Fungal Spoilage of Smoked-Dried Fish in Okitipupa, Ondo State, Nigeria

Running Title: Mycotic Spoilage of Nigerian Smoked Fish

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ABSTRACT

This study aimed to identify the major fungi associated with the spoilage of smoked fish sold in Okitipupa Market, Ondo State, Nigeria, and to assess their public health implications. Smoked fish samples of Oreochromis niloticus, Gadus morhua, and Scomber scombrus were purchased, stored under ambient conditions, and examined microbiologically after visible spoilage. Fungal isolates were obtained using standard culturing techniques and characterized morphologically. All samples exhibited spoilage within 72 hours, with total fungal counts ranging from 3.5×10^4 to 8.7×10^6 cfu/g, and only filamentous moulds were detected. O. niloticus showed the highest fungal load, while G. morhua had the least. The predominant spoilage fungi identified included species of Aspergillus, Chaetomium, and Penicillium, indicating that smoked fish sold in the area may harbour potentially toxigenic moulds. These findings underscore the need for proper handling, hygienic processing, and adequate storage to prevent fungal contamination and safeguard consumer health.

Keywords: *Fungi, Smoked Dried Fish, Fish Spoilage, Oreochromis niloticus, Scomber scombrus, Gadus morhua, Mycotoxins, Public Health and Food Safety*

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INTRODUCTION

The burden of malnutrition remains a persistent challenge in low and middle-income countries, and fish have the capacity to alleviate this problem (Hasselberg, 2020). Fish is a globally preferred seafood and serves as a primary source of high-quality protein in Nigeria (Anihouvi et al., 2019; Okunlola, 2011). It accounts for about 60% of the total protein intake among Nigerians (Samuel et al., 2015). Fish muscle, which contains approximately 15–25% of total protein, provides essential macronutrients and micronutrients, such as proteins, fats, vitamins, and minerals that are indispensable for human metabolic activities (Ryu et al., 2021; Ahmed et al., 2022). It also represents a vital source of micronutrients in low- and middle-income nations (Hasselberg, 2020). Fish and fish oil are the principal sources of omega-3 fatty acids, essential nutrients that humans cannot synthesize and must obtain through diet (Dhillon, 2025; Demets & Foubert, 2021). The low cholesterol content and presence of beneficial fatty acids have contributed to the growing consumption of fish across various cultural and socio-economic groups (Samuel et al., 2015; Anihouvi et al., 2019; Sani et al., 2016).

Fish is, however, highly perishable due to its biochemical composition, necessitating effective preservation methods (Samuel et al., 2015). Smoking is one of the most common traditional preservation techniques used to enhance flavour, inhibit microbial growth, and extend shelf life (Wogu et al., 2011; Racioppo et al., 2023). Despite consumer preferences for fresh fish, smoked and dried products remain widely popular across Nigeria (Liverpool-Tasie et al., 2021). The smoking process dehydrates fish, reduces enzymatic activity, and imparts desirable sensory characteristics. However, smoked fish products may still pose health risks if improperly processed or stored (Rahman & Al-Farsi, 2020).

In tropical countries like Nigeria, smoke-drying remains a traditional method used by fishing communities to preserve fish, enhance flavour, and extend shelf life (Amponsah et al., 2022). This process reduces bacterial spoilage and oxidation; however, contamination can still occur during handling and storage, particularly in unhygienic conditions. Fungal colonisation of smoked or dried fish is particularly concerning, as it not only speeds up spoilage but also poses serious public health risks through potential mycotoxin production (Ikeh et al., 2024; Adeyeye, 2016). Fungal spoilage compromises the nutritional quality and may lead to foodborne illnesses, representing an important yet often overlooked aspect of food safety in fish products. Factors such as poor handling, high humidity, and inadequate storage facilities further promote fungal growth. Additionally, fungal spores can survive harsh conditions, allowing them to persist in the supply chain and contribute to outbreaks of foodborne illness. This study aimed to identify fungi species present in smoked and dried fish samples, emphasizing their relevance for food safety and public health.

The specific objectives of this study were to:

1. Isolate and characterize fungal species contaminating smoked and dried fish sold in selected markets.
2. Determine the prevalence and distribution of fungal genera in relation to fish type and handling conditions.
3. Evaluate the potential public health risks associated with fungal spoilage and possible mycotoxin contamination in smoked fish products.

