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Occurrence of mycotoxins in some domestic vegetable oils of Northern Nigeria

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Abstract
An investigation was carried out on the species of fungi associated with domestic vegetable oils commonly consumed in some selected states of Northern, Nigeria. Samples of indigenous vegetable oils were collected using random sampling techniques from three different States in Northern, Nigeria. Three samples of each domestic vegetable oils (coconut oil, cotton seed oil and peanut oil) were collected from three States within the study areas. The experimental samples were plated out on Malt Extract Agar (MEA) using 30 culture plates for each sample. The resultant culture plates were then incubated at room temperature 25^0C for the isolation of different fungal species. The incubated culture plates were examined after 4-7 days for the development of fungal species. Moreover, the culture plates were re-examined after 14 days for the appearance of additional fungal species. Result of fungal counts from the domestic products ranges between $11.22x10^4$ cful/ml, to $7.33x10^3$cfu/ml. A total of thirteen (13) species of fungi were isolated from the domestic vegetable oils. *Aspergillus spp* were found in all the domestic products and *Rhodotorula spp* is the least. This study shows that *Aspergillus spp* is the most predominant fungal species in the domestic products. Different types and levels of mycotoxins were also detected and determined appropriately using mycotoxin testing kits.

Keywords: domestic vegetable oils, mycotoxins, northern Nigeria

Introduction
Mycotoxins are fungal metabolites produced by fungi that are capable of causing diseases such as cancer, bone marrow failure and bleeding, birth defects and even death in humans and other animals, particularly livestock's (Hussein *et al.*,2001) [2]. Due to their pharmacological activity, some mycotoxins or their derivatives have found use as antibiotics (Kendra, 2008). On the other hand, mycotoxins are metabolites of fungal origin that are toxic to humans and can be present in stored foods not properly preserved. The most common food borne mycotoxins are aflatoxins and ochratoxins. Aflatoxins are known to be produced by many species of *Aspergillus*, and these fungi can be found in soil and foods (such as groundnuts, peanut butter, olive oil) and some cosmetics. Ochratoxin is produced by *Aspergillus* and *Penicillium* species, and can be found in cereal, coffee and wine (Shephard, 2008) [8]. 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).
Cottonseed oil is similar to canola corn, soya bean and sunflower oils in terms of its polyunsaturated fat oil composition. In its non-hydrogenated form, it can be used for deep frying and lower the amounts of fat fried foods (Dutch *et al.* 2001). Cottonseed oil is known for its culinary purposes. It is used for frying or baking, and added to salad dressing, baked foods, cereals and mayonnaise. Because of its neutral taste, cottonseed oil donot mask or over power the other flowers in many dishes, unlike other oils. Cottonseed oil can be added to margarines icings and whipped topping, because of its potentials to help form beta prime crystalwhich give the food products a smooth and creamy appearance and consistency. Coconut oil is 99% fat, composed mainly of saturated fats, which supplies 890 calories. Disputes its high saturated fat content, coconut oil is commonly used in bake goods, pastries and santes, having a "haunting nutty" flavor with a touch of sweetness (Clark M., 2011). Moreover, coconut have been used for hair grooming and has been found to reduce protein loss in hair. Coconut oil has been tested for used as an engine lubricant (Romares S.V., 2010). The oil derivatives such as coconut fatty acid are used as raw materials in the manufacture of surfactants such as Cocamidopropyl betaine, cocamide MEA and cocamide DEA.The acid derivatives from coconut can be used as herbicides. The oil is also an important ingredient for the manufacture of soap. Soap made from coconut oil to end to be hard, though it retain more water than soap made with other oils and therefore increased manufacturer yield. It is more soluble in hard water and salt water than other soaps allowing it to lather more easily.

Significance of the Work
This investigation will help in the identification of various fungal species that could be associated with domestic vegetable oils sold in some selected states of northern, Nigeria.

Statement of Problem

The need to study the mycotoxins associated with domestic vegetable oils such as coconut oil, cotton seed oil and peanut oils respectively, 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's. Jibrin and Paul (2001) reported that most cases of natural deaths in Nigeria, are due to the ingestions of high concentrations of mycotoxins in some foods and indigenous domestic oils. In the last few years, it has been established that it is very necessary to study the levels and effects of mycotoxins in indigenous fermented foods (Jiang and Ma, 2008).

Materials and Method

Sample Collection

The samples were collected from Bauchi, Jigawa and Plateau States of Northern, Nigeria. A preliminary field survey was carried out to identify the domestic vegetable oils from three different states of Northern Nigeria. These were Bauchi of North Eastern part, Jigawa North Western part and Plateau State of north central respectively. Samples of the indigenous vegetable oils were collected from these three different states using random sampling techniques (Harvard, 2001).

