VARIOUS RANGES OF LIPASE ACTIVITY (Diameter Zones of Clearance) DETECTED FROM DIFFERENT SPECIES OF FUNGI PRODUCING MYCOTOXINS.

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ABSTRACT

A study was carried out to isolate and identify various species of fungi associated with deteriorated vegetable oils. 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 flavus and 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). These are termed aflatoxin B_1 and G_1 being the most abundant, and their hydro derivatives B_2 and G_2 occurring in lesser amounts. The compounds have a blue fluorescence in ultraviolet light which forms the basis of most of the simple chemical assay producers used at present to detect the aflatoxins in food.

The need to study the mycotoxins associated with deteriorated vegetable oils, such as garlic oil, olive oil and soya bean oil cannot be overemphasized as these are among the major toxic substances in foods which may lead to serious diseases and even dead in both humans and livestocks. Jibrin and Paul (2001) reported that most cases of natural death in Nigeria, are due to the ingestion of high level of mycotoxins in some essential oils, in the last few years, it has been established that it is necessary to study the levels and effects of mycotoxins in edible essential oils (Jiang and Ma, 2008).

In view of the need to study the levels and effects of mycotoxins present in foods, the importance of the comparative analysis of the various mycotoxin levels in olive oil, garlic oil and soya bean oil consumed within Bauchi metropolis is of vital importance and these will help to ascertain the presence or otherwise of mycotoxins in these deteriorated vegetable oils sold in Bauchi town.

MATERIALS AND METHOD

The samples were collected from Bauchi Metropolis of Bauchi State, Nigeria. 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 allowed to 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 Petri dishes 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).

3.7 Determination of Lipases activity

Lipase activity was determined using the methods of Briquet *et al.*,(1999). The zone of clearance was used as a measure of the lipase activity on tributyrin medium. The colonies of lipolytic microorganisms have the ability to hydrolyse lipids and develop clear zones on the medium.

RESULTS AND DISCUSSION

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.

Table 1: Lipase Activity of Fungal isolates

Isolate	Zone of clearance (mm)
Aspergillus flavus	19.8
Aspergillus fumigatus	16.4
Rhizopus stolonifer	12.9
Fusarium sporotrichioides	17.5
Fusarium verticillioides	14.0
Aspergillus niger	18.1
Fusarium solani	14.2
Rhodotorula rubra	12.9

CONCLUSION AND RECOMMENDATIONS

From the result of this study, all the eight fungal isolates were found to produce lipase activity (diameter zones of clearance) which enables them to produce mycotoxins from the deteriorated vegetable oils.

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

1. The processors of vegetable oils and other food products should intensively pasteurize the products at the approved standard sterilization temperature (121°c) for 15 mins, before sealing.
2. Periodic monitoring of the vegetable oils with improved screening techniques for monitoring fungi and mycotoxin levels is required.
3. A primary focus for continuing research is the development of management strategies to reduce the incidence of aflatoxigenicity strains, in vegetable oils is necessary.
4. 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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