MATERIALS AND METHODS

Study area and sample collection

This study was conducted at the Okitipupa Market, located in Okitipupa Local Government Area, Ondo State, Nigeria. Three commercially available smoked fish species *Oreochromis niloticus*, *Gadus morhua*, and *Scomber scombrus* were selected for the smoked fish shelf-life study. A total of 10 kg of each species was procured directly from market vendors and was set up in duplicates. Immediately after purchase, the fish samples were transported to the Microbiology Laboratory at Olusegun Agagu University of Science and Technology (OAUSTECH), Okitipupa. Transportation was carried out using sterile polythene bags to minimize contamination. Upon arrival, the samples were stored at ambient conditions, with temperature ranging from approximately 22.9 °C to 33.5 °C (73.2 °F to 92.2 °F) and relative humidity fluctuating between 58% and 86%, with an average of 70%. The samples were monitored under these conditions until visible signs of decomposition were observed.

Sample preparation and serial dilution

The smoked fish samples were monitored over several days to assess spoilage progression. Deterioration was identified based on observable changes in appearance, discolouration, and the development of a disagreeable odour. Once spoilage was evident, the affected samples were subjected to microbiological analysis. Each sample was diluted with sterile 90% normal saline. For each fish species, 10 g of visibly spoiled smoked fish was weighed onto sterile aluminium foil. The samples were macerated in 90 mL of sterile normal saline using a Waring blender to obtain an initial 10^{-1} dilution, following the method described by Teniola and Odunfa (2002). Subsequent serial dilutions were prepared as needed to reach suitable dilution levels for microbial enumeration.

Isolation and enumeration of fungi

Potato Dextrose Agar (PDA; Oxoid CM0139) was prepared according to the manufacturer's instructions. The pour plate technique and standard plate count method, as outlined by Cheesbrough (2005), were employed for fungal isolation and enumeration. Aliquots of appropriately diluted samples were pipetted into sterile Petri dishes. Molten PDA supplemented with penicillin and streptomycin was aseptically poured into each dish and allowed to solidify. All plates were prepared in duplicate and incubated at 28 °C for three days. Daily observations were made, and colony counts were recorded. Distinct fungal colonies were sub-cultured to obtain pure isolates. Identification of fungal species was carried out using morphological characteristics and microscopic examination (wet mount technique), in accordance with standard references including the *Practical Manual of Mycology* (Frey et al., 1979; Funder, 1961).

RESULTS

The microbial count of the specimen

The results obtained from this study indicated spoilage within 72 h for all samples tested. The fungal count of the three species of fish investigated ranged from 3.5 x 10^4 to 8.7 x 10^6 cfu/g. *Oreochromis niloticus* (Tilapia fish) has the highest microbial population (8.7 x 10^6 cfu/g), followed by *Scomber scombrus* (9.6 x 10^4 cfu/g) ('Titus' fish), while the lowest is *Gardus morhua* ('Panla' fish) with a count of 3.5 x 10^4 cfu/g as presented in (Table 1).

Morphological characteristics and the occurrence of fungal isolates

Table 2 illustrates the morphological characteristics of the fungal isolates found in the fish samples studied. The fungal isolates identified include *Aspergillus fumigatus*, *A. niger, A. flavus, Chaetomium* sp. and *Penicillium chrysogenum.* The prevalence of fungi in various fish samples differs in composition and frequency. Occurrence of different fungi in spoiled Panla fish (*Gadus morhua*) is shown in (Table 3), revealing the isolate with the highest number of fungal isolates of four (4) or eighty per cent of all the isolated fish fungal isolate species. Only *Penicillium chrysogenum* was absent in the spoilt 'Panla' fish. However, it was isolated and represented in the 'Titus' fish (*Scomber scombrus*). *Aspergillus fumigatus* and *A. niger* were the other fungi isolated in the same fish. *Aspergillus fumigatus, A. flavus* and *Chaetomium* sp. were the only isolated fungi in 'Tilapia' fish (*Oreochromis niloticus*).

Percentage occurrence of isolated fungal species

The fish, *Gadus morhua* ('Panla'), exhibited the largest concentration of fungal isolates, as previously noted, and was analyzed to influence the percentage occurrence of the isolated fungal species (Figure 1). As depicted, *Aspergillus fumigatus* is the most dominant and accounted for 40% of the isolates, while other fungal isolates of *A. niger*, *A. flavus*, and *Chaetomium* sp. followed at the same levels of 20% each, all occurring in only one spoiled fish sample (*Gardus morhua*).

