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Primary Screening for Fungi Isolates that Produce Mycotoxin from Sun-Dried Meat

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

This study aims to screen fungal isolates from sun dried meat samples to identify fungal presumed producing mycotoxins in the sun dried meat. A total of 8 fungal isolates from sun dried meat were used for the study. They are Aspergillus (2), *Penicillium spp* (3), Fusarium spp, Mucor spp and Rhizopus spp. The fungal isolates from eight sun dried-meat samples were sub-cultured on Potato Dextrose Agar and Sabouraud Dextrose agar in order to obtain pure colonies for further analysis. The detection of mycotoxins in the sun dry meat samples was carried out by the use of Coconut Agar Media (CAM), by which the fungal isolates were cultured and plates incubated for 3-7days at 28C. The results obtained show that some of the fungal isolates had the ability to produce mycotoxins. From the mycotoxin screening, only four (4) species (Aspergillus spps, and Penicillium spp) were positive (+) and Rhizopus spp, Fusarium spp and Mucor spp, were negative (-). Mycotoxin contamination level varied in different market locations in which Academy Market had the highest level of mycotoxin (71.4%) followed by Oje Market (14.3%) and Oremiji Market (14.3%). Mycotoxigenic producing fungi and mycotoxin levels in the sun dried meat samples are public health concern. Also these results calls for more sensitization for safety of sun dried meat.

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1. INTRODUCTION

"Meat is one of the most popular and known food items which come from flesh of animals that are suitable as food" [1] "Meat surface is usually heavily contaminated with a wide range of microorganisms due to its chemical composition which includes; water content, peptides, sugars, amino acids, nucleosides, mineral and vitamins". Ostry and Ruprich [2] "This composition makes the meat a suitable medium for the growth of microorganisms" [2].

"Due to the high perishable nature of meat, its freshness and shelf life could be greatly affected by a large number of factors such as temperature, moisture, enzymes, microorganisms among several others which can make it unhealthy for human consumption" [3]. Sun-dried meat is widely commonly consumed and cherished for its distinct flavor in many cultures worldwide particularly in Africa, Asia, and South America, it is also recognized as a possible source of mycotoxins [4].

There are many types of fungi, this includes yeast, mould and mushroom, and each having its distinct action or activities on substances they feed and live on Peck, [5]. Fungal loads on sun-dried meat can increase the risk of food poisoning and illness due to the various toxins produced by this organism Omojokun, [4]. "Some of the food poisoning that occur from sun-dried meat include nausea, vomiting, abdominal pain and diarrhea, but severe cases may lead to hospitalization" [6].

"Mycotoxins are toxic secondary metabolites produced by fungi that can contaminate a wide range of food including sun-dried meat. Mycotoxins are known to cause a variety of negative health effect to both humans and animals, such as cancer, liver damage, renal failure, and immune suppression, making their presence in food products a critical public health concern" [7].

Meat is susceptible to fungal contamination because of its high moisture content, relatively low acidity level and exposure to warm temperatures during the drying, storage processes all of which creates an ideal environment for fungal growth. Fungi are the primary source of mycotoxins, and studies have shown that the incidence of mycotoxin contamination in food is closely associated with the presence of fungi in that commodity [8]. The contamination of meat products with various fungi 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 risk associated with these fungi and their metabolites in food make it necessary to always to screen for their presence. Therefore, this study, focus on screening fungi for their mycotoxins production because identification is an important step towards their control and reduce their economic and uphold food safety in the society.

2. MATERIALS AND METHODS

2.1 Sample Collection

The samples were isolates obtained from eight samples of sun-dried meat randomly obtained from three (3) markets, (Oremeji Market), (Oje Market), (Academy Market) in Oyo state, Ibadan, Nigeria.

2.2 Media Preparation

"Potato dextrose agar and Sabouraud Dextrose agar were employed. They was made according to the manufacturer's instructions. Thirty nine grams (39g) of dehydrated powder (PDA) and (SDA) were weighed and suspended in 1 litre of distilled water separately, or 1000 ml, in a conical flask. The conical flask was then heated on a hot plate to completely dissolve the agar. The conical flask's mouths were sealed with cotton wool, and the mouths were covered with aluminum foil. The media was then sterilized using an autoclave 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" [9].

