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Assessment of Fungi Associated with Bakery Products in Port Harcourt Metropolis

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Authors' contributions

This work was carried out in collaboration among all the authors. Author WJO designed the study and wrote the protocol. Author DSI performed the statistical analysis. Author WSP managed the analysis of the study, wrote the first draft of the manuscript. and managed the literature searches. Authors WJO, DSI and WSP read and approved the final manuscript.

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ABSTRACT

Bakery products such as bread, cakes, etc are staple foods consumed by both the poor and rich. Bread and other bakery products are subject to fungal contamination. This study is aimed at identifying the different fungi associated with bakery products in Port Harcourt metropolis, Nigeria. Sampling was done once monthly for three months, January to March for twenty-three (23) bakery products. Various bakery products were collected from different locations (Abacha Road, Ikwerre Road and Mile 3) in Port Harcourt Metropolis for enumeration, isolation and identification of different fungi that cause spoilage of the bakery products. In the month of January, bread samples had the following fungal counts: 2.0×10^6 sfu/g, 1.0×10^6 sfu/g, 2.0×10^6 sfu/g, 1.0×10^6 sfu/g, 1.0×10^6 sfu/g and 3.0×10^6 sfu/g. The lowest count was 1.0×10^6 sfu/g and the highest count was 3.0×10^6 sfu/g with mean values of 1.07 ± 1.00^a and 2.5 ± 0.55^a , respectively. During the study, ten (10) different species of fungi were identified. The species are in eight (8) genera, *Aspergillus* (39.2%), *Fusarium* (10.5%), *Penicillium* (5.7%), *Mucor* (13.4%), *Eurotium* (8.1%), *Cladosporium* (8.1%), *Alternaria* (3.3%), and *Rhizopus* (11.5%) species. Statistically, there was a significant difference

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comparing the growth of the organisms in Abacha Road (2.00 ± 0.89^a), Ikwerre Road (5.80 ± 83^b) and Mile 3 (4.03 ± 1.23^a). It was observed that Abacha Road had the lowest counts of the fungi. There was an increased count in Ikwerre Road as well as Mile 3 compared to Abacha Road. Therefore, Abacha Road < Ikwerre Road \geq Mile 3 based on the analysis of variance.

Keywords: Bakery products; *Aspergillus*; *Cladosporium* sp.; Abacha Road; et al., Ikwerre Road; Mile 3.

1. INTRODUCTION

Bakery products like bread are significant food items acknowledged all over which is an exceptionally helpful type of food source wanted by all populace including the rich and poor for both the rural and urban metropolitan regions [1]. Bread and other pastries or bakery products undergo different deterioration issues which could be physical, chemical or microbial [2].

Various organisms involved in bread and other bakery products spoilage include *Rhizopus*, *Mucor*, *Penicillium*, *Eurotium*, *Aspergillus* and *Monillia* [3]. The organisms however, can cause food poisoning [5]. Food poisoning can occur through the consumption of food contaminated by organisms which secrete toxins [4]. Effect of contaminated bread in the society can lead to an epidemic of gastrointestinal disorder whereby that particular environment that consumed the contaminated bread become affected thereby developing stomach upset [4]. As a result of this, a lot of consideration should be given to bread that is sent into the community to be sold and this should be done by the standardized system called Hazards Analysis Critical Control Point (HACCP). This method is now generally considered as a choice for ensuring safety of foods. Hazard Analysis Critical Control Point involves identifying places in the production process where hazard could occur, that is, critical control point and putting monitoring procedures in place to prevent these hazards from occurring [2]. Even with this system in place, samples still need to be tested for the presence of microorganisms [5].

