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ORIGINAL RESEARCH ARTICLE

Isolation and Identification of Fungi Associated with Dried Meat Sold at Sokoto Metropolis

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ABSTRACT
Thin sheets of high-quality, lean beef are partially dried in the sun to create dried meat made from animals. The duration of the solar drying phases and the infusion period of the ingredients rely on the ambient conditions, which are the primary sources of microbial contamination, notably fungi. This study's objective was to recognize and collect fungi connected to the dried meat offered for sale in Sokoto city. During the course of the research, dried meat samples were gathered from four different Sokoto metropolis locations, including Kusha Shagalinku on Atiku Road, Gidan Man Ada, Abdullahi Fodio Road, and Sasakawa. The samples were examined for microorganisms using serial dilution, and fungi were identified based on the morphology of the colony, conidia, and conidiogenous cells. The research's findings showed that four species of fungi were isolated, with *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, and Aspergillus terreus* having the highest and lowest percentages of occurrence, respectively, among the isolated species. According to the location, the dried meat retrieved from Atiku road has the lowest frequency occurrence (1.94) and the fungi isolated from it has the highest frequency occurrence (30.56%). These results suggest that eating dry meat may increase the risk of exposure to aflatoxins in humans. The preparation and storing of the dried meat should be done with the utmost safety and cleanliness.

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INTRODUCTION
Sheep, cattle, goats, and pigs all produce meat, which is animal flesh used as food (Hassan *et al.*, 2014; Mohammed *et al.*, 2017). Because it contains so many nutrients and so much moisture, meat is one of the most perishable foods because it encourages the colonization and growth of bacteria. Due to the rising population in developing nations, demand for its consumption is gradually increasing. Due to their great nutritious value, meat products improve diets (Olusola *et al.*, 2010). The most popular dried meats in Nigeria are Kilishi, Tinco, and Kundi, which are primarily made by people from the north. Others include Ndariko, Jiorge, and Banda, who used buffalo, donkey, asses, horses, camels, and horses' flesh after the bones were removed (Segun *et al.*, 2016). Protein is abundant in dried beef, according to Ogonsola and Omojpla, (2008). Foods that have been processed using the Hurdle Technology are more safe and microbiologically stable while being stored, especially at room temperature because to auto sterilization (Segun *et al.*, 2016).

It is still far from normal for a large portion of the Nigerian populace to have access to and afford meat. Dried meat has a long shelf life, is easy to carry in quantity, and is fortified with extra nutrients. The primary method of meat preservation brought to West Africa by medieval Arabic sources was sun drying. Dried meat is processed in such a way that it is particularly vulnerable to major microbial infection, endangering its suitability for human eating. It contains about 46% meat and 54% non-meat ingredients. A finished product contains about 50% protein, 7.5% moisture, 18% lipid and 9.8% fibre /ash respectively. Dried meat is a common gift from the northerners to visitors especially the youth corps members from other parts of Nigeria. Adding spices to dried meat ingredients is also of health importance this-could be a check to stomach disorders, rheumatics and act as relaxers of the alimentary system (Segun *et al.*, 2016). Meat is regarded as a significant source of minerals, B complex vitamins, vital amino acids, and proteins. It provides a particularly favorable environment for the growth of microorganisms because of its rich content (Abdel Sater *et al.*, 2017). Because of the ways that different types of meat are prepared and preserved, there are several types of meat. Due to its chemical makeup, the surface of meat is typically extensively infected with a variety of microbes. Due to its composition, beef might serve as a favorable

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environment for the development of microbes (Salau *et al.*, 2020).

The condition of the products is impacted by fungi that enter meat products through meat, spices, and other components, as well as through the environment, equipment, and handlers during processing (Morshdy *et al.*, 2015; Perron *et al.*, 2019; Abd El-Tawab *et al.*, 2020). According to Asefa *et al.* (2010) and Abdel-tawab *et al.* (2020), contamination of meat products with various mold species is a genuine risk since it increases the likelihood that the products will decay and deteriorate, which has an impact on their quality. The objectives of this study are to isolate and identify the fungi that cause spoilage in dried meat, determine how frequently they occur, suggest control measures to lessen the harm that the fungi cause, and raise public awareness of the prevalence of fungi in food.

