Various Levels of Mycotoxins Detected from Different Deteriorated Vegetable Oils (Garlic oil, Olive oil, and Soya bean oils) Sold in Bauchi Metropolis of Bauchi State, Nigeria.

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ABSTRACT: A study was carried out to isolate and identify the various species of fungi associated with deteriorated vegetable oils (Garlic oil, Olive oil and Soya bean oil) sold in Bauchi metropolis, Bauchi state, Nigeria, at the Department of Plant Science and Technology Laboratory. Samples of deteriorated vegetable oils were collected using random sampling techniques from three open market in Bauchi metropolis. Five samples each of garlic, olive and soya bean oils were collected from each of the three markets. The deteriorated oils were screened for the presence of aflatoxins, ochratoxins and zearalenone. Fungi associated with the deteriorated oils were isolated and characterized for their identification using standard methods. Result of fungal counts from garlic oil, olive oil and soyabean oil were $12.75x10^5$cful/ml, $9.22x10^5$cfu/ml, and $7.38x10^6$cfu/ml, respectively.A total of eight species of fungi were isolated from the three different deteriorated oils. Aspergillus flavus and Fusarium sporotrichioides were found in all the three deteriorated oils. Aspergillus fumigatus and Fusarium verticillioides were isolated from garlic oil and soya bean oil, whereas Aspergillus niger was isolated from olive oil and soya bean oil, respectively. Rhizopus stolonifer was isolated from only olive oil while Fusarium solani was isolated from only soyabean oil. Similarly, Rhodotorula rubra was isolated from only garlic oil. All the eight different fungal isolates were found to have lipases activity. Aspergillus flavus had the highest lipases activity, followed by Aspergillus niger which had diameter zones of clearance of 19.8mm and 18.1mm respectively. This study shows that Aspergillus flavus is the most predominant fungal species in the three deteriorated oils and this was demonstrated by its relatively high lipase activity.

INTRODUCTION

Mycotoxins are secondary metabolities produced by certain strains of filamentous fungi such as *Aspergillus*, *Penicillium* and *Fusarium*, which invade crops in the field and may grow in foods during storage under favourable conditions of temperature and humidity. They are regularly implicated in toxic syndromes in humans and animals (Smith *et al*., 1995). Due to the diversity of their toxic effects and their synergitic properties, mycotoxins are considered as risky to the unsuspecting consumers of contaminated foods and feeds (Yiannikouris and Jonany, 2002; Amede, 2008). Mycotoxins have been detected in various food commodities from many parts of the world and are presently considered as one of the most dangerous contaminants of foods in humans and animal feeds (Cast, 1989; Okoli 2005; Okoli *et al*., 2007). Animals may have varying susceptibilities to mycotoxins depending on physiological, genetic and environmental factors. Mycotoxins occur sporadically, both seasonally and geographically. Production of mycotoxins by fungi in foods is considered a global problem. However, in certain geographical areas of the world, some mycotoxins are produced more readily than others (Lawlorband *et al.*, 2005).

The most common mycotoxins are a group of compounds called Aflatoxins, which are fluorescent compounds that are chemically classified as diflurocoumarolactones. Aflatoxins are the most well known mycotoxins, which

are classified into four major types produced in feed stuffs, and these are B_1, B_2, G_1 and G_2 respectively (Cortyl, 2008). It is presently generally agreed that only four species of fungi so far been reported produce aflatoxins, and these are: *Aspergillus flavus, Aspergillus parasiticus*, *Aspergillus nomius* and *Aspergillus pseudotamari* in which *Aspergillus flavusand Aspergillus parasiticus* are of industrial importance (Cortyl, 2009). Aflatoxins are produced when adequate substrate and favourable conditions are present.

Aflatoxins are group of related difurano-coumarin secondary metabolites produced by certain strains of *Aspergillus flavus* and *Aspergillus parasiticus*. These compounds were isolated from groundnut meal, following outbreaks of liver diseases among pheasants, turkeys, pigs, and calves during 1960 in great Britain. However, the great interest in these compounds has been stimulated by the observation that they are hepato-carcinogenic to several species and that they may be present in some human foods. Four Aflatoxins were isolated and their chemical structures determined (Asao *et al*., 1965).

MATERIALS AND METHOD

Sample Collection

The samples were collected from Bauchi Metropolis of Bauchi State, Nigeria during the early rainy season from April to June, 2014. A preliminary field survey

was carried out to identify the deteriorated vegetable oils from three different open markets in Bauchi Metropolis. These were Mudalawal market, Wunti market and Central market. Samples of the deteriorated vegetable oil were collected from these three markets using random sampling techniques (Harvard, 2001).

