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Determination of Fungi and Aflatoxins Levels in Unbranded Palm Oil Sold in Port Harcourt Metropolis

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Abstract

Fungi and aflatoxins levels in unbranded palm oils sold in Port Harcourt Metropolitan markets were determined. The samples were obtained from Mile 1 and Mile 3 Markets. The microbial analysis and aflatoxin concentration determination were done employing standard methods. The study revealed that the mean total heterotrophic fungal counts of palm oil obtained from Mile 1 and Mile 3 markets were 3.89 Log_{10}cfu/ml and 3.93 Log_{10}cfu/ml respectively. The fungi isolated and occurrence percentages are *Aspergillus flavus* (75 %); *Aspergillus fumigates* (50 %); *Aspergillus niger* (75 %); *Aspergillus parasiticus* (75 %); *Fusarium* spp. (50 %); *Mucor* spp. (25 %), and *Penicillium* spp. (50 %). The palm oil samples from Mile 1 Market 1 had the highest mean aflatoxin concentration of 4640.5 µg/L and Mile 3 Market 1 lowest mean aflatoxin concentration of 2179.5µg/L The fungal counts and aflatoxin concentrations exceed the permissible limit of 2×10^4 cfu/ml and 20 µg/kg respectively even as the organisms identified are capable of deteriorating oil quality and mycotoxins production. The oil samples investigated are not safe for human consumption based on the potential health risk involved. Regulatory agencies should ensure routine analysis of edible oils; more education and enlightenment of the processors is highly advocated while end users are strongly advised to heat the oil before consumption.

Keywords: Aflatoxin, Aspergillus, fungi, market, unbranded palm oil,

Introduction

Palm oil forms an important diet of most Nigeria homes. It is a good source of energy and vitamins with its unique flavour (Undiandeye & Otaraku, 2017). The quality of palm oil is a function of many factors like weather, soil, harvesting condition, storage and process of extraction (Davie & Vincent, 1980). There are reports that palm oil has become the world's most important edible oil since 2006 (Frank et al., 2013). However, palm oil is subject to deterioration and its consequence is very harmful to health. The quality of palm oil sold in many Nigerian markets has been a source of concern for many years. Olonrunfemi et al. (2014) investigated the quality of palm oil sold in major markets in Ibadan, Oyo State. The results were not pleasant. According to the report, aflatoxin contents exceeded permissible limits, indicating that the quality of palm oil in the sampled markets was poor. The study recommended the promotion of improved processing processes as well as good handling and storage practices. However, in their study to assess the quality of palm oil sold in major markets in Abia State, Udensi and Iroegbu (2007) showed that the quality of palm oil examined had properties that were within the standards recommended by Standard Organisation of Nigeria and Nigerian International Standard.

Unbranded vegetable oil (UVO), according to Chabiri et al. (2009), refers to the locally produced and laboratory extracted edible vegetable oil without branding. Food borne diseases are a critical public health problem and microbial contamination of food such as edible oil is the most common health risk. This problem is repeatedly observed in many edible oil market areas (Tesfaye et al., 2015). The number and type of microbes present on the products are important indicators of their

deterioration. Most commonly isolated genera of fungi include *Aspergillus flavus*, *Aspergillus niger*, *Mucor* spp., *Penicillium* spp. and *Rhizopus* spp. (Onawo & Adamu, 2018). The quality of palm oils may be affected by several factors, from the choice of raw material to the methods of processing, refining, bottling and storage (Shahidi, 2005). Therefore, appropriate control throughout the production chain is important to ensure the quality of vegetable oils delivered to food industries and final consumers. Oil quality and its stability are therefore very important for the consumers and in applications to industries (Wali et al., 2015).

Palm 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). According to Yousif et al. (2010), ingestion of these contaminated materials could lead to liver, kidney or nervous system damage, immunosuppressant and carcinogenesis. Since the retailers and consumers in Mile 1 Market and Mile 3 Market are not aware of the existence of aflatoxins in edible oils, this study was undertaken to determine the fungi and aflatoxin levels in unbranded palm oil sold in two major Port Harcourt Metropolitan markets.

Materials and Methods

The study was carried out within Diobu axis of Port Harcourt metropolis where the two major markets are located. The two major and popular markets within Diobu, Mile 1 (Rumuwoji) Market and Mile 3 Market were selected for this study as they are prominent places where edible oil marketing processes takes place. The GPS coordinates of Mile 1 Market is Lattitude 4º471'.287376"N and Longitude 6º59'53.7756"E, while Mile 3 Market had coordinate of Latitude 4'48'8.0604"N and Longitude 6º59'27.3912"E.