In most cases, the mould spoilage of bread is due to post processing contamination. When freshly prepared bread or other bakery products are brought out of the oven, they are free from molds or mould spores due to the intensity of heat or thermal inactivation during the baking process [6]. Bread and other bakery products became contaminated after baking from the mold spores present in the atmosphere surrounding loaves during cooling, slicing, packaging and

storage (Para and Megan, 2004). The most common source of microbial spoilage is due to mould growth. Previous studies have proven that bread molds like *Mucor* and *Rhizopus* are found or identified to grow first during bread spoilage [7]. Other fungi like *Aspergillus*, *Penicillium* and *Fusarium* spp. will follow suit.

Mycotoxins are secondary metabolites of fungi. These fungal metabolites can be found in foods such as bakery products. They have the potential to cause diseases in humans, livestock or domestic animals, therefore, are of public health importance [8]. There are environmental factors that stimulate the production of mycotoxins. Therefore, the extent of contamination will differ with the agricultural methods, geographic location and the susceptibility of the commodities to the penetration of fungi during storage and processing periods [5].

Examples of mycotoxins causing human and animal illness include aflatoxin, citrinin, flumonins, ochratoxin A, patulin, trichothecenes, ergot alkaloids [9].

Some of the health effects that are found in animals and humans include death, identifiable diseases or health problems, weakened immune system without specificity to a toxin and allergens or irritants [10].

Mould infection is severe in people with existing lung illnesses. Mould exposure can also cause respiratory conditions in otherwise healthy people [11].

This includes symptoms such as upper respiratory tract problems, coughing and wheezing and shortness of breath. It is also linked to developing respiratory illnesses like asthma in certain people who are likely to be more susceptible [4]. This particularly concerns healthy children who may go on to develop asthma or other types of respiratory illnesses. For those who currently suffer from asthma, mold exposure could intensify symptoms and cause asthma attacks [12].

2. MATERIALS AND METHODS

2.1 Sample Collection

Different bakery products such as bread, cake, meat-pie, biscuit and doughnut were aseptically collected at random from three different locations in Port Harcourt Metropolis into sterilized bags and were transported into the Microbiology Laboratory, Rivers State University, Port Harcourt, Nigeria for Microbiological analysis.

2.2 Preparation of Culture Medium

Sabouraud Dextrose Agar (SDA) was used during the study. One gram of each sample was dissolved in 9 ml of normal saline. It was prepared according to the Manufacturer's specification. Antibiotic (chloramphenicol) 500 mg concentrations was added to the medium before pouring the plates. This was done to inhibit the growth of bacterial contaminants. The plates were allowed to dry, inoculated with the solution containing the bakery products, aseptically spread on the surface of the plates and were incubated at $27\pm 2^{\circ}\text{C}$ for four days [13].

2.3 Characterization and Identification of Fungal Isolates

The fungal isolates were characterized based on their colonial and cellular morphology. The shape, colour, spores and surface texture characteristic of the isolates were identified macroscopically. Lactophenol cotton blue (LPCB) was used for the microscopic examination of the fungal isolates. Few drops of the lactophenol cotton blue were aseptically placed on a clean grease-free microscope slide and a fragment of the fungus was added and carefully spread with the aid of a sterilized needle. It was properly covered with a cover slip avoiding air bubbles. It was observed microscopically using x40 and x100 objective lens, respectively [13].

3. RESULTS

The result of the Fungal Counts obtained during the study from the bakery products, meat pie, doughnut, biscuit, bread and cake are as follows: Table 1 shows the month of January having the following fungal counts; bread sample had

