

## Mycoflora and Aflatoxin Levels in Stale Retailed Pepper Sold in Abeokuta Nigeria

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### Abstract

Fungi constitute a major problem in the production, storage and processing of agricultural products, recent concern about the consumption of stale retail pepper in Abeokuta necessitated the need to determine the fungal population and also to quantify Aflatoxin produced by the fungi. Fungi species were isolated from 20 pepper samples (*Capsicum annum*) bought from different markets in Abeokuta using standard microbiological procedures and High Performance Liquid Chromatography (HPLC) was used to quantify the aflatoxin present. Isolated fungi were *Aspergillus flavus* (55%), *Mucor sp* (10%), *Sacharomyces cerevisiae* (20%), *Aspergillus fumigatus*, *Rhizopus sp* and *Penicillium sp* (5%) respectively. Results shows that fourteen of the pepper samples had no detectable aflatoxin and the aflatoxin content in the remaining six samples was 23ng/kg, 18 ng/kg, 15 ng/kg, 9 ng/kg, 6 ng/kg and 2 ng/kg,. five of these samples had aflatoxin content above the European Union maximum tolerance level of 5ng/kg. The presence of toxin producing *Aspergillus flavus* capable of causing food poisoning raises concern over public health risks that may be associated with the consumption of stale pepper.

**Keywords:** Aflatoxin, Fungal contamination, Capsicum annum, stale foods, High Performance Liquid chromatography, public health

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### Introduction

Pepper (*Capsicum annum*) is considered the most known and nutritive legume. It is rich in nutrients and can be used as a spice and as colorant due to its colour properties (Mueller *et al.*, 2010). It is usually abundant in the month of August-September. Spoilage is the change in which the food tends to become less palatable with bad smell, appearance or texture (Akinmusire 2011). Stale pepper are fruits that have lost some of their quantity and quality attributes such as size, appearance, shape, texture, moisture content, nutritional value and may not be safe for consumption depending on the degree of their deterioration. Stale pepper, in local areas in

Nigeria are called "Esa". Retailers of peppers sort out and sell these rotten pepper at cheap rates.

Microbial spoilage of food is a limiting factor for shelf life of fruit (Akinmusire, 2011). In order to keep fruits and vegetables from losing their turgidity, farmers or marketers often times sprinkle water on them after harvesting and the low temperature condition encourages the growth of microorganisms and this cause vegetable and fruits to spoil if not sold immediately (Murray *et al.*, 2017). Fruits including pepper are known to undergo self-integration known as autolysis, which leads to softening and results in loss of weight, colour, flavor changes and reduced nutritional

value (Bhattacharya *et al.*, 2010). Pepper contains a variety of microorganisms on their surfaces, this is not surprising since microbes are ubiquitous (Manonmani *et al.*, 2005). Spoilage of pepper by fungi can lead to mycotoxin contamination which pose high health risk to the consumers.

Aflatoxins negatively impact agricultural produce in all parts of the globe and their accumulation in food and feed represent a major threat to human and animal health (Oluwafemi and Taiwo, 2004). Aflatoxins are poisonous secondary metabolites produced mainly by *Aspergillus flavus*, *A. parasiticus* and *A. nomius*. The four major aflatoxins, B1, B2, G1 and G2 are the most important mycotoxins in foods and feeds because of their high prevalence in nature and toxicity (Makun *et al.*, 2012).

There is dearth of information on fungi associated with pepper spoilage in Nigeria according to Wogu and Ofuase (2014). This study thus aimed to assess the safety of consumption of stale retail pepper by determining the mycoflora present in the pepper and quantify the aflatoxins if present.

## Materials and Methods

### *Collection of Samples*

Twenty stale pepper samples were purchased from different markets in Abeokuta, Nigeria. The samples were collected into sterile polythene bags and transported to the laboratory for analysis.

### *Preparation of Pepper Sample*

Stale pepper samples were first sterilized by washing under running tap water in order to remove sand and surface dirt. A part of the stale portion of the samples from each market was excised and crushed into paste form using sterile mortar and pestle.

### *Isolation of Fungi in the Stale Pepper*

Pour plate method was used to enumerate fungal load of the samples. Serial diction of each pepper sample was obtained by pipetting of pepper paste into a test tube containing 9 ml of peptone water and 1ml was pipetted again from the 10 ml that was made up to another test tube containing 9 ml of peptone water and was done in 10 folds. The  $10^3$ - $10^3$  dilutions were plated on Sabouraud Dextrose Agar (SDA) at 28°C in an incubator for five days each dilution was replicable five times, colonies were counted and expressed in colony forming unit/gram (CFU/g) of samples.

