



(RESEARCH ARTICLE)



Mycological and mycotoxicological quality assessment of dried meat (Kilishi) sold in Kebbi state, Nigeria

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Abstract

This study assessed the mycological quality of dry meat products in three Emirates of Kebbi, Nigeria. A total of 63 dry meat samples were collected through systematic random sampling; twenty one samples from each Emirate. The standard method of isolating micro-organisms was adopted. The total viable count obtained ranged from 1.0×10^3 - 3.7×10^3 . Eight different fungi were found associated with the dried meat samples sold in the three different Emirates. The associated fungi were *Aspergillus flavus*, *Aspergillus fumigatus*, *Absidia* sp., *Rhizopus* sp., *A. niger*, *Mucor* sp., *Cladosporium* sp, *Penicillium* sp. *Aspergillus flavus* and *A. niger* had the highest rate of occurrence among the isolated fungi. Mycotoxicologically, Ochratoxin were positive for 71% samples and concentration ranged of 0.2 – 14.0 µg/kg, Fumonisin were positive for 52.4% samples at concentration ranged of 0.2 – 31.0 µg/kg, while 19% samples had mycotoxin concentrations above the European Union maximum tolerance level of 4µg/kg. It is obvious that dried meat products sold in Kebbi are potentially contaminated; proper hygiene practices should be observed during handling, marketing and calls for concerted efforts on the part of relevant authorities to check the trend, since it is a public health challenge.

Keywords: Kebbi Emirates; Dried meat; Mycotoxin, Fumonisins; Ochratoxin and Fungi

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]. Different kinds of meat exist as a result of their methods of preparation and preservation. In Nigeria, the most common name for dried meats are Tinko, Kilishi and Kundi majority prepared by peoples of Northern Nigeria [2]. 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 [3]. In this part of the world (Nigeria), meat is preserved by sun-drying after cooking or smoking. Dried meat "Tinko" can be preserved by regular sun-drying, salting and prevention of moisture being absorbed into the meat product. Traditionally, dried meat microbiology involves a natural development of wild fermentation in which microbial successions occur, uniform salting over the entire surface is most critical to inhibit pathogens and spoilage organisms [2].

Moreover, dried meats displayed in the market are found to be visibly mouldy (high level of discolouration). Most of these meats are produced from the Northern part of Nigeria and are transported to the South in small packages such as woven sisal bags, jute sacks and baskets. They invariably become mouldy before reaching the market. The presence of moulds in the meat products usually causes a decrease in their biological and nutritional values due to the enzymatic degradation of meat components [4]. These moulds or yeasts could be spotted in different forms and colours on dried

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meats [5]. Earlier work by Okafor [6] revealed that a number of mould species has been isolated from dried meat and fish in Nigeria. Also, Berwal [7] and [8] reported that numerous strains of microscopic filamentous fungi isolated from the surface of various meat products showed in-vitro ability to produce toxic substances called mycotoxins and which may remain in the product for years, long after the mould has died.

Mycological quality of meat products plays an important role in an increasing public health issue all over the world. Microbial analysis must be carried out in dried meat aimed for human consumption because of its high mycoflora contamination due to inadequate handling practices, as well as fungi contamination during preservation [9].

It is imperative to note that the tremendous growth in the production and consumption of dried meat in the Kebbi state of Nigeria has made it a great concern to study and to know its mycological quality. This study therefore examines the mycological quality of dried meat sold in three (3) Emirates of Kebbi State, Nigeria.

2. Material and methods

2.1. Sampling

The State has four emirates namely: Argungu, Gwandu, Yawuri and Zuru. Each Emirate has between 4 - 10 local governments' areas. For the purpose of this study, three emirates (Argungu, Gwandu and Yawuri) were randomly selected by balloting; a system random sampling technique was used for sampling of meat in the markets of the selected local governments in the emirates.

2.2. Selection of markets/Stores

Markets from selected local government areas were identified through local government departments' of agriculture and traditional authorities.

2.3. Collection of Samples

A total of sixty three (63) samples of dried meat were obtained from markets. The samples (21 each) were collected from three agricultural zones, at different selling shades and centres respectively. Approximately 25g representative sample was collected in small polyethylene packs according to Whitaker [10].

2.4. Fungal isolation

The isolation of fungi was carried out according to the agar dilution method as described [11]. One (1) grams 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 (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 [12, 13].

2.5. Identification of Mold

Identification of fungal Genera and the determination of each species of fungi were done using the keys of Klich [14] for *Aspergillus* spp. and Pitt and Hocking [12] for *Penicillium* sp. This was done by observing both microscopic characteristics and morphology of the colonies on PDA and SDA medium.

