

The Mycotoxin Risks Lurking in Breads Sold in Abuja Metropolis

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Bread is one of the most essential food products that is universally accepted as a very convenient form of food that has desirability to all population rich or poor, rural or urban dwellers. A staple food prepared by baking dough of flour, water and/or sugar. It is a good source of nutrients, such as macronutrients and micronutrients that are all essential for human Bread like other bakery products are subject to microbial contamination and spoilage by molds irrespective of the intrinsic composition of the product. The present study was carried out to identify the fungi associated with bread spoilage sold in Abuja metropolis, Nigeria and the possible health risks. Twenty-four (24) bread products from different locations within FCT, Abuja, Nigeria covering the six regions; Abaji, AMAC, Bwari, Gwagwalada, Kuje and Kwali were sampled and the molds associated with them were isolated using spread plate method. Based on the cultural and morphological characterizations using standard identification techniques, the mold namely *Aspergillus* sp. (29.7%), *Mucor* sp. (17.4%), *Penicillium* sp. (17.1%), *Fusarium* sp. (14.7%), *Rhizopus* sp. (8.0%), *Cladosporium* sp. (7.4%) *Alternaria* sp. (3.4%) and *Geotricum* sp. (2.3%) were found. The presence of mold is a signal to the possibility of mycotoxin build-up and other food safety risks. Therefore, bread manufacturer and distribution vendors need to periodically validate their preventive measures to control potential hazards associated with fungi-laden breads.

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1. INTRODUCTION

Historically, bread has been an essential part of human diets and feels like it has literally been around forever. This product is present everywhere, no matter where one travel to and in many different taste, shapes or colors. Bread has been given its own culture through different countries over thousands of years. Bread is a pleasantly soft baked diet that can be found almost anywhere and in almost anything. Although the origin of bread is not exactly known, however, it is known that the bread eaten then was undeniably different from the bread we eat today.

Bread making is a dynamic process with continuous physicochemical, microbiological, and biochemical changes induced by the mechanical–thermal action and the activity of the yeast and some Lactic Acid Bacteria together with the activity of the endogenous enzymes. Many challenges have been recorded and attempted to solve regarding the shelf stability of the product. For instance, low-temperature technology has been initially applied to bakery products to solve the economic losses associated with the bread staling problem that produces a decrease of consumer acceptance. Recently, the technology of frozen dough, par-baked bread, and frozen bread is being incorporated as routine processes [1]

The major component of bread is a starchy-based carbohydrate that makes up about 70% of flour by weight. Basically, starch granules are attacked by enzymes present in the flour, they release the sugars that yeast feeds on. Starch also reinforces gluten and absorbs water during baking, helping the gluten to contain the pockets of gas produced by the yeast [2,3].

Yeast, on the other hand, is a live, single-celled fungus. There are about 160 species of yeasts around us. Of these, only few strains are genetically fit for bread production. The organism feeds on the sugars in flour, and releases the carbon dioxide that makes bread rise. Yeast also adds many of the distinctive flavors and aromas we associate with bread [4].

Microbial spoilage of bread and the consequent waste problem causes large economic losses for both the bakery industry and the consumer. Furthermore the presence of mycotoxins due to

fungus contamination in cereals and cereal products remains a significant issue. The use of conventional chemical preservatives has several drawbacks [5].

The most common source of microbial spoilage of bread is mould growth. Less common, but still causing problems in warm weather, is the bacterial spoilage condition known as 'rope' caused by growth of *Bacillus* species. Least common of all types of microbial spoilage in bread is that caused by certain types of yeast. Mould spoilage of bread is due to post-processing contamination. Freshly baked bread from the oven is free of moulds or mould spores due to their thermal inactivation during the baking process [4]. However, bread becomes contaminated after baking from the mould spores present in the atmosphere surrounding loaves during cooling, slicing, packaging and storage. The environment inside a bakery is not sterile because dry ingredients, especially flour, contain mould spores, and flour dust spreads easily through the air. It has been estimated that 1 g of flour contains as many as 8000 mould spores. In some bakeries a similar number of spores settle on 1m² of surface every hour. Production operations such as weighing and mixing of ingredients increase the mould count in the air. In larger bakeries where segregation is possible, the flour handling areas are separated from the cooling and packaging area of the finished bread. Previous studies by Marian, 2015, bread molds like *Mucor* and *Rhizopus* are found to grow first during bread spoilage [5]. This is followed by some other fungi like *Aspergillus*, *Penicillium* and *Fusarium* sp. Among these *Penicillium* sp is the most common one, though *Aspergillus* sp may be of greater significance in tropical countries according to the study.

