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Different types and levels of mycotoxins detected in some indigenous fermented foods commonly consumed in Bauchi State, North- Eastern, Nigeria

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Abstract
Mycotoxins are fungal metabolites found in foods including fermented products and other vegetable oils consumed by average Nigerian. The fungal metabolites are capable of causing different diseases in humans and other animals, such as liver cancer, kidney dysfunction, vomitting among other. The aim of this study was to determine different types and levels of mycotoxins associated with indigenous fermented foods in Bauchi state, North-Western, Nigeria. A total of 30 different samples comprising (dry meat, dry fish, soya-cake, cassava flour, akamu-pap, iru-dadawa and cowmilk) were collected into sterile bottles in triplicates using random sampling techniques from open market places which included; Mudalawal, Wunti, Central Market Sabon Kasuwa and Bogoro open market places in Bauchi State. The experimental samples were plated out on Malt Extract Agar (MEA) using 10 culture plate for each sample. The resultant culture plates were incubated at room temperature 25^0C for 4-7 days for the isolation of different fungal species. The incubated culture plates were examined after 4-7 days for the development of fungal species. The culture plates were re-examined after 14 days for the appearance of additional fungal species. Result of fungal counts from the indigenous fermented ranged between $13.22x10^6$ cful/ml, to $6.44x10^3$cfu/ml. A total of thirteen species of fungi namely Aspergillus flavus, Aspergillus niger, Aspergillus fumugatus, Aspergillus terreus, Fusarium verticilliodes, Fusarium sporotrichiodes, Penicillium citrinum, Rhizopus stolonifer, Saccharomyces cerevisiae, Alternaria altanata, Mucor indicus, Neurospora crassa and Rhodotorula rubra were isolated from the indigenous fermented foods. The total of three different types of mycotoxins were detected and these included; total aflatoxins, ochratoxins and patulin respectively. Therefore, the presence of these fungal metabolites in the fermented foods and oils might pose to serious health hazards, such as liver cancer and kidney dysfunction. It is therefore recommended that, processors of indigenous fermented foods and other domestic vegetable oils should ensure proper fermentation process in order to prevent fungal contamination and associated mycotoxins.

Keywords: Indigenous fermented foods, mycotoxins, fungal metabolites

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). 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). 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 [25]; 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). Mycotoxins are secondary metabolites produce by contain strains of filamentous fungi such as *Aspergillus, Penicellium*, and *Fusarium*, which inrade crops in the field and may grow in food during storage under favourable condition of temperature and humidity (Smith *et al.*,2000). They are regularly implicated in toxic syndromes in humans and other animals (Anderson T., 2001) [6]. Occurrence of mycotoxin contamination in foods is more prevalent in the tropical and subtropical countries resulting in cute and chronic mycotoxicoses in human and animals. Atanda *et al.*, 2002 [7] reported that many west Africa countries, over 98% of the tested people were positive to aflatoxin DNA aducts indicating aflatoxin exposure in the population.

Fermentation is effected by the natural microbiota of raw materials, micro organisms attached to the fermentation equipments or from externally added starter cultures. Yeast, especially *Saccharomycetes cerevisiae* and *Candida krusei*, and lactic acid bacteria. The elimination of absorbable mycotoxins is possible to be done through adsorption. However for the elimination of the toxicity of non-absorbable mycotoxins, such as zearalenone, ochratoxins and trichothecenes biotransformation is crucial. Biotransformation, which is enabled by enzyme producing microorganisms, allows with non-toxic, harmless metabolites. Mycotoxins frequently contaminate the food raw materials such as cereals, fruits, nuts, spices, milk, and meat at various levels.

1. Statement of problem

The need to study the mycotoxins associated with indigenous fermented foods such as dried meat, dried fish, soya cake, cassava flour, pap-ogi, pounded yam, iru-dadawa and cowmilk 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 and edible essential oils (Jiang and Ma, 2008).

2. The justification of the study

In Nigeria, Hepatic problems are now in the increase in Nigeria, such level of increase in lives of liver disease has been attributed to the consumption of decayed foods with the associated mycotoxins contents. On the earliest reports of mycotoxins contents of foods included that recorded in Nigerian peanuts.

