

ISOLATION AND IDENTIFICATION OF FUNGI ASSOCIATED WITH SMOKED *Oreochromis niloticus* IN WUKARI MARKETS, NIGERIA

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ABSTRACT

The contamination of food products with microorganisms presents a problem of global concern, since the growth and metabolism of microorganisms can cause serious food borne intoxications and a rapid spoilage of the food products. The study aimed at investigating fungi present on smoked Tilapia (*Oreochromis niloticus*) sold in the old and new markets of Wukari. A total of fifty fish samples were collected, and fungal types and frequencies were analyzed using standard keys. Five fungal genera were identified: *Aspergillus niger*, *Aspergillus flavus*, *Mucor* spp, *Rhizopus stolonifer*, and *Penicillium* spp. Smoked Tilapia from the New Market showed a higher fungal load (2.50×10^5 CFU/g) compared to the Old Market (1.8×10^5 CFU/g). In the Old Market samples, *Aspergillus flavus* and *Aspergillus niger* were equally prevalent (24% each), with *Aspergillus niger* being the most dominant overall at 52%. The study recommends improved drying and storage methods for preservation and raising awareness among fishermen, marketers, and consumers about the health risks of fungal contamination and mycotoxins in fish.

Keywords: Fungi; smoked fish; contamination; mycotoxins; food safety.

INTRODUCTION

Food quality and safety is an increasingly important public health issue. Nowadays, the topics “food quality” and “food safety” are very close and two important issues in the food sector, due to the globalization of the food supply and the increased complexity of the food chain (Fung *et al.*, 2018). Consumers need to purchase safe products that do not involve any kind of risk for health. Food safety is a fundamental aspect of public health and economic stability. It encompasses a wide range of policies, practices, and technologies designed to prevent food-borne illnesses and ensure the safe consumption of food products (Yang, 2019). More so, assessment of food quality helps to ensure that all attributes that influence the value of a product for the consumer; this includes negative attributes such as spoilage, contamination with filth, discoloration, off-odors and positive attributes such as the origin, color, flavor, texture and processing method of the food (Böhme *et al.*, 2012). The contamination of food products with microorganisms presents a problem of global concern, since the growth and metabolism of microorganisms can cause serious food borne intoxications and a rapid spoilage of the food products. Fungi, molds and bacteria are the agents responsible for various types of food spoilage and food borne intoxications (Akinsemolu, 2024).

Preserving fish involves processes that impede growth of microorganisms either by the addition of growth inhibiting ingredients or adjusting storage conditions by freezing or drying (FAO, 2016). Processing methods affect the microorganisms in fish in different ways, resulting in different types of micro-flora and different risks from spoilage organisms and pathogens. Generally, the different preservation methods are: drying, smoking, freezing, chilling and brining. Despite the fact that smoking is the most common method of fish preservation in Nigeria (Latha and Mohan, 2013; Rokibul *et al.*, 2013; Mojisola, 2014; Ibemenuga and Okeke, 2014; Jalal *et al.*, 2017; Shahriar *et al.*, 2019), its effectiveness as an international source of foreign exchange is gradually losing ground (Schwartz, 2000). This is attributed to the exportation of smoked fish to developed countries which is becoming increasingly stringent due to the emergence of food safety and agricultural health standard, along with the fact that buyers keep changing their requirements. Food poisoning organisms can multiply profusely in foods without initially altering the appearance, taste or odor (Hernández *et al.*, 2017). A large number of people suffer from gastrointestinal upsets annually, as a result of eating contaminated food which leads to a considerable loss of man-hour with accompanying consequences (Jere *et al.*, 2019). The incidence of food-borne illness in developing countries is less well understood and occurs extensively than in the developed countries, where the consumption of unhygienic food and water are the leading causes of death. Sometimes, death occurs. In many instances, outbreaks result from lack of understanding of food hygiene in the preparation, cooking and care of food (Mohammad *et al.*, 2018). These problems might hinder the attainment of Sustainable Development Goals’ objectives. In order to avert such outbreaks, safety evaluation is necessary (Morse *et al.*, 2018).