The purpose of this study was to empirically determine the molds associated with this ready-to-eat staple food in order to add to knowledge as well as create awareness on the dangers of mycotoxin and other related health risks associated with the consumption of such foods.

2. MATERIALS AND METHODS

2.1 Location and Study Area

The study was conducted in FCT comprise of Gwagwalada, Abaji, Kuje, Bwari, Kwali, and

Abuja Municipal Area Council. The map is as shown below;

2.2 Sample Collection

Twenty loaves of bread were purchased from different shops within the FCT, Abuja, Nigeria between January-March, 2021. The respective samples were brought in their original polythene bags to the laboratory for analysis. They were exposed to the laboratory environment for seven days [6].

2.3 Preparation of Culture Media for Isolation of Fungi

As a routine, all glass ware used during the study were sterilized in a hot air oven at 160 °C for 1 hour. Other materials requiring steam sterilization were sterilized by autoclaving at 121 °C for 15 mins. Potato Dextrose Agar (PDA) was used for the isolation and routine growth of the fungi in the study. The medium preparation involved dissolving 39g of PDA in 100ml distilled water, autoclaving at 121 °C for 15 mins, allowing to cool down to about 50 °C, then dispensing about 20 ml of the medium into each Petri dish previously sterilized in an oven. The medium in Petri dish were allowed to solidify [7].

2.4 Isolation of Fungi

Bread sample (10g) was mixed with distilled normal saline (90ml) and a homogenate was prepared. Serial dilution was made using 1ml

from the solution into 9ml sterile distilled normal saline and an aliquot portion was used to prepare spread plate for the enumeration of fungi from the breads. The working surface was routinely sterilized using ethanol. 1ml was taken from the above homogenate. Tween80 was added to the normal saline to ensure even distribution of fungal spores because fungal spores sediment quickly than bacteria [7]. Aliquot 0.1 ml of the inoculum dilution 10^{-4} was added on the surface of the PDA medium and spread evenly over the surface of the PDA using a sterile spreader (bent glass rod). The plates were incubated in an upright position at 30°C for 7days. The same procedure was carried out for all the samples. The fungal count was recorded. The different types of colonies were used as inocula to obtain pure cultures by sub culturing in PDA [8].

2.5 Identification of Fungi

A small portion of each sub-cultured colony was cut using a sterile scalpel and placed on a sterile glass slide. The slide was covered with a cover slip and placed in a Petri dish. These Petri dishes were left at 30 °C for 5days. Then the cover slips were taken with forceps and placed on slides containing Lacto-phenol cotton blue. Excess stain was removed and the preparations were observed under the microscope. The morphology, that is, shape, structure of conidia, conidiophores, pigmentation, shape of sporangia, sporangiophores were recorded. The identification was based on the standard keys used by previous researchers [9].