Five samples each of the garlic, olive and soya bean oils were collected from the three markets and transported to the Department of Plant Science and Technology Laboratory University of Jos for analysis.

Preparation of Medium

The fungal medium used was Malt extract agar (MEA), which was prepared according to manufacturer's instructions and thereafter sterilize by autoclaving at 121^{o}C for 15 minutes and then allowedto cool to 45^{o}C. Then 0.01g/l of chloramphenicol powder was added to the sterilized media to suppress bacterial growth (Weschoff, 1998). The medium was then aseptically dispensed into sterile Petridishes and allowed to solidify under laminar air flow.

Isolation/Enumeration of Fungi

Isolation of fungi was carried out by a modification method of Olowolafe and Jonathan (2001). A 0.5ml concentrate of each deteriorated vegetable oil was

suspended separately in 0.5ml of sterile distilled water and then introduced into the Petri dishes containing solidified Malt Extract Agar (MEA) incorporated with 0.01g/l of chloramphenicol. The inoculated suspension was aseptically spread with an L- shaped glass spreader and then incubated at room temperature (25°C) for 7 days. The colonies that developed were counted and expressed as colony forming unit(cfu/ml).

The frequency of occurrences of the fungal isolates was determined as described by the standard method described by Robert (1992), which shows that as the number of fungus concerned was divided by the total fungi obtained x100.

Purification/ Identification of Fungal Isolates

The fungal colonies that grew on the plates were sub cultured into fresh malt extract agar plates using sterile wire loop to obtain pure isolates. Stock of fungal isolates were preserved on malt extract agar (MEA) slants in McCarthney bottles and stored in the refrigerator (4^{o}C) prior to further use (Klich, 2000).

The fungal isolates were identified on the basis of their cultural and morphological features and reference was made to descriptive standard illustrations (Barnett and Hunter, 1998).

Determination of Mycotoxin Levels in the Deteriorated Vegetable Oils

The mycotoxins were detected and determined using High Performance Liquid Chromatography-HPLC as recommended by ICMSF, (2001). This analytical

method enable different groups of mycotoxin to be detected at very low levels as low as part per billion (ppb). The High Performance Liquid Chromatography (HPLC) relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components leading to the separation and detection of the components as they flow out from the column

Using High Performance Liquid Chromatography, each mycotoxin type was detected and quantified appropriately by collecting a little quantity of each deteriorated vegetable oil and mounted in Analytical Detector Machine (UV-Light) which detect and quantified after 15 mins (μmol/ml).

Statistical Analysis

All data generated were subjected to statistical analysis using 2-way analysis of variance.

RESULTS AND DISCUSSION

The results obtained shows that the average fungal counts from the deteriorated oilswere $12.75x10^5$cfu/ml, $9.22x10^5$cfu/ml, and $7.38x10^5$cfu/ml for garlic oil, olive oil and soyabean oil respectively (Table 1). A total of eight species of fungi were isolated from the three different deteriorated oils.*Aspergillus flavus* and *Fusarium sporotrichioides* were found in all the three deteriorated oils. *Aspergillus fumigatus* and *Fusarium verticillioides* were isolated from garlic oil and soya bean oil, whereas *Aspergillus niger* was isolated from olive oil and soya bean oil. *Rhizopus stolonifer* was isolated from olive oil only, while *Fusarium solani* was isolated from soyabean oil. Similarly, *Rhodotorula rubra* was isolated only from garlic oil. Cultural and morphological characteristics of fungal isolates were presented in. Percentage of occurences of the fungal isolates was also determined were *Aspergillus flavus* had the highest percentage of occurrence .

The various levels of mycotoxins detected from the deteriorated products showed that garlic oil had 3.0µmol/ml total aflatoxins, 2.0µmol/ml ochratoxins,

4.0µmol/ml zearalenone, olive oil had 2.0µmol/ml total aflatoxins, 3.0µmol/ml ochratoxins, 2.0µmol/ml zearalenone, while soya bean oil had 3.0µmol/ml total aflatoxins, 1.0µmol/ml ochratoxins and 3.0µmol/ml zearalenone (Table 5).

Fungi isolated from Cultural and Morphological Characteristics of Deteriorated Vegetable oil samples sold in Bauchi metropolis of Bauchi state, Nigeria.

