# Microbial Contamination of Smoked Tilapia Fish Sold in Kure Market, Minna, Niger State, Nigeria

### Abstract

This study assessed the microbial load and diversity in smoked tilapia fish sold in Kure Market, Minna, Niger State, Nigeria. A total of 40 samples were analyzed using standard microbiological methods. The results revealed a mean bacterial colony count ranging from  $120 \pm 13.03$  to  $189 \pm 11.03$  CFU/ml across different parts of the fish. Seven bacterial species, including pathogenic strains such as *Salmonella sp.* and *Escherichia coli*, were isolated alongside four fungal species, including *Aspergillus flavus*, a known producer of aflatoxins. The presence of these microorganisms poses serious public health risks, highlighting the urgent need for improved fish handling, processing, and storage practices to safeguard consumer health. 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 for 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 (Adamu et al., 2022; Akinwumi & Adegbehingbe, 2015). 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 (Adamu et al., 2022; Mohammed et al., 2019). Fish contains most of the important essential amino acids, particularly, lysine, methionine and tryptophan that are lacking in plant proteins (Adamu et al., 2022). It is also an important source of vitamins and minerals, which are important for good living (Abolagba & Melle, 2008). Fish are perishable food which are prone to fast spoilage and its spoilage are caused by enzymatic and bacterial breakdown of the flesh, especially in the tropic region, which is characterized with high temperature (Nwachukwu & Madubuko, 2010). There is a need to preserve fish after capturing both from wild and artificial environments to preserve loss due to spoilage and the major process of preservation employed in the tropics is smoking (Ibrahim et al., 2022).

Smoking is the process in which meat or fish flesh is exposed to thermal combustion of wood, and it penetrates their flesh (Simko, 1991). 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 (Akinwumi & Adegbehingbe, 2015; Ibrahim et al., 2022; Simko, 1991). Smoking of fish gives the fish and its product a desirable taste, and it also extends its shelf life using its antibacterial and oxidative effects, lowering the pH, the drying process, and acting as an antagonist to spoilage agents (Ibrahim et al., 2022). Fish contamination caused by microorganisms often results in food spoilage, causing life-threatening health implications like food poisoning (Yusuf & Hamid, 2017). 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 (Ayeloja et al., 2018).

In many developing nations, Nigeria included, 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 a potential source 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 to assessing the level of their public health importance.

#### Methodology

#### 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 the North-Central Eco region of Nigeria, which is characterized by two distinct seasons, i.e., the wet and dry seasons (Mohammed et al., 2021). 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

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 distilled 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 (Cheesbrough, 2006).

#### Media preparation and inoculation of sample

28 g of nutrient agar was dissolved in 1000 ml of distilled water and mixed till the suspension was uniform. The media was heated to boiling point to dissolve the medium completely, and the medium was sterilized by autoclaving at 121°C for 15 minutes. The media was dispensed accordingly in a sterilized Petri dish, and the Petri dishes were inverted to prevent condensation; droppings from the lid of the plate onto the surface of the media were allowed to gel by cooling; and all the Petri dishes were carefully labeled. 1 ml of the samples was taken with the aid of a pipette from the serially diluted samples and was placed over a prepared solidified nutrient, agar, in a Petri dish. The Petri dishes were incubated at 37°C for 24 hrs (Cheesbrough, 2006). Each bacterial colony that appeared on the culture plates was 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 tests were conducted to identify the isolates based on the method described by Cheesbrough (2006).

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 (100 mg/kg) to prevent incubation of bacteria and inoculated at  $28.00 \pm 2.00^{\circ}$ C for 5 days before subculturing. Pure cultures of the different fungal species isolated were morphologically characterized following procedures described by Senanayake (2020). 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 analyse 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  $(189 \pm 11.03)$  in the muscle of the sampled Tilapia, followed by the head  $(154 \pm 10.30)$  and lowest mean colony  $(120 \pm 13.03)$  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., *Salmonella*

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 1	Tilapia I	Fish sold i	n 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 <sup>-4</sup>
Muscle	189 ± 11.03	$1.89 \pm 11.03 \times 10^{-4}$
Tail/fin	120 ± 13.03	$120 \pm 13.03 \times 10^{-4}$

Table 2: Biochemical characterist	ic of bacteria isolated	l from smoked Tilapia fisl	h sold in Kure
Market Minna Niger State Nigeria			
Market Minna Niger State Nigeria			

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

	+	Rod	+	+	+	+	+	-	+	+	+	Bacillus sp.
	-	Rod	+	-	-	-	+	-	-	+	-	Salmonella sp.
	-	Rod	+	-	+	-	+	-	+	+	+	Pseudomonas sp.
Tail	+	Rod	+	+	+	+	+	-	+	+	+	Bacillus sp.
	-	Rod	+	-	-	-	+	-	-	+	-	Salmonella sp.
	-	Rod	+	-	+	-	+	-	+	+	+	Pseudomonas sp.
	+	Cocci	+	-	+	+	+	-	+	+	+	Staphylococcus aureus
	-	Rod	+	-	-	-	+	-	+	+	+	Escherichia coli