MATERIALS AND METHOD

Study area

The study was conducted at Mycology Laboratory of the Department of Biological Sciences Usmanu Danfodiyo University Sokoto. Sokoto is situated in the extreme northwest of Nigeria, near to the confluence of the Sokoto River and the Rima Rivers. It is situated geographically at a height of 272 m above sea level, between latitude 13°349°N and longitude 5°1489°E. Farming, trading, fishing, and leatherworking are among the city dwellers' occupations (Moi, 2008). The town, Sokoto is bordered by sand dunes and isolated hills in the parched Sudanese savannah. Rainfall begins in June and finishes in early September; however, it occasionally continues into October. With a peak in August, annual precipitation is 550 m. The hot season's peak temperature of 45°C occurs between March and April, and Harmattan occurs between November and February. Ohunakin *et al.* (2014).

Materials

Potato dextrose agar (PDA), cotton wool, aluminum foil, masking tape, conical flask, petri dishes, distilled water, ethanol, clean glass slide and cover slip, weighing scale, bouncing burner, inoculating needle, microscope, autoclaving machine, test tubes, streptomycin, hand gloves, and face mask are among the supplies used for the research project.

Collection of samples

Four separate Sokoto metropolis locations were used in the dried meat samples collection, including Kusha Shagalinku on Atiku Road, Gidan Man Ada, Abdullahi Fodio Road, and Sasakawa (located in the UDUS). The sample was taken to the mycology laboratory of Usmanu Danfodiyo University, Sokoto for analysis after being labeled A, B, C, and D, respectively, to denote the collection centers.

Sterilization of glass wares

To completely remove all traces of detergent, all glassware used in this practical washed with detergent solution first, then rinsed with tap water. As instructed by Kumar *et al.* (2019), they were completely dried before being sterilized for an hour in a hot air oven at 160°C. Then, before being put to use, they were allowed to cool to ambient temperature.

Preparation of culture media

As described by Ravimannan *et al.* (2014), potato dextrose agar was employed. It was made in accordance with the manufacturer's instructions, which call for 39 g of dehydrated powder (PDA) to be weighed and suspended in 1 litre of distilled water, or 1000 ml, in a conical flask. Also weighed and suspended in the distilled water was 1g of streptomycin, and the conical flask was then heated on a hot plate to completely dissolve the agar. The conical flask's mouth was sealed with cotton wool, and the mouth was covered with aluminum foil. The media was then sterilized using an autoclave (model YX-2804) at 121°C for 15 minutes. After allowing the media to cool, 20 ml was removed and poured into 90 mm sterilized petri dishes, where it would stay for 24 hours to undergo sterilization and solidification.

Isolation of fungi from dried meat

The samples (A, B, C, and D) were weighed before being subjected to serial dilution in accordance with the technique described in (Orogu *et al.*, 2018). Each sample was weighed and put in a test tube with 9 ml of distilled water, totaling 1g. After sterilizing it in an autoclave at 121°C for 15 minutes, it was allowed to cool before performing the serial dilutions (10^{-1} to 10^{-3}). Using a steromitizer to spread and cover the media's surface, 1ml of a 10^{-2} dilution was spread-plated onto PDA medium. The media was then incubated for five days at a temperature of between 28 °C and 35 °C. After incubation colonies of different shape and colour were observed on the plates. Each colony was sub-cultured twice in a fresh PDA in order to obtain a pure culture. These colonies were isolated and subjected to identification.

Identification of isolated fungi

According to Kova, *et al.* (2020), morphological traits of the colony, conidia, and conidiogenous cells were used to identify the fungus. With the aid of a sterile pipette and a sterilized inoculating needle, a drop of distilled water was applied to a grease-free surface to identify the isolates. In this instance, a tiny bit of the mycelium from the fungi cultures was taken out and dipped into the distilled water drop. To make viewing easier, the mycelium was evenly dispersed throughout the surface using 2 sterile inoculating needles. Air bubbles were removed by lightly pressing a cover slip onto the surface. After that, the slide was mounted on an electric microscope (Model XSZ21), and X10 and then 40 objective lenses were used to see it. The mycological atlas by Robert and Ellen (1988) was used

to help identify the isolates, and Tafinta *et al.* (2014) obtained certification of the identification from trained mycologists, technologists, and technicians working in the mycology laboratory.

