IDENTIFICATION OF MYCOTOXINS-PRODUCING FUNGI SPECIES ASSOCIATED WITH SUYA MEAT SOLD IN PORT-HARCOURT METROPOLIS

ODIKE, ORIDIKITORUSINYAA

DEPARTMENT OF BIOLOGY, FACULTY OF NATURAL AND APPLIED SCIENCES

IGNATIUS AJURU UNIVERSITY OF EDUCATION, RUMUOLUMENI, PORT-HARCOURT,

RIVERS STATE, NIGERIA.

Abstract

The study investigates mycotoxins-producing fungi species associated with suya meat sold in Port-Harcourt Metropolis and the Mycotoxins associated with these species. Three (3) different types of ready-to-eat meat (suya) (Balangu, Tsire, and Kilishi) were sampled, collected at four (4) locations in Port-Harcourt, Rivers State, Nigeria. The areas are Trans-Amadi, Diobu, GRA, and Borokiri. In a preliminary studies, a totaJ of eight (8) fungi species were isolated from twelve (12) samples of suya collected from these areas mentioned, they are Aspergillus niger; Aspergillus flavus; Penicillium sp.; Rhizopus stolonifer; Candida sp.; Fusarium sp.; Alternaria sp.; and Mucor sp. The identification of mycotoxins was done by ELISA method, lateral flow kits obtained from Bio-Check, UK, was used, ten (10) mycotoxins were identified, they are Citrinin; Patulin; Ochratoxin A; Fumonisin B1, Moniliformin; Deoxynivalenol (DON); Zearalenone (ZEA); T-2 toxin; Aflatoxin B1; Aflatoxin G1; and Aflatoxin M1. With the result obtained means suya meat sold in Port-Harcourt Metropolis requires thorough investigation by health authorities because of the spices used and exposure of the product to pathogenic microorganisms, also more research work is required.

Keywords: Borokiri, Diobu, ELISA, Fungi, GRA, Mycotoxins, Port-Harcourt, Suya, Trans-Amadi.

Introduction

Mycotoxins, is a term derived from the Greek word- Mikes meaning Fungi and Toxicon meaning poison, which are produced by Moulds during the final growth phase or early stationary phase of the organisms. Mycotoxins are poisonous substances commonly produced by moulds (fungi) that contaminate agricultural produce by growing on them (Shepherd *et al* 2010). Toxigenic fungi are everywhere in nature and frequently contaminate a wide range of agricultural products due to infestation both before and after harvest whenever there is sufficient humidity and temperature, despite all effort to minimize and control contamination. Thousands of mycotoxins exist in nature, though very few are considered important in food safety hazards- Aflatoxins, Deoxynivalenol (DON), Zearalenone (ZEA), Ochratoxin, Fumonisin, and Patulin produced by these fungal species: Aspergillus, Fusarium, and Penicillium (Gaag *si al* 2003). Although it is tropical climates (wide range of temperatures, humidity and rainfall) that favor mycotoxins contamination, nevertheless is poor hygiene during post-harvest treatment that promotes the growth of moulds leading to the production of mycotoxins (Martins et *al.,* 2001). Both ochratoxin A and Aflatoxins are the most dangerous mycotoxins with worldwide occurrence and pose a high risk to human health because they are resistant to heat (e.g. roasting and cooking), and damage specific tissues and organs (Galvano *et al.,* 2005). It has been reported globally that the contamination of spices used in suya by mycotoxins has been significant in various countries such as Turkey, Portugal, Italy, Egypt and Morocco (Aziz *et al.,* 2008, Gurbuz *et al.,* 2000, Martins *et al,* 2001, Zinedine *et al.,* 2006 and Romagnoli *et al.,* 2007).