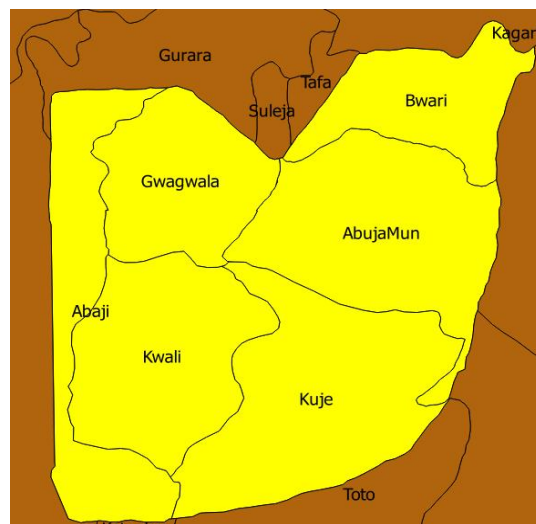


Fig.1. Map of Abuja Where Bread Were Collected



Plate A1. Bread sample after incubation



Plate A2. Isolation of fungi



Plate A3. Culture of fungi



Plate A4. Culture of aspergillus



Plate A5. Fungi culture on PDA slant



Plate A6. Fungi culture slant in air tight bags

Plate A1 – A6. Isolation, Identification and Storage of Fungal Isolates

Plate 1. Isolation, Identification and Storage of Fungal Isolates

3. RESULTS AND DISCUSSION

The table below, Table 1 shows the isolated fungi species based on cultural and morphological characterization.

From the study, breads from Gwagwalada had the highest number of visible colony count of *Aspergillus* being Forty-Three (43) while the lowest were found in Abuja Municipal Area Council (29). Generally, *Aspergillus* spp had the

overall highest count with a total of 226 out of the overall 760 visible count representing 29.7%.

Geotricum spp had the lowest count of 2.3% with loaves from Abaji, Kuje and Kwali having no record of visible fungal colony count.

4. DISCUSSIONS

A total of 24 loaves of bread were collected from FCT where 760 visible fungal colonies were noted and enumerated after the incubation period, which is by allowing the loaves of bread collected to remain unrefrigerated for ten days. The total fungal count of bread samples are shown in Table 1.

It had a fungal range of eight species. There was no count of *Geotricum* spp on the bread collected from Kuje, Abaji and Kwali in the study of the twenty-four (24) samples used while *Aspergillus* had the highest in term of occurrence. However, all the samples showed positive fungal growth from the fourth day till the tenth day. This agrees with several researchers that fungal colonization of bread is responsible for staling and spoilage [5,10, 6].

Table 2 shows the percentage of occurrence of fungal isolates on the enumerated bread samples. *Aspergillus* spp. Had the highest occurrence (29.7%) followed by *Mucor* spp. (17.4%) and the least being *Geotricum* spp. (2.3%). The fungal count generally ranged from 2.5×10^4 to 5.1×10^4 cfu/ml. The highest count was from AMAC area while the lowest count was from Kuje area council. The high count recorded might be due to poor post-baking handling especially during slicing of the loaves because it is expected that microorganisms are killed by the baking heat. Fungal spores are present both in the air around the production areas as well as on the various utensils used for the processing [2,7].

Fig. 1 shows relative distribution of fungi using pie-chart. *Aspergillus* has the highest occurrence. This agrees with the work of Unachukwu and Nwakanma in the study of fungi associated with spoilage of bread in Enugu, Nigeria [5].

Fig. 2. Comparative Occurrence of Fungi using Bar-Chart. From the chart, *Aspergillus* had the highest occurrence while *Geotricum* had the

lowest. The presence of *Aspergillus* has been enumerated earlier [7].

Table 3 shows the site-wise visible fungal colony count and percentage occurrence on the 10th day of shelf storage (Allowed for incubation spores).

Aspergillus spp are usually found in old damp, musty houses, in onions as black mold, damp hay, leather goods, spoiled foods and decaying vegetation. Both bread factory employees and vendors interact often with these environments. As such, the presence of these organisms in abundance might be due to cross contamination during post-oven processing of the bread such as slicing [2,3].

This study also showed that the mold growth increased with the number of days. This was responsible for the build-up of mycelium which eventually produced the spores.

Plate A shows fungal colonies on bread sample, isolation and characterization procedures, growth isolated fungi on Potato Dextrose Agar (PDA) and stored fungal cultures on PDA slants in airtight bags. It is an indication that the fungal sp. can grow well on PDA media [9], though it shows variations in the growth rate (growth/day) and sporulation.

The results of this research show that bread sold within the study area are associated with serious post-oven fungi contamination. However, the prevention of mycotoxin build-up is one of the best food safety defense mechanisms for consumer's protection. Therefore, awareness of possible route of contaminations is obviously necessary giving the results of practical field studies like this.