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

	brown with irregular ridges	
Yello-green in colour with	Septatehyphae, divided by	Aspergillus flavus
light green pigmentation	cross wall called septa	
	conidospores	

#### 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  $\pm$  11.03 CFU/ml), followed by the head (154  $\pm$  10.30 CFU/ml) and the tail (120  $\pm$  13.03 CFU/ml). This pattern aligns with previous studies of Ayeloja et al. (2018), 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 (Senanayake et al., 2020; Joseph et al., 2020; Mohammed et al., 2023). *Salmonella sp.*, including *S. Enteritidis* and *S. Typhi*, offers considerable dangers, with *S. Typhi* producing typhoid fever and non-typhoidal *Salmonella* contributing to gastroenteritis (Joseph et al., 2020). These findings underscore the necessity for safe food handling, adequate cooking, and enhanced sanitation to decrease contamination risks (Gourama, 2020; Bhat et al., 2022). The presence of these pathogens in smoked tilapia fish indicates probable fecal contamination, possibly introduced during handling or processing. Akinwumi & Adegbehingbe (2015) 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 (Abdallah & Sulieman, 2024). *Pseudomonas sp.*, though not normally pathogenic, is a prominent spoiling bacteria in perishable foods, resulting in sensory and economic losses through slime generation, discoloration, and off-odors (Krell & Matilla, 2024). The presence of these bacteria suggests post-processing contamination, likely due to inappropriate handling and inadequate storage conditions (Sheng & Wang, 2021; Quintieri et al., 2021). These findings are consistent with Ibrahim et al. (2022), 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 tilapia fish. The prevalence of *Aspergillus species* is similar to Nwachukwu & Madubuko (2010), who observed substantial fungal contamination in smoked fish stored under suboptimal conditions. Of particular concern is *A. flavus,* which produces aflatoxins, carcinogenic chemicals associated with liver cancer, especially in locations with heavy fish consumption (Benkerroum, 2020). The discovery of *Penicillium sp.* also implies poor storage conditions, as these fungi flourish in wet environments (Smiri et al., 2021). This fungal species has also been reported in water samples subjected to different anthropogenic activities (Adamu et al., 2022).

The measured microbial load (120–189 CFU/g) in this study exceeds the values reported by Yusuf & Hamid (2017) 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 (Ibrahim et al., 2022).

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 (Afreen & Bağdatlı, 2021). 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

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* raises worries about long-term health effects, the presence of pathogenic bacteria like *Salmonella sp.* and *E. coli* poses major health dangers, including foodborne diseases.

#### Recommendation

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.

# **Disclaimer (Artificial intelligence)**

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

# Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

- <mark>1.</mark> 2.
- <u>3.</u>

# Reference

- Abdallah, E. M., & Sulieman, A. M. E. (2024). Staphylococcus aureus. *Microbial Toxins in Food Systems: Causes, Mechanisms, Complications, and Metabolism*, 235.
- Abolagba, O. J., & Melle, O. O. (2021). Chemical composition and keeping qualities of a scaly fish tilapia, Oreochromis niloticus smoked with two energy sources. *African Journal of General Agriculture*, 4(2).
- Adamu KM, Aliyu-Paiko M, Mohammed YM, Adebola TT, Hafsat M, Iloba KI (2022)
  Bacteria and fungi analyses of fish diets with grasshopper and cockroach meals: the potential replacement of fishmeal in fish diets. *Journal of Fisheries* 10(2): 102203.
  DOI: 10.17017/j.fish.324