Good hygiene practice is the main control measure of possible microbial contamination during production, harvesting, transportation and post-harvest processing such as drying and preservation (Romagnoli *et al.,* 2007).The contamination of food

by fungi may be one of the more pervasive and seldom recognized causes of diseases. Fungi produce mycotoxins that are versatile and potent causes of diseases. A very large number of moulds produce toxic substances designated as mycotoxins. Some are mutagenic and carcinogenic; some display specific organ toxicity and some are toxic by other mechanisms. At least 14 mycotoxins are known to be Carcinogens, with aflatoxin being the most potent (Toth *et al.,* 2004). It is generally accepted that 93% of mutagenic compounds are carcinogens with mycotoxins, microbial assay system reveal 85% level of 80% level of correlation between Carcinogenicity and mutagenesis (Degger *et al.,* 2006).

Mycotoxins are produced as secondary metabolites. The primary metabolites of fungi as well as for other organisms are those compounds that are essential for growth. Secondary metabolites are formed during the end of the exponential growth phase and have no apparent significance to the producing organism relative to growth and metabolism. In general, it appears that they are formed when large pools of primary metabolic precursors such as amino acids, acetates, pyruvate etc., accumulate. The synthesis of mycotoxins represents one way the fungus has of reducing the pool of metabolic precursors that is no longer required for metabolism. (Degger *et al.,* 2006).

Aflatoxins are the most deadly mycotoxins; they are produced by Aspergiltus species and are known to be one of the most deadly carcinogens due to detrimental effects they can exert on their consumers, and this is also confirmed by the International Agency for Research on Cancer (IARC), which explained further that there is sufficient evidence in humans for the carcinogenicity of naturally occurring aflatoxins that exist in nature, they are: Aflatoxin Bl (AfBl), Aflatoxin B2(AfB2) Aflatoxin Gl (AFG1), Aflatoxin G2 (AfG2), Aflatoxin Ml (AfMl) respectively. Aflatoxins are toxins produced mainly by two Aspergillus species, that's *Aspergillus flavus and Aspergillus parasiticus,* and the categories of foods they contaminate are cereals and cereal products, poultry products, meat, animal feeds, milk and milk products. "suya spices" fall into group of foods targeted by aflatoxins (Domijan *et al.,* 2005). All toxins produced by these fungal species mentioned are poisonous and cause adverse health effects such as kidney and liver damage, mutagenic and teratogenic effects, birth defects, and cancer, especially liver cancer (Benneth and Klich, 2003). Aflatoxin Bl (AFB1), and Fumonisins are known human carcinogens and Patulin is also suspected as human carcinogens, Ochratoxins are produced by *Aspergillus ochraceus* and *Penicillium vercosum;* Fumonisins are produced by *Fusarium* moulds- B1 by *Fusarium verticilloides* and *F. proliferatum;* Patulin is produced by species of *Penicillium, Aspergillus,* and *Byssochylamys;* Citrinins produced by species of the genera *Aspergillus, Penicillium,* and *Monascus;* Sterigmatocystin is produced by *A. versicolor;* and Ergot Alkaloids is produced by *Claviceps* sp.(Wang *et al.,* 2010).

The effects of some food-borne mycotoxins are acute with symptoms of severe illness appearing quickly after consumption of food products contaminated with mycotoxins while others occurring in food have been linked to long-term effects on health including the induction of cancer and immune deficiency (Kriskaetal. 2008). It is in view of the foregoing that this research was set up and of course timely with the aim of isolating and identifying the mycotoxins-producing fungi associated with suya, also to determine the extent of the risks involved in the consumption of such kind of suya (meat), in Port-Harcourt metropolis. So recommendations would be made based on the results and findings obtained.

Materials and Methods

Identification of Fungal Isolates: Fungal isolates were identified based on their morphology recommended by Frazier and Westhoff (2006), viewed under microscope- x 40 objective lens, with the use of cotton-blue-lactophenol as stain.

Cultivation of Isolates for Mycotoxins Production: Culture of isolates was incubated for seven (7) days and was later used to obtain a spore suspension in 0.2% (v/v) aqueous solution (water/methanol) from the kit pack. Further inoculation and incubation of isolates were done according to the manufacturers of the kit.