The presence of fungal contamination and growth within a short time as observed in the work means that, one, spores of these organisms were already present on the bread, two, that significant levels of mycotoxin might be recorded as such product stays under the favorable conditions for fungal growth [3]. It is thus expected that there must have been a pattern of contamination either at the industry level or during distributions. Since bread is one of the main staple, ready-to-eat food, the need to enforce food safety regulatory laws cannot be overemphasized.

Table 1. Cultural and morphological characteristics of the isolated fungi

S/N	Cultural characteristics	Morphological characteristics	Identified Fungal Isolate
1	Black, cottony with yellow or hallow region, powdery, sooty surface becoming completely black with age.	Globule vesicles and phialides are produced directly from the vesicle surface (uniserate). The phialides give rise to short chains of spherical conidia.	<i>Aspergillus</i> spp
2	Colony usually green in colour with the edges being white with yellow back side	Presence of septate hyphae and hyaline. Conidiospore elongation halted by a brush-like structure (a classical morphological characterization for <i>Penicillium</i> species (Forbes et al., 2007).	<i>Penicillium</i> spp
3	Rapidly growing wooly (lemon/yellow) colony, initially white (snow-white) on PDA but changed to pink as the culture became old	Septate hypha, Rod-like and lightly bent macroconidia (Multicellular distinctive sickle shaped macro conidia).	<i>Fusarium</i> spp
4	Creamy-white/large fluffy white colonies covering the whole surface in 3 days and later turned to gray. Colony reverse is whitish	Sporangium comes out directly from the hyphal without stolon or rhizoids columella.	<i>Mucor</i> spp
5	Slow-growing, olivaceous-brown to blackish-brown Colonies, sometimes grey, buff or brown, suede-like to floccose, often becoming powdery due to the production of abundant conidia. The reverse is olivaceous-black.	Produces erect, dark, septate hyphae. Conidiophores are also darkly pigmented, may be septate and show tree-like branching. Fragile chains	<i>Cladosporium</i> spp
6	Large fluffy white milky colonies which later turns grey-black as culture ages. Black reverse side. Covers plate in 3 days	Long, Non-septate hyphal with uptight sporangiophore connected by stolon	<i>Rhizopus</i> spp
7	Lettuce green to olive green with white margin. Colony texture sometimes felty to woolly, pale olive gray to olive gray. May be cottony dark olive gray, iron gray to castor gray in color.	Formation of conidial chains 6 to 18 conidia in length and the uncommon occurrence of secondary chains 1 to 4 conidia in length. Chain branching occasionally occurred in a sympodial manner	<i>Alternaria</i> spp
8	Colonies are raised and pale white, with a neat edge and visible hyphae at the edge. Colonies friable, with no pigments, opaque and not shiny; vigorous sporulation, and the colony surface white and powdery. Yeast-like, white, dry, and dusty colonies.	Single or multiple short hyphae often adhered to the septa at the base of the hyphae	<i>Geotricum</i> spp

Table 2. Frequency of occurrence of fungi

SN	FUNGAL ISOLATE	OCCURRENCE (%)
1	Aspergillus spp.	29.7
2	Penicillium spp.	17.1
3	Fusarium spp.	14.7
4	Mucor spp.	17.4
5	Cladosporium spp.	7.4
6	Rhizopus spp.	8.0
7	Alternaria spp.	3.4
8	Geotricum spp.	2.3

