

Isolation and Identification of Fungi from Dried Fishes sold at Garindau, Wudil Local Government Area Kano State

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Abstract: This study was aimed at isolating and identifying fungal contamination of dried fish sold at Garin dau Market, Wudil Local Government Area, Kano State, Nigeria. A total of ten samples of dried fish were collected and analyzed using standard microbiological techniques to determine fungal load and species identification. The results revealed fungal counts ranging from 9.00×10^3 to 2.90×10^4 CFU/ml, significantly exceeding the safety threshold set by NAFDAC/WHO, which is 1.00×10^2 CFU/ml. Five fungal species were isolated from the samples, including *Aspergillus niger* (33.33%), *Mucor* spp. (29.63%), *Aspergillus flavus* (14.81%), *Candida* spp. (14.81%), and *Penicillium* spp. (7.41%). The presence of *Aspergillus flavus*, a known producer of aflatoxins, poses a serious public health concern due to its carcinogenic potential. These fungi such as *Mucor* and *Candida* species are also associated with infections, particularly in immunocompromised individuals. These findings highlight the need for improved preservation methods, including more effective drying techniques and proper storage practices, to reduce fungal contamination. These measures are vital for safeguarding public health and maintaining the economic value of dried fish in local markets.

Keywords: Dried fish, fungal contamination, aflatoxins, food safety, microbial load.

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Introduction

Fish is a vertebrate animal living in fresh and seawater. The importance of fish in human and animal nutrition cannot be overemphasized. According to Mei *et al.*, (2019), fish is a low fat food, a great source of protein, vitamins and minerals. Fish harvesting, handling, processing and distribution provide livelihood for millions of people. It is the most important animal protein food available in the tropics, and it represents about 14% of all animal protein on a global basis. In Nigeria, fish is eaten fresh, preserved or processed (Dried) and form a much-relished meal that spans socio-economic, age educational and religious barriers. Abebe *et al.* (2020) reported that the demand for fish in Nigeria greatly exceeds supply. This problem is aggravated by the low level of domestic fish production against the increase in human production. Fish is highly nutritious food of about 60-80% water, 15-25% protein, 11-22% fat, 20% mineral and 1% carbohydrate (Iacumin *et al.*, 2022). Most of the catches come from oceans, seas, rivers and lately man-made ponds. It is often cheaper than meat and so it is a rich protein source for both the poor and wealthy (Dehghani *et al.* 2018).

The preservative effect of salt has been recognized as being due to a decrease in water activity, less availability to microbial attack and enhancement of functional properties, leading to an increase of the shelf life time. Meanwhile, dried fish and shellfish products have been reported to be a source of microbial hazards including *Listeria monocytogenes*, *Salmonella* spp. and

Clostridium botulinum. Outbreaks of botulism, listeriosis, and salmonellosis resulting from dried fish have been reported for over 30 years. Also, Chitrakar *et al.*, (2019) reported that dried fish samples from four local Markets in Kainji Lake area of Nigeria were dominated by gram-positive bacteria, potential pathogens, coagulase-positive *Staphylococcus* and *Escherichia coli*(Abebe *et al.* 2020). Delay or prevention of microbial spoilage of fish may be achieved by different preservative methods that include the use of dried and brining (Hicks, 2016).

In certain instances, sodium chloride is added mainly as a flavoring and functional ingredient and hence in these cases the effect could be "indirect" (Kuley *et al.*, 2020). Another reason that the antimicrobial effect of sodium chloride may be called indirect is that it reduces the water activity in many foods and this by indirectly prevents microbial growth (Kuley *et al.*, 2020). The steps in the dried process are necessary not only for safe preservation, but also to produce good flavour and aroma. Hence dried fishes are less prone to microbial spoilage than fresh fish. However, spoilage still occurs as a result of growth of microbes due to partial dehydration during dried (Bratt, 2020). Dried fish products can be a source of microbial hazards including *Listeria monocytogenes*, *Salmonella* spp., *Clostridium botulinum* etc. Abraha *et al.* (2018) due to unhygienic handling, marketing and storage or due to the partial removal of water activities during production. Raw Dried fish are generally eaten in many countries (Ikape, 2017). If the

Dried fish are contaminated with pathogenic microbes, this can cause the fatal diseases in the human body (Cheng *et al.*, 2015).

In many developing communities, dried fish are usually hawked without taking cognizance of the microbial contamination from the environment. In Nigeria, Dried 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 potential sources of microbial contamination (Howell *et al.* 2015). For this reason, it is necessary to estimate the bacterial load along with some pathogenic bacteria and fungal contamination in fresh and stored dried fish (Arason *et al.*, 2014). Despite the widespread consumption and economic importance of dried fish in many regions, particularly in developing countries, spoilage remains a significant challenge. This spoilage is primarily caused by various microorganisms that thrive even in low-moisture conditions, leading to substantial food losses, economic setbacks, and potential health risks due to toxin production.

