Assessment of the Fungal Contaminants of Dried Fishes Sold in Open Markets in Awka, Nigeria

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Posted Date: 29 May 2024

doi: 10.20944/preprints202405.1880.v1

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Article

Assessment Of the Fungal Contaminants of Dried Fishes Sold in Open Markets in Awka and Nigeria

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ABSTRACT: Fish is a very healthy food that is a great source of proteins, vitamins, minerals, and essential fatty acids. For many people in developing nations, like Nigeria, smoked and dried fish serves as a significant source of protein and is an integral part of the traditional diet. However, smoked and dried fish are susceptible to fungal growth, especially when the storage conditions are not right, and it has been discovered that many products contain fungi that are easily visible on the surface. This study was conducted to investigate the diversity and occurrence frequency of contaminated fungi species in dried fishes sold in local markets of Awka in Anambra state, Nigeria. Twenty-four fungal isolates with varying colony morphology were isolated from the various dried fish products.The contaminating species were further subjected to lactophenol cotton blue (LPCB) staining and Ten (10) Unique fungal morphology was detected. Carbohydrate utilization test was used to select unique isolates and further molecular identification showed successful PCR amplification of the isolates. The current study identified various fungal groups contaminating dried fish products sold in Awka Markets. The fungal genera' Aspergillus Penicillium and Fusarium were the three dominant genera. These results suggest that most dried fish being sold in the Markets are contaminated with multiple fungi which pose a critical health risk to individuals. Thus, there is a need for attention to be paid to the packaging and storage of dried fish to lessen the likelihood of air exposure, lower water activity, and prevent microbiological growth on the dried fish.

Keywords: dried fish; molecular identification; PCR; aspergillus; penicillium and fusarium

1. INTRODUCTION

Fish is a very healthy food that is a great source of proteins, vitamins, minerals, and essential fatty acids. Fish consumption has gained popularity over time as a result of two factors: first, its popularity as a lean meat substitute; and second, the health benefits it offers as a rich source of omega-3 fatty acids, which lower cholesterol levels, the risk of heart disease, and the likelihood of preterm birth [1]. For many people in developing nations, smoked and dried fish serves as a significant source of protein and is an integral part of the traditional diet. They are frequently used as raw ingredients for seasoned foods like soups and sauces and are frequently enjoyed for their distinct flavour.

The earliest techniques for preserving fish that are still in use today include salting, smoking, and drying. Traditional production methods are still utilized, despite advancements in techniques over time. To produce a product that is stable for transport and storage, smoked fish is typically processed using a combination of curing/salting, smoking, and drying steps. Raw fish is either dry salted, pickled, or boiled in salted water before being smoked at temperatures between 40 and 100 °C and dried. However, the process can vary significantly depending on the species of fish, the desired product, and local customs in various regions [2]. Direct sun drying is frequently the method of choice for tropical nations, where most production occurs. While improving the flavour and textural qualities, the process creates a product with less water activity and better microbial stability than the raw material.

 doi:10.20944/preprints202405.1880.v1

However, smoked and dried fish are susceptible to fungal growth, especially when the storage conditions are not right, and it has been discovered that many products contain fungi that are easily visible on the surface. It is known that the growth of some fungi improves product acceptability while the growth of others denotes product spoilage [3]. However, there is concern that the existence of toxic fungi and their toxic byproducts may have detrimental effects on both human and animal health [4,5]. Various fungi species and other microorganisms have toxic secondary metabolites that can contaminate food materials [6,7]. These food products are sold in open markets throughout Nigeria and the rest of the world, putting them at risk for mycotoxin infection and nutritional content loss. When food is handled and stored improperly, it frequently results in quality degradation, decreased market value, and the production of mycotoxins [8]. The tropics, where the warm, humid climate is stable for microbial (fungal) proliferation and subsequent establishment in a variety of substrates, make this terrible situation even worse.

In the southeast of Nigeria, such as in Awka, Anambra state, it has been observed that dried fish is used in almost all food preparation. Because mycotoxins can have serious acute and long-term effects on both human and animal health, it is necessary to look into the contamination of dried fish purchased from local markets in the Awka metropolis. Research is required to confirm certain theories regarding fungi contamination in dried fish because the situation is still largely unknown. Additionally, laws and regulatory organizations frequently demand this. Mycotoxins, a product of certain fungi groups have been shown to cause DNA damage and cause cell death when they are present in food that is consumed by the general public. This study was conducted to investigate the diversity and occurrence frequency of contaminated fungi species in dried fishes sold in local markets of Awka in Anambra state, Nigeria.

2. MATERIALS AND METHODS

2.1. Study Area

The study was carried out in strategic markets of Awka, Anambra state, Nigeria. The four selected markets are the Eke-Awka market, Ifite market, Amaenyi market and UNIZIK Junction market.

2.2. Sample Collection

A total of forty (40) dried fish samples, ten (10) from each of the four markets were purchased, at random. The dried fish samples were packaged in a sterile specimen bag, labelled properly and transported to the Microbiology Laboratory of Nnamdi Azikiwe University Awka, where they were to be prepared for analysis immediately.