Fungi are omnipresent in the environment, been found wherever water, suitable organic nutrients and an appropriate temperature occur (Divya *et al.*, 2021). They secrete enzymes outside their body structure and absorb the digested foods (MacFarlane, 2018). The smoking of fish from smoldering wood for preservation dates back to civilization (Adeyeye 2016; Kumar, 2018). It was also noted that apart from giving the product

desirable taste and odour, smoking provides longer shelf-life through its anti-bacterial and oxidative effects, lowering of pH, imparting desirable colouration as well as accelerating the drying process and acting as antagonist to spoilage agents (Sani *et al.*, 2016). Contamination may be primarily by fungi (the initial agents) or as a secondary invasion of tissues already damaged by viral, bacteria or mechanical agents. Several foodborne moulds and possibly yeasts may also be hazardous to human health because of their ability to produce toxic metabolites known as mycotoxins. Most mycotoxins are stable compounds that are not destroyed during food processing or home cooking (Bullerman, 2014). The food industry stands to lose production if the food has a shorter shelf-life; therefore, technologies are used to curb the effect of food spoilage on the economy and company profits (Bashir *et al.*, 2017).

This study is aimed at the isolation and identification of fungi associated with smoked (Tilapia fish) sold in New and Old Market within Wukari Metropolis, Wukari Local Government Area, Southern Taraba State.

MATERIALS AND METHODS

Study area

The isolation and identification of fungal contamination was carried out on smoked dried fish gotten from Old and New Market of Wukari Local Government Area, Taraba State. Wukari is the headquarter of Wukari Local Government Area of Taraba State, it is located between latitude 7°51'N to 7°85'N and longitude 9°46'E to 9°78'E of the Greenwich meridian. Wukari Local Government area is situated in the southern part of Taraba State. The Local Government is bounded by Plateau State in the North, Benue State in the Southwest, Northeast by Karim Lamido, Bali, and Takum Local Government Area (LGA). It has an area of about 4308 km² (1663 sq mi). (Musa, 2025)

Wukari LGA covers an area of approximately 6500 sq. Km. The whole area is a gently undulating plain, with a mean altitude of 200 m above sea level. The drainage systems drain northward and serve as tributaries network to the River Benue while eastward discharge of rivulets and other smaller tributaries from Wukari town drains towards the Donga River, which is a major tributary of River Benue.

The study area is characterized with a tropical continental climate. The mean annual rainfall value ranges from 1000 - 1500 mm. The onset of the raining season is usually around April while the offset period is October. This means the rainy season usually last for seven months and about five month of dryness from November to March. The mean maximum temperature is being experience around April at about 40 °C while the mean minimum temperature occurs between the period of December and February at about 20 °C. Relative humidity also exhibited temporal variability. It is higher during the night hours in the rainy season than during the day hours in the dry season respectively. By location, the climate of the area is being influenced by the prevalence of two opposing air masses, the tropical maritime air mass (MT, south westerly's trade) and the tropical continental air mass (CT, north easterlies trade). The tropical continental air mass (CT) is a dry air that blows across the Sahara Desert to the West African region (Dada, 2006).

Sample Collection and Preparation

A total of Fifty (50) samples of smoked-dried Tilapia Fish were bought from the old and new market of Wukari in a sterile specimen bag and labeled. The total weight of the samples was measured in grams. The skins were removed and blended until well chopped

and mixed. The blended samples were kept in airtight containers until ready for analysis.

Moisture Analysis

About 10 grams of ground sample was added to pre-weighed aluminum foil and total weight was recorded. Samples was placed in 105°C oven and dry for one hour to constant weight. Sample was weighed several times (record time and weight) making sure sample had dried to desired weight. Percentage moisture was computed as weight lost divided by sample weight multiplied by 100.

$$\% \text{ moisture} = \frac{\text{weight loss}}{\text{sample weight}} \times 100$$

Isolation of fungi

Dilution (Pour plate) method

Ten grams (10g) of each sample was aseptically weighed into a sterile bottle containing 90 ml of sterile peptone water. The mixture was shaken vigorously using vortex mixer to dislodge all fungal contaminants, 5-fold serial dilutions was prepared to 10^5 (Samson *et al.*, 2010). One milliliter of each dilution was dispensed in duplicate in sterile Petri dishes. Molten sabouraud dextrose agar to which Chloramphenicol (0.5g/l) was added to the Petri dishes, which was gently rotated to ensure even dispersion. The plates were allowed to solidify and incubated at ambient temperature (room temperature) for 3-7 days.