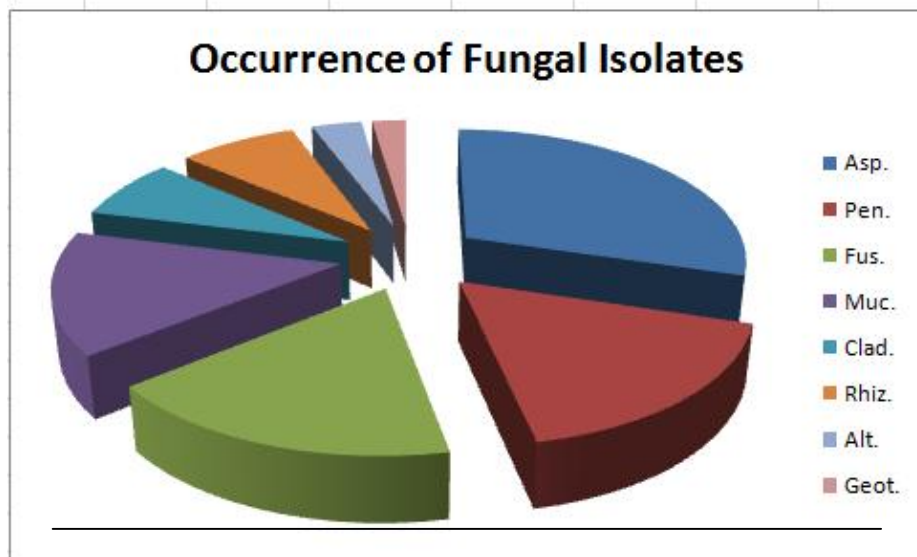


Fig .1. Relative distribution of fungi using pie-chart

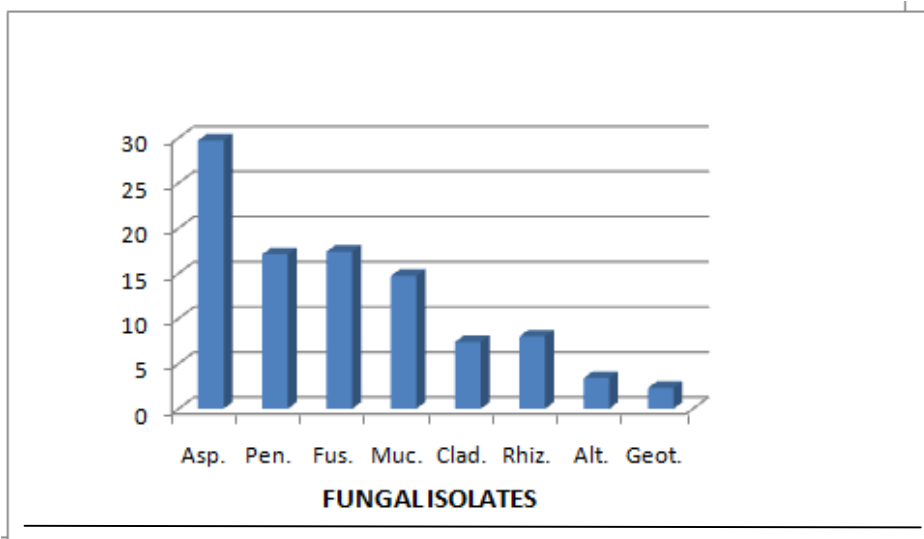


Fig. 2. Comparative occurrence of fungi using bar chart

Table 3. shows the site-wise visible count of fungal colonies and percentage occurrence on the 10th day of storage of the bread product obtained from the regions that made-up the FCT

REGION	Aspergillus sp.	Penicillum sp	Mucor sp	Fusarium sp	Cladosporium sp-	Rhizopus sp	Alternaria sp	Geotricum sp	Total
Abaji	38	28	32	23	14	12	3	0	150
AMAC	29	33	29	20	10	21	8	5	155
Bwari	37	11	24	25	7	4	6	4	118
Gwags	43	28	21	16	13	7	0	7	135
Kuje	38	17	11	15	5	9	5	0	100
Kwali	41	13	15	13	7	9	4	0	102
TOTAL	(226) 29.7P%	(130) 17.1%	(132) 17.4%	(112) 14.7%	(56) 7.4%	(62) 8.0%	(26)3.4%	(16)2.3%	760 (100%)

5. CONCLUSIONS

Spoilage of bread by mold has been reportedly associated with post-processing contamination (Seiler, 1994). Initially the molds namely *Mucor* sp and *Rhizopus* sp were found to be the cause of bread spoilage. This was followed by *Aspergillus* sp and *Penicillium* sp. In this study, however, *Aspergillus* sp was found to be the most common fungus during the spoilage of bread in FCT.

Bread and other bakery products are subjected to various spoilage problems, viz., physical, chemical and microbial; the latter is the most serious one particularly bacterial (*Bacillus* sp.) and mold growth. However, being a food product that is universally accepted as a very convenient form of food that has desirability to all population rich—and poor, rural and urban, post-oven handling to avoid fungal and other forms of contaminations becomes very significant giving the result of this study. Various molds involved in spoilage of bread in FCT have been enumerated and appropriate control measures are hereby recommended.

