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Molds and Mycotoxins in Food Value Chain: A Challenge to Food and Nutrition Security

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ABSTRACT

Due to postharvest losses on fresh produce, most farmers apply simple and affordable open sun-drying in-between harvest and the final consumers but this method causes the growth of mold and fungi in processed food. Hence, the objective of this study was to isolate and characterize molds associated with the drying of plantain chips, and post-drying handling of catfish and cassava peels using standard laboratory procedures. One gram (1gm) of respective samples of plantain chips, dried fish, and cassava peel was taken and crushed in a mortar, 1g of each was serially diluted up 10^{-6} fold, and 0.1mL of each was pour-plated in potato dextrose agar modified with chloramphenicol and incubated for 72 hours at 28^{0}C. Thereafter, discrete colonies were counted and characterized using standard laboratory procedures. A total of eight fungal isolates were identified which were *Aspergillus flavus, Rhizopus sp., Aspergillus niger, Fusarium sp., Penicillium digitatum, Aspergillus fumigatus, Mucor sp., Neurospora sp.* The mean fungal counts of cassava peels revealed that the samples recorded the highest mean fungal counts which were by *Mucor sp.* ($24.1x10^{6}$ Cfu/g) while Catfish had the lowest overall mean counts ($3.2x10^{6}$ Cfu/g). But *Aspergillus niger* had the lowest counts of ($2.7x10^{6}$ Cfu/g) in plantain chips. The total mean fungal count at ratio: catfish (31.4), plantain chips (37.6), and cassava peel (111). In conclusion, several findings from this work showed that samples dried under the sun in the studied area were contaminated with mycotoxin-producing microbes that could lead to their early decay, deterioration and loss of nutrients.

INTRODUCTION

In developing countries like Nigeria, farming activity is increasing due to the daring need to feed the ever-growing population. Several of these population lives in urban areas requiring a lot of food and material supplies for themselves and their livestock. Fish is one of the most nutritious food sources for humans due to their high protein content. As such, they are very suitable for microorganisms as a growth medium (Lina *et al.*, 2021). The growth of these organisms coupled with lipid degradation often leads to the spoilage of this important product. High environmental temperature and moisture often create conducive conditions for this deterioration which eventually reduce the quality of fish and its keeping time and shelf life (Sean T. Hammond *et al.*, 2015). Plantain chips are known to be an attractive nourishing essential food in certain areas of African countries slated due to their nutritional content. The immature, plantain is developed into a powdery form/state using the usual local sun-drying procedure and method to process, in other reduce/maintain the moisture content to extend its shelf life and retain the quality (Marina *et al.*, 2019).

Microbial population growth rates depend on environmental conditions, Temperature is key to metabolic rates/action and growth of food-spoiling microbes (Sean T. Hammond *et al.*, 2015).

The continued growth of mold and fungi in processed food produces mycotoxins in its food resulting in spoilage via spores that germinate and form visible mycelium before the end of processing (P. Battalini, 2008).

Mycotoxins are poisonous toxic secondary metabolites produced by fungi naturally; they are of low molecular weight not contagious or infectious but can be found on plant/food products generally, they are capable of causing acute/intoxication and spoilage (Mohammed S. S. D. *et al.*, 2013).

In this part of the world, farm produces are usually in abundance during the rainy season thereby making foods surplus, leading to either wastage or farmers being forced to sell at cheap prices leading to serious loss of input capital, profit, and effort. Slow drying especially during the high-humid period results in mold build-up by different types of microflora and subsequent associated health risks. Controlling moisture can prevent fungi growth. Therefore, this study aimed to assess fungi associated with dried catfish, plantain chips, and cassava peels during open sun-drying and storage, as well as the health risks from mycotoxin-producing molds.

MATERIALS AND METHODS

Study Area

The study was carried out at the microbiology laboratory of the Food and Industrial Biotechnology Department of the National Biotechnology Development Agency,

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Lugbe, Federal Capital Territory, Nigeria. I tried, but see below for best google map location, thanks

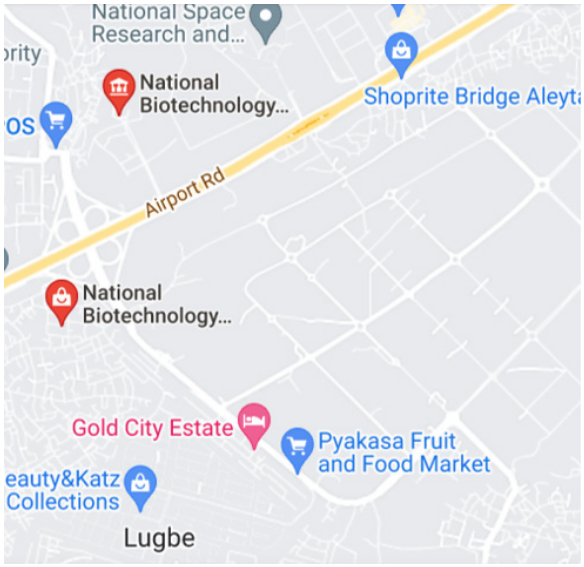

Figure 1: New view of study area using google map

Sample Collection

A total of ten (10) African catfish were randomly obtained from River Niger bank, Marine Road in Lokoja where drying of fish was done by some local processors. Five (5) plantain chips undergoing open sun-drying were also randomly collected and were carefully packed into polythene bags separately and labelled. Open sun-drying cassava peels were obtained from Garri Processing Centre at Lokongoma Phase II. All materials were transported to the microbiology laboratory for mycological analysis.