Isolates were identified using morphological and cultural characteristics as highlighted by Klich (2002).

### *Screening for aflatoxigenic Potential of fungal isolates*

Isolates were screened using Bright Greenish-Yellow Fluorescence experiment (BGYF). Strains were inoculated at a central point on a 6 cm diameter Petri dish containing 10 ml of Coconut Agar Medium (CAM) supplemented with 0.3%  $\beta$ -cyclodextrin for a preliminary screening of aflatoxins production, (Fente *et al.*, 2001) and incubated for 5 days in the dark at 28°C. Cultures were tested for 365 nm UV light fluorescence and for bright green-yellow colony reverse coloring expression under daylight.

**Results**

**Table 1:** Identification of fungal isolates in spoilt pepper samples

S/N	Isolates Code	Macroscopy	Microscopy	Suspected microorganisms
1	M1	Brown filamentors colonies	Smooth walled conidiospores	<i>A fumigatus</i>
2	M2	Creamy large white colonies	Conidiphores	<i>Sacharomyces cerevisiae</i>
3	M3	Creamy white colonies	Budding yeast cell	<i>Sacharomyces cerevisiae</i>
4	M3	Creamy white colonies	Budding yeast cell	<i>Sacharomyces cerevisiae</i>
5	M4	White cotton like colonies	Hyphae without rhizoid spp	<i>Mucor spp</i>
6	M5	Yellowish Filaments Colonies	Glohose Conidiophores	<i>Aspergillus flavus</i>
7	M5	Yellowish Filaments Colonies	Glohose conidiophores	<i>Aspergillus flavus</i>
8	M6	Filamentous creamy	Large conidiphores	<i>Penicillium</i>
9	M6	Yellowish Filaments Colonies	Glohose conidiophores	<i>Aspergillus flavus</i>
10	M8	Yellowish Filaments Colonies	Glohose conidiophores	<i>Aspergillus flavus</i>
11	M9	Creamy white colonies	Scudding yeast cell	<i>Sacharomyces cerevisiae</i>
12	M10	Yellowish Filaments Colonies	Globose conidiospores	<i>Aspergillus flavus</i>
13	M11	Yellowish Filaments Colonies	Globose conidiospores	<i>Aspergillus flavus</i>
14	M14	Cotton like colonies	Hypha	<i>Rhizopus spp</i>
15	M15	White cotton like colonies	Hyphae without rhizoid spp	<i>Mucor spp</i>

Table one Shows the identification of fungal isolates in stale pepper samples. The isolated moulds were characterized with yellow to

brown and white filamentous colonies, smooth walled conidiophores to budding yeast cells.

**Table 2:** percentage of pepper samples containing identified fungal genera

Fungal isolates	number	Percentage (%)
<i>Apergillus fumigatus</i>	1	5.0
<i>Aspergillus flavus</i>	11	55.0
<i>Sacharomyces cerevisiae</i>	4	20.0
<i>Mucor spp.</i>	2	10.0
<i>Penicillium spp.</i>	1	5.0
<i>Rhizopus spp.</i>	1	5.0

Table two shows Percentage of Pepper samples containing identified fungal genera. It revealed six organisms isolated from pepper samples. *Aspergillus flavus* has the highest

percentage of occurrence with (55%) followed by *Saccharomyces cerevisiae* (20%), *Mucor spp* (10%) with *Penicillium spp*, *Aspergillus fumigatus* and *Rhizopus* having (5% each).

**Table 3:** Aflatoxigenic potentials identified in fungal isolates

S/N	Fungal isolates	Aflatoxigenic Properties
1	<i>Aspergillus fumigatus</i>	++
2	<i>Sacharomyces cerevisiae</i>	-
3	<i>Aspergillus flavus</i>	+++++
4	<i>Mucor spp</i>	-
5	<i>Rhizopus spp</i>	-
6	<i>Penicillium spp</i>	-

Key

+ = Fluorescence

+++++ = Fluorescence five times

- = Non Fluorescence

++ = Fluorescence twice

Table three reveals that out of the six fungi sample, *Aspergillus flavus* and *A. fumigatus* are the moulds that shows potential to be aflatoxigenic with *A. flavus* being the

predominant. *Sacharomyces cerevisiae*, *Mucor spp*, *Rhizopus spp*, and *Penicillium spp* shows no potential to produce aflatoxins