DISCUSSION

The findings of this investigation indicated that the fungal count of the three fish species examined varied from 3.5 × 10^4 to 8.7 × 10^6 cfu/g. *Oreochromis niloticus* ('Tilapia' fish) exhibited the highest fungal load (8.7 × 10^6 cfu/g), indicating advanced decomposition (ICMSF, 2011; PHLS, 2000; FAO/WHO, 2020), while *Scomber scombrus* ('Titus' fish), falls close to the spoilage threshold., showing moderate fungal activity (Gram & Huss, 1996) and *Gadus morhua* ('Panla' fish) had the **lowest count**, suggesting relatively better preservation or natural resistance to fungal colonization (9.6 × 10^4 cfu/g and 3.5 × 10^4 cfu/g, respectively (Table 1). The variation in fungal load may be linked to the nutritional composition of each species, as higher lipid and protein content can support greater microbial proliferation. Thus, *O. niloticus* likely provided more favourable growth substrates. Similar trends were observed by Ogunyebi et al. (2025), who reported species-dependent fungal loads in smoked *Micromesistius poutassou* in Lagos markets.

All the smoked fish samples exhibited fungal contamination, representing a 100% incidence rate. This aligns with Junaid et al. (2010), who recorded complete contamination among smoke-dried fish samples in Jos, and Akwuobu et al. (2019), who reported contamination rates between 67.6% and 84.8%. Such high microbial prevalence can be attributed to multiple contamination points during handling, smoking, and marketing. Unhygienic drying racks, exposure to moist air, and inadequate packaging create ideal conditions for fungal growth. Dike-Ndudim et al. (2014a) and Dike-Ndudim et al. (2014b) earlier emphasized that poor pre- and post-smoking handling contributes significantly to spoilage. Recent work by Adebami et al. (2024) and Rady (2024) supports these observations, highlighting how inadequate smoke temperature control and ambient humidity accelerate fungal colonization and mycotoxin formation in smoked fish.

Five fungal species were isolated: *Aspergillus fumigatus*, *A. niger*, *A. flavus*, *Chaetomium* sp., and *Penicillium chrysogenum*, all associated with the spoiled samples (Table 4). *A. fumigatus* was predominant (40%), followed by *A. niger*, *A. flavus*, and *Chaetomium* sp. (20% each), while *P. chrysogenum* occurred only in *S. scombrus* (10%). The dominance of *Aspergillus* species suggests airborne contamination from the processing or retail

Table 1: Total fungi count for spoiled fish with interpretation of results (cfu/g).

Fish samples	Mean Fungi Count (cfu/g)	Relative to standard	Interpretation	Spoilage Remark	References
Tilapia fish *(Oreochromis niloticus)*	8.7×10^6	Approximately 87× higher than 1×10^5 limit	Severely spoiled; microbial overgrowth; unsafe for consumption	Very high	ICMSF (2011); PHLS (2000); FAO/WHO (2020).
Panla Fish (*Gardus morhua*)	3.5×10^4	Within marginal range	Early signs of spoilage; short shelf life	Moderate	Gram & Huss (1996).
Titus Fish (*Scomber scombrus*)	9.6×10^4	Near upper spoilage limit	Poor quality; approaching spoilage threshold	High	Gram & Huss (1996).

Table 2: Morphological Characteristics of Fungal Isolates.

Fungal Isolate Code	Macroscopy	Microscopy	Probable Isolate
TLFS3 ,PFS8 ,TiFS9	Gray-green fluffy ,colonies	Long, erect, non-septate conidiophores	*Aspergillus fumigatus*
PFS1, TiFS6	Black colonies	Long, smooth, erect, septate conidiophores	*A. niger*
TLFS5 , PFS7	Light yellow-green and powdery	Long, erect, septate conidiophores	*A. flavus*
TLFS2 , TLPFS4	Brownish black fluffy/hair-like hyphae	Long, erect, septate conidiophores	*Chaetomium sp.*
TiFS	Rapid growing, white and densely woolly fungus	Hyaline, septate, branched hyphae	*Penicillium chrysogenum*

Keys: TLFS: 'Tilapia' Fish Sample (*Oreochromis niloticus*), PFS: 'Panla' Fish Sample; (*Gadus morhua*), TiFS: 'Titus' Fish Sample (*Scomber scombrus*).

