

SPECIES OF FUNGI PRODUCING MYCOTOXINS ISOLATED FROM DETERIORATED VEGETABLE OILS (GARLIC OIL, OLIVE OIL AND SOYA BEAN OILS) SOLD IN BAUCHI METROPOLIS OF BAUCHI STATE, NIGERIA

Ibrahim R¹, Ogonna C.I.C², Tahir H³, Mukhtar Muhammad Warrah

¹Department of Forestry Technology, Bauchi State College of Agriculture, Bauchi, Nigeria

[*rabs007@yahoo.com](mailto:rabs007@yahoo.com) Mobile No: +234-08067061477

²Department of Science and Technology, University of Jos, Nigeria

³Department of Science and Technology, Abubakar Tatari Ali Polytechnic, Bauchi, Nigeria

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 12.75×10^5 cfu/ml, 9.22×10^5 cfu/ml, and 7.38×10^6 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 metabolites 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 synergistic 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 difluorocoumarolactones. Aflatoxins are the most well known mycotoxins, which are classified into four major types produced in feed stuffs, and these are B₁, B₂, G₁ and G₂ 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 B₁ and G₁ being the most abundant, and their hydro derivatives B₂ and G₂ 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 livestock. 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°C for 15 minutes and then allowed to cool to 45°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 McCartney bottles and stored in the refrigerator (4°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).

RESULTS AND DISCUSSION

The results obtained shows that the average fungal counts from the deteriorated oils were 12.75×10^5 cfu/ml, 9.22×10^5 cfu/ml, and 7.38×10^5 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.

Table 1: Mean fungal counts from deteriorated vegetable oil samples

Deteriorated vegetable oil samples	Fungal count (cfu/ml)
Garlic oil	12.75×10^5
Olive oil	9.22×10^5
Soya bean oil	7.38×10^5

Table 2: Cultural and Morphological Characteristics of the Fungi isolates.

Fungal isolates	Cultural characteristic	Morphological characteristic
<i>Aspergillus flavus</i>	Yellow to green colonies reverse gold to red brown	Conidiophores vary in length, spiny and rough septate
<i>Aspergillus fumigatus</i>	Dark green to gray, reverse white to tan	Septate hyphae, short or long conidiophores
<i>Aspergillus niger</i>	Black powdery growth, reverse white to yellow	Septate hyphae, long conidiophores
<i>Rhizopus stolonifer</i>	Whitish to whitish-gray colonies with smooth stolons colourless to yell- owish-brown	Sporangiospores solitary, globose with spinulose wall dark brown to black brown
<i>Fusarium sporotrichioides</i>	Floccose or cottony, whitish, later becoming yellow, pink red to purple, reverse to brownish shades	Conidiophores branched, bearing cylindrical phialides
<i>Fusarium verticillioides</i>	Peach, pale, cream, violet to lilac	Conidiophores unbranched, septate clavate

		with truncate base
<i>Fusarium solani</i>	Leathery, grayish-white cream	Verticillate conidiop-
	to buff, sometimes green to blu-	hores, septate fusif
<i>Rhodotorula rubra</i>	Red- colouration	

Table 3: Distribution and percentage occurrence of Fungi in the Deteriorated Vegetable oil Samples

Fungal Isolates	<u>Percentage of Occurrence</u>		
	Garlic oil	Olive oil	Soya bean oil
<i>Aspergillus flavus</i>	(8.0)	(7.0)	(6.0)
<i>Aspergillus fumigatus</i>	(8.0)	(0.0)	(6.0)
<i>Rhizopus stolonifer</i>	(0.0)	(8.0)	(0.0)
<i>Fusarium sporotrichioides</i>	(7.0)	(6.0)	(4.0)
<i>Fusarium verticillioides</i>	(9.0)	(0.0)	(7.0)
<i>Aspergillus niger</i>	(0.0)	(8.0)	(7.0)
<i>Fusarium solani</i>	(0.0)	(0.0)	(5.0)
<i>Rhodotorula rubra</i>	(5.0)	(0.0)	(0.0)

CONCLUSION AND RECOMMENDATIONS

From the result of this study, fungal loads of the deteriorated garlic oil as 12.75×10^5 cfu/ml, and those for olive oil as 9.22×10^5 cfu/ml, while those for soya bean oil was 7.38×10^5 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.

1. From the finding of this research work, it is important to recommend that processors of vegetable oils should ensure 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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