In view of this, it has become imperative to carry out a survey on the presence of mycotoxins in various Nigerian fermented foods which are widely consumed and also in various commonly used domestic oils.

3 Aim and objectives

3.1 Aim

The aim of the study is to determine the various mycotoxin types and levels associated with some selected fermented foods commonly consumed in Bauchi State, Nigeria

3.2 The specific objectives include

1. The determination of different species of fungi associated with the indigenous fermented foods.
2. The determination of types and levels of Mycotoxin present in such Fermented foods.

Sample Collection

The samples of indigenous fermented foods (Iru-Dadawa, Pap-Ogi, Dried Meat, Soya Cake and Cow Milk) were collected at random from some selected open market places within Bauchi State. A preliminary field survey was conducted in order to identify the indigenous fermented foods.

Media Preparation

The fungal media used included; Malt Extract Agar (MEA), and Sabouraud Dextrose Agar (SDA) in which the fungal media were prepared according to manufacturer's instruction manuals.

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 indigenous fermented foods was suspended separately in 0.5ml of sterile distilled water and then were introduced into the Petri dishes containing solidified Malt Extract Agar (MEA) and Sabouraud Dextrose Agar (SDA) incorporated with 0.01g/l of Streptomycin Sulphate Powder. The inoculated suspension was then be aseptically spread with an L- shaped glass spreader and were 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).

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 and Sabouraud Dextrose Agar (SDA) plates using sterile wire loop in order 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).

Determination of Mycotoxin Types and Levels in the Indigenous Fermented Foods

Mycotoxins Detection using Rida Kit

Rida kit quick mycotoxin/aflatoxins were done by using Rida quick scan, which is a modern optical reading device that is 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.

Sample Preparation for Mycotoxin/Aflatoxin Detection

a. The mobile solvent for sample preparation were brought and kept at room temperature (25^{0c}) prior use.
b. The samples were stored in cool dried place and was protected from sunlight.
c. A representative samples was grounded and thoroughly mixed prior to proceedings with the extraction procedure.
d. About 10g of the ground sample were deposited into a suitable container. Thereafter, 20ml of methanol (70%) were added.
e. Therefore, the samples were blended for two minutes and then filtered with what Man No. 1 filter paper.

f. About 100µl of the temperate solvent was added.
g. 50µl of the clear supernatant filtrate solution to 100µl of the temperate mobile solvent.
h. The samples were then mixed along with 100µl in the test.

Test Procedure

1. About 100µl of the samples solution were applied on the application area of the test strip. However, 5 minutes after, the test strip were inserted into the Rida quick scan for detection. The results was interpreted after 16 minutes.
2. The results was read by pressing a start scan on the Rida quick scan machine.
   Finally, the results were then displayed and printed accordingly.

Qualitative Assuarance Certificate of Analysis
Samples: Indigenous Fermented Foods
Parameters: Aflatoxins, Ochratoxins and Patulin
Results: The results were in Part Per Billion (ppb)

Table 1: Mean Fungal Counts of Fermented foods Commonly Consumed in Bauchi State, North Eastern, Nigeria

Fermented Foods	Coliform Forming Unit CFU/ml /(g)
Dry Fish	$12.55x10^4$ CFU/g
Dry Meat	$11.23x10^5$ CFU/g
Cassava Flour	$10.66x10^2$ CFU/g
Soya cake	$11.52x10^4$ CFU/g
Iru-Dadawa	$12.33x10^2$ CFU/g
Pap-Ogi	$8.66x10^4$ CFU/ml
Cow milk	$10.22x10^3$ CFU/ml
Coconut oil	$9.30x10^2$ CFU/ml
Cottonseed oil	$8.23x10^3$ CFU/ml
Peanut oil	$12.24x10^3$ CFU/ml
Pito-Burukutu	$11.66x10^2$ CFU/ml

Table 2: Mycotoxin Types and Levels Part Per Billion (ppb) in the Indigenou Fermented Foods in Bauchi State, North Eastern, Nigeria.