Eight samples of the unbranded palm oil each were bought from the vendors in Mile 1 and Mile 3 markets, making a total of sixteen (16) samples. All the samples were collected in already labelled 100ml sterile bottles and transported to the Biology Department Laboratory, Ignatius Ajuru University of Education for microbiological (fungal) and aflatoxin analyses. Potato Dextrose Agar (PDA), prepared according to the manufacturer's instructions was used for the isolation and enumeration of total fungal counts after serial dilution up to 10^{-1} according to methods described by Ogbulie et al. (2001). Pure isolates were identified on the basis of their cultural, morphological and physiological characteristics. Slides of fungi were prepared in lactophenol cotton blue and examined microscopically (x40). Identification to species was done using the taxonomic keys provided by Watanabe (2020).

The Association of Analytical Chemists (AOAC) method (2008) was followed for the purification of aflatoxins from collected samples in brief: 250 ml of methanol-water (55-45) were added for each oil sample (50 g), then 50 mL 0.1 N HCl, were added to the mixture, blended and then centrifuged, then filtered through 24 cm whatman No. 1 filter paper. Fifty milliliters of the filtrates were transferred into a 250 mL separator; 50 mL 10% NaCl solution were added and swirled; 50 ml of hexane were added and gently shaken for 30 seconds. the separated lower aqueous layer phase was drained into another 250 mL separator; then extracted by 3-25 ml dichloromethane and were added to aqueous phase and shook vigorously for 30 seconds. Then the phases separated and the lower dichloromethane layer was drained, collected and evaporated on a boiling water bath to dryness. Derivatization hexane (200 mL) were added to extract and 50 mL Tri-Floro-Acetic acid (TFA) and mixed on a vortex for 30 seconds; allowed to stand for 5 minutes then 1.950 mL water – acetonitrile (9 + 1) was added. Mixed vigorously for 30 seconds and allowed layer to separate for 10 minutes; and lower layer of acetonitrile water phase was taken in vial for HPLC determination. High Pressure Liquid Chromatography (HPLC) aflatoxins were analysed at wavelength of 360 nm as described by Cora *et al*. (2005). The aflatoxin concentrations in the samples extract were determined and quantified by the retention time and peak areas respectively.

Quantification of the actual aflatoxin in µg/kg is based on the following formula:

Aflatoxin content (µg/kg) = S x Y x VW x Z

Where:

S = volume of standard with colour intensity as sample (µl)

Y = concentration of mycotoxin standard used in µg/ml

V = volume of solvent required to dilute sample contained in final extract

W = effective weight of original sample contained in final extract
Z = volume of spotted sample equivalent to standard (µl)
The data obtained from this study were subjected to statistical analysis (Analysis of Variance, ANOVA).

Results
The fungal load in palm oil samples obtained from the investigated markets in Port Harcourt metropolis are presented in Figure 1. The figure shows that palm oil from Mile 3 Market 2 had the highest mean fungal load of 3.99 Log_{10}cfu/ml while Mile 1 Market 1 had the lowest mean fungal load of 3.64 Log_{10}cfu/ml. The analysis of variance (ANOVA) at $p \leq 0.05$ of fungal counts indicates that there was a significant difference between the fungal counts from samples in Mile 1 and Mile 3 Markets.

