ABSTRACT

Spices are parts of plants or plant substances primarily used for flavouring and preservation of food. Spices can be exposed to microbial contamination and toxic substances such as mycotoxins during processing, storage and distribution. The aim of the study was to determine the incidence of toxigenic moulds in food spices sold in open markets in Benin City. A total of 35 samples were obtained from three markets. Potato dextrose agar (PDA) and Aspergillus flavus and parasiticus agar (AFPA) were employed for the isolation, characterization and identification of fungal isolates using standard microbiological procedures. The pH was determined using an electronic pH meter, titratable acidity and moisture content were also determined using appropriate methods. Fungi isolated in this study included: Fusarium sp., Alternaria sp., Rhizopus sp., Geotrichum sp., Cladosporium sp., Aspergillus niger and Penicillium sp. The most occurring fungus was Fusarium sp. (26%) while Alternaria sp. (2%) was the least occurring fungus. The fungal load ranged from 0.33 - 28.67 x 10³ sfu/g and the pH values of samples ranged from 4.83 – 5.67. The titratable acidity ranged from 0.037 – 0.521 mg/l, while the moisture content ranged from 2.0 – 28.0%. The study revealed the presence of toxigenic moulds such as Fusarium and Penicillium species in food spices sold in open markets. This may have resulted from improper storage, handling and poor sanitary conditions in the open markets.

Keywords: food spices, incidence, moulds, mycotoxins, open markets, toxige
INTRODUCTION
Spices (seed, fruit, bark and root of plants) primarily used for flavouring, colouring and preserving food. Food spices enhance flavours in food with no detrimental effects on the organoleptic quality of the food. Spices have also been long recognized to possess physiological effects beneficial to human health (Kaefer and Milner, 2008). Over one hundred varieties of food spices are produced throughout the world, with Asia being the largest producer (Prasad et al., 2011). Spices can be exposed to a wide range of microbial contamination and toxic substances such as mycotoxins during processing, storage and distribution. Traditional drying of spices on the floor associated with unhygienic conditions expose them to microbial contamination (Prakash et al., 2011). Mycotoxins are low-molecular weight products produced as secondary metabolites by filamentous fungi. According to the FAO, one-fourth of the worlds’ crop is affected by mycotoxins (Pankaj et al., 2018). Mycotoxins appear in cereals, fruit, vegetables, spices, animal feed and food products (Tola and Kebede, 2016). Generally, agricultural products that are improperly stored for prolonged periods can be subject to mould growth and mycotoxin contamination (Bennett and Klich, 2003).

The production of mycotoxins is stimulated by certain environmental factors; therefore the extent of contamination differs with geographical location, agricultural methods and the susceptibility of commodities to the penetration of fungi during storage and processing periods (Jonathan and Esho, 2010). Tropical and sub-tropical areas of the world have been reported to be the highest areas for aflatoxin contamination of food products (Strosnider et al., 2006).

Some species of moulds referred to as toxigenic moulds are capable of producing mycotoxins such as aflatoxins, ochratoxins and fumonisins which when transmitted to food can adversely affect human health (Ashiq, 2015). Mycotoxins are highly toxic to humans when ingested at high concentrations and have been reported to be carcinogenic, hepatotoxic, immunosuppressive and embryotoxic (Bezerra da Rocha et al., 2014). The aim of the study was to determine the prevalence of toxigenic moulds in food spices sold in open marketplaces in Benin. The particular goals were to count, isolate, and characterize fungal isolates from food spices, as well as determine the samples' physicochemical properties (pH, titratable acidity, and moisture content).

METHODOLOGY
SAMPLE COLLECTION
Samples comprising of factory-packaged and unbranded food spices (curry, thyme, shallot, dried onions and nutmeg) were purchased from open markets in Benin City, Edo State, Nigeria.

ENUMERATION, ISOLATION AND IDENTIFICATION OF FUNGAL ISOLATES
The culture media used for fungal isolation in this study were potato dextrose agar (PDA) and Aspergillus flavus and parasiticus agar. Serial dilutions were performed using sterile distilled water as diluent prior to plating by pour plate method. All plates were incubated at 28±2°C for 5-7 days. Fungal colonies were enumerated and isolates identified based on colonial and morphological characteristics.
PHYSICOCHEMICAL ANALYSES
Physicochemical parameters including pH, titratable acidity and moisture content were determined. The pH of samples was determined using an electrode pH meter (JENWAY 3020) and titratable acidity was determined by titrating 0.1M sodium hydroxide against 10ml of supernatant of homogenized sample, using phenolphthalein indicator. The moisture content was determined by oven drying method as described by AOAC (2016).