Fermented Foods	Total Aflatoxin (ppb)	Ochratoxin (ppb)
Dry Fish	3.2	3.0
Dry Meat	2.9	2.7
Cassava Flour	2.3	2.4
Cow milk	2.3	2.2
Soya cake	2.0	2.2
Iru-Dadawa	2.0	2.1
Pap-Ogi	2.3	2.4
Pito-Burukutu	2.9	2.7

Table 3: Mycotoxin Types and Levels (Patulin) Part Per Billion (ppb) in the Cowmilk in Bauch State, North-Eastern, Nigeria.

States	Patulin M_1 (ppb)	Patulin M_2 (ppb)
Bauchi	24	2.2

Discussion

Thirteen fungal species were isolated from the indigenous fermented foods. The species isolated were *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Fusarium verticillioides, Fusarium sporotrichioides, Rhizopus stolonifer, Penicillium citrinum, Aspergillus terreus, Saccharomyces cerevisiae, Alternaria altanata, Mucor indicus, Neurospora crassa* and *Rhodotorula rubra*. *Aspergillus flavus* and *Fusarium sporotrichioides* had the highest frequencies of occurrence, which is in agreement with the work of (Nyam *et al*., 2020) that isolated *Aspergillus, Penicillium, Rhizopus, Rhodotorula* and *Fusarium* species from groundnut cake and oils. Rabiu *et al*., (2017) also isolated different species os *Aspergillus, Penicillium, Fusarium, Rhizopus, Mucor* and *Alternaria* species among others from indigenous fermented foods in Bauchi State.

Various types and levels of mycotoxins were obtained from eleven different indigenous fermented foods. The levels detected were found to be higher than those reported on palm oil (Bacaloni *et al*., 2012). The total aflatoxin levels detected in soya cake and cassava flour of 2.8ppb were significantly higher than those in agreement with NAFDAC and SON of 2.5ppb in cereal products, 2022. For pito-burukutu, the total aflatoxin levels detected were also significantly higher of 3.9ppb in line with NAFDAC and SON maximum permissible limits of 3ppb in beverages, 2022. However, the total aflatoxin levels detected in dry fish and dry meat of 3.6ppb were also above maximum permissible limits as stipulated by NAFDAC and SON of 2ppb in frozen foods, 2022. The patulin M_1 and M_2 levels in cowmilk detected 2.7ppb has also exceeded maximum permissible limits in line with the National Agency for Food and Drug Administration and Control (NAFDAC), and Standard Organization of Nigeria (SON) regulations of 1.0 ppb in dairy products, 2022.

Conclusion

From the result of this study, fungal loads of the indigenous fermented foods and other domestic vegetable oils ranges between $13.22x10^6$ to $6.44x10^3$. The study also showed that all the indigenous fermented foods had high fungal counts above 10^3 cfu/ml beyond acceptable limits (ICMSF, 2007). It was also observed that fungal isolates from all the indigenous fermented foods had shown ability for aflatoxins, ochratoxins, and patulin production.

Recommendations

1. From the findings of this research work, it is important to recommend that processors of indigenous should ensure proper fermentation process in order to prevent fungal loads associated with the products.
2. The processors/sellers of indigenous fermented foods should cover the fermented products properly during transportation; distribution or sales. This is due to the ubiquitous nature of microorganisms, particularly fungi, which may found to contaminate the fermented products.
3. Periodic monitoring of the indigenous fermented foods 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 indigenous fermented foods is necessary.
5. It is required that strict monitoring of indigenous fermented foods processors should be enhanced by the monitoring organization to ensure strict compliance to quality.

