Original Research Article

PRELIMINARY SCREENING FOR MYCOTOXIN-PRODUCING FUNGI ISOLATES FROM SUN DRIED MEAT

Comment [H1]: PRIMARY SCREENING FOR FUNGI ISOLATES THAT PRODUCE MYCOTOXIN FROM SUN-DRIED MEAT

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 were sub-cultured on Potato 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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Keywords: Food Safety; Mycotoxicosis; Aflatoxin; Ochratoxin; Ready-to-eat food

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INTRODUCTION

Meat is a rich source of protein which contains amino acids that are essential for the human body. 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. This composition makes the meat a suitable medium for the growth of microorganisms (Ostry 2001).

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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 (Zhou *et al.*, 2010).
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. Mycotoxins are toxic secondary metabolites produced by fungi that can contaminate a wide range of food including sun-dried meat. These toxins 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 (Rocha *et al.*, 2014).

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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 (Magan *et al.*, 2010). The contamination of sun-dried meat with mycotoxins is a potentially serious public health concern, as these meats are often consumed in high quantities and have a long shelf life. Mycotoxin contamination in sun-dried meat can occur through various pathways, from fungal growth during drying and storage to cross-contamination and fungal diversity. Therefore, it is crucial to conduct preliminary screening of mycotoxins in sun-dried and also important to investigate the potential mycotoxin-producing fungi in order to ensure it is safe for human consumption. Thus, by isolating the potential fungi and determining their ability to produce mycotoxins, we can gain insights into the extent of contamination, risk factors, and take preventive measures to safeguard public health (Bennett and Klich, 2003). Therefore this study aimed at the preliminary screening of mycotoxins in sun-dried meat isolates.

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MATERIALS AND METHODS

Media Preparation

All media were prepared according to the manufacturer's instructions and autoclaved at 121ºC for 15minutes. Both the pure culture selection of the organisms and the enumeration of the fungi were conducted using these media. All glassware, including Petri-dish, test tubes, pipettes, flasks and bottles used in the analysis were sterilized in a hot oven at 170˚C ± 5˚C for at least two hours. After preparation, the media were allowed to cool before pouring into the sterile petri-dishes.

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Fungal Isolation

Fungal isolation

One (1) gram of each sample were homogenized with 90 ml of buffer peptone water and serial decimal dilutions (10^{-1} to 10^{-5}) 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 (Oxoid, UK) and incubated for 5 to 7 days at 25°C for purification. Fungi were identified by using taxonomic schemes based on microscopic observation and culture appearance. The total fungal count for each plate was expressed as colony-forming units per gram of sample (CFU/g). Each genus or specie identified was then expressed as percentage (%) of the total isolated fungi. The total colonies of fungi were enumerated and results were reported in mean and average fungal counts as described (Pitt and Hocking 1997).

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Identification of fungi

Identification of fungal Genera and the determination of each species of fungi were done using the method of Klich, (2002). This was done by observing both microscopic characteristics and morphology of the colonies on PDA and SDA medium.

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*. (2018) for AF and by Zhang *et al.* (2016) 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. The production of a yellow-orange pigmentation of fungal hyphae was observed in toxigenic strains. AF- and OTA-producing isolates showed green fluorescence on the reverse sides of the plates.

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RESULTS AND DISCUSSIONS

The cultural and microscopic observation showed five different fungal species in the 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 1: **Frequency of Isolated Fungal Species**

S/N	Fungal Species	No of Isolates	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

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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(Jeswal, and Kumar2015).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%). Previous studies in Algeria, Burkina Faso and Chad also reveal *Aspergillus* and *Mucor* spp as the major pathogenic fungal in dried food (Tidjani *et al*., 2008; Mahibeb, 2015; Compaore *et al*., 2016). The frequency of fungal kinds here found is also similar to some previous studies of Rebbouh (2016) and Laaid *et al*. (2009). Other fungal species such as *Aspergillus falvus*, *Aspergillus aculeatus*, and *Aspergillus tubingenisis* have been isolated in dried fish by Ni *et al.* (2018). *Aspergillus* kind protect itself throughout spores. Its spores is then spread in several areas and contaminate seral foods. *Aspergillus* have an important growth factor in an unappropriate storage conditions according to Hocking (2006). There is high percentage of contaminated among the samples is very high. Fungal growth in relative high humidity area, 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

Table 2: Mycotoxin preliminary screening of fungi isolated from sun-dried meats

S/N	Sample location	Probable organism	Mycotoxin-Producing Ability (Green Fluorescence Intensity)	
			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

The representative fungal isolates were tested for their ability to produce mycotoxins (AF and OTA), as shown in Table 2. The isolates of *Aspergillus spp* and *Penicillium spp* showed varying intensities of 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 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 (Duarte *et al.*, 2010)

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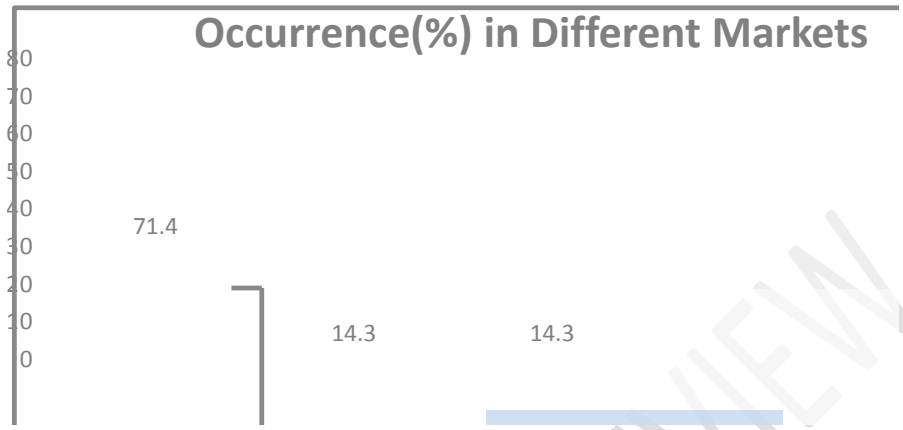

Figure 1. Occurrence of Mycotoxins in Different Market Locations

Mycotoxins varied differenty in the various market locations, which includes Academy Market, Oremiji and Oje as show in Fig 1.

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, 2004). Some species of genus *Penicillium* might induce pulmonary infection, external otomycosis, mycotic keratitis and endocarditis (Birera *et al*., 1998). 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. The members of the *Aspergillus* genus are of huge importance in the synthesis of chemicals, biosynthetic transformation and enzyme production, but their involvement into the decomposition of a large number of foodstuffs and their production of mycotoxins, have an immense economic and social impact (Pitt and Hocking, 2009). Furthermore, the genus is frequently reported to be both human and animal pathogen (Samson *et al.*, 2014). 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.

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.

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Comment [H37]: Conclusions

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