2.3 Fungal Isolation

The isolation of fungi was carried out according to the agar dilution method as described by Pal et al., [10]. One (1) gram from each sample were homogenized with 90 ml of buffer peptone water and serial decimal dilutions (10^{-1} to 10^{-4}) were performed. Fungal species were isolated on the Potato dextrose agar. The medium was poured into sterile Petri dish and 0.1 ml of each sample suspension was spread-plated onto the PDA

media. The plates were incubated for 5 to 7 days at 25°C. Fungal isolates were sub-cultured on Sabouraud Dextrose agar and incubated for 5 to 7 days at 25°C for purification. The total fungal count for each plate was expressed as colony-forming units per gram of sample (CFU/g). Each genus or species identified was then expressed as percentage (%) of the total isolated fungi.

2.4 Identification of Fungi

Identification of fungal Genera and the determination of each species of fungi were done using the method of Klich, [11]. This was done by observing both microscopic characteristics and morphology of the colonies on PDA and SDA medium.

2.5 Mycotoxigenic Potential of Fungal Isolates

The mycotoxigenic potential of the fungal isolates was determined using coconut agar Media (CAM) following a method described by Norlia et al. [12] for AF and by Zhang et al. [13] for OTA, with a slight modification. Coconut Agar (CAM) was used. For the preparation of Coconut Agar Medium, using 300 ml of hot distilled water, a 100 g piece of coconut was homogenized for 5 minutes. After passing through layers of cheesecloth, the homogenate was filtered, and 2 N NaOH was used to bring the filtrate's pH down to 7.0. After adding 20 grams of agar per liter, the mixture was autoclaved for 15 minutes at 120 degrees Celsius to sterilize it. When the media was solid, the pure fungal isolates were cultured on Coconut Agar Media (CAM) and plates incubated for 10 days at 30˚C. When fungal strains grew on Coconut Agar Medium (CAM) they were first screened for the production of Aflatoxin by looking for the emission of blue or green fluorescence at 365 nm following UV light. Extracted mycotoxins were analysed using chromatographic techniques. The production of a yellow-orange pigmentation of fungal hyphae was observed in toxigenic strains. AF- producing isolates showed green fluorescence on the reverse sides of the plates and a blue-green fluorescence for OTA.

3. RESULTS AND DISCUSSIONS

The cultural and microscopic observation of the isolates showed five different fungal species which was gotten from eight sun dried-meat samples from three different markets in Oyo state as shown in Table 1. All the isolated fungal belong to four kinds of pathogenic fungal. It's *Penicillium spp*, *Aspergillus spp*, *Mucor spp*, *Rhizopus spp*, *Fusarium spp*.

Table 2, shows fungal frequency occurrence in three markets and eight sampling point.

Table 1. Identification of fungi isolated from sun-dried meats

S/n	Sample code	Microscopic characteristics	Probable organism
1	Academy Market 1	Hyphae are smooth, aseptate and polynuclear. Sporangiospores are globose	*Mucor spp*
2	Academy Market 2	Stolons present, rhizoids, Sporangiophores sprouting from columellae. Striated sporangiospores	*Rhizopus oryzae*
3	Academy Market 3	Conidial heads are biseriated and radiated. Hyaline, smooth brown coloured conidiophores and conidia	*Aspergillus niger*
4	Oje Market 1	Filamentous and septate hyphae with conidia. Hyphae are colourless, slender, tubular and branched	*Penicillium chrysogenum*
5	Oje Market 2	Colonies green to gray in colour. Radial furrow absence. Distinctive brush-shaped conidiophores	*Penicillium* spp
6	Oje Market 3	White colonies with velvety surfaces and branching hyphae. Filaments are hyaline and septate	*Fusarium* spp
7	Oremiji market 1	Branching hyphae. Powdering masses of yellowish-green spores on the upper surface and reddish-gold on the lower surface	*Aspergillus flavus*
8	Oremiji market 2	Colonies green to gray in colour. Radial furrow absence. Distinctive brush-shaped conidiophores	*Penicillium* spp

Table 2. **Frequency of isolated fungal species**

S/N	Fungal Species	No of Isolates CFU/g	Percentage (%) of isolation
1	*Mucor spp*	1	12.5%
2	*Aspergillus spp*	2	25%
3	*Rhizopus spp*	1	12.5.3%
4	*Penicillium spp*	3	37.5%
5	*Fusarium spp*	1	12.5%
	Total	8	100