Isolation and Characterization of Fungi

One gram (1g) of the respective sample was measured and mashed using a mortar and a pestle and was added to nine milliliters (9mL) of pre-sterilized sterile peptone water and, serially diluted up to 10^{-6} fold. Afterwards, 1mL from 10-2, 10-4, and 10^{-6} suspensions of the samples (Catfish, Plantain chips, and cassava peels) were pour-plated using freshly prepared potato Dextrose Agar previously modified by adding Chloramphenicol antibiotics to suppress bacteria growth. The mixture was then gently swirled to mix and allowed to gel after which the plates were incubated at 28^{0}C for three to five (3-5) days. After the incubation period, the discrete colonies that emerged were counted, characterized, and purified by sub-culturing several times and then the pure cultures were maintained on the modified potato dextrose agar slants as stock until further use, media suitable for the enumeration, isolation, and identification of fungi from food (Pitt, J.I., Hocking, A.D., 2009). The fungi isolated were identified microscopically using simple staining techniques where two to three (2-3) drops of Lactophenol Cotton Blue stain were added to a cut portion of the growing fungi under a cover slip and observed with oil immersion under the microscope. Biochemical tests were carried out according to the methods described by Harrigan and McCance (1976) as well as Seeley and Van Demark (1972) on morphology, motility, spore staining, catalase, coagulase production, starch hydrolysis, and sugar fermentation. (Onyeze *et al.*, 2019).

Personal Protective Equipment (PPE) such as respirators/ nose masks, goggles, gloves, and lab coats was used considering the number one laboratory rule, which is safety first so that researchers were well protected. Also putting into consideration spore from the fungal. The usage of Laminar Flow hood during pour-plating. The fungi were killed via proper decontamination Burgener, J. (2006) before discarding to avoid exposing the environment to hazardous and pathogenic organisms.

RESULTS

Biochemical Test

Test	Results
Spore Staining	Sporangia
	Conidiospores
	Sporangiospores
Sugar Fermentation	Glucose (+)
	Maltose (+)
	Galatose (+)
Hydrolysis	(+)
Spore staining	Fungi spore
	Hyphae
	Fruiting structure stains blue while the background stains blue
Catalase	Most are a catalyst (-ve)
Motility	non-motile
Morphology	Generally the hyphae grew and form a network on known mycelia
Starch hydrolysis	(+)

Enumeration of Fungi Colonies that developed after incubation were subjected to counting. The total fungal counts were expressed as spore/g.

Fungi Load of The Selected Food/Feed Samples

Table 1: The Occurrence of Fungal Isolates Associated with the Samples

Fungal Isolate	Fungal Counts on Sample (x10^{6} Cfu/g)		
	Catfish	Plantain chips	Cassava peels
Aspergillus flavus	4.2	5.3	-
Rhizopus sp.	-	4.9	23.2

Aspergillus niger	4.0	2.7	23.6
Fusarium sp.	6.2	3.8	2.1
Penicillium digitatum	4.3	5.2	1.2
Aspergillus fumigatus	-	-	21.3
Mucor sp.	3.2	6.2	24.1
Neurospora sp	3.3	-	3.2
Total	**31.4**	**37.6**	**111**

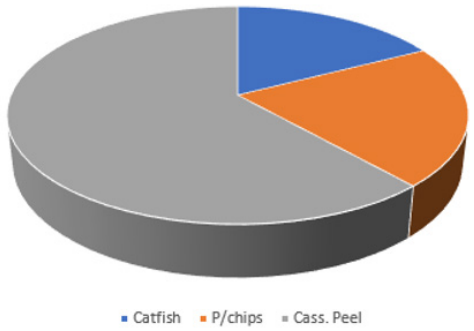

Figure 2: Relative Occurrence of Fungi on the Samples

A close observation of Table 1 for total fungal counts and prevalence shows that cassava peels had the highest. (Fig. 2) shows relative occurrence in samples comparison.