References

1. Abila OR, Food Safety in Food Security and Food Trade. Case Study in Kenya Fish Exports 2010 Vision for Food Agriculture and Environmental Focus 10, 2003.
2. Abraca ML, Accens IF, Bragulate MR, Cabanes FJ. Current Importance of Ochratoxin A Producing Aspergillus Species. Journal of Food Protection,2005:64:903 – 906.
3. Adrio DS, Demain KY. Protease microbes playing vital role in differentiation. *Journal of Enzymology*,2004:16(2):248-249.
4. Akande KE, Abubakar MM, Adegbola TA, Bogoro SE. Nutritional and a. health implications of mycotoxins in animal feeds. *A Review Journal of Nutrition*,2006:*5:*398-403
5. Ali AK, Nisha B. Enzymes and genetic manipulation for biotechnological application. *Journal of Biotechnology and Microbiology*,2016:13(4):124-129.
6. Anderson T. Mycotoxins occurrence in human feeds & health Implications. *Journal of African Biotechnology*, 2001¸ 406-409.
7. Atanda DS, Jibrin BD. Mycotoxins Incidence in West African food and feeds. *Journal of life sciences*, 2002, 306-309.
8. Aguilo PL, Esperenc A, Mbau E, Palmer P, Patel A, Sparkman T. Attracting investment to Kisumu: Opportunities and Challenges. Report Prepared for Earth Institute, Millenium Cities Initiative, Columbia University, 2007.
9. Aish JL, Rippon EH, Barrlow T, Hattersley SJ. Ochratoxin A. In: Magan, N., Oslen, M. editors. Mycotoxins in food: Detection and control. Boca, Fla: CRC press, 2004, 307-338.
10. Association of Nutrition and food Service Professionals (ANFP) (2011). ANFP Practice
11. Standards: Food Storage Guidelines, Accessed February 2012.
12. Barnett HL, Hunter B. Illustrated Genera of Imperfect Fungi. Saint Paul; The American Phytopathological society, 1998.
13. Babajide JM, Oyewoye OB, Obadina OA. An Assessment of the Microbiological Safety of Dry Yam (gbodo) processed I south west Nigeria. *African Journal of Biotechnology*,2006:*5:157-161*.
14. Barrett J. Mycotoxins of moulds and maladines. *Environmental Health a. Perspective,*2000:*108*:20-23 Medline
15. Bashir SM, Chellapin S, Beena PS. Lipase from marine *Aspergillus*: Production, partial purification and application in oil effluent treatment. New Biotechnology,2011:28(6):627-638.
16. Bautaiba S, Hacene H. Preliminary characterization of lipolytic activity archeon. *Journal of Molecular Catalysts Enzyme*,2006:41(2):21-26.
17. Bhat FC, Vsanthi JB. Mycotoxins and Health Implication. *Journal of Medical Sciences*,2003:7:172-180
18. Bellí N, Marín S, Sanchis V, Ramos AJ. Influence of Water Activity and Temperature on Growth of Isolates of Aspergillus section Nigri Obtained from Grapes. International *Journal of Food Microbiology*,2004:*96:19-27.*
19. Bennett JW, Klich M. Mycotoxins. Clinical Microbiology Review,2003:16(3):497-516.
20. Berger KJ, Guss DA. Mycotoxins, Revised Part (ii). Blackie, London, 2005, 289.
21. Bille GP, Shemkai HR. Process Development, Nutrition and Sensory Characteristics of Spiced Smoked and Sun Dried Dagaa (Rastrineobola argentea) from Lake Victoria, Kenya. In: African Journal of Food Agriculture Nutrition and Development,2006:6(2):98-102.
22. Borelli GM, Trono D. Recombinant lipases and phospholipases and their use as biocatalyst for industrial applications. *International Journal for Molecular Science*,2015:16(9):20774-20840.
23. Campbell T. Food safety: The case study of aflatoxin. In: Occurrence and Health Risks, 2013. www.cip.cornell.edu/../1200-428161. Accessed on 14/01/2013.
24. Cano J. European Commission of Regulation (EC). No. 472/2002 of 12th March 2002. Amending Regulation (EC) no.466/2002 Setting Maximum Levels for Certain Contaminants in food Stuffs, 2002.
25. Cast. Mycotoxins: Economic and health risk. Task force report 16. Council for a. Agricultural Science and Technology, Ames, IA, 1989.
26. Chamberlain T, Titili G. Sea Food Processing. Community Fisheries Training. USP Marine Studies Programme, Pacific, 2001, 6.
27. Chartered Institute of Environmental Health (2010). Food hygiene, Health and Safety. Bpp Learning Media.
28. Chu FS, Li GY. Simultaneous occurrence of fumonisin B1 and other mycotoxins in moldy corn collected from the People's Republic of China in regions with incidences of esophageal cancer. Journal of Applied Environmental Microbiology,1994:60:847–852.
29. Codex, Mycotoxin limits in foods. *European Society for Biotechnology*, 2018, 13(6).
30. Copetti MV, Santurio JM, Cavalheiro AS, Alves SH, Ferreiro L. Comparison of different culture media for mycological evaluation of commercial pet food. Acta Scientia Veterinariae.2009:37(4):329- 335.
31. Diener-UL. Epidemiology of aflatoxin formation by. *Aspergillus flavus. Annual revision Phytopathology*,1987:*25*:249-270.
32. Dinrifo RR, Babatundo SO, Bankolo YO, Demu QA. Physicochemical properties of rain water collected from some industrial areas of Lagos state, Nigeria. *European Journal of Scientific Research*,2010:41(3):383-390.
33. Dugje IY, omoigui LO, Ekeleme F, Bandyopathy R, Lara Kumara P. Guide to soya bean production in Northern Nigeria. *International Institute of Tropical Agriculture, Ibadan, Oyo Nigeria*, 2009, *21.*
34. Eaton DL, Grooman JD. The Toxicology of Aflatoxins. Acadenmic Press, New York, 1994.
35. Edema MO, Omemu AM, Fapetu OM. Microbiology and physicochemical analysis of different sources of drinking water in Abeokuta, Ogun state, Nigeria. *Nigerian Journal of Microbiology*,2001:5:57-61.
36. Ehizibolo DO, Perez AM, Carillo C, Alkhamis M, Umoh JU, Metwally SA. Epidemiological Analysis, Serological Prevalence and Genotypic Analysis of foot and Mouth Diseases in Nigeria. *Journal of*