- Adamu, K. M., Mohammed, Y. M., Ibrahim, U. F., Abdullahi, I. L., & Jimoh, Y. O. (2022).
  Assessment of some physical, chemical and biological parameters of Lake Dangana, Niger State, Nigeria. *Zoologist (The)*, 20(1), 133-140.
- Afreen, M., & Bağdatlı, İ. (2021). Food-borne pathogens in seafood. Eurasian Journal of Agricultural Research, 5(1), 44-58.
- Akinwumi, F.O. & Adegbehingbe, K.T. (2015). Microbiological Analysis of Three of Smoked Fish Obtained from the Ondo State, Nigeria. *Food and Public Health*, 5(4): 122-126 DOI: 10.5923/j.fph.20150504.04
- Ayeloja, A. A., George, F. O. A., Jimoh, W. A., Shittu, M. O., & Abdulsalami, S. A. (2018).
  Microbial load on smoked fish commonly traded in Ibadan, Oyo State, Nigeria. *Journal of Applied Sciences and Environmental Management*, 22(4), 493-497.
- Benkerroum, N. (2020). Aflatoxins: Producing-molds, structure, health issues and incidence in Southeast Asian and Sub-Saharan African countries. *International journal of environmental research and public health*, 17(4), 1215.
- Bhat, K. A., Manzoor, T., Dar, M. A., Farooq, A., Allie, K. A., Wani, S. M., ... & Shah, A. A. (2022). Salmonella infection and pathogenesis. In *Enterobacteria*. IntechOpen.
- Cheesbrough, M. (2006). *District laboratory practice in tropical countries, part* 2. Cambridge university press.
- Gourama, H. (2020). Foodborne pathogens. In *Food safety engineering* (pp. 25-49). Cham: Springer International Publishing.
- Govindarajan, D. K., Viswalingam, N., Meganathan, Y., & Kandaswamy, K. (2020). Adherence patterns of Escherichia coli in the intestine and its role in pathogenesis. *Medicine in Microecology*, 5, 100025.
- Ibrahim, M. I., Ibrahim, Y., Abdullahi, A. M., Najibullah, B. A, Obi, P. U. & Mohammed, Y. M. (2022). Bacteria associated with smoked catfish sold at Bida Modern market, Northcentral Nigeria. *International Journal of Fisheries and Aquatic Studies*, 2022; 10(2), 38-40. <u>https://doi.org/10.22271/fish.2022.v10.i2a.2654</u>
- Joseph, A., Cointe, A., Mariani Kurkdjian, P., Rafat, C., & Hertig, A. (2020). Shiga toxinassociated hemolytic uremic syndrome: A narrative review. *Toxins*, *12*(2), 67.

- Krell, T., & Matilla, M. A. (2024). Pseudomonas aeruginosa. *Trends in microbiology*, 32(2), 216-218.
- Mohammed, H., Adamu, K.M., Kasim, A.A., Mohammed, Y.M. & Dadi-Mamud, N.J. (2023). Bacteriological Analysis of Water from Emu-Stream, Kusotachin, Nigeria. *Lapai Journal of Applied and Natural Sciences*, 8(1): 38-44
- Mohammed, Y. M., & Adamu, K. M. (2019). Bacteria associated with some freshwater fishes in dangana lake Lapai, Nigeria. *Jewel journal of scientific research*, 4(1&2), 83-90.
- Mohammed, Y. M., Arimoro, F. O., Ayanwale, A. V., Adamu, K. M., Keke, U. N., Abubakar, M. D., & Achebe, A. C. (2021). The current state of water quality and benthic invertebrate fauna in Chikke Stream (North-Central Nigeria). Ukrainian Journal of Ecology, 11(3), 26-34.
- Nwachukwa, V. N., & Madubuko, C. U. (2010). Microflora associated with processing and storage of the white catfish (Chrysichthys nigrodigitatus).
- Quintieri, L., Caputo, L., Brasca, M., & Fanelli, F. (2021). Recent Advances in the Mechanisms and Regulation of QS in Dairy Spoilage by Pseudomonas spp. *Foods*, *10*(12), 3088.
- Senanayake, I. C., Rathnayaka, A. R., Marasinghe, D. S., Calabon, M. S., Gentekaki, E., Lee,
  H. B., & Xiang, M. M. (2020). Morphological approaches in studying fungi: Collection, examination, isolation, sporulation and preservation. *Mycosphere*, 11(1), 2678-2754.
- Sheng, L., & Wang, L. (2021). The microbial safety of fish and fish products: Recent advances in understanding its significance, contamination sources, and control strategies. *Comprehensive Reviews in Food Science and Food Safety*, 20(1), 738-786.
- Simko, P. (1991). Changes of benzo (a) pyrene contents in smoked fish during storage. *Food chemistry*, 40(3), 293-300.
- Smiri, M., Kheireddine, A., Hammami, R., Rouissi, M., Espeso, E. A., & Sadfi-Zouaoui, N. (2021). An assessment of the air quality in apple warehouses: new records of Aspergillus europaeus, Aspergillus pulverulentus, Penicillium allii and Penicillium sumatraense as decay agents. Archives of Microbiology, 203, 5975-5992.

- Yusuf, M. A., & Hamid, T. A. (2017). Isolation and identification of bacteria in retailed smoked fish, within Bauchi Metropolis. *Journal of Pharmacy and Biological Sciences*, 3(1), 01-05.
- Yusuf, M. A., & Hamid, T. A. (2017). Isolation and identification of bacteria in retailed smoked fish, within Bauchi Metropolis. *Journal of Pharmacy and Biological Sciences*, 3(1), 01-05.

UNDER PER MILLIN