



## Extent of Microbial Contamination of Refined and Unrefined Vegetable oils sold in South-west Nigeria

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### ABSTRACT

Oils constitute a major source of plant-based protein. A major limitation to optimal oil consumption in sub-tropical region is fungal infestation and consequent mycotoxin contamination. Ten refined and eight unrefined vegetable oils were randomly purchase from open markets and screened for microbial contamination using standard microbial procedures. Twenty six fungi isolates were obtained from the vegetable oil samples, the isolates were identified as *Aspergillus fumigatus* (43.0%), *Mucor* (17.9%), *Saccharomyces cerevisiae* (10.7%), *Aspergillus niger* (7.1%), *Aspergillus flavus* (7.1%), *Penicillium* spp (7.1%), *Aspergillus oryzae* (3.6%), *Mucor* (17.9%) and *Rhizopus* spp (3.6%). Five out of the ten refined vegetable oil samples had no fungal contamination. *A. flavus* and *A. oryzae* were absent in all the refined oil samples while *A. niger* was absent in all the unrefined oil samples. Isolation of mycotoxigenic fungi such as *Aspergillus* spp. is of vital importance in the food industry. Education and training of processors and consumers is recommended.

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### Introduction

Agricultural products such as oils make an excellent substrate for the growth of mould, fungus and other microbiological forms. As these moulds grow and derive their cell carbon from these oils, they cause deterioration in oil quality by producing free fatty acids under local storage conditions. It has been reported that most tropical edible oils are heavily infested by moulds (Manonmani et al., 2005).

Moulds are part of the natural environment, they are the most typical form of fungus on earth and comprise approximately 25% of the earth biomass. Apart from moulds capable of digesting and exhausting nutrient content of crops, more to be feared is the added danger of aflatoxin production in the oils (Tagoe, 2008).

Mycotoxins are considered an important problem throughout the world in terms of public health, agriculture and economics. They are natural poisons produced by fungi as secondary metabolites (Baskaya et al., 2006). Three genera are responsible for the majority of the mycotoxins with which FDA is concerned: The *Aspergillus* spp *Penicillium* spp, and *Fusarium* spp.

The term mycotoxin is derived from the greek word "mykes" meaning fungus and the latin word "toxicum" meaning poison. Accumulation of mycotoxins in food and feeds represent a major threat to human and animal health Mupunga et al., (2014) and Oluwafemi and Taiwo (2004).

Aflatoxins are secondary metabolite of mycotoxin produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* which commonly infect food produce such as maize, oilseed, peanut and tree nuts (Mmongoyo et al., 2017)

Contamination of food with aflatoxin is more prevalent in tropical and sub tropical areas where environmental conditions such as high temperature and humidity prevail (Klich, 2007). Many vegetable oils are consumed directly or used as ingredients in food and they are primarily from seeds of oilseed plants (Behrman and Venkat, 2005).

Vegetable oils are majorly used for cooking, processing in the food industry and meeting dietary demands. Often times they are contaminated by mycotoxins and heavy metals (Ma et al., 2015).

Refined oils are purified oils obtained from oil cakes using a process of solvent extraction. Refined cooking oils are made by highly intensive mechanical and chemical (solvent extraction) process to extract the oil from the seeds and vegetable products. The crushed seeds are heated to a temperature between 110°C-180°C in a steam bath to start the oil extraction process.

However, unrefined oils are obtained by a process of pressing the seeds or other vegetable materials. High level of mould in oil seed and their products are undesirable due to their capability of digesting and exhausting the nutrient contents of crops as well as the added danger of aflatoxins in the oils. In view of this assertion, this study assessed the quality of marketed vegetable oils in South-west Nigeria.

## Materials and Methods

### Sample Collection

Eight (8) samples of unrefined oils were collected from vegetable oil factories in Lagos, Ibadan and Abeokuta. The samples were aseptically collected in sterile glass bottles, capped and labelled properly and transferred immediately to the laboratory for analysis.

Ten (10) brands of vegetable oils (refined) were also purchased from various markets in Lagos, Ibadan and Abeokuta and taken to the laboratory for further analysis.