2.6. Data Analysis

The One-way ANOVA test was used, Means for the distribution of concentrations of Mycotoxins, comparison of means of TFC across sampling and overall (%) for fungal species. The means were separated for test of significance by the Duncan's Multiple Range Test at $P = 0.05$.

3. Results

3.1. Occurrence and distribution of fungi

The Meat products samples analyzed in this study had fungal contamination at varying levels. A total of 164 fungal isolates belonging to 8 identified species (*Aspergillus niger*, *A. flavus*, *A. fumigatus*, *Fusarium*, *Cladospori*, *Rhizopus Absidia* and *Mucor*) were recovered from the analyzed samples. Meat samples from three emirate of Kebbi State had the highest total fungal count of 3.4×10^4 cfu/g while those from Isa zone had the least fungal load, 1.1×10^3 cfu/g (Table 1). *Aspergillus* species were recovered from most of the samples in all locations with *A. flavus* (31.1%) has the highest percentage, followed by *A. niger* (22.6%) and *Fusarium* species (16.6%). The least in occurrence were isolates of *Rhizopus stolonifer* and *Absidia candidus* with 4.9% each. *Aspergillus* species were recovered from both samples site and in all locations, while *Absidia candidus* were detected. On the overall, the incidence of *Aspergillus flavus* was the highest (31.1%) being significantly ($P < 0.05$) higher than the proportion of all other fungal species. *Mucor racemosus* with 5.5%, although its incidence was not significantly ($P < 0.05$) different than the incidence of *Rhizopus stolonifer* and *Absidia candidus*. The results are presented in table 1.

Table 1 Occurrence and distribution of fungi contaminating Meat products within three Emirates in Kebbi state, Nigeria.

Products	Dried Meat				Overall (%)
	Zone	Gwandu	Argungu	Yawuri	
*TFC (cfu/g)		4.7×10^4	1.5×10^4	3.8×10^4	3.2×10^4
Percentage (%) of fungal species occurring in Meat products samples					
<i>Aspergillus flavus</i>		32.6	36.1	23	31.1
<i>Aspergillus niger</i>		14	19.4	31.4	22.6
<i>Aspergillus fumigates</i>		14	11.1	5.7	9.1
<i>Fusarium solani</i>		4.7	8.3	17.1	14.6
<i>Cladosporium spp.</i>		6.9	5.6	11.4	7.3
<i>Mucor racemosus</i>		9.3	11.1	----	5.5
<i>Rhizopus stolonifer</i>		11.6	5.6	----	4.9
<i>Absidia candidus</i>		6.9	2.8	11.4	4.9

*TFC (cfu/g): Total fungal count in colony forming units per gram
Overall (%) Mean with different letters in the same row are statistically different ($p < 0.05$) according to Duncan's test

3.2. Fumonisin and Ochratoxin A Contaminating Meat Products

The contaminated dried meat samples were found to contain Fumonisin B1 (FB₁) at concentrations ranging from 0.2 to 31.0 µg/kg. The least concentration of FB₁ was found in Gwandu emirate and highest concentrations were detected in samples obtained from Yawuri Emirate (Table 2). The incidence of the FB₁ in Kebbi state shows that, Yawuri emirate has the highest incidence with 57.1%, and the least were Gwandu emirate with 38.1% only. However, the mean of FB₁ concentration in samples analyzed by locations were not significantly ($p < 0.05$) different from each other. Out of 63 samples, only 4 (6.3%) were contaminated with > 20 µg/kg FB₁ (maximum acceptable limit (MAL) recommended by the Standard Organization of Nigeria). FB₁ levels were not significantly ($P < 0.05$) different. Overall, 33 (52.4%) of the 63 samples were contaminated with FB₁, while 12 (19.1%) of the samples had FB₁ levels above the stipulated EU limit 4 µg/kg.

Table 2 Incidence of Fumonisin B1 Concentrations in Dried Meat in Three Emirates of Kebbi States,

Sampling location	N (%)	Fumonisin B1 concentration ($\mu\text{g}/\text{kg}$)			
		Range	Mean	N (%) >4	N (%) >20
Gwandu	12/21(57.1%)	0.8-21.2	4.56 ^a	7/21 (33.3%)	2/21 (9.5%)
Argungu	8/21(38.1%)	0.2-29.6	2.73 ^a	3/21 (14.3%)	1/21 (4.8%)
Yawuri	13/21(61.9%)	0.2-31.0	2.38 ^a	2/21 (9.5%)	1/21 (4.8%)
Total	33/63(52.4%)	0.2-31.0	3.22	12/63 (19.1%)	4/63 (6.3%)

Means followed by the same letter(s) do not differ significantly according to Duncan Multiple Range Test (DMRT) at 5% level of significance

N = Number of samples contaminated with Fumonisin B1.

