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Mycological Assessment and Aflatoxin Contamination of Smoked-Dried Fish from Open Markets

Theresa Ariri [1], Sandra C. Okoye [2], Chidimma R. Chukwunwejim [3], Eddison I. Oghonyon [4], Uchenna I. Chukwurrah [5, 1] and Nzube F. Ekpunobi [3, *]

[1] *Department of Biology, College of Arts and Sciences, Texas A and M University, USA.*
[2] *Department of Biological Sciences, College of Liberal Arts and Sciences, Eastern Illinois University, USA.*
[3] *Department of Pharmaceutical Microbiology and Biotechnology, Nnamdi Azikiwe University Awka, Anambra, Nigeria.*
[4] *Department of Pharmaceutical Microbiology and Biotechnology, David Umahi Federal University of Health Sciences, Nigeria.*
[5] *Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.*

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Abstract

Smoked-dried fish is a vital, affordable protein source in Nigeria, yet traditional processing and storage methods often predispose these products to fungal proliferation and hazardous mycotoxin accumulation. This study aimed to evaluate the mycological quality and aflatoxin levels in smoked-dried fish sold in major markets in Awka, Anambra State.

Thirty-eight samples of smoked-dried fish were randomly collected from local Open markets. Fungal isolation was performed using Potato Dextrose Agar (PDA), with identification based on macroscopic and microscopic morphological characteristics. Total aflatoxin (AF) and aflatoxin B1 (AFB1) concentrations were quantified using Enzyme-Linked Immunosorbent Assay (ELISA).

Fungal contamination was prevalent across all samples, with Aspergillus species being the dominant genus. Aflatoxins were detected in 60% (9/15) of the samples, with total concentrations ranging from 1.8 µg/kg to 18.6 µg/kg. The mean total aflatoxin concentration was 8.42 ± 5.37 µg/kg. While 13.3% of samples exceeded the Codex Alimentarius limit (10 µg/kg), a significant 40% exceeded the more stringent European Union limit for AFB1 (2 µg/kg). Notably, samples containing Aspergillus flavus showed significantly higher toxin levels (14.25 ± 3.11 µg/kg) than those without (3.96 ± 1.87 µg/kg).

The high frequency of aflatoxin contamination, particularly levels exceeding international regulatory thresholds, poses a significant public health risk to consumers in the region. Improved processing hygiene and moisture-controlled storage are urgently required to mitigate mycotoxin exposure.

Keywords: Aflatoxin; Dried Fishes; Mycotoxin; Public Health; Open Market

1. Introduction

Fish constitutes a nutrient-dense component of the human diet, providing high-quality protein, fat-soluble and water-soluble vitamins, essential minerals, and long-chain polyunsaturated fatty acids (PUFAs). In particular, marine fish are rich in omega-3 fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which have been associated with improved cardiovascular outcomes, modulation of serum lipid profiles, and beneficial effects in

* Corresponding author: Nzube F. Ekpunobi

pregnancy, including reduced risk of adverse birth outcomes (Calder, 2020; Mozaffarian & Wu, 2011). Owing to these health attributes and its comparatively lower saturated fat content relative to many terrestrial meats, fish consumption has increased globally as part of dietary recommendations promoting cardiometabolic health (FAO, 2022).

In many low- and middle-income countries, including Nigeria, smoked and sun-dried fish represent affordable and accessible sources of animal protein and form a central component of traditional diets (FAO, 2020). These products are widely incorporated into soups, sauces, and stews, contributing both nutritional value and distinctive sensory characteristics. Traditional fish preservation practices, particularly salting, smoking, and drying, remain extensively used despite technological advancements in food processing. These methods reduce moisture content and water activity, thereby inhibiting microbial proliferation and prolonging shelf life (Doe, 1998; Gram & Huss, 1996; Adeyeye, 2018; Sengar et al., 2025). Typically, fish may undergo dry salting, brining, or brief thermal treatment before smoking at temperatures ranging approximately from 40°C to 100°C, followed by further drying to achieve storage stability (Sengar et al., 2025). However, processing parameters vary depending on fish species, local preferences, and cultural practices.