2.0×10^6 sfu/g, 1.0×10^6 sfu/g, 2.0×10^6 sfu/g, 1.0×10^6 sfu/g, 1.0×10^6 sfu/g and 3.0×10^6 sfu/g. The lowest count was 1.0×10^6 sfu/g and the highest count was 3.0×10^6 sfu/g with mean values of 1.07 ± 1.00^a and 2.5 ± 0.55^a respectively. Doughnut had counts of 1.0×10^6 sfu/g, 2.0×10^6 , 1.0×10^7 sfu/g, 1.0×10^7 sfu/g, 3.0×10^7 sfu/g and 2.0×10^7 sfu/g. The doughnut recorded the lowest count of 1.0×10^6 sfu/g and the highest count of 3.0×10^7 sfu/g with the mean values of 1.07 ± 1.00^a and 2.5 ± 0.55^a . Cake had fungal counts of 3.0×10^6 sfu/g, 2.0×10^6 sfu/g, 3.0×10^6 sfu/g, 2.0×10^6 sfu/g, 2.0×10^6 sfu/g and 3.0×10^6 sfu/g. The minimum count was 2.0×10^6 sfu/g and maximum count, 3.0×10^6 sfu/g with the mean values of 1.38 ± 1.45^a and 2.5 ± 0.55^a . Biscuit recorded the following counts, 1.0×10^6 sfu/g, 1.0×10^6 sfu/g, 1.0×10^6 sfu/g, 1.0×10^6 sfu/g and 1.0×10^7 sfu/g. the lowest count of biscuit was 1.0×10^6 sfu/g and the highest count was 1.0×10^6 sfu/g with the mean values of 1.0 ± 0.89^a and 1.38 ± 1.45^a . However, table 1 demonstrated that meat pie had the following fungal counts; 2.0×10^6 sfu/g, 3.0×10^6 sfu/g, 2.0×10^6 sfu/g, 3.0×10^6 sfu/g, 3.0×10^6 sfu/g and 2.0×10^6 sfu/g. The lowest count of meat pie was 2.0×10^6 sfu/g and 3.0×10^6 sfu/g as the highest count with the mean values of 2.00 ± 89^a and 2.67 ± 1.03^a . This result obtained was enumerated as described by Williams et al. [10]. Following the results of the fungal counts, biscuit had the lowest count compared to other bakery products, bread, cake doughnut, and meat pie.

Moisture promotes or enhances fungal growth. Since the biscuit is produced, packaged in low water density and a polyethylene bag and stored in ambient temperature (27°C - 28°C), fungal growth is minimized or reduced.

Table 1 shows the different fungi isolated and identified during the study. These organisms were identified macroscopically and microscopically.

On Plates 1 to 4 are the pure culture of some of the fungi: *Penicillium notatum*, *Mucor heimaalis*, *Aspergillus flavus* and *Rhizopus* identified macroscopically.

Fig. 1 shows the fungal counts in various locations (Abacha Road, Ikwerre Road and mile 3) of Port Harcourt Metropolis. Abacha Road had less counts compared to Ikwerre Road and Mile 3.