DISCUSSION

From the study shown in Table 1, the mean fungal counts of cassava peels revealed that the samples recorded the highest mean fungal counts which were by *Mucor sp.* ($24.1x10^6$ Cfu/g) while Catfish had the lowest overall mean counts overall but *Aspergillus niger* had the lowest counts of $2.7x10^6$ Cfu/g in plantain chips. A total of eight fungal isolates were identified which *Aspergillus flavus, Rhizopus sp., Aspergillus niger, Fusarium sp., Penicillium digitatum, Aspergillus fumigatus, Mucor sp., Neurospora sp.* and *Aspergillus fumigatus* was not found on Catfish and plantain chips. Most of the organisms found in these samples are those commonly found in air and soil environments as should be expected and reported, *Aspergillus niger* is most commonly found in mesophilic environments such as decaying vegetation or soil and plant materials (Belli *et al.*, 2004). In general terms, fungi grow and survive in optimal conditions most especially in humid environments. Hence, they require a certain moisture level which if provided by foods during processing could lead to their growth and eventual spoilage (Essono *et al.*, 2007). A close observation of Table 1 for fungal counts and prevalence shows that cassava peels had the total highest fungal count (Fig. I) and prevalence for *Aspergillus niger* when compared with other samples. This revealed that all is not well with the food materials studied owing to the fungi quality present in the three products; Catfish, plantain chips, and cassava peels processed in the area selected. The isolation of pathogenic and spoilage fungi such as Aspergillus flavus in this work raises public health concerns about safety in consuming the products because of the mycotoxins they might contain. The presence of the identified fungi in the products is attributed mainly to the high moisture contents of the atmosphere followed by poor handling by the processors. The mean fungal counts of cassava peels revealed that the samples recorded the highest mean fungal counts which was by *Mucor sp* ($24.1x10^6$ Cfu/g) while Catfish had the lowest overall mean counts overall but *Aspergillus niger* had the lowest counts of $2.7x10^6$ Cfu/g in plantain chips. This result exceeded the range of specified microbiological limits recommended for products meant for human consumption (Roberts *et al.*, 1996).

The results of this study were in no contradiction but similar to previous studies, such that - The observation might be due to the fact that *Aspergillus niger* is common in the soils from where cassava roots were drugged out (Awuah and Akrasi, 2007). Also, cassava tubers are usually not washed thoroughly before being peeled. This might be the reason for the high incidence of the organism on the peel. This account agrees with several reports such as those of (Arotupin and Akinyosoye, 2006) in their work on the Microbiological and Physicochemical Characteristics of Cassava Cultivated Soils. In a similar observation, *Aspergillus niger* was the dominant fungal on plantain chips ($6.2x10^6$ Cfu/g). Conversely, however, *Fusarium sp.* recorded the highest occurrence of Catfish ($6.2x10^6$).

The obtained result might be attributed to poor post-drying handling of the fish samples and supported by the fact that fungi have the ability to grow and survive in a wide range of temperatures such as 6-47°C and pH range of 1.4-9.8 (Morya and Yadav, 2009).

(Ajay *et al.*, 2011) also reported *Aspergillus niger* as one of the commonest microorganisms associated with smoked fish.

It was also reported that *Mucor sp.* thrives in soil, plants, manure, decaying fruits, and vegetables; hence, they are regarded as a common contaminant of stored and processed foods in the kitchen (Adebayo-Tayo *et al.*, 2008). The incidence of *Mucor sp*ecies causing disease in man has been reported because of their ability to grow and thrive at high temperatures (thermo-tolerant status).

Table1 shows that *Mucor sp.* was present in all the samples. This could be attributed to the storage conditions of the food and feed materials by the fish handlers especially the facilities used for their processing as well as the exposure to the environment because all the fungi produce spores that make them ubiquitous in nature (Krijgsheld *et al.*, 2013).

Generally speaking, the growth and high occurrence of fungi populations and mycotoxins in dried fish products

cause spoilage and pose a potential health threat to consumers as reported earlier by (Yijia Deng *et al.*, 2021). Several findings from this work showed that samples dried under the sun in the studied area were contaminated with mycotoxin-producing microbes that could cause their early decay, deterioration and loss of nutrients, which pose a potential health threat to consumers

Awareness has been created with the result of this research work that slows drying in humid environments aids molds and mycotoxins production that constitutes health and nutrition challenges, hence hindering food security. This research work is relevant in providing a solution to the challenge of food and nutrition security by alerting food processors to the danger of the slow drying process involved in the humid environment.

Further implementation – This will be to conduct further research on different drying temperature range to ascertain the best drying temperature that can minimize the growth of mycotoxin-producing microbes for each sample; catfish, cassava peel, and plantain chips respectively.

CONCLUSION

It was concluded that the foods and feed products processed in the Lokongoma, market/centers in Lokoja, Kogi State Nigeria were contaminated with pathogenic and spoilage microbes that thrive due to the presence of high humidity via slow drying. The findings from this work show that smoked catfish dried under the sun in the studied area were contaminated with fungi that could lead to their deterioration and loss of nutrients. Exposure of cassava peels to conditions that accelerate fungal growth poses a danger to the health of animals since little or no processing is done afterwards.

RECOMMENDATION

Based on the findings it was recommended that:

1. Drying of human food materials/food stuff be done using a hot air oven to prevent the growth and multiplication of mycotoxin-producing microbes
2. Centralized food processing equipment be established in the hinterlands for the rural folks.

Conflict of Interest

No conflict of interest.

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