### Culturing of Refined and Unrefined Oil Samples

Serial dilution of homogenized oil was done, by pipetting 1 ml of oil into a test tube containing 9 ml of peptone water and 1 ml 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  $10^3$ - $10^5$  dilutions were plated on Sabouraud Dextrose Agar (SDA) at 28°C in an incubator for five days. Each dilution was replicated five times and the fungal counts estimated in colony forming unit/gram (CFU/g) of samples.

### Identification of Fungal Isolates

Fungal isolates were identified based on morphological and cultural characteristics described by Barnett et al. (2003). Additional characterization tests were carried out according to standard methods of Tsuneo (2010) and Klich (2002).

## Results

### Identification and Characterization of Fungi Isolates from The Oil Samples

Table 1 shows the identification of unrefined vegetable oil fungal isolates. The characteristics of the mould isolated were yellow to brown, green filamentous colonies, smooth walled conidiophores to budding yeast cells. It revealed that twelve mould were isolated of which *Aspergillus fumigatus* (8) had the highest occurring percentage, followed by *A. flavus* (2), *A. oryzae* (1) and *Saccharomyces cerevisiae* (1).

Table 2 shows the identification of refined vegetable oil fungal isolates. It revealed that fourteen fungi were isolated and identified as *Rhizopus* spp. (1), *Mucor* spp. (4), *A. niger* (2), *A. fumigatus* (3), *Penicillium* spp. (2) and *Saccharomyces cerevisiae* (2). All the yeasts identified were budding yeast. Moulds included hyphae without rhizoids and large conidiophores. The colour of the colonies included creamy-white, bluish-green, green, brownish-black and green filamentous colonies.

### Percentage of Fungal Genera in The Oil Samples

Table 3 shows the frequency of occurrence (%) of fungi in oil samples. Eight fungi isolated from refined oil samples were *Aspergillus fumigatus* (43.0%), *Mucor* spp (17.9%), *S. cerevisiae* (10.7%), *A. niger*, *A. flavus* and *Penicillium* spp (7.1%) while *A. oryzae* and *Rhizopus* spp are 3.6% respectively.

### Distribution of Fungi on Refined and Unrefined Vegetable Oil Samples

Table 4 presents the distribution of fungal isolates in the refined vegetable oil samples studied. Five (Cotton, Canola, Sunflower, Soya and Palm oil) out of the ten refined vegetable oil samples analysed had no fungal contamination. Of the eight mould identified, *A. flavus* and *A. oryzae* were absent in all the refined oil samples. *A. fumigatus* was found only in corn oil and palm kernel oil, *S. cerevisiae* occurred only in coconut oil while *Mucor* spp was present in both corn oil and olive oil. *Rhizopus* spp. and *A. niger* were present in groundnut oil while *Penicillium notatum* was present in palm kernel oil only.

Table 1 Characteristics of fungal isolates of unrefined vegetable oil

S/N	Isolate code	Macroscopy	Microscopy	Organism
1	UR1	Brownish filamentous colonies	Smooth walled conidiophores	<i>Aspergillusfumigatus</i>
2	UR2	Brownish filamentous colonies	Smooth walled conidiophores	<i>Aspergillusfumigatus</i>
3	UR1	(a)Brownish filamentous colonies (b) White cotton-like colonies	(a)Slender conidiphore (b) Hyphae without rhiziod sp.	(a) <i>Aspergillusoryzae</i> (b) <i>Mucor</i> sp.
4	UR3	Brownish filamentous colonies	Smooth walled conidiophores	<i>Aspergillusfumigatus</i>
5	UR4	Brownish filamentous colonies	Smooth walled conidiophores	<i>Aspergillusfumigatus</i>
6	UR5	Brownish filamentous colonies	Smooth walled conidiophores	<i>Aspergillusfumigatus</i>
7	UR6	Green filamentous colonies	Smooth walled conidiophores	<i>Aspergillusfumigatus</i>
8	UR7	Green filamentous colonies	Smooth walled conidiphores	<i>Aspergillusfumigatus</i>
9	UR8	Green filamentous colonies	Smooth walled conidiophores	<i>Aspergillusfumigatus</i>
10	UR4	Creamy white colonies	Budding yeast cell	<i>Saccharomyces cerevisiae</i>
11	UR6	Yellowish filamentous colonies	Globose conidiophores	<i>Aspergillusflavus</i>
12	UR3	Yellowish filamentous colonies	Globose conidiophores	<i>Aspergillusflavus</i>