Table 1. Macroscopic and microscopic characteristics of the fungal isolates

Isolate	Colonial/ Structural morphology	Microscopic morphology	Probable organism
1	Whitish colony at the underlying. Later become earthy colored to dark.	Non-septate mycelia, with sporangiospore, ovoid fit as a fiddle with sub-globose columella.	<i>Rhizopus stolonifer</i>
2	Dark green with white cover at the edge and light profound yellow on the converse.	Conidiospores are for some time, expanded branches spread at the tip taking after a brush with conidia in long chains.	<i>Penicillium notatum</i>
3	Whitish green which turns dark green with time, has cottony surface	Hyphae is septate, unbranched conidiophores expanded at the tip. The phialids that produce conidia is available.	<i>Aspergillus fumigatus</i>
4	Profound pink or pink-red underside ethereal mycelium.	Hyphae are septate, conidia and blastoconidia fusiform, adjusted and tightened towards the base.	<i>Fusarium chlamydosporum</i>
5	Dull yellow-green and whitish spores at the middle, yellow on the converse, and granular to stuff.	Septate with transmit conidial head, hyalinated conidiophores coarsely harsh, more perceptible close to the vesicle conidia globose.	<i>Aspergillus flavus</i>
6	Enormous white provinces at starting which turns dark later.	Erect sporangiospores are shaped. Sporangiospores swells at the tip to frame sporangia which are globular shape. Columella is available.	<i>Mucor hiemalis</i>
7	Dark to dim earthy colored with yellow on the opposite.	Phialids orne straightforwardly on the globes vesicle sclerotia and erect conidiopores and are septate.	<i>Aspergillus niger</i>
8	Have smooth walled ascospores and dab have edges or flangs. The hyphal and opposite are yellow to orange. Develops between 28°C to 30°C.	The hyalinated conidiophores is coarsely harsh. It is septate.	<i>Eurotium</i>
9	Have level colony and fleece to wooly and was secured with dark grayish hypha.	Under the microscope, demonstrated septate hyphae and conidiospores with septate that is earthy colored in shading. Have huge conidia which have both cross over and longitudinal septations. The conidia produced germ tube. The finish of the conidium closest to the conidiospores is round while it tightens towards the pinnacle. This gives the average or club-like appearance.	<i>Alternaria alternata</i>
10	The opposite side on the agar plate is dull greenish to dark. The living being generally structure back spots on nourishments polluted. The living being develops best at low temperature.	The mycelia are dull which gives off an impression of being earthy colored to blackish earthy colored. The creature typically delivers one-celled conidia.	<i>Cladosporium sphaerospermum</i>



Plate 1. Pure culture of *Penicillium notatum*



Plate 2. Pure culture of *Mucor heimalis*



Plate 3. Pure culture of *Aspergillus flavus*



Plate 4. Pure culture of *Rhizopus stolonifera*

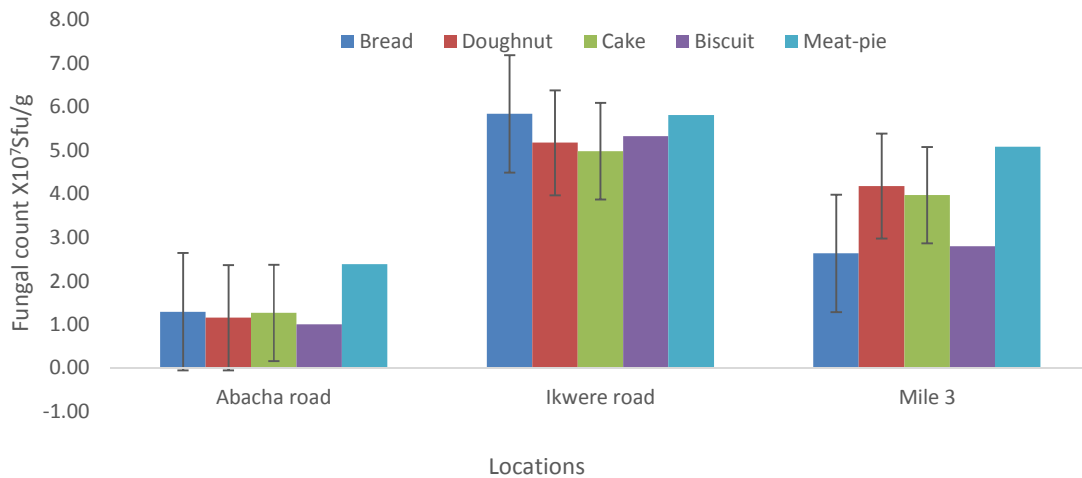


Fig. 1. Fungal counts in the food samples in the three locations in January, February and March

4. DISCUSSION

The study demonstrated that these bakery products were contaminated by fungi. Table 1 shows that the fungi associated with the bakery products were *Aspergillus niger*, *Aspergillus fumigatus*, *Mucor heimalis*, *Penicillium notatum*, *Aspergillus flavus*, *Rhizopus stolonifer* etc. This is in agreement with the findings of Nirmala et al. [14]. These fungi that contaminated the food samples are of public health importance because they have the capacity to cause infections like aspergillosis, penicilliosis, fusariosis, zygomycoses etc) [8]

Aspergillus niger, *Mucor heimalis* and *Aspergillus flavus* are wide spread in nature and these are commonly found in foods such as bakery products and other substrates since they can tolerate high salt and sugar concentrations. This agrees with the findings of Chozhavendhan, [15] who earlier reported that *Aspergillus niger*, *Aspergillus flavus* etc. produce mycotoxins which can cause food intoxication in human beings and animals.

