudomonas sp.*) were detected. The fish muscle had the largest microbial load, most likely as a result of its high moisture content. While aflatoxin-producing fungus like *Aspergillus flavus* raise worries about long-term health effects, the presence of pathogenic bacteria like *Salmonella sp.* and *E. coli* poses major health dangers, including foodborne diseases. These results highlight the pressing need for actions to enhance food safety procedures in the handling and processing of smoked fish. There is an urgent need to enhance sanitation procedures in order to address the high level of microbiological contamination found in smoked tilapia fish sold in Kure Market.

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