UR1 – Unrefined mat 1-Soya extract; UR2 – Unrefined mat 2-Palm stearin; UR3 – Unrefined Raw mat 3-Palm kernel; UR4 – Unrefined Raw mat 4-Soya extract; UR5 – Unrefined mat 5-Palm olein; UR6 – Unrefined mat 6-Palm oil; UR7 -- Unrefined mat 7-Palm oil; UR8 -- Unrefined mat 8-Palm oil

Table 2 Characteristics of fungal isolates of refined vegetable oil

S/N	Isolate names	Macroscopy	Microscopy	Organism
1	Groundnut oil	Cotton-like colonies	Hyphae with rhizoids	<i>Rhizopus</i> spp.
2	Groundnut oil	Cotton-like colonies	Hyphae without rhizoids	<i>Mucor</i> spp
3	Cotton oil	Creamy white colonies	Budding yeast cell	<i>Saccharomyces cerevisiae</i>
4	Palm oil	Cotton-like colonies	Hyphae without rhizoids	<i>Mucor</i> spp
5	Olive oil	Cotton-like colonies	Hyphae without rhizoids	<i>Mucor</i> spp
6	Corn oil	Cotton-like colonies	Hyphae without rhizoids	<i>Mucor</i> spp
7	Groundnut oil	Black filamentous colonies	Large/globoseconidiphores	<i>Aspergillus niger</i>
8	Corn oil	Green filamentous colonies	Smooth walled conidiophores	<i>Aspergillus fumigatus</i>
9	Corn oil	Green filamentous colonies	Smooth walled conidiophores	<i>Aspergillus fumigatus</i>
10	Palm kernel oil	Green filamentous colonies	Smooth walled conidiophores	<i>Aspergillus fumigatus</i>
11	Palm kernel oil	White filamentous colonies	Large conidiphores	<i>Penicillium</i> spp
12	Palm oil	Whitefilamentouscolonies	Large conidiphores	<i>Penicillium</i> spp
13	Corn oil	Black filamentous colonies	Large/globoseconidiphores	<i>Aspergillus niger</i>
14	Olive oil	Creamy white colonies	Budding yeast cell	<i>Saccharomyces cerevisiae</i>

Table 3 Frequency of occurrence (%) of fungi in oil samples

Fungal isolates	Frequency	Percentage
<i>Aspergillus fumigates</i>	12	43
<i>Aspergillus niger</i>	2	7.1
<i>Aspergillus oryzae</i>	1	3.6
<i>Aspergillus flavus</i>	2	7.1
<i>Mucor</i> spp	5	17.9
<i>Penicillium</i> spp.	2	7.1
<i>Rhizopus</i> spp.	1	3.6
<i>Saccharomyces cerevisiae</i>	3	10.7

Table 4 Distribution of fungal isolates in the refined vegetable oil samples

Isolates	COT	SUN	CAN	COR	COC	OLI	SOY	PALK	PAL	GRO
<i>Aspergillus fumigatus</i>	-	-	-	+	-	-	-	+	-	-
<i>A. flavus</i>	-	-	-	-	-	-	-	-	-	-
<i>A. oryzae</i>	-	-	-	-	-	-	-	-	-	-
<i>Sacharomyces Cerevisae</i>	-	-	-	-	++	-	-	-	-	-
<i>Mucor</i> spp	-	-	-	+	-	++	-	-	-	-
<i>Penicillium notatum</i>	-	-	-	-	-	-	-	+	-	-
<i>Rhizopus</i> spp	-	-	-	-	-	-	-	-	-	+
<i>A. niger</i>	-	-	-	-	-	-	-	-	-	++

+ = One occurrence; ++ = Two occurrence; - = No occurrence; COT: Cotton oil, SUN: Sunflower oil, CAN: Canola oil, COR: Corn oil, COC: Coconut oil, OLI: Olive oil, SOY: Soya oil, PALK: Palm kernel oil, PAL: Palm oil, GRO: Groundnut oil

Table 5 Distribution of fungal isolates in the unrefined vegetable oils from oil factories