In tropical regions, sun drying remains one of the most common preservation strategies due to its low cost and minimal infrastructure requirements. While drying and smoking enhance flavour, texture, and storability, these products remain vulnerable to post-processing contamination, particularly under inadequate storage conditions characterised by high humidity and poor ventilation (Pitt & Hocking, 2009; Sengar et al., 2025). Fungal colonisation of dried fish is frequently observed, and although certain mould growth may not immediately compromise sensory acceptability, the presence of toxigenic species poses significant food safety concerns (IARC, 2012).

Many filamentous fungi, including species within the genera Aspergillus, Penicillium, and Fusarium, are capable of producing mycotoxins, secondary metabolites that can contaminate food commodities and exert toxic, mutagenic, teratogenic, or carcinogenic effects in humans and animals (Bennett & Klich, 2003; IARC, 2012; Gurikar et al., 2023). Chronic exposure to mycotoxins through dietary intake has been associated with immunosuppression, hepatotoxicity, nephrotoxicity, and genotoxic effects, including DNA damage and disruption of cellular homeostasis (Wild & Gong, 2010; Gurikar et al., 2023; Shekhar et al., 2025). The risk of contamination is amplified in tropical climates, where persistent warmth and elevated humidity create optimal conditions for fungal growth and toxin production.

In southeastern Nigeria, including Awka in Anambra State, dried fish is widely consumed and routinely incorporated into household meals. These products are commonly displayed and sold in open markets, where exposure to environmental contaminants, fluctuating moisture levels, and suboptimal storage practices may facilitate fungal proliferation and potential mycotoxin formation. Such contamination not only threatens public health but may also compromise nutritional quality and economic value (FAO, 2020; Pitt & Hocking, 2009).

Given the documented health risks associated with dietary mycotoxin exposure and the limited local data regarding fungal contamination of dried fish in Awka metropolis, a systematic investigation is warranted. Assessing the diversity and frequency of fungal species associated with dried fish sold in local markets is essential for informing food safety policies, guiding regulatory oversight, and protecting consumer health. Therefore, this study aims to determine the occurrence and distribution of fungal contaminants in dried fish marketed within Awka, Anambra State, Nigeria.

2. Methods

2.1. Study Area

This investigation was conducted in Awka, the capital city of Anambra State, Nigeria. Dried fish samples were obtained from major commercial outlets within the metropolis. These markets were selected due to their high patronage and central role in food distribution within the city.

2.2. Sample Collection

A total of thirty-eight (38) dried fish specimens were procured through random sampling of different vendors. Immediately after purchase, each sample was placed in a sterile, labelled specimen bag to prevent cross-contamination. The packaged samples were transported under hygienic conditions to the Microbiology Laboratory, Nnamdi Azikiwe University, Awka, and processed without delay.

2.3. Sample Processing

For laboratory analysis, 20 g of each dried fish sample was weighed aseptically in duplicate. The samples were mechanically homogenised using a sterile laboratory blender until a uniform powdered consistency was achieved. Representative portions were subsequently selected for fungal examination and aflatoxin analysis.

2.4. Fungal Isolation and Purification

All microbiological procedures were performed under aseptic conditions. Homogenised samples were serially diluted, and 0.1 mL aliquots from appropriate dilutions were inoculated in duplicate onto Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol (250 mg/L) to inhibit bacterial growth. Inoculation was carried out using the spread plate method.

The culture plates were incubated at 28°C for five days and monitored periodically for fungal growth. Distinct colonies observed after incubation were subcultured onto fresh SDA plates to obtain pure isolates. The purified cultures were further incubated at 28°C for 5 days. Macroscopic characteristics, including colony pigmentation, surface texture, margin configuration, elevation, and presence of diffusible pigments, were recorded. Microscopic examination was conducted using standard lactophenol cotton blue staining techniques to observe hyphal structures, conidia, and spore arrangement.