The cultures were examined for growth at regular intervals and all observed colonies were sub-cultured to obtain pure colonies, which were subsequently isolated and identified using morphological characteristics, spore formation, the production of fruiting bodies and biochemical reactions (Alkenz *et al.*, 2015) and compares with mycology color atlas.

Identification of fungi

Macroscopy

The growth rate, color, texture, colonial morphology and diffusible pigments of each sample was examined macroscopically.

Microscopy

The microscopic morphological features of most fungi are stable and exhibit minimal variation. The definitive identification was based on the morphology of the spores and hyphae. A drop of lactophenol cotton blue stain was placed on a clean grease-free slide. A small fragment of (vegetative) colony, woolly or powdery colony was picked using a sterile needle and teased in the stain until a homogenous blue mixture of stain and culture was obtained. A clean cover slip was applied avoiding air bubbles. Excess stain was removed with blotting paper and the preparation was examined using X10 and X40 objectives of the microscope respectively.

Statistical analysis

The frequencies and market distributions of fungal isolates were determined using proportion and rates. The results were presented in tables and figure.

RESULTS AND DISCUSSION

Samples from 43 of the 50 smoke-dried Tilapia fish examined showed fungal contamination on potato dextrose agar (PDA) with some morphological characteristics. The fungi isolated from the two (2) locations has a total of 5 genera which are *Aspergillus niger*, *Aspergillus flavus*, *Mucor* sp, *Rhizopus stolonifer* and *Penicillium* spp., Table 1 presents the isolated fungi and their morphological characteristics.

The prevalence of fungal isolate of fungal flora on the smoked-dried *Oreochromis niloticus* vended in New Market (Table 2) revealed the presence of four (4) different fungal species associated with the smoked-dried *Oreochromis niloticus* in the market. *Aspergillus niger* was the most predominant constituting 52%, followed by *Aspergillus flavus* accounting for 20%, *Penicillium* spp. with 16% and the least abundant fungal species is *Mucor* spp. occupying 12% on the fish.

Meanwhile for Old Market, there are equal numbers of species prevalent with the samples gotten from the market amounting to four (4) different fungal isolates associated with the fish (Table 3), it was revealed that *Aspergillus flavus* (24%) and *Aspergillus niger* (24%) both with the same prevalence being the highest abundant species, followed by *Rhizopus stolonifer* accounting for 16% and lastly *Penicillium* spp. occurring by 18% making the least abundant species on the smoked-dried Tilapia Fish.

The total fungal count of the samples from both markets is presented in Table 4. The results from this study revealed that smoked-dried Tilapia procured from Old Market had the lowest fungal count of 1.8×10^5 CFU/g while the highest fungal count of 2.5×10^5 CFU/g was found from the smoked-dried Tilapia fish of New Market.

The rates of fungal contamination of smoked-dried Tilapia fish sold were very high, recording Twenty-five (58.1%) fungal isolates for New market while eighteen (41.9%) fungal isolates for Old market.

Table 1: Morphological characteristics of isolated fungi on potato dextrose agar

Fungi	Morphology	Mycelia
<i>Aspergillus niger</i>	Dark appearance	Present
<i>Aspergillus flavus</i>	Dark green appearance	Present
<i>Mucor</i> spp.	White/brown appearance	Present
<i>Rhizopus stolonifer</i>	Ash/brown	Present
<i>Penicillium</i> spp.	Pale green	Present

Table 2: Prevalence of isolated fungi from smoked-dried Tilapia fish samples from New Market

Fungi Percentage (%)	Number of fungi isolated	Total number of samples
<i>Aspergillus flavus</i>	5	25
<i>Aspergillus niger</i>	13	25
<i>Mucor spp.</i>	3	25
<i>Penicillium spp.</i>	4	25