RESULTS

The result of this study indicated that, the dried meat is infected with fungi either during the processing stage or packaging. From the samples obtained in four (4) difference location within Sokoto metropolis four species of fungi were isolate *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus and Aspergillus terreus*. Table 1 shows the colonies and morphological characteristics of fungi isolated from dried meat. Table 2 shows Distribution of fungi isolated from four sampling point, while Table 3 shows the number of isolates per sampling point. Table 4 shows the frequency of occurrence of the fungal isolate of all sampling points. Table 5 shows the % of occurrence of the isolate. *A.niger* has the highest % of occurrence (80%) and *A.terreus* has the lowest % of occurrence (20%). Table 4 shows the frequency occurrence in four sampling point, the total number of organisms isolated from each sample and frequency occurrence of each of the total number of organisms. Fungi isolated from dried meat obtained from Abdullahi fodio road has the highest frequency occurrence whereas the dried meat obtained from Atiku road has the lowest frequency occurrence. Table 5 on the other hand shows the percentage occurrence of the fungal organisms in totality irrespective of the four locations. *A. niger* has the highest % occurrence followed by *A. flavus* and then *A. fumigatus*. *A. terreus* has the lowest % occurrence.

TABLE 1. Morphological and microscopic characteristics of the fungal isolate

Organisms	Morphological	Microscopic
A. flavus	Colonies are commonly powdery masses of yellowish green on the upper surface and reddish gold on the lower surface. Colonies appears downy in texture.	The conidiophores were colorless, thick-walled, roughed, and bearing vesicles. The diameter of the conidiophores ranged from 800 to 1200 µm. The conidia were globose, thin-walled, slightly roughed, and ranged from 250 to 450 µm in diameter.
A. niger	Have cottony appearance; iniatially white to yellow and then turning black. It growth rapidly.	Smooth colored conidiophores and conidia. The conidiophores are protrusions from septate and hyaline hyphae. The phialides produce conidia that have a rough texture, are dark brown colored, and have a diameter of 4-5um.
A. fumigates	The colonies have distinct margin appeared greenish brown in colour. The surface has powdery appearance	Conidiophores are smooth-walled, uncolored, up to 300 µm long, and terminate in a dome-shaped vesicle that is 20- 30 µm in diameter. This species is uniseriate with closely compacted phialides (5-10 x 2-3 µm) occurring only on the upper portion of the vesicle.
A. terreus	Colonies have the condition to grow rapidly and have smooth-like wall. The surface colour is pinkish, cinammom to deeper wih age. It has entire margin and it grows moderate to rapid.	The conidial heads that is compact, biseriate, and densely columnar, reaching 500 × 30–50 µm in diameter. The conidia of A. terreus are small, about 2 µm in diameter, globose-shaped, smooth-walled, and can vary from light yellow to hyaline

KEY; M= Morphological characteristics, M= Microscopic characteristics

TABLE 2. Number of fungi isolated from dried meat in Sokoto, Nigeria

Organisms observed	Samples			
	A	**B**	**C**	**D**
Aspergillus flavus	2	3	2	4
Aspergillus niger	4	1	6	2
Aspergillus terreus	0	0	2	1
Aspergillus fumigatus	1	5	1	2
Total	7	9	11	9
Mean	1.75	2.25	2.75	2.25

KEY; A= Atiku road, B= Gidan man Ada, C= Abdullahi Fodio road, D= Sasakawa

The table above shows the total number of fungal organisms from each sampling point where Abdullahi fodio road has the highest number of organisms.

TABLE 3. Number of isolate per sampling point

Sites	*A. flavus*	*A. niger*	*A. terreus*	*A. fumigatus*	Total
A	2	4	0	1	**7**
B	3	1	0	5	**9**
C	2	6	2	1	**11**
D	4	2	1	2	**9**
Total	**11**	**13**	**3**	**9**	**36**

KEY; A= Atiku road, B= Gidan man Ada, C= Abdullahi Fodio road, D= Sasakawa

The table above shows the total number of organisms isolated from each sampling point. *Aspergillus niger* has the highest number where as *Aspergillus terreus* had the lowest number.