Fungal isolate	**Cultural characteristic**	**Morphological characteristic**
Aspergillus flavus	Yellow to green colonies reserve gold to red brown	Conidiophores vary in length, spiny and rough septate
Aspergillus fumigatus	Dark green to gray, reverse white to tan	Septate hyphae, short or long conidiophores
Aspergillus niger	Black powdery growth, reverse white to yellow	Septate hyphae, long conidiophores
Rhizopus stolonifer	Whitish to whitish-gray	Sporongiospores solitary,

	colonies with smooth stolons colourless to yell-owish-brown	globose with spinulose wall dark brown to black brown
Fusarium sporotrichioides whitish, later becoming	Floccose or cottony, bearing cylindrical phialides yellow, pink red to purple, reverse to brownish shades	Conidiophores branched,
Fusarium verticillioides	Peach, pale, cream, violet to lilac	Conidiophores unbranched, septate clavate
	with truncate base	
Fusarium solani	Leathery, grayish-white cream to buff, sometimes green to blu-	Verticillate conidiop- hores, septate fusifo

Table 3: Distribution and percentage occurrence of Fungi in the Deteriorated Vegetable oil Samples sold in Bauchi metropolis of Bauchi state.

Percentage of Occurrence			
Fungal Isolates	**Garlic oil**	**Olive oil**	**Soya bean oil**
Aspergillus flavus	(8.0)	(7.0)	(6.0)
Aspergillus fumigatus	(8.0)	(0.0)	(6.0)
Rhizopus stolonifer	(0.0)	(8.0)	(0.0)

Fusarium sporotrichioides (7.0) (6.0) (4.0)

Fusarium verticillioides (9.0) (0.0) (7.0)

Aspergillus niger (0.0) (8.0) (7.0)

Fusarium solani (0.0) (0.0) (5.0)

Rhodotorula rubra (5.0) (0.0) (0.0)

Mycotoxin levels (µmol/ml) in Deteriorated Vegetable Oil Samples.

Vegetable oil	Total Aflatoxins	Ochratoxins	Zearalenone
Garlic	3.0	2.0	4.0
Olive	2.0	3.0	2.0
Soya bean	3.0	1.0	3.0

DISCUSSION

Eight fungal species were isolated from the deteriorated vegetable oil samples.The species isolated were *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Fusarium verticillioides, Fusarium sporotrichioides, Fusarium solani, Rhizopus stolonifer* and*Rhodotorula rubra*.*Aspergillus flavus* and *Fusarium sporotrichioides* had the highest frequencies of occurrences. The average fungal counts of the deteriorated oils in this study ranged from $12.75x10^5$ to $7.38x10^6$cfu/ml. The counts in this study were very high compared to those reported by Akande *et al.,* (2001) on cottonseed oils ($6.76x10^5$cfu/ml). Eight species of fungi were isolatedand identified. Various levels of mycotoxins were obtained from the three different oils. The levels detected were found to be higher than those reported on palm oil (Bacaloni *et al.,* 2012). Moreover, species of the genera *Aspergillus, Penicillium* and *Fusarium* have been known to produce aflatoxins (Cortyl, 2008). The mycotoxins detected are among the major toxic substances that cause serious diseases and even death in humans and other animals (Smith *et al.,* 1995). Rashid

et al., (2008) reported one toxigenic isolate of *Aspergillus parasiticus* out of 157 *Aspergillus flavus.*

Bhat *et al.,* (2003) reported that ingestion of aflatoxins in an essential oils is capable of causing acute and chronic effects in humans and other animals ranging

CONCLUSION

From the result of this study, fungal loads of the deteriorated garlic oil as $12.75x10^5$cfu/ml, and those for olive oil as $9.22x10^5$cfu/ml, while those for soya bean oil was $7.38x10^5$cfu/ml. The study also showed that all the deteriorated vegetable oil samples had high fungal counts above 10^3 cfu/ml beyond acceptable limits (ICMSF, 1970). The lipase activity for fungal isolate was recorded ranging from 19.8 to 12.9 respectively. Moreover, it was also observed that fungal isolates from all the deteriorated vegetable oils had shown ability for aflatoxins, ochratoxins and zearalenone production, and the various levels of mycotoxins was detected appropriately.

RECOMMENDATIONS

1. From the finding of this research work, it is important to recommend that processors of vegetable oils should ensured proper sealing of containers to prevent fungal loads associated with the products.

2. The processors of vegetable oils should intensively pasteurize the products at the approved standard sterilization temperature (121°c) for 15 mins, before sealing.
3. Periodic monitoring of the vegetable oils with improved screening techniques for monitoring fungi and mycotoxin levels is required.
4. A primary focus for continuing research is the development of management strategies to reduce the incidence of aflatoxigenicity strains, in vegetable oils is necessary.
5. It is required that strict monitoring of vegetable oil processors should be enhanced by the monitoring organization to ensure strict compliance to quality.

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