The study shows that *Aspergillus* species were present in most of the cultured plates. These organisms, for example, *Aspergillus*, *Mucor*, *Penicillium* as well as other fungi can produce mycotoxins causing diverse illnesses in humans when consumed [3].

Fig. 1 shows the Fungal Counts in the different locations, Abacha Road, Ikwerre Road and Mile 3, Diobu, Port Harcourt. Abacha Road (1.0×10^6 sfu/g) was observed to have the lowest count followed by Mile 3 Diobu (3.9×10^8 sfu/g) while Ikwerre Road (5.2×10^8 sfu/g) had the highest count. This suggests that the bakery products collected from Ikwerre Road were the most contaminated products during the study. This could be as a result of the exposure of the products to the atmosphere which contained fungal spores. Fungi contaminated the bakery products and multiplied in number compared to other ones in the other locations. The study showed that *Aspergillus* species was the most relatively abundant species amongst others. Hence, bakery products should be well protected or preserved to discourage the contamination and multiplication of the *Aspergillus* species and other fungi.

The Analysis of Variance (ANOVA) at 5% confidence level demonstrated that there was no

significant difference of the growth of fungi across the same location.

Statistically, there was a significant difference comparing the growth of the organisms in Abacha Road, Ikwerre Road and Mile 3. It was observed that Abacha Road (1.07 ± 1.00^a) had the lowest count of fungi. This result agrees with the report of Pitt and Hocking, [14]. There was an increased count in Ikwerre Road (4.90 ± 0.86^b) as well as in Mile 3 (2.67 ± 1.03^a) compared to Abacha Road (1.07 ± 1.00^a). Therefore, Abacha Road < Ikwerre Road \geq Mile 3 based on the analysis of variance. This could be as a result of the environment of the various locations. It is possible that the location such as Abacha Road had less fungal spores in the atmosphere compared to the other locations [5].

Penicillium species are molds that are ubiquitous in nature and cause food deterioration. A large portion of the types of *Penicillium* sp create extremely high poisonous mycotoxins. A portion of the animal groups are blue in colour and generally develop on old bread and give a blue fluffy surface. A portion of the types of the *Penicillium* have been demonstrated to be pathogenic to animals and humans [3]. Penicilliosis is an infection brought about by *Penicillium* species. Most of these infections are discovered mostly in immunocompromised individuals or hosts. *Rhizopus* is a mold and a common saprophytic fungus on plants and specialized parasites on animals. *Rhizopus* species are found on numerous natural substances including natural foods grown from the ground, peanuts, cowhide and bread including other pastry items, for example, doughnut, cake, biscuit, meat pie and so on [7]. A portion of the *Rhizopus* species, for example, *Rhizopus stolonifer* (dark bread form) is an astute specialist of ailments. Zygomycosis (fungal infection) is the primary illness that may be brought about by this organism, *R. stolonifer*. This ailment is extremely dangerous and may cause death [16].

5. CONCLUSION AND RECOMMENDATIONS

The study demonstrated that some fungi can contaminate bakery products, despite the fact that the products are hygienically prepared and are properly stored in a good environment. Many fungi were isolated, enumerated and identified from the pastry items. This suggests that bakery products should not be stored for a long period of time before consumption because of the public

health importance. The pastry items should not be exposed since the atmosphere may contain fungal spores which may contaminate the products. The environment where these products are prepared and packaged should be properly cleaned and sanitized to reduce contamination to the barest minimum.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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