Isolates	A	B	C	D	E	F	G	H
<i>Aspergillus fumigatus</i>	++++	+	-	-	-	+	+	+
<i>A. flavus</i>	-	-	+	+	-	-	-	-
<i>A. oryzae</i>	-	-	+	-	-	-	-	-
<i>A. niger</i>	-	-	-	-	-	+	-	-
<i>Sacharomyce cerevisae</i>	+	-	-	-	-	-	-	-
<i>Mucorspp</i>	-	-	++	-	-	-	-	-
<i>Penicillium</i> spp	-	-	-	+	-	-	-	-
<i>Rhizopus</i> spp	-	-	-	-	-	-	-	-

+ = One occurrence, ++ = Two occurrence, ++++ = Four occurrence, - = No occurrence, A – Unrefined mat 1-Soya extract, B – Unrefined mat 2- Palm stearin, C – Unrefined mat 3-Palm kernel, D – Unrefined mat 4–Soya extract, E – Unrefined mat 5-Palm olein, F – Unrefined mat 6-Palm oil, G – Unrefined mat 7-Palm oil, H – Unrefined mat 8- Palm oil

Table 5 presents the distribution of fungal isolates in the unrefined vegetable oil samples studied. *A. fumigatus* was present in unrefined oil samples C, D and E while *A. flavus* was present only in samples C and D. *A. oryzae*, *S. cerevisiae* and *Penicillium* spp. were present in only A, C and D unrefined oils respectively while *Rhizopus* spp and *A. niger* were absent in all the oil samples.

## Discussion

It has been estimated by the Food and Agriculture Organization (2002) that 25% of the world's crops are affected by mycotoxins produced by moulds. It is one of the most potent naturally occurring mutagens and carcinogens known. Global view of aflatoxin-

contaminated food items revealed that virtually all foods are vulnerable including edible oil seeds.

In agreement with Umeh et al., (2000) and Bankole et al., (2004), the present study showed that members of *Aspergillus* spp. was highly prevalent. *Aspergillus* isolated from the vegetable oils has been known to produce aflatoxin B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub> and G<sub>2</sub>, (CDC, 2006).

Ragab et al. (2001) had reported that mycotoxins mainly formed by certain filamentous fungi belonging to the genera *Aspergillus*, *Penicillium*, *Alternaria* and *Fusarium* species, may grow on a number of food commodities which are the major contributors of food spoilage. The high frequency and abundance of *Aspergillus* spp. in the findings could be due to failures during food production and storage.

The results also agrees with the findings of IARC (2002) that maize, corn, cotton seeds, oil seed and most crops are frequently contaminated with aflatoxin. It is also in line with the work of Ngoko et al. (2001) who reported that *Fusarium* and *Aspergillus* were the most prevalent fungi on the sampled commodities with isolation frequency varying from 20 to 100%.

This work has been able to establish that unrefined oil has more fungal load than the refined oils which are in accordance with the findings of Elzupit et al. (2010) and Mariod and Idris (2015) and that consumers should limit the rate at which they consume unrefined oils because they are more susceptible to aflatoxins contamination. Hence, protective measures must be followed during the refining processes.

It could be deduced that the significant amount of impurities obtained in this oils could be linked to the method of storage. These storage facilities are mostly metallic and thus rust with time, thereby releasing toxic chemicals into the oil and this is in accordance with the findings of Odoh et al. (2017).

This work has been able to establish that refining reduces aflatoxin level in vegetable oil which corroborates the findings of Banu (2004).

Elzupir and Abdulaziz (2014) recommended keeping oil seed at freezing temperature until production will reduce or arrest proliferation of mould invariably aflatoxin thereby ensuring food safety.

Technologies such as biological control, improved packaging, irradiation and mechanism of ozone to inhibit microbial populations in food via the progressive oxidation of vital cellular components can also minimise aflatoxigenic mould contamination in agricultural products Udomkun et al. (2017).

## Conclusion

Fungal contamination rate should not be neglected. Hence, isolation of mycotoxigenic fungi such as *Aspergillus* species is of vital importance in the food industry therefore, it is feasible to decrease fungal contamination by sufficient education in the field of food industry. Regulatory agencies in Nigeria should equally monitor mould growth in oils meant for human consumption to avoid food poisoning and contamination.

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