Preparation of Sample for Mycotoxins Analysis: Each sample of the inoculate was mixed with 10 ml of 25:75 (v/v) water/methanol obtained from J.T. Baker (Deventer, Netherlands) and homogenized in a high speed blender (16000 rpm) and centrifuged at 8500 xg for 10 min., supernatant was collected
and filtered and used for the analysis. 70% methanol was used for the extraction of the toxins.

Identification of Mycotoxins: The identification was done by ELISA method without the need of clean-up. Lateral flow device test kit was obtained from Bio-Check. UK. For the identification of Aflatoxins (AFB1, AFG1, AFM1,); Citrinin; Patulin; Ochratoxin A; Fumonisin B1; Moniliformin; Deoxynivalenol (DON); Zearalenone (ZEA); and T2 toxin. The test strip was used as instructed by the manufacturers and read positive or negative.

Results and Discussion

Analyses of twelve (12) samples of three (3) different types of suya for frequently occurring mycotoxins, showed all contaminated with different mycotoxins which in agreement with the work done by Salari, *et at.* (2012). This study also agrees with Azziz *et al,* (2004) that some toxicogenic filamentous fungi have been identified that possesses the ability to produce mycotoxins from suya and the possible damage this will cause to human's health, these poisons are also able to cause liver cancer in humans. Table 1 shows the general characteristics of Moulds development and mycotoxins production in suya meat. Results obtained in table 2 shows the fungal species isolates from different suya samples, that of table 3 shows the morphological characteristics of the fungal isolates. Finally table 4 shows the identification of mycotoxins in various suya types.

Several works has been done concerning fungal contamination of food items and mycotoxins production involving spices reported in various countries by several scholars such as Aziz *et al.,* (2008), in Egypt Gottlich *et al.,* (2002), in Turkey, Martins *et al.,* (2001), in Portugal, Zinedine *et al.,* (2006), in Morocco and Romagnoli *et al.,* (2007), in Italy. All of them pointed out to the fact that species of fungi isolated from the spices used on meat products and other food items are able to produce mycotoxins.

Buttinger *et* a*l*., (2003), noted that the rnycotoxins that have received the most attention in scientific literature in food products from West-African sub-region are Aflatoxin. While there are few researches carried out on other mycotoxins like Fumonisin and Ochratoxin.

The most important rnycotoxins are the ones produced by moulds of the family of *Aspergillus, Penicillium,* and *Fusarium.* Also Galvano *et al.,* (2005), confirmed that Ochratoxin A and Aflatoxins are the most dangerous rnycotoxins with worldwide occurrence and poses a high risk to human health because they are resistant to heat (e.g. roasting and cooking) and damage specific organs and tissues. Amadi *et al.,* (2009), supported the work done by Buttinger *et al.,* in 2003 and posit that the only rnycotoxins that has received the most attention in scientific literature in food production in West Africa sub-region is Aflatoxin while there are other rnycotoxins that needed such attention like Fumonisin and Ochratoxin. To support the work done by Azziz *et al.,* (2004), Milicevic *et al.,* (2010), advice that the finding of Azziz *et al.,* (2004), should form the scientific basis for promulgation of regulations important in the decision making process to establish meaningful limits for mycotoxins in food, most especially meats and meat products.

Aflatoxins are group of toxic compounds produced by some mould species under favourable temperature and humidity. They are

more commonly found in peanuts and corn. Aflatoxins can lead to liver cancer especially for Hepatitis B carriers, after long term investigation. Consumers are advised not to consume food that looks mouldy or damage to avoid excessive intake of the toxin (Sangare-Tigori, *et al.* 2006).

The results of this study depict that, mycotoxins contamination of suya is mainly due to the application of spices after preparation of the suya. Spices are added into suya (ready-to-eat) for flavour and aroma. Even the suya type known as kilishi, in which spices are added before processing and preparation is still contamination with mycotoxins-producing fungal species because heat treatment or sun drying does not affect the potency of mycotoxins especially aflatoxins which are completely heat-stable, neither cooking nor freezing destroys the toxin.