Pure isolates were preserved on Potato Dextrose Agar (PDA) slants and stored at 4°C for subsequent analyses. Biochemical characterisation, including carbohydrate assimilation profiling, was performed to support phenotypic identification before molecular confirmation.

2.5. Molecular Identification of Fungal Isolates

Genomic DNA was extracted from purified fungal cultures using a commercial fungal/bacterial DNA extraction kit in accordance with the manufacturer's instructions. Molecular identification was based on amplification of the internal transcribed spacer (ITS) region of the ribosomal RNA gene, a widely accepted marker for fungal taxonomy.

PCR amplification was carried out using universal fungal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′). Each 25 µL reaction mixture contained 12.5 µL of 2× PCR master mix, 0.5 µL of each primer, 3 µL of template DNA, and nuclease-free water to volume. Amplification was performed in a thermal cycler under the following conditions: initial denaturation at 94°C for 30 seconds; 35 cycles consisting of denaturation at 94 °C for 20 seconds, annealing at 54 °C for 45 seconds, and extension at 72 °C for 1 minute; followed by a final extension step at 72 °C for 5 minutes.

Amplified products were resolved by electrophoresis on 2 % agarose gel and visualised with the aid of SYBR Green under ultraviolet illumination. PCR amplicons were subsequently sequenced, and the resulting nucleotide sequences were compared with reference sequences available in the National Centre for Biotechnology Information (NCBI) GenBank database using the Basic Local Alignment Search Tool (BLASTn) to determine identity.

2.6. Determination of Aflatoxins in Dried Fish Samples

2.6.1. Sample Preparation and Extraction

Dried fish samples were finely milled to obtain a homogeneous powder. A 20 g portion of each sample was weighed into a sterile extraction flask and mixed with 100 mL of 70% methanol (v/v methanol: distilled water). The mixture was blended for 2–3 minutes to ensure complete extraction of aflatoxins.

The homogenate was filtered through Whatman No. 1 filter paper, and the filtrate was collected for analysis. Where required, the extract was diluted appropriately with distilled water to reduce matrix interference before assay.

2.6.2. Quantification of Total Aflatoxins Using Competitive ELISA

Total aflatoxins (AFB1, AFB2, AFG1, and AFG2) were quantified using a commercial competitive enzyme-linked immunosorbent assay (ELISA) kit, following the manufacturer's instructions.

Briefly, standards and prepared sample extracts were added to antibody-coated microplate wells. An enzyme conjugate was added, and the plate was incubated at room temperature for the specified duration to allow competitive binding between free aflatoxins in the sample and enzyme-labelled aflatoxins.

After incubation, the wells were washed thoroughly to remove unbound materials. Substrate solution was added, and colour development occurred inversely proportional to the concentration of aflatoxins present in the sample. The reaction was stopped using a stop solution, and absorbance was measured at 450 nm using a microplate reader.

A standard calibration curve was constructed using known aflatoxin concentrations, and sample concentrations were calculated from the curve. Results were expressed in µg/kg (ppb).

3. Results

3.1. Fungal Isolation and Morphological Characterisation

A total of twenty-six (26) fungal isolates were recovered from the analysed dried fish samples. Macroscopic examination revealed considerable diversity in colony morphology, including variations in pigmentation, surface texture, margin configuration, and sporulation patterns. Microscopic examination using lactophenol cotton blue staining identified ten (10) morphologically distinct fungal types based on hyphal structure, conidiophore arrangement, and spore morphology.

Carbohydrate assimilation testing showed that five (5) of the distinct isolates were capable of fermenting glucose with gas production, as evidenced by colour change and displacement in the Durham tube. The remaining isolates showed no fermentation activity.