Table 3: Prevalence of isolated fungi from smoke-dried Tilapia fish samples from Old Market

Fungi Percentage (%)	Number of fungi isolated	Total number of samples
<i>Aspergillus flavus</i>	6	25
<i>Aspergillus niger</i>	6	25
<i>Rhizopus stolonifer</i>	4	25
<i>Penicillium spp.</i>	2	25

Table 4: Total Fungal Counts from smoked-dried fish obtained from Old and New Markets of Wukari LGA

MARKETS	FUNGAL (CFU/g)	COUNT
Old Market	1.8 x 10 ⁵	
New Market	2.5 x 10 ⁵	

The results from this study revealed a highest fungal count (2.5 x 10⁵ CFU/g) from smoked Tilapia obtained from new market. However, the highest and lowest value of fungal counts obtained by this study is lower than those obtained by many researches in Nigeria. Adegunwa *et al.* (2013) studied the microbial quality of smoked herring (*Sardinella eba*) obtained from Odder local government, Ogun state, Nigeria and reported highest fungal counts of 3.50×10³ CFU/g. Abolagba (2010) Also reported the highest fungal count of 4.98×10⁶ CFU/g from smoked fish vended in Benin City, Nigeria. Similarly, Adebayo-Tayo *et al.* (2008) Reported fungal counts of smoked fish vended in Oyo, Nigeria as 7.6 x 10³CFU/g, 8.4×10⁴CFU/g and 9.0×10²CFU/g. Wogu and Iyayi (2011) who reported an appreciably higher microbial load 14 x10³ – 20 x 10³ cfu/g. But it concurred and in range with the study of Jere (2019) revealing a highest fungal count of 2.50 x 10³ CFU/from

smoked fish obtained from the second retailer (Retailer B) obtained from Sabon gari market, Kano state.

However, the least fungal count was found from smoked Tilapia obtained from old Market Wukari (1.8×10^5), and the result is consistent with the findings of Jere (2019) reporting the least fungal count to be $1.85 \pm 0.15 \times 10^3$. The range of fungal counts observed in the smoked fish samples is also in consistency with the range of values ($1.693 \times 10^5 - 9.043 \times 10^5$ CFU/g) reported by Udochukwu *et al.* (2016) From smoked and fresh fish sold in Benin City, Nigeria.

The presence of *Aspergillus flavus* and *Aspergillus niger* in the fish samples can cause serious health concern because of their mycotoxigenic potentials Essien *et al.* (2005) reported that *Aspergillus flavus* and *Aspergillus niger* produced aflatoxins, which destroyed the liver and kidney in man resulting to death.

Furthermore, the result of the prevalence of isolated fungi from smoke-dried Tilapia fish samples vended in New Market and old market are presented in Table 2 and 3, For old market it was found that *Aspergillus flavus* (24%) and *Aspergillus niger* (24%) both with the same prevalence and they are the highest abundant species, followed by *Rhizopus stolonifer* accounting to 16% and lastly *Penicillium* spp. occurring by 18% making the least abundant species on the smoked-dried *Oreochromis niloticus*. The mycotoxigenic potential of the mould genera implicated in the study have been reported by Obemeata *et al.* (2011) and Esseini *et al.* (2005) with *Aspergillus flavus* and *A. niger* as dominant mould flora in the smoked fishes. The existence of these moulds on cold smoked fish is a pointer to the potential health risk associated with consumption of such ready to eat products. This is similar to observation of (Essein *et al.*, 2005).

According to these authors such presence of microbes can indicate unhygienic conditions during the processing, because the product contamination could be the result of a combination of improper handling, storage and cross contamination. Ayelaja *et al.* (2012) also reported some of the microbial load indicated in this study in smoked fish sold in Ibadan Nigeria which were *Aspergillus flavus*, *Penicillium* spp., *Fusarium oxysporum*, *Trichoderma* spp. and *Ceotrichium albidium*. Most of the organisms found in these smoked fish are those commonly found in soil and water.