RESULTS
The total fungal counts of unbranded food spices ranged from 0.33 - 21.67 x 10^3 sfu/g while no fungal counts were recorded for factory-packaged spices (control) as shown in Table 1. Fungi genera isolated in this study included; Fusarium sp., Penicillium sp., Saccharomyces sp., Aspergillus niger, Alternaria sp., Rhizopus sp., Cladosporium sp. and Geotrichum sp. (Table 2). Table 3 shows the moisture content of food spices which ranged from 2.0 - 28.0% for re-packaged food spices while the moisture content for factory-packaged food spices ranged from 2.0 to 18.0%. The pH values of unbranded food spices ranged from 4.827 - 5.670 while the pH values of control samples ranged from 4.751 - 5.552 (Table 3). Titratable acidity of samples ranged from 0.037mg/l - 0.521mg/l while values for control samples ranged from 0.085 - 0.573mg/l (Table 3).

Table 1: Total fungal count of food spices sold in open markets in Benin City (sfu/g)

| Markets   | Sample A | Sample B | Sample C     | Sample D | Sample E |
|-----------|----------|----------|--------------|----------|----------|
| Oba       | 6.00±2.66| 1.00±0.68| 28.67±15.64  | 1.67±0.42| 2.33±0.88|
| Uselu     | 21.67±12.12 | 1.67±0.62 | 1.33±0.21   | 2.67±0.99| 17.67±10.25|
| New Benin | 1.67±0.49 | 0.33±0.21 | 1.00±0.37   | 1.00±0.63| 6.33±3.38 |
| Control   | 0        | 0        | 0            | 0        | 0        |

Key: Sample A = Shallot, Sample B = Thyme, Sample C = Nutmeg, Sample D = Dried Onion and Sample E = Curry

Table 2: Frequency of occurrence of fungal isolates in food spices sold in open markets in Benin City

| Organism          | Frequency | Percentage (%) |
|-------------------|-----------|----------------|
| Penicillium sp.   | 7         | 18             |
| Saccharomyces sp. | 9         | 23             |
| Aspergillus niger | 5         | 13             |
| Alternaria sp.    | 1         | 2              |
| Rhizopus sp.      | 3         | 8              |
| Fusarium sp.      | 10        | 26             |
| Cladosporium sp.  | 2         | 5              |
| Geotrichum sp.    | 2         | 5              |
Table 3: Physicochemical qualities of food spices sold in open markets in Benin City

| Markets   | pH      | Titratable acidity (mg/L) | Moisture content (%) |
|-----------|---------|---------------------------|----------------------|
| Oba       | 5.34±0.14 | 0.11±0.07                 | 12.16±7.34           |
| Uselu     | 5.13±0.29 | 0.26±0.13                 | 7.60±6.37            |
| New Benin | 5.16±0.17 | 0.23±0.10                 | 12.80±7.86           |
| Control   | 5.09±0.26 | 0.23±0.18                 | 11.00±5.65           |

DISCUSSION
Findings from this study showed that samples from unbranded food spices had fungal counts ranging from 0.33 to 21.67 × 10^3 cfu/g. These values were above the recommended limit (10^1 to 10^3 cfu/g) for fungal contamination in food spices, according to the international microbiological standards. These findings were similar to work carried out by Omorodion et al. (2014) who reported fungal counts ranging from 3.60 × 10^4 to 7.10 × 10 cfu/g in unbranded food spices sold in Port Harcourt. Also, fungal counts of 1.00 × 10^2 to 3.00 × 10^3 cfu/g have been reported by Khalil et al. (2015) in food spices. The factory-packaged food spices were of better mycological quality which could be attributed to proper processing and packaging under better sanitary conditions. The presence of fungi in the unbranded food spices may have been due to improper storage and/or handling during the process of re-packaging in the open markets (Asta, 1999).

The occurrence of species of the genera Penicillium and Fusarium which are known producers of mycotoxins such as ochratoxin, zearalenone, deoxynivalenol and fumonisins, have been reported to have toxic and carcinogenic effects on humans and therefore places consumers at risk (Elshafie et al., 2002). The high moisture content and the pH values of the samples showed that the spices were slightly acidic to neutral, revealed that the spices could permit the growth of fungi.

CONCLUSION
This study has shown that unlike the factory-packaged food spices, re-packaged food spices sold in open markets had the presence of toxigenic moulds capable of producing mycotoxins which when ingested could lead to deleterious effects in human health. It is therefore necessary to ensure that hygienic and proper drying conditions are employed during the processing and packaging of these food spices. Proper storage, minimal handling and exposure of these spices in the open markets is advised.
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