Isolation And Enumeration of Public Health Fungi Associated with Bakery