Three samples each of the indigenous vegetable oils were collected from the three different states and transported to the laboratory for analysis.

Preparation of Medium

The fungal medium used was Malt Extract Agar (MEA), which was prepared according to manufacturer's instructions and thereafter sterilized by autoclaving at 121ºC for 15 minutes and then allowed to cool to 45ºC. Then 0.01g/l of Streptomycin Sulphate 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 0.5g concentrate of each domestic vegetable oils 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 Streptomycin Sulphate Powder. 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) / (cfu/g).

Mycotoxins Detection Using Rida Kit

Rida kit quick mycotoxin/aflatoxins is done by using Rida quick scan, which is a modern optical reading device that were purely developed in order to perform the interpretation of the bands not by naked eye but electronically by an optical unit. However, the Rida quick scan enables you to obtain qualitative results.

Results and Discussion

The results of total fungal counts of domestic vegetable oils (coconut oil, cotton seed oil and peanut oils) are presented in table 1. The results obtained show that fungal counts range from $11.22x10^4$CFU/ml to $7.33x10^3$CFU/ml. However, the total of thirteen (13) different fungal species were isolated and identified with *Aspergillus spp* as the most dominant fungi in the indigenous vegetable oils and yeast (*Saccharomyces cerevisiae*) was the least on both samples. Occurrence on fungal isolates on the indigenous vegetable oils sampled were indicated accordingly, which shows that *Aspergillus spp* had the highest occurrence as the dominant fungi on the domestic products. Various types and levels of different mycotoxins detected from domestic vegetable oils were also presented.

Table 1: show mean fungal count CFU/ml in the domestic vegetable oils

Domestic Vegetable oils	Coliform Forming Unit CFU/ml /(g)
Coconut oil	$9.30x10^2$ CFU/ml
Cottonseed oil	$7.33x10^3$ CFU/ml
Peanut oil	$11.242x10^4$ CFU/ml

Table 2: Distribution and Percentage Occurrence of Fungi in the Domestic Vegetable oils.

Isolates	D-M	D-F	S-C	C-F	P-O	I-D	C-M	C-O	CT-O	P-O	B-P	TOTAL	FREQ. (%)
Aspergillus flavus	+	+	-	-	+	+	-	-	+	+	+	07	10.00
A.niger	+	-	+	+	+	-	-	-	+	-	+	06	8.75
A. fumigatus	-	+	+	-	+	-	+	-	+	+	+	07	10.00
Fusarium sporotri.	+	+	-	+	-	+	-	+	+	-	+	07	10.00
F. verticilliodes	-	+	-	-	-	+	-	+	-	+	-	04	5.71

Penicillium citrinum	-	+	+	-	-	+	-	+	-	+	+	06	8.57
Aspergillu terreus	-	+	-	-	-	+	+	-	+	+	+	06	8.57
Rhizopus stolonifer	-	+	-	-	-	+	-	-	-	-	+	03	4.28
Saccharomyces cerevisiae	-	-	+	-	+	-	+	-	-	-	-	03	4.28
Rhodotorula rubra	+	+	-	+	-	-	+	-	-	+	+	06	8.75
Alternaria spp	+	-	-	-	+	-	-	+	-	-	-	03	4.28
Mucor spp	+	+	-	-	+	-	+	-	+	-	+	06	8.75
Neurospora spp	+	-	+	-	+	-	+	-	+	-	+	06	8.75
Total No. of Isolates:	07	09	05	03	07	06	06	04	07	06	10	70	100

Table 3: Mycotoxin Types and Levels Part Per Billion (ppb) in the Domestic Vegetable Oils.

Domestic Vegetable oils	Total Aflatoxin	Ochratoxin
Coconut oil	2.0	2.2
Cottonseed oil	1.8	1.7
Peanut oil	8.6	5.0

Conclusion

From the result of this study, fungal loads of the domestic vegetable oils ranges between $11.22x10^4$ to $7.33x10^3$ Cfu/ml. The study also showed that all the indigenous vegetable oils had a high fungal counts above 10^3 cfu/ml beyond acceptable limits (ICMSF, 2007).

Recommendations

1. Periodic monitoring of the domestic vegetable oils with improved screening techniques for monitoring fungi and mycotoxin levels is required.
2. A primary focus for continuing research is the development of management strategies to reduce the incidence of aflatoxigenicity strains, in indigenous vegetable oils are necessary.
3. It is required that strict monitoring of domestic vegetable oils processors should be enhanced by the monitoring organization to ensure strict compliance to quality.

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