Table 3: Occurrence of fungi in different spoiled fish (cfu/g).

Fish samples	*Aspergillus fumigatus*	*A. niger*	*A. flavus*	*Chaetomium sp.*	*Penicillium chrysogenum*
'Tilapia' fish (*Oreochromis niloticus*)	+	-	+	+	-
'Panla' fish (*Gardus morhua*)	+	+	+	+	-
'Titus' fish (*Scomber scombrus*)	+	+	-	-	+

Key: +, Positive; -, Negative

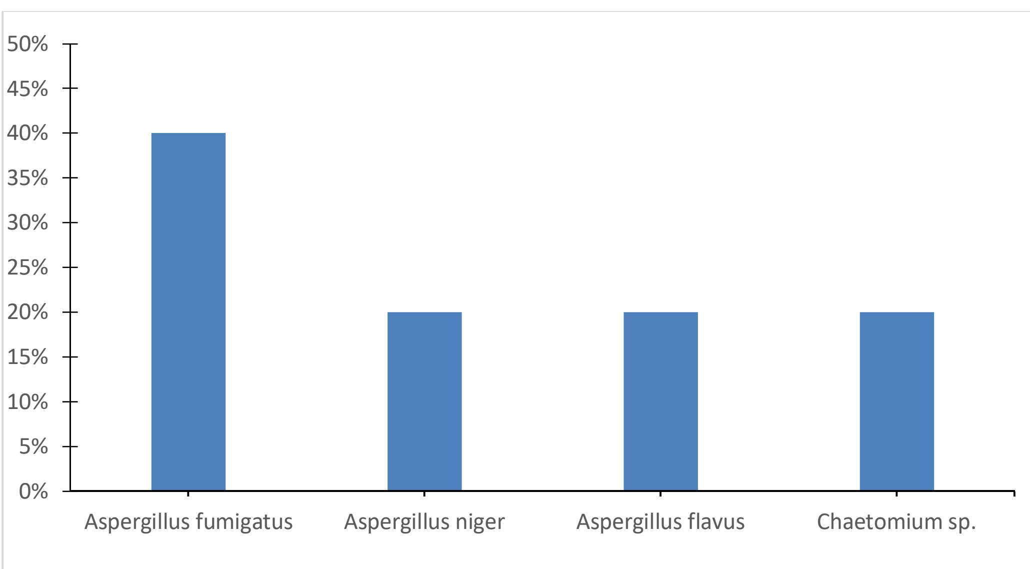

Figure 1: Percentage occurrence of isolated fungal species in spoilt 'Panla fish' (*Gardus morhua*).

environment, as these fungi thrive in tropical humidity and adhere to unsmoked or rehydrated surfaces (Kortei et al., 2022). Their presence poses public health concerns, especially *A. flavus*, known for aflatoxin production, which has been linked to hepatotoxicity and carcinogenic effects (Oni et al., 2020). Comparatively, Junaid et al. (2010) and Sani et al. (2016) also reported heterogeneous fungal growth, corroborating the mixed contamination patterns observed here. To mitigate these risks, adherence to good manufacturing practices, reduced manual handling, and improved packaging, such as vacuum sealing, have been shown to reduce fungal diversity in smoked fish (Ogunyebi et al., 2025).

Conclusion

This study establishes a clear correlation between fungal contamination and the deterioration of smoked-dried fish. The predominant fungal species identified were *Aspergillus fumigatus*, *A. niger*, *A. flavus*, *Chaetomium* spp., and *Penicillium chrysogenum*. The presence of these fungi poses significant public health risks, as they are capable of producing harmful mycotoxins, including aflatoxin B_1, ochratoxin, and zearalenone. These compounds are known to induce a range of toxicological effects, such as hepatotoxicity, nephrotoxicity, and immunosuppression, and may contribute to foodborne illnesses when contaminated fish products are consumed.

Recommendations

In light of the high prevalence of fungal contamination in smoked-dried fish, it is recommended that public health authorities and market regulators intensify awareness campaigns to educate fish vendors and consumers on proper hygiene and handling practices. Such initiatives should emphasize the potential for cross-contamination by spoilage organisms, including those capable of producing mycotoxins. Additionally, future research should explore the antimicrobial resistance profiles of the fungal isolates associated with the spoilage of smoked-dried fish. This will provide deeper insights into their survival mechanisms and support the development of effective control measures to enhance food safety and reduce health risks associated with fungal contamination.

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