2.3. Sample Preparation

Twenty (20) grams each of aseptically weighed dried fish samples purchased from the different market locations were taken in duplicates. The samples were pulverized to powder using a Warring blender until well minced and mixed [9]. Fifteen (15) randomly chosen portions were then used for microbiological culture and isolation.

2.4. Isolation and purification of Fungi

A microbiological analytical culture procedure was carried out under aseptic conditions. Following a 5-fold dilution, 0.1 ml diluent was cultured out in duplicate by spread plate technique on the Sabouraud Dextrose Agar (SDA) plate containing 250 mg/l of chloramphenicol to suppress the growth of bacteria. The plates were incubated at 28^0C for five (5) days as described by [10]. The cultures were examined for growth at regular intervals. After the incubation period, all observed distinct colonies were subcultured out on the Sabouraud Dextrose Agar (SDA) medium, and incubated for five (5) days at 28^0C [10]. The colony colour, size, appearance, texture, colonial morphology and diffusible pigments of each pure isolate were observed and compared with the

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mycology colour atlas text [11]. Pure cultures obtained were stored in a Potato Dextrose Agar (PDA) slant at 4^0C for further use. The obtained pure isolates were routinely verified microscopically [12], followed by a Carbohydrate assimilation test [13] and molecular characterization.

2.5. Molecular Identification/Characterization of Fungi Isolates

Molecular identification was conducted based on the nucleotide sequence of DNA. Genomic DNA was extracted using Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research), according to the recommended protocol. The ribosomal RNA gene was amplified using universal fungal primers: internal transcribed spacer 1 (ITS1: 5′- TCCGTAGGTGAACCTGCGG -3′), and internal transcribed spacer 4 (ITS4: 5′- TCCTCCGCTTATTGATATGC -3′). The PCR reaction mixture was prepared as follows: 12.5µl of One Taq Quick-Load 2X Master Mix with Standard Buffer (New England Biolabs Inc.); 0.5µl each of forward and reverse primers (ITS1 5′- TCCGTAGGTGAACCTGCGG -3′ and ITS4 5′- TCCTCCGCTTATTGATATGC -3′); 8.5µl of Nuclease free water and 3µl of DNA template was used to prepare 25µl reaction volume of the PCR cocktail. The reaction was gently mixed and transferred to a thermal cycler (Eppendorf nexus gradient Mastercycler (Germany)).

PCR amplification was performed with the following cycling parameters: Initial denaturation for 30secs at 94°C, followed by 35 cycles of denaturation at 94°C for 20secs, primer annealing at 54°C for 45secs and strand extension at 72°C for 1 min. Final extension at 72°C for 5 min. PCR products were separated on a 1.5% agarose gel and DNA bands were visualized with Ethidium bromide under a UV transilluminator. Amplification products were sequenced and the resulting sequence was compared with other ITS rDNA gene sequences obtained from the NCBI GenBank database. Sequencing results were individually inputted online into the nucleotide BLAST program (BLASTN 2.2.29) through the NCBI database to identify the isolates [14].

3. RESULTS

3.1. Isolation and diversity of contaminated fungi

Twenty-four fungal isolates with varying colony morphology were isolated from the various dried fish products. Twenty-two isolated colonies were filamentous fungi and 2 colonies showed a yeast-like morphology in PDA agar.

The contaminating species were further subjected to lactophenol cotton blue (LPCB) staining and Ten (10) Unique fungal morphology was detected. A carbohydrate utilisation test was carried out on the unique isolates and only Five (5) of the isolates were able to ferment glucose.

The species of five (5) contaminating fungi that could utilize carbohydrates required further molecular identification. Agarose electrophoresis results showed successful PCR amplification of 5 strains.

Table 1. Carbohydrate Utilization by Different Isolates.

Samples	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	INTERPRETATION
1a	NR	Slight Colour Change	Slight colour change with space in DT	Colour change with space in DT	Colour change with space in DT	Glucose Fermented with Gas production
1b	NSG	NSG	NSG	NSG	NSG	Glucose not Fermented
2	NR	Slight Colour Change	Slight colour change with space in DT	Colour change with space in DT	Colour change with space in DT	Glucose Fermented with Gas production
4	NR	NR	NR	NR	NR	Glucose not Fermented
5b	NR	Slight Colour Change	Colour change with space in DT	Colour change with space in DT	Colour change with space in DT	Glucose Fermented with Gas production

 doi:10.20944/preprints202405.1880.v1

6b	NR	Slight Colour Change	Colour change with space in DT	Colour change with space in DT	Colour change with space in DT	Glucose Fermented with Gas production
7a	NR	NR	NR	NR	NR	Glucose not Fermented
10	NR	Slight Colour Change	Colour change with space in DT	Colour change with space in DT	Colour change with space in DT	Glucose Fermented with Gas production
11	NR	NR	NR	NR	NR	Glucose not Fermented
18	NR	NR	NR	NR	NR	Glucose not Fermented

NR; No Reaction NSG; No Significant growth DT; Durham Tube.