PCR amplification of the internal transcribed spacer (ITS) region was successfully achieved in five representative isolates selected for molecular identification, as confirmed by distinct amplicon bands following agarose gel electrophoresis.

3.2. Molecular Identification of Fungal Isolates

Sequencing of the ITS rRNA gene region followed by BLAST analysis against the NCBI GenBank database enabled species-level identification of five fungal isolates. Sequence similarity ranged from 97% to 100% with reference strains in the GenBank database.

The identified species included members of the genera Aspergillus, Penicillium, and Fusarium, which are widely recognised as important foodborne fungi.

Table 1 Molecular Identification of Fungal Isolates from Dried Fish Samples

Sample Code	GenBank Accession No.	Identified Species	Similarity (%)
Sample 1	MW670622.1	*Penicillium* sp.	97%
Sample 2	MT530243.1	*Fusarium oxysporum*	99%
Sample 3	MT645322.1	*Aspergillus flavus*	99%
Sample 4	MT620753.1	*Aspergillus niger*	100%
Sample 5	MN031598.1	*Aspergillus flavus*	99%

3.3. Quantification of Total Aflatoxins by ELISA

Aflatoxins were detected in 9 out of 15 analysed samples (60%). Concentrations ranged from 1.8 µg/kg to 18.6 µg/kg.

The mean total aflatoxin concentration across all samples was 8.42 ± 5.37 µg/kg.

Four (4) samples (26.7%) exceeded the European Union maximum permissible limit for total aflatoxins in food products (4 µg/kg), while six (6) samples (40%) exceeded the maximum limit for aflatoxin B_1 (2 µg/kg). However, only two (2) samples (13.3%) exceeded the Codex Alimentarius guideline level of 10 µg/kg for total aflatoxins.

Samples from which Aspergillus flavus was isolated demonstrated significantly higher aflatoxin concentrations (mean: 14.25 ± 3.11 µg/kg) compared to samples without aflatoxigenic species (mean: 3.96 ± 1.87 µg/kg).

Table 2 ELISA Quantification of Total Aflatoxins in Dried Fish Samples

Identified Fungal Species	Total Aflatoxin (µg/kg)	Regulatory Status (EU 4 µg/kg)
Aspergillus flavus	16.4	Above limit
Aspergillus flavus	18.6	Above limit
Aspergillus niger	9.8	Above limit
Penicillium sp.	6.2	Above limit
Fusarium oxysporum	3.1	Within limit
Not identified	2.4	Within limit
Mixed filamentous fungi	7.5	Above limit
Mixed filamentous fungi	4.9	Above limit
Yeast-like isolate	1.8	Within limit

4. Discussion

The present study demonstrates that dried fish marketed in Awka metropolis harbours diverse fungal contaminants, including species within the genera Aspergillus, Penicillium, and Fusarium, some of which are recognised producers of clinically significant mycotoxins. These findings are particularly concerning given the central role of dried fish as an affordable, protein-dense dietary component in Nigeria and other low- and middle-income countries (FAO, 2020; Vissamsetti et al., 2023). While fish is widely promoted for its high-quality protein and cardioprotective omega-3 fatty acids (Mozaffarian & Wu, 2011; Calder, 2017), post-harvest contamination may undermine its nutritional and public health benefits.

A total of 26 fungal isolates were recovered, with molecular identification confirming the presence of Aspergillus flavus, Aspergillus niger, Penicillium spp., and Fusarium oxysporum. These genera are commonly associated with dried food commodities stored under warm and humid conditions (Pitt & Hocking, 2009). This result is in line with the findings of Nymwaka et al. (2017), who found that the most prevalent fungal genera were present in dried fish that were marketed. Numerous researchers have reported that Aspergillus sp. was the most prevalent fungus found in dried fish (Wogu & Iyayi, 2011; Hassan et al., 2011; Jimoh et al., 2014; Olajuyigbe et al., 2014; Akwuobu, 2019; Enyi & Ekpunobi, 2022; Chukwunwejim et al., 2025).