Dried fish is a significant source of protein and of these essential nutrients, particularly in regions where access to fresh fish is limited. However, despite its reduced moisture content, dried fish is susceptible to spoilage, primarily due to microbial activity. Understanding the microorganisms associated with the spoilage of dried fish are crucial for developing effective preservation methods, ensuring food safety, and minimizing economic losses.

Material and Methods

Study Area

Garindau Market Wudil local government area, Kano was coordinated on the latitude 11° 8.2127'N and longitude 8°5'E of the Greenwich Meridian. It is surrounded by Wudil Local Government to the North and East, Gaya to the East, Dawakin Kudu to the West. The 2006 census puts the population of the area at 115,189 with an estimate land mass of 332 km² (NIPOST, 2006).

Sample Collection

According to Chakraborty and Chakraborty (2017), ten (10) sample of dried fish were randomly collected from different vendors from Garindau market, Wudil L.G.A. the sample dried fish were carefully packed and transported to the laboratory of department of microbiology, Aliko Dangote university of science and technology Wudil, where they were properly identified according to the identification keys described by Chakraborty and Chakraborty (2017), weighed individually and stored in a refrigerator prior to mycological analysis.

Enumeration of Fungi

Sample preparation

For isolation of fungi, pour plate technique was used in which a portion of 10ml of the sample was added into a flask

containing 90ml of distilled water that was mixed carefully. A portion of 1ml of the homogenate was transferred into a test tube containing the 9ml of the diluents, it was mixed carefully with a fresh pipette by aspirating 10 times then from the second dilution with the same pipette another 1ml was transferred into the next tube containing 9ml of the sterile diluents, it was then mixed with a fresh pipette and repeated with the same steps using 1st, 2nd, 3rd, 4th and 5th tubes of the diluent. A portion of 1ml of the sample dilution was pipetted from the 1st, 2nd, 3rd, 4th and 5th tube into each of the appropriately marked sterile petri-dish. CFU/ml (Anihouvi *et al.*, 2019).

Isolation and Identification of Fungi

A portion of 15ml of acidified PDA (kept at 44 to 46°C in water bath) was poured into each of the Petri dish, the sample dilution and agar medium were mixed thoroughly and uniformly by swirling the petri dishes gently, allowed to set and solidified. The prepared petri dishes were inverted and incubated at 32-37°C (in the cupboard) for 3 to 5 days, growth were counted and the results were recorded as CFU/g (Anihouvi *et al.*, (2019).

Sub-culturing

A pure culture of each of colony type were obtained and maintained. The maintenance was done by sub-culturing each of the different colonies on to the SDA plates and incubated at room temperature again for 5 days. In sub-culturing, baiting method which involves the use of a sterile wire loop to pick up growth on the culture and then transferred to the subculture media was implemented, after which the petri dish were labeled according to the color of growth that was transferred into it (Iacumin *et al.*, 2022).

Wet mount microscopy of the Fungi isolates

A portion of 72 hours culture from the plates were picked and place on a clean glass slide. A few drops of lactophenol were added and heated to steam while gently teasing the culture then a few drops of lactophenol cotton blue stain were added and this was also heated to steam for few seconds and removed from heat. The glass slide were covered with a cover slip and the edge of the cover slip was gently pressed and finally observed under a microscope (Abebe *et al.*, 2020).

Results

Fungal Plate Count of the dried fish sample

The fungal plate count, reveals that the fungal contamination levels of the dried fish samples ranged from 9.00x10³ to 2.90x10⁴ CFU/ml. These values are significantly higher than the recommended safety limit set by the NAFDAC/WHO, which is 1.00x10² CFU/ml. This indicates a high level of fungal contamination in all the samples, posing potential health risks to consumers (Table 1).

Table 1: Fungal Plate Count of the dried fish sample

Sample ID	Fungal Counts (CFU/ml)
A	2.90x10 ⁴
B	1.65x10 ⁵
C	1.30x10 ⁴
D	2.30x10 ⁵
E	1.50x10 ⁴
F	2.15x10 ⁵
G	1.30x10 ⁴
H	1.20x10 ⁵
I	1.45x10 ⁴
J	9.00x10 ⁵

Key words; CFU/ml – colony forming unit per mill of sample, A – J Samples from different locations.

Cultural and Morphology Characteristics of the Fungal Isolates

Five different fungal species were identified across the fish samples: *Aspergillus niger*, *Mucor* spp., *Aspergillus flavus*, *Candida* spp., and *Penicillium* spp (Table 2).