>4 = Maximum limit for Fumonisin B1 and Total Aflatoxin concentrations, respectively (EU)

>20 = Maximum limit for Fumonisin B1 concentration in foods (NAFDAC)

The presences of Ochratoxin A in meat products obtained are presented in Table 3. The frequencies of contamination are 71.4%, 47.6% and 47.6% for Gwandu, Argungu and Yawuri emirates respectively, concentration of the sample analysed ranged between 0.6 – 11.4, 0.2 – 12.1 and 0.4 – 14.0 $\mu\text{g}/\text{kg}$ in the respective emirates, with mean of 3.36, 1.95 and 2.95 for the Gwandu, Argungu and Yawuri emirates respectively. There are no significant differences between the emirates.

Table 3 Ochratoxin A Concentration ($\mu\text{g}/\text{kg}$) of Dried Meat Collected from three Emirates

Ochratoxin A	Emirates	Frequency of Contamination	Range of concentration of positive samples ($\mu\text{g}/\text{kg}$)	Mean \pm Standard deviation
DRIED MEAT	Gwandu	15/21(71.4%)	0.6-11.4	3.36 \pm 3.57a
	Argungu	10/21(47.6%)	0.2-12.1	1.95 \pm 3.51a
	Yawuri	10/21(47.6%)	0.4-14.0	2.95 \pm 4.72a

Means followed by the same letter(s) do not differ significantly according to Duncan Multiple Range Test (DMRT) at 5% level of significance

4. Discussion

Meat surface is usually contaminated with a wide range of microorganisms. Due to its beneficial chemical composition (the content of water, proteins, peptides, amino acids, nucleotides, sugars, minerals and vitamins), the meat is a suitable medium for the development of all microorganisms [15].

The observed viable microbial count in Meat product samples analysed, were in line with reports by Osho, [16], Inyang, [17], and Egbebi and Seidu, [18], that "Suya processed in Abeokuta (South Western Nigeria), Markurdi (Northern Nigeria), and Ado and Akure (South West Nigeria) respectively, have microbial contaminations. Similarly, this finding are in line with the reports by [19, 20] on the microbial hazards of poorly processed meat, the microbial hazards associated with, processing of suya meat, reported that processing water, meat processing slabs, utensils, spices and raw meat revealed contamination with potential pathogens such as *A. flavus* group that produce aflatoxins include *A. flavus*, *A. parasiticus*, *A. nomius*, *A. tamari* and *A. bombycis*.

Since no published work on the contamination of meat with fumonisins in Nigeria is available, it's going to be compared with other cereals investigated in Nigeria by other researchers. [21] Detected fumonisin B1 in 51% (55 out of 104 samples) of maize samples analysed with concentration range of 65 to 1830 $\mu\text{g}/\text{kg}$, and mean value of 390 $\mu\text{g}/\text{kg}$. In a similar study on the natural occurrence of fumonisins in pre-harvest maize in south western Nigeria, [22] reported *F. verticillioides* occurring in 89.3% of samples. Fumonisin B1 was reported to be the predominant toxin (frequency at 76.8%, concentration between 70 and 1780 $\mu\text{g}/\text{kg}$, and mean of 495 $\mu\text{g}/\text{kg}$), while Fumonisin B2 was detected in 66% of the samples with a mean of 114 $\mu\text{g}/\text{kg}$.

Detection of OTA in the samples can be a result of contamination by *Aspergillus* specie Production of OTA by black *Aspergillus* species has been reported [23]. Ochratoxin A has been found in wheat, corn, and oats having fungal infection and in cheese and meat products of animals consuming ochratoxin-contaminated grains [23]. *A. ochraceus* found on

dry foods, such as dried meat and smoked fish, soybeans, garbanzo beans, nuts, and dried fruit. OTA is immunosuppressive, teratogenic, genotoxic and mutagenic, and IARC [24] has classified it in group 2B as possibly carcinogenic to humans. OTA is a genotoxic carcinogen [25], and it was proposed that its levels in foods should be reduced to the lowest level that can be technologically attained. The Joint Expert Committee on Food Additives of the WHO [25] and FAO [26] set a provisional maximum intake of 100 µg/kg body weight (bw), while the Scientific Committee on Food of the European Union proposed that the maximum daily intake of OTA should not exceed 5 µg/kg bw [25].

Therefore Food producers must follow the principles of good manufacturing practice and take preventive measures in order to reduce the growth of microscopic filamentous fungi and the production of their toxic metabolites in the final products.

5. Conclusion

In conclusion, the present work indicated that the examined dried meats were contaminated with several moulds especially of the genus *Aspergillus*. Many of these moulds 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. Strict hygienic measures must be applied during the processing and storage of the meat samples and calls for concerted efforts on the part of relevant authorities to check the trend, since it is a public health challenge.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no competing interests.

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