3.2. Genetic identification of contaminated fungi

Sequenced fungal sequences were used as BLAST queries against the NCBI database. It shows the fungi isolated from dried fish samples had 97–100% similarity compared with closely related fungi in the GenBank. Through BLAST alignment analysis, the molecular identification results of 5 fungal strains are shown in Table 2.

Table 2. Identification of fungal isolates recovered from different dried fish types by sequencing the ITS rRNA gene region and comparing with sequences listed in the GenBank for similar species.

Sample No.	Ascension No. In Gene Bank	Fungi	Similarity Index
2a	MW670622.1	*Penicillum Sp.*	97%
1b	MT530243.1	*Fusarium oxysporon*	99%
1a	MT645322.1	*Aspergillus flavus*	99%
7a	MT620753.1	*Aspergillus niger*	100%
6b	MN031598.1	*Aspergillus flavus*	99%

4. DISCUSSION

The current study identified various fungal groups contaminating dried fish products sold in Awka Markets. Numerous fungal genera' isolates were identified; *Aspergillus* sp., *Penicillium* sp., and *Fusarium* sp. were the three dominant genera. This result is in line with the findings of Nymwaka et al. [15], who found that the most prevalent fungal genera were present in dried fishes that were marketed. Numerous researchers have reported that *Aspergillus* sp. was the most prevalent fungus found in dried fish [16–20].

The fungal contamination of dried fish with *Saccharomyces* sp., *Mucor* sp., *Schizosaccharomyce* sp., *Acremonium* sp., *Rhizopus* sp., *Trichoderma* sp., *Candida* sp., *Cladosporium* sp., and *Alternaria* can be regionally specific, according to a study by Olajuyigbe et al. [20]. In contrast to fungi that resemble yeast, filamentous fungi were found to be more frequently contaminated with dried fish in the current investigation. This prevalence may be explained by a variety of factors, one of which is filamentous fungi's greater capacity for diffusion and reproduction. Filamentous fungi that produce spores find it simple to attach to and endure in high-protein food sources like dehydrated fish.

Two species of *Aspergillus* sp. were identified by genetic analysis as *A. flavus* and *A. niger*, which were isolated from dried fish (Table 2). The majority of sun-dried fish have been found to be contaminated by *Aspergillus flavus*, the most prevalent type of fungus [20–22]. The risk of fungal production in dried fish is increased by *Aspergillus* sp., which can produce a large number of conidia and spores that are encouraged to germinate by the high moisture content. Apart from the Aspergillus species, other species that have been found in dried salted fish include *A. tamari*, *A.*

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sydowii, *A. versicolor*, *A. Fumigatus*, *A. aculeatus*, *A. tubingensis*, *A. tereus*, *A. ochraceus*, and *A. wentii* [22,23]. Isolation of fungi that produce aflatoxin indicates the possibility of mycotoxin contamination. According to Liu et al. [24], exposure to aflatoxins can result in long-term health problems such as hepatocellular carcinoma and hepatitis B, both of which pose a serious risk to human health.

This current study also identified *Penicillium* sp. This is in line with a study conducted in Egypt [17] that found *Penicillium citrinum* in dried fish samples and that was also reported in dried fish samples sold in Giza, Egypt. Since higher water activity and storage temperatures are ideal for *Penicillium* spp. growth, it is possible that Penicillium spp. was first discovered in dried fish. Additionally, isolates from the *Fusarium* species were found in the samples, indicating that dried fish products were contaminated with Fusarium. These findings are consistent with those published by Nyamwaka et al. [15] and Fafioye et al. [25], who reported Fusarium contamination of sun-dried fish in Kenya and smoke-dried fish in Nigeria, respectively.

It's interesting to note that, despite being frequently isolated from dried fish, *Rhizopus* sp. and *Mucor* sp. were not found in our samples. According to our research, the presence of fungal isolates in dried fish samples may have resulted from improper temperature and humidity control measures or an unhygienic processing environment.

These results suggest that most dried fish being sold in the Markets are contaminated with multiple fungi which pose a critical health risk to individuals. Thus, there is a need for attention to be paid to the packaging and storage of dried fish to lessen the likelihood of air exposure, lower water activity, and prevent microbiological growth on the dried fish. There is also the need for regulatory bodies to pay more attention to the food materials being distributed in various markets in Nigeria as they directly impact human health [26].

5. CONCLUSION

Results of this study show the presence of toxigenic fungi in the dried fish samples. The fungal genera isolated in this study (*Aspergillus, Penicillium*, and *Fusarium*) are health-risk organisms occurring as a result of storage conditions of dried fish. These fungal genera are represented in our study as the ability to produce aflatoxins that pose health concerns to consumers of such contaminated dried fish.

Funding: This research received no external funding and was self-sponsored by all the authors.

Data Availability Statement: The authors confirm that the data supporting the findings of this study are available within the article.

Conflicts of Interest: The authors declare no conflict of interest

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