Table 2: Cultural and Morphology Characteristics of the Fungal Isolates

Cultural Characteristics	Morphological Characteristics	Isolates
Non-branched conidiophores with bulb end carries conidia like sunrays	Blackish colonies on SDA or pin like black growth	<i>Aspergillus niger</i>
Non-branched conidiophores with bulb end carries conidia	Pin like green growth	<i>Aspergillus flavus</i>
Unicellular cocci or orbid shape, larger than bacterial cell	Flat, smooth large colonies	<i>Candida</i> sp.
Sporangia contain spores, do not have rhizoids	Cotton like white growth spotted with black color	<i>Mucor</i> spp.
Brush-like conidiophore carries conidia	Green or green greyish color colonies growth over fruits	<i>Penicillium</i> sp.
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Frequency of Occurrence of Fungal species

The frequency of occurrence of fungal species showed *Aspergillus niger* was the most prevalent, occurring in 33.33% of the samples, followed by *Mucor* spp. at 29.63%. *Candida* spp. and

Aspergillus flavus both had an occurrence rate of 14.81%, while *Penicillium* spp. was the least frequent at 7.41%. These fungal species are known for their potential to cause spoilage and pose health risks, with some, such as *Aspergillus flavus*, being capable of producing harmful mycotoxins (Table 3).

Table 3: Frequency of Occurrence of Fungal species

Samples	<i>Candida</i> spp.	<i>Mucor</i> spp.	<i>Aspergillus</i> <i>flavus</i>	<i>Aspergillus</i> <i>niger</i>	<i>Penicillium</i> spp.
A	+	+	+	+	+
B	+	+	-	+	-
C	-	+	+	+	-
D	-	+	-	-	-
E	-	+	+	+	-
F	-	+	-	+	-
G	+	+	+	+	-
H	+	-	-	+	-
I	-	+	-	+	-
J	-	-	-	+	+
Total	4 (14.81%)	8 (29.63%)	4 (14.81%)	9 (33.33%)	2 (7.41%)

Key words; CFU/ml – colony forming unit per mills of sample, + = Positive - = Negative. A – J Samples from different locations

Discussion

Findings from this study shows that fungi, mold and yeast contaminate dried fish. The traditional method of preserving is often employed to produce commonly acceptable dried fish for the consumption. The fish can be transported or stored. Results from this study shows that 10 sample of dried fish were contaminated with fungi species. Fungi produce spores which are moderately resistance to drying and therefore easily implicated in the contamination of spoilage of dry and semi dry materials. This is an agreement with the finding of Eaton and Groopman, (1994) that molds have the ability to survive harsh conditions and low moisture content. The dominant occurrence of *mucor* spp obtained in this research is similar to worker reported by Ahemed *et al.* (2004), Essein *et al.* (2005). Where *candida* spp, *Rhizopus* spp, *A. fumigatus*, *A. Flavus* and *A. niger* in decreasing sequential order the distribution of these fungi probably resulted from the ubiquitous nature of fungi as elaborated by Ibeh *et al.* (1991). Molds produces numerous air bone conidia which easily disperse by air movement and possibly insects.

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. *Penicillium* spp produce aflatoxin and citrin. Mari and Riccioli (2004) reported *A. niger* and *A. fumigatus* as being allergenic. The presence of these fungi in the dried fish samples might probably make it consumption hazardous to health, Adebayo Tayo *et al.* (2008) because they might contain metabolites produced by fungi. Although some *A. Flavus* strains can produce elevated levels of aflatoxins, while other strain produces negligible levels, Abbas *et al.* (2009). Based on literature myotoxin production by *A. Flavus*, account for 30-40% of known isolates is believed to produce Aflatoxins Frisvad *et al.*, (2007). The strain variability is also dependent on several factors such as substrate, the geographical location and the environmental factors (Horn, 2005, Klich, 2007), the potency of these metabolites is not affected by cooking and may cause severe or fatal damage to the liver and kidney (Mitchell, 2007). FACT,(2004) noted that where the relative humidity is above the critical value of 80-90% most *Penicillium* spp and *Aspergillus* spp would grow easily on dried fish .The study also observed mixed growth of fungi in various combinations of two or three fungi from all the market similar to the work of Junaid *et al.*, (2010) the

data obtained were statistically analyzed for comparison, however there is no significant relationship between the fungal organism isolated and market places where the samples were sourced.

Conclusion

The study aimed to determine the fungal load associated with dry fish sold at Garindau market in Wudil Local Government Area, Kano State. A total of 10 samples were collected from different vendors and analyzed using standard microbiological techniques. The result revealed that *Aspergillus niger* was found with highest number of occurrence of 33.3 % and *Penicillium* spp. with lowest number of occurrence of 7.4%. It will be therefore useful to develop and establish public health standards in processing good hygienic environmental condition in market places as consumption of these fungi exposes the consumers to the possible toxic metabolites produced by some of the isolates. Based on the findings of this study, it is recommend that proper drying techniques, such as mechanical drying, to reduce moisture and inhibit fungal growth in dried fish should be employed. Natural preservatives like salt and organic acids should be used to extend the shelf life and reduce fungal contamination in dried fish.

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