In this study the ability of the fungal species to produce mycotoxins was observed and is very obvious that these organisms are toxigenic and are mycotoxins-producing species capable of causing health hazards to humans, therefore it is important to avoid any factor that will necessitate the contamination of spiced suya or to make sure of good manufacturing practice is maintained in every settings especially ready-eat-food products. All of these fungal species have been previously reported to have the ability to produce mycotoxins (Reddy *et al.,* 2009).

Table 1: The general characteristics of moulds development and the production of Mycotoxins in ready-to - eat meat (suya).

Factor	Growth of Molds	Production of Mycotoxins
Temperature	From 12 to 55°C	From +4 to 44°C
PH-Value	From 1.7 to 102,5,	Optimum between 5 & 7
Water activity	Min. of 0.62	Min. of 0.8-0.85
Additives e.g. salt.	Up to 20%	Up to 14%
Influence of spices	Min. of 0.85	Min. of 0.97

Adopted from Gaag er *al.*, (2003)

Table 2: Fungi Isolated From Different Suya Samples

Fungi	Balangu	Tsire	Kilishi
Aspergillus niger	+	+	-
Aspergillus flavus	-	+	+
Penicillium sp	+	+	-
Rhizopus stolonifer	+	+	+
Candida sp.	-	+	-
Fusarium sp.	-	+	-
Alternaria sp.	-	+	+
Mucor sp.	-	+	+

(+): Isolated (-): not isolated

Table 3: Characterization and Identification of the Isolates

Isolate	Characteristics & morphology	Microscopic Features
Aspergillus niger	colonies appear black with fast growing mycelium with spores.	smooth conidiophores, hyaline has spherical spore heads, double sterigmata, Conidia head uniseriate.
Aspergillus flavus	light yellow mycelium	has spherical spore heads, conidiophores are heavy walled, hyaline coarsely roughened.
Penicillium sp.	conidiophores are borne from Subsurface or surface, hyphae colonies appear grayish green and later turns white.	have spherical conidia, long septate, hyaline sporangiophore bears terminal sporangium.
Rhizopus stolonifer	spherical sporangia initially white but later black with numerous spores, stolon hyaline becoming brown towards nodes, light brown mycelium.	sporangia resembles black pin heads and are widely interspersed in cotton wood-like mycelium. Non-septate hyphae, dark-spherical sporangia at terminal.
Candida sp.	colonies appear yellowish-green	hyphae are septate with conidiophores arranged at the top hyphae.
Fusarium sp.	produces abundant micro-conidia, fluffy spread creating white mycelium	they are hyaline, abundantly uniform micro-conidia are formed In long chains.
Alternaria sp.	colonies appear pink	chains of conidia are produced at the beak of a spore
Mucor sp.	appears white with numerous fluffy spores, hyaline septate	sporangia bear black heads, conidia heads are in chains and dispersed

Table 4: Identification of Mycotoxins in Suya Samples

Mycotoxins	Suya (meat) samples		
	balangu	kilishi	tsire
Aflatoxin BI	+	-	+
Aflatoxin GI	+	-	+
Aflatoxin MI	-	+	+
Citrinin	-	+	-
Patulin	-	-	+
Ochratoxin A	+	+	-
Deoxynivalenol	-	-	+
Zearalenone	+	-	+
Moniliformin	-	-	+
Fuminisin	+	-	+
T_2 Toxin	-	+	+

(+):Positive (-): Negative

Conclusion

The results of this study confirmed the presence of mycotoxins-producing fungi in the suya sold in Port-Harcourt Metropolis and also various mycotoxins were identified from the different types of suya (balangu, kilishi, tsire), therefore, strict measures should be adopted regarding the sales of suya by vendors using unwashed hands, wrapping the product with old newspaper, exposing the product to dust, and using contaminated spices *on* the product. This study recommends periodic investigation of suya (meat) product to determine the microbiological safety and quality of this product by health authorities.

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