6. RECOMMENDATIONS

Bread has been reported to be the second most widely consumed non-indigenous food product after rice. Therefore, having studied one of the challenges associated with the production and distribution of the important product in FCT, the following recommendations are hereby made;

- ❖ The shelf-life of bread kept at room temperature ranges from 3–7 days but becomes moldy by the tenth day, though may vary depending on ingredients, type of bread, and storage method. Therefore, overstayed bread should not be consumed because some mold can produce harmful and invisible poisons called mycotoxins. These may spread through bread, particularly when mold growth is heavy;
- ❖ The order of particular fungal growth may be related to the constituents available in the substrate (Bread) and the physical parameters which necessitate further studies;
- ❖ Although some fungal colonies might not be visibly sighted on unrefrigerated bread, a fungi structure such as hyphae often penetrate, which gives off-flavor and may be harmful to health;

- ❖ It is impossible to know what kind of mold is growing on the bread just by looking at it, it is therefore best to assume that over stayed, unrefrigerated bread is harmful and not to be eaten;
- ❖ Additionally, smelling moldy bread by way of testing its spoilage status should be totally avoided, especially by allergic patients, as spores from the fungus might be inhaled which could lead to breathing problems, including a life-threatening anaphylaxis and asthma;
- ❖ The Food Safety and Inspection authorities such as NAFDAC should advise and maintain that moldy bread are discarded along with the entire batch if one has developed mold;
- ❖ Moisture encourages mold growth. Therefore, bread should be kept dried always. If there is any visible moisture inside the bread package, paper towel or a clean cloth could be used to dry the package before sealing it;
- ❖ Bread should be packaged in clean environment or in a hood to shield it from spores in the air. Also, bread should not be packaged until it is thoroughly cooled.
- ❖ Freezing bread stops the growth of mold without altering the texture of bread. Also, refrigeration slows their growth. Therefore bread should be refrigerated to keep it cool and fresh by avoiding heat, humidity and light which are the favourable conditions for fungal growth.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Selomulyo VO, Zhou W. Frozen bread dough: Effects of freezing storage and dough improvers. *J. Cereal Sci.* 2007;45:1-17.
2. Oyeleke SB, Manga SB. *Essentials of Laboratory Practice in Microbiology.* Tobest Publisher, Minna, Nigeria. 2008;36-75.
3. Smith JP. Bakery products. In: *Principles and Application of Modified Atmosphere Packaging of Food* (ed. R. T. Parry). Blackie Academic and Professional, Glasgow, UK. 1993;134-169.
4. Marcelo V, Garcia AB, Andrieli SM, Venturini C. Spoilage fungi in a bread

- factory in Brazil: Diversity and incidence through the bread-making process; Food Research International. 2019;126.
5. Marian Unachukwu. The fungi associated with the spoilage of bread in Enugu state; International Journal of Current Microbiology and Applied Sciences. 2015;4:989-995.
 6. Okoko FJ, Ogbomo O. Amylolytic Properties of Fungi Associated with the Spoilage of Bread, Continental J. Microbiology. 2010;4:1-7.
 7. Ricardo A, Acacio GR, Cidalia P. A fast, practical and reproducible procedure for the standardization of the cell density of an Aspergillus suspension; Journal of Medical Microbiology. 2004;53:783–786.
 8. Nirmala R, Pathmanathan S, Selvaratnam S. Study on fungi associated with spoilage of bread; Int. J. Adv. Res. Biol. Sci. 2016;3(4):165-167 165.
 9. Richard A. Humber Fungi: Identification, USDA-ARS Plant Protection Research Unit, US Plant, Soil & Nutrition Laboratory, Tower Road, Ithaca, New York USA. 2017;14853-2901.
 10. Onuorah O, Obika I. Filamentous Fungi Associated with the Spoilage of Commercial Bread in Akwa, Nigeria, Am. J. Life. Sci. Res. 2015;3(2):163-168.

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