**Table 4:** Aflatoxin levels (ng/kg) in stale retail pepper in Nigeria.

S/N	SAMPLE	AFB1	AFB2	AFG1	AFG2	TOTAL AF
1	Sample 1	0.002	0.000	0.000	0.000	0.002
2	Sample 2	0.000	0.000	0.000	0.000	0.000
3	Sample 3	0.000	0.000	0.000	0.000	0.000
4	Sample 4	0.000	0.000	0.000	0.000	0.000
5	Sample 5	0.009	0.000	0.000	0.000	0.009
6	Sample 6	0.005	0.000	0.000	0.001	0.006
7	Sample 7	0.000	0.000	0.000	0.000	0.000
8	Sample 8	0.013	0.000	0.000	0.002	0.015
9	Sample 9	0.000	0.000	0.000	0.000	0.000
10	Sample 10	0.008	0.000	0.000	0.000	0.008
11	Sample 11	0.023	0.000	0.000	0.000	0.023
12	Sample 12	0.000	0.000	0.000	0.000	0.000
13	Sample 13	0.000	0.000	0.000	0.000	0.000
14	Sample 14	0.000	0.000	0.000	0.000	0.000
15	Sample 15	0.000	0.000	0.000	0.000	0.000
16	Sample 16	0.000	0.000	0.000	0.000	0.000
17	Sample 17	0.000	0.000	0.000	0.000	0.000
18	Sample 18	0.000	0.000	0.000	0.000	0.000
19	Sample 19	0.000	0.000	0.000	0.000	0.000
20	Sample 20	0.000	0.000	0.000	0.000	0.000

Pepper samples 2,3,4,7,9,12,13,14,15,16,17,18,19 and 20 had no detectable aflatoxin while samples 1,5,6,8,10 and 11 had 0.002, 0.009, 0.006, 0.015, 0.008 and 0.023 (ng/kg) aflatoxin respectively.

### Discussion

Due to ubiquity of fungi, it contaminates food by colonizing them at several stages of food chain (Manonmani *et al.*, 2005). This study has demonstrated that the infiltration of human pathogens into pepper can occur and that survival and growth

can occur under certain conditions leading to spoilage. Peppers acidic pH also creates good growth condition for yeast and fungi (Korkutata 2015). Occurrence of *Rhizopus* specie in food samples has been found to cause spoilage of tropical crops and food poisoning (Frazier and Westoff, 2005).

This study is in agreement with the findings of Arias *et al.*, (2002) who stated *Aspergillus* specie as the most prevalent fungal in spoilt pepper. Contamination of pepper could be as a result of poor processing method which may involve washing with faecal contaminated water (Frazier and Westoff, 2005). Aflatoxin B1 was the most prevalent of all aflatoxins (0.060ng/Kg) in the six positive pepper samples while Aflatoxin G1 was not found in the samples and this is in agreement with the findings of Alam *et al.*, (2010) who reported that amongst all fractions, AFB1 is normally the most predominant in food and feed products. Aflatoxin contamination is a serious problem in tropical countries of the world where relative humidity is very high and temperatures conducive for its growth and production by moulds.

### Conclusion

Human health is often adversely affected by aflatoxins in diets hence, much attention should be given to what we consume. This research therefore recommended that stale pepper consumption should be discouraged. However, this might not always be ensured or possible especially in urban areas due to transport challenges of moving the pepper from the farm to the urban centres or markets. To reduce postharvest losses in pepper, harvesters and handlers should be careful not to store pepper on the floor instead it should be kept in crates to avoid microbial infiltration while damaged pepper should not be sold at all. It is also recommended that market women selling pepper should be enlightened on the dangers of those pepper getting contaminated and possibility of microbial contamination by some toxin producing organisms which may not be inactivated by cooking heat especially aflatoxin which are heat resistant. To overcome global problem of aflatoxin, even though out of the 6 samples that tested positive for aflatoxin in this research, one of the samples was within the European commission standard regulatory limits of 5ng/kg, while five were above regulatory limit.

It should be well noted that there is no tolerable daily intake for aflatoxin and that there is no threshold at which no harmful effect is observed. It is therefore recommended that regulatory agencies in Nigeria should monitor aflatoxin in pepper

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