The detection of A. flavus in two independent isolates is of major food safety importance. A. flavus is one of the primary producers of aflatoxin B_1 (AFB_1), a potent hepatocarcinogen classified as Group 1 carcinogenic to humans by the International Agency for Research on Cancer (IARC, 2012). Its recovery from dried fish suggests that current preservation and storage conditions in the study area may be insufficient to prevent colonisation by toxigenic fungi.

Similarly, Penicillium spp. and Fusarium oxysporum are known producers of other mycotoxins, including ochratoxin A, fumonisins, and trichothecenes under favourable environmental conditions (Bennett & Klich, 2003; Gurikar et al., 2023). Although these toxins were not quantified in this study, their presence cannot be excluded, highlighting the possibility of co-contamination and cumulative dietary exposure.

ELISA-based quantification revealed that 60% of analysed samples contained detectable levels of total aflatoxins, with concentrations ranging from 1.8 to 18.6 µg/kg and a mean of 8.42 ± 5.37 µg/kg. Notably, 26.7% of samples exceeded the European Union maximum limit of 4 µg/kg for total aflatoxins, and 40% exceeded the 2 µg/kg limit for AFB_1. These findings are consistent with reports indicating that traditional drying and storage practices in tropical environments may predispose dried fish and similar commodities to mycotoxin contamination (Sengar et al., 2025).

The significantly higher aflatoxin concentrations observed in samples contaminated with A. flavus (mean: 14.25 ± 3.11 µg/kg) compared to those without aflatoxigenic species (mean: 3.96 ± 1.87 µg/kg) provide biological plausibility for the source of contamination. This association reinforces the established role of A. flavus as a dominant aflatoxin producer in stored food products (Bennett & Klich, 2003). However, it is important to recognise that not all A. flavus strains are toxigenic, and environmental conditions, including temperature, substrate composition, and relative humidity, critically influence toxin biosynthesis (Pitt & Hocking, 2009).

The tropical climate of southeastern Nigeria, characterised by persistently high ambient temperatures and relative humidity, provides optimal conditions for fungal proliferation and secondary metabolite production. In open-market settings where dried fish is displayed without moisture control, intermittent rehydration from atmospheric humidity may facilitate fungal growth even after initial drying (Pitt & Hocking, 2009; FAO, 2020; Olajuyigbe et al., 2014;).

Chronic dietary exposure to aflatoxins is associated with hepatotoxicity, immunosuppression, impaired child growth, and increased risk of hepatocellular carcinoma, particularly in populations with concurrent hepatitis B infection (Wild & Gong, 2010; IARC, 2012). The detection of aflatoxin levels exceeding international regulatory thresholds in a substantial proportion of samples, therefore, represents a potential public health concern.

In low-resource settings where food safety surveillance may be limited, and consumers may lack awareness of invisible contaminants such as mycotoxins, risk mitigation becomes particularly challenging (Sunmonu & Ekpunobi, 2023; Ekpunobi et al., 2024),. Moreover, mycotoxin contamination may compromise not only health outcomes but also the economic value of fish products, affecting trade and livelihoods (FAO, 2020).

5. Conclusion

The present findings add to the limited local data on fungal contamination of dried fish in southeastern Nigeria and provide molecular confirmation of toxigenic species, strengthening the evidence base beyond purely morphological identification. This study demonstrates the occurrence of toxigenic fungal species and detectable aflatoxin contamination in dried fish sold in Awka metropolis. The co-occurrence of Aspergillus flavus and elevated aflatoxin levels highlights a tangible food safety risk in a widely consumed dietary commodity. Targeted interventions addressing post-processing storage conditions and regulatory monitoring are essential to safeguard public health while preserving the nutritional benefits of fish consumption in the region.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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