Aspergillus spp. and *Penicillium* spp. were the most frequent genera isolated from all of the samples from this study. The high frequencies of these genera indicated that the contamination might occur from the storage, as these genera are commonly found in low-moisture crops during storage. Our findings were in line with previous studies that reported the predominance of these two genera in spices [14]. The frequency of each fungal is 37.5% for *Penicillium spp* , followed by *Aspergillus* spp, 25% *Fusarium* spp (12.5%), *Mucor* spp (12.5%) and Rhizopus spp (12.5%). The isolation of toxigenic fungi of the genus *Aspergillus* in meat agree with the work done by Adjovi et al*.* [15] who characterized "*Aspergillus flavus* isolated from smoked, fermented and dried fishes sold inmain markets of Cotonou (Benin)". "This survey also revealed the occurrence of *Fusarium* species in the analysed samples, these 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" [16]. "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" [17]. High contamination reveal an inappropriate process of sun dried meat and storage condition, also the habit of displaying the dried meat for sale in dirty containers or heaping carelessly on the floor in unventilated stores can increase contamination.

"The representative fungal isolates were tested for their ability to produce mycotoxins (AF and OTA), as shown in Table 3. The isolates of *Aspergillus spp* and *Penicillium spp* showed varying intensities of blue and green fluorescence under UV light (365 nm), while the other species did not fluoresce. Although the amounts of AF and OTA were not determined in this study, the chromatography employed in this research revealed the mycotoxins in meat samples however, quantification is challenging since this technique is often not sensitive enough for more detail analysis. Therefore, it is used specifically for initial screening and for accurate quantification, more sensitive and advanced techniques will be needed. The presence of these mycotoxigenic fungi has confirmed the potential risk of aflatoxin (AF) and ochratoxin A (OTA) contamination in sun dried meat. The fluorescence was observed under UV light (365 nm) after 10 days of incubation. While no fluorescence was detected on the non-producer isolates. OTA poses as the major contaminant of meat products, while other mycotoxins are seen less often and in lower concentrations [18].

Table 3. Mycotoxin preliminary screening of fungi isolated from sun-dried meats

S/N	Sample location	Probable organism	Mycotoxin-Producing Ability (Blue-Green Fluorescence Intensity Malachova, *et al*., 2015)	
			AF	**OTA**
1	Academy Market 1	*Mucor spp*	-	-
2	Academy Market 2	*Aspergillus spp*	+	-
3	Academy Market 3	*Penicillium spp*	-	+
4	Oje Market 1	*Rhizopus spp*	-	-
5	Oje Market 2	*Penicillium spp*	-	+
6	Oje Market 3	*Aspergillus spp*	+	-
7	Oremiji market 1	*Fusarium spp*	-	-
8	Oremiji market 2	*Penicillium spp*	-	+

Note: AF – aflatoxin; OTA - ochratoxin A

"Penicillium occupy a wide spectrum of habitats in our environment. As a consequence, many have become economically important in either harmful or useful roles. Some species cause deterioration of wide range of stored products" Frisvad and Samson, [19] "The Aspergillus genus is a genus consisting of a few hundred species spread worldwide, mostly in tropical and subtropical rather than moderate climates, primarily present in the soil and various stored products, such as cereals, nuts, spices, but also dry-cured meat, and frequently reported to be both human and animal pathogen [19]. The most important species belonging to this genus are Aspergillus flavus and Aspergillus parasiticus, which are AFs-producers, *Aspergillus ochraceus* and *Aspergullas niger*, which are OTA-producers, and Aspergillus versicolor, which is a STC- and CPA- producer [20,21].

4. CONCLUSION

Sun dried Meat products were highly contaminated with *Aspergillus* spp. and *Penicillium* spp. which may gain access during the drying process leading to a public health hazard due to the production of mycotoxins. The result demonstrates the fact that the unhygienic and poor sanitary conditions under which sun dried meat products are handled and processed are not acceptable from sanitary point of view. It has further evidence that the undesirable level of contamination which might have been acquired from the environment and agents. To obtain wholesome safe and sound meat products, the principles Good Manufacturing Practices (GMP) and Hazard Analysis, Critical Control Point (HACCP) must be adopted.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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