In new market, the sample revealed the presence of four (4) different fungal species associated with the smoked-dried Tilapia fish in the market. *Aspergillus niger* was the most predominant constituting 52% followed by *Aspergillus flavus* accounting to 20%, *Penicillium* spp. with 16% and the least abundant fungal species is *Mucor* spp. occupying 12% on the fish. This is in agreement with microorganisms reported by Adesokan *et al.* (2011) and Olawale *et al.* (2005) and reported *Aspergillus niger* as part of the commonest microorganisms associated with smoked fish. Similar microorganisms were also reported by Abolagba and Igbinevbo (2010) in smoked fish (*Clarias* spp.) sold in Benin metropolis. The occurrence of *Aspergillus*, *Rhizopus*, and *Penicillium* species could be due to reabsorption of moisture during storage, the stored fish might have reabsorbed moisture from the environment which then supported the growth of the microorganisms in addition to the contamination during processing, handling and display on the market stall (Ayolabi and Fagade, 2010). The fungi found in the study aligned with that of Edema and Agbon (2010), they isolated *A. flavus*, *A. niger*, *Penicillium* sp, and *Rhizopus* spp from

smoked cured fish from open market in south western Nigeria. The growth of these fungi species in the fish is as a result of favourable condition that supports their growth.

Fungi isolated from this present study are in consonant with findings by others authors (Firouz *et al.*, 2005; Refai, *et al.*, 2010 and Alkasim *et al.*, 2025). However, Refai *et al.* (2010) reported that *Penicillium* spp., *Aspergillus* spp., and *Rhizopus* spp. are normal mycoflora present in most fish. Notwithstanding, many fungal genera have virulence factor which cause toxin elaboration under favourable predisposing environment. Ecology is also an important factor which influences the diversity of fungus genera on fish and their eggs, this affects other species that uses fish for food such birds (Abdul *et al.*, 2025).

Fungal contamination occurs probably during hawking where the fish is exposed to fungal and fungal spore contamination as reported by Dike *et al.* (2014). Any handling of fish and the associated sanitary practice from the point of harvesting can potentially contribute to the micro flora on the final product.

The presence of toxigenic fungi, for example some species of *Aspergillus*, *Penicillium* and *Candida*, in foods as contaminants increases the risk for mycotoxins production which could induce gastrointestinal and metabolic disturbances when contaminated foods like smoke-dried fish are consumed (Casadevall, 2019). Some of the moulds isolated are common in the air and soil and have been linked with the production of various types of toxins under various conditions. *Aspergillus* spp has been proven to produce aflatoxin and ochratoxin, *A. Penicillium* spp. produces aflatoxin and citrinin. Mari and Riccioli (2004) reported *A. niger* and *A. fumigatus* as being allergenic. The presence of these fungi in the smoked-dried Tilapia samples might probably make it consumption hazardous to health. Adebayo-Tayo *et al.* (2008) because they might contain metabolites produced by the fungi. Although some *A. flavus* strains can produce elevated levels of aflatoxins, while other strain produces negligible levels, Abbas *et al.* (2009)

CONCLUSION

Food safety is the scientific discipline describing handling, preparation, and storage of food in ways that prevent food-borne illness to avoid potentially severe health hazards. Generally, the density of fish and fishery products are related to the environmental factors such as water pollution, hygienic condition of processing environment, processing in appropriate methods, handling, transportation, commercialization and storage condition. Good Aquaculture Practice (GAP), Sanitation Standard Operating Procedure (SSOP), hazard analysis and critical control points (HACCAP) etc. should be maintained in every step from culturing through processing to marketing of product so as to ensure food safety. This work therefore recommend that to prevent the incidence of food contamination, there is a need to educate the related people such as fish processors, handlers, retailers or vendors about the importance of sanitation, hygienic measures and good food handling practices. Proper hygienic condition should be maintained at every step of culturing, catching, landing and transportation, processing, storing and marketing following HACCAP steps for producing good quality of fish and fishery products. And this good quality of fish and fishery products can play a significant role in fulfilling the nutrient demand of Wukari people and earn foreign currency to improve the economic condition of the locality, state and the nation

Conflict of Interest: The Authors declares no Conflicts of Interest.

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