TABLE 4. Fungal frequency occurrence in four sampling point

Sites	Fungal Occurrence	Fungi % Frequency
A	7	19.44
B	9	25
C	11	30.56
D	9	25
Total	**36**	**100**

KEY; A= Atiku road, B= Gidan man Ada, C= Abdullahi fodio road D= Sasakawa

TABLE 5. Percentage occurrences of the Isolated fungi irrespective of their locations

Isolates	Occurrences	Percentage of Occurrence
A. flavus	11	30.56
A. niger	13	36.10
A. terreus	3	8,30
A. fumigatus	9	25.0
Total	**36**	**100**

The above table shows the percentage occurrence of the fungal organisms of four location. *A. Niger* has the highest % occurrence followed by *A. Flavus* and then *A. fumigatus*. *A. terreus* has the lowest % occurrence.

DISCUSSION

This study shows that *A. Niger, A. flavus, A. fumigatus* and *A. terreus* were found in dried meat sold in Sokoto state, Nigeria. Out of the fungi isolated *A. Niger* has the highest percentage (36%) followed by *A. flavus* (30%) *A. fumigatus* (25%) and *A. terreus* (8%). The total highest fungi count was recorded in died meat samples obtained from Abdullahi fodio road dried meat and the lowest from Kusha shagalinku Atiku road. The isolation of these species in the samples agreed with the findings of some researchers who isolated similar organisms from smoked-dried fish, dried meats and Suya spicy in Nigeria (Tayo *et al.*, 2015; Tayo *et al.,* 2008; Mohammed, *et al.,* 2017; Amal 2011).

Different fungal strains were isolated from the dried meat samples which are of major public health concern. A major public health risk with long term health implication is the contamination of food and feed with fungi that may be aflatoxigenic (Adeyeye, 2016).

Aspergillus niger popularly known as the black mould and several other species cause decay of foodstuffs. *A. flavus and A. niger* parasitizes man and animals. They cause a number of diseases grouped under the name Aspergillosis. Most people breathe in *Aspergillus* spores every day without getting sick. However, people with weakened immune systems or lung diseases are at a higher risk of developing health problems due to *Aspergillus*. Health issues related to *Aspergillus* include infection in the organs of the body such as the lung as well as allergic reaction. This infection may also be seen in human ear and is called Otomycosis. *A. flavus* is reported to produce mycotoxin known as Aflatoxin which is a potent carcinogen and has been directly correlated with adverse health effects, such as liver cancer, in many animal species (Alhaji *et al.,* 2020; Kutama *et al.,* 2022).

Particularly common clinical syndromes associated with *A. flavus* include chronic granulomatous sinusitis, keratitis, cutaneous aspergillosis, wound infections and osteomyelitis following trauma and inoculation (Hedayati *et al.*, 2007; Li *et al.*, 2009; Pasqualotto, 2009). *Aspergillus flavus* outbreak differs from A. fumigates outbreak in that outbreak that are of *A.flavus* is found to be related to just one strain or closely related strains (Hedayati *et al.*, 2007). The isolated fungi are of economic and public health importance. Some species of these fungi have been reported to produce potent mycotoxins called ochratoxins that can be harmful to human beings and animals (Alhaji *et al.,* 2020).

The low water activity as a result of drying reduces the competitive effects of most bacteria. Several physical factors including moisture, humidity, ambient temperature, storage time, pH and oxygen affect fungal growth and mycotoxins production in dried meat. The isolated fungi can be classified as storage fungi. The storage fungi could have contaminated the dried meat sample during any of the phases of processing. The fungal species that colonize the dried meat samples must have been present in the atmosphere in the form of spores during the processing or gained entrance during storage period as a result of inadequate storage facilities as well as in the market and also during transportation (Adeyeye, 2016).

CONCLUSION

Based on the finding of this study, it was found that *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus* and *Aspergillus terreus* are detected in spoilt dried meat. The present work indicated that the examined dried meats were contaminated with several fungi. Many of these fungi are capable of producing mycotoxin such as aflatoxins, ochratoxin and fumonisins. These findings indicate that there may be a risk of human exposure to mycotoxins through the consumption of the dried meat.

RECOMMENDATIONS

1. Strict hygienic measures must be applied during the processing and storage of the meat samples
2. Concerted efforts on the part of relevant authorities to check the trend, of these public health challenge needed.

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