Transboundary and Emerging Diseases,2003:61:500-510.
37. Eskola M, Parikka P, Rizzo A. Trichothecenes, ochratoxin A and zearalenone contamination and Fusarium infection in Finnish cereal samples in 1998. Food Additives and Contamination,2001:18:707–718.
38. European Commission. EC No 123/2005 of 26th January 2005 Amending Regulation. Journal of the European Communities,2005:25:3-5.
39. Evander B. How to Determine the Moisture Content of Foods. Available online (www.ehow. Com/ how 7844565 determine moisture content food). Accessed on16th October, 2012.
40. Eyo AA. Traditional and Improved Fish Handling, Preservation and Processing Techniques NAERLS/NIFER. National Workshop on Food Processing, Storage, Marketing and Utilization, 1992, 15.
41. Egmond van HP, Schothorst RC, Jonker MA. Regulations relating to Mycotoxins a. in food: *Perspective in a Global and European contex Annual Bioanalytical-Chemistry*,2007:*389*(1):147-57
42. Felicia WU, Clare MT, Liu Y. The health economics of aflatoxin: Global burden of disease. Working, 2011, 4.
43. Finley JW, Robinson SF, Armstrong DJ. Food Safety Assessment. American Chemical Society, Washington, D.C, 1992, 261-275.
44. Fisheries Annual Report. Department of Fisheries, Ministry of Livestock and Fisheries. Kenya, 2010.
45. Food and Agriculture Organization (FAO). Fishery country profile: Republic of Kenya. FID/CP/KEN FAO, Rome, 2007, 91.
46. Food and Agriculture Organization (FAO), (2008). Manual on simple methods of meat preservation, animal production and health paper110. Available online (http://www.fao.org/docrep/ 003/x 6932e/ X6932E00.htm). Accessed on 29th May, 2012.
47. Fox EM, Howlett BJ. Secondary Metabolism; Regulation and Role in Fungal Biology. *European Journal of Applied Microbiology*,2008:19:67-78.
48. Fred DH. How to Preserve Food by Salting. Precautions and Effects. Journal of Applied Microbiology,2007:16:1853-1858.