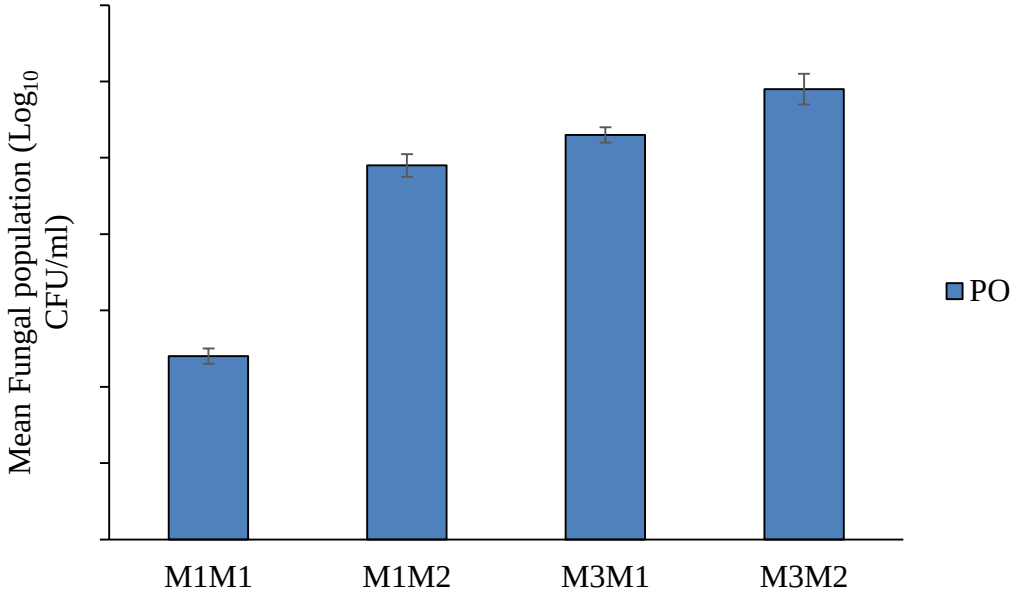

Figure 1 Fungal population in palm oil from investigated markets in Port Harcourt

Key:
PO: Palm oil
M1M1: Mile 1 Market 1 M1M2: Mile 1 Market 2 M3M1: Mile 3 Market 1
M3M2: Mile 3 Market 2

The fungi isolated and identified in the palm oils samples are presented in Table 1. They are *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus parasiticus*, *Fusarium* spp., *Mucor* spp., and *Penicillium* spp.

Table 1 Characterisation and identification of fungi isolated from obtained from Palm Oil Samples

Isolate Number	Colonial Description	Mycelial structure/ microscopic morphology	Spore formation	Organism identified
1.	Greenish mass with white edge, powdery and pin-head structure	Non-septate	Conidiophore	*Aspergillus flavus*
2.	Black velvety with	Septate	Conidiophore	*Aspergillus fumigatus*
3.	Black, powdery, Pin-head structures	Non-septate	Conidiophore	*Aspergillus niger*
4.	Dense Dark greenish	Non-septate	Conidiophore	*Aspergillus*

	mass			*parasitic* spp.
5.	Pinkish-white, fluffy (cottony)	Septate	Conidiophore	*Fusarium*
6.	Black and fluffy colony	Non- septate	Sporangio-phore	*Mucor* spp.
7.	Grayish, velvety, furrowed, brownish reverse (base)	Septate	Conidiophore	*Penicillium* spp.

The occurrence (detection) percentage of fungal isolates for palm oil from investigated markets in Port Harcourt is presented in Table 2. The table shows that the occurrence (detection) percentage for the palm oil samples are as follows: *Aspergillus flavus* (75 %); *Aspergillus fumigates* (50 %); *Aspergillus niger* (75 %); *Aspergillus parasiticus* (75 %); *Fusarium* spp. (50 %); *Mucor* spp. (25 %), and *Penicillium* spp. (50 %).

Table 2Detection of Fungal Isolates and Aflatoxin Concentration Levels in Palm Oil from Mile 1 and Mile 3 Markets in Port Harcourt Metropolis

Isolates	**Markets**				**%**
	M1M1	**M1M2**	**M3M1**	**M3M2**	
Occurrence					
Aspergillus flavus	present	present	ND	present	75
Aspergillus fumigatus	present	ND	ND	present	50
Aspergillus niger	ND	present	present	present	75
Aspergillus parasiticus	present	present	present	ND	75
Fusarium spp.	present	ND	ND	present	50
Mucor spp.	present	ND	ND	ND	25
Penicillium spp.	ND	ND	present	present	50

Key: ND Not Detected % Percentage

Aflatoxin concentrations in palm oil samples from the investigated markets in Port Harcourt metropolis are presented in Table 3. It shows that palm oil samples from Mile 1 Market 1 had the highest aflatoxin concentration of 4,640 µg/L while Mile 3 Market 1 had the lowest concentration of 2179.5 µg/L. The analysis of variance (ANOVA) at $p \leq 0.05$ of aflatoxin concentration indicates that there was significant difference between the aflatoxin concentration from samples in Mile 1 and Mile 3 Markets.

Table 3: Aflatoxin Concentration Levels in Palm Oil Samples from Selected Markets in Port Harcourt

Market	**Aflatoxin Concentration (µg/L)**
	Mean
M1M1	4640.5
M1M2	3395.0
M3M1	2179.5
M3M2	2511.0

Key: M1M1: Mile 1 Market 1 M1M2: Mile 1 Market 2
M3M1: Mile 3 Market 1 M3M2: Mile 3 Market 2

Discussion

One key constraint to optimally consuming edible oil within the sub-tropical region is fungi infestation and resultant mycotoxin contamination (Flora et al., 2018). The focus of this study was to determine the fungi and aflatoxins level in unbranded palm oils sold in two major markets in Diobu Area of Port Harcourt metropolis.

The study reveals that for the palm oil analysis, 3.89 Log_{10}cfu/ml (7.65×10^3cfu/ml) and 3.93 Log_{10}cfu/ml (8.55×10^3cfu/ml) were the highest total heterotrophic fungi count for Mile 1 Market 2 and Mile 3 Market 1 respectively. The counts were generally above the permissible limit of 2×10^4 cfu/ml as recommended by SON (2000) for edible oils. However, Odoh et al. (2017) reported mean mould count ranging from 3.18×10^4 cfu/ml - 4.56×10^4 cfu/ml for palm oil samples analysed. The variation in the fungi count could be attributed to difference in time of sample collection, quality of each sample based on the producers, storage and packaging conditions. Okwelle and Nwabueze (2020) noted that differences in fungi counts of oil samples could be attributed to the fungi species unable to metabolise oil effectively.

The study further revealed that both palm oil samples obtained from Mile 1 and Mile 3 Markets recorded reasonable number of fungi genera. The fungi isolated included *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus parasiticus*, *Fusarium* spp., *Mucor* spp. and *Penicillium* spp. Odoh et al. (2017) had reported *Aspergillus* sp., *Fusarium* sp. and *Mucor* sp. while Tesfaye et al. (2015) isolated *Penicillium chrysogenum*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Fusarium* spp. and *Mucor* spp. from locally produced oils. The prevalence of these organisms is a reflection of the poor conditions of the markets and the unhygienic practices adopted by the processors and vendors (Okwelle & Nwabueze, 2020). The specific identification of *Aspergillus* species, especially showing 75 % occurrence in the oils is noteworthy; this is because CDC (2006) has noted that they are known to release Aflatoxin B1, B2, G1 and G2 when involved in studies *in vitro*.

Also, the presence of *Penicillium* and *Fusarium* in oils likely suggests that contamination of several mycotoxin is a possibility (Abdul et al., 2017). Odoh et al. (2017) opined that these fungi (moulds) are believed to facilitate the quick deterioration of edible oil such as palm oil and produce toxin (aflatoxin) that could cause health challenges when eventually taken in by consumers; also, these fungi can remain viable in palm oil as they produce lipase enzyme, form spores which therefore make them resistant to heat, either when cooking or ultra-violet radiation exposure, hence becomes threat to life of the consumers (Ekwenye, 2005; Enemour et al., 2012).

The study confirms that aflatoxins are in some of the samples analysed. Palm oil samples from Mile 1 Market 1 showed the highest mean aflatoxin concentration while Mile 3 Market 1 had the lowest mean aflatoxin. According to Ezekiel et al. (2013), in Nigeria, the regulatory authority, National Agency for Food Drugs Administration and Control (NAFDAC) has set the maximum permissible limit of 20 µg/kg for total aflatoxins in all food that should be consumed by humans, which the aflatoxin values in this study far exceeds.

The aflatoxin concentrations values obtained in this study were also above the 1.23-6.87 ng/g reported by Undiandeye and Otaraku (2017) for palm oils marketed at Oil Mill Market, Port Harcourt. With reference to aflatoxin concentration levels in this study, Salau et al. (2017) stated that due to scanty information about aflatoxins in oils consumed in the country, this verity of the risk to human health as result of the continuous consumption of contaminated oil is unidentified. The high aflatoxin concentration indicates the presence of highly toxic fungi and therefore requires the adoption of appropriate measures during collection of the seeds, processing and good storage practice before and during sales.

Conclusion and Recommendations

The study concluded that the fungi counts obtained were above the permissible limit of 2×10^4 cfu/ml for edible oils. The high counts could result to potential hazards to the final consumers. The fungal species are capable of deteriorating oil quality and the production of mycotoxins which is harmful to human health. The aflatoxin level observed in this study are very high and exceed the maximum permissible limit of 20 µg/kg as recommended by NAFDAC, although, the samples from Mile 1 Market 1 and Mile 3 Market 2 had no aflatoxin. It is feasible to minimise microbial contamination by

adequate education and enlightenment of the producers and marketers of palm oil. Thereafter, there should be application of good manufacturing practice (GMP) and hazards analysis critical control points (HACCP). Regulatory agencies in the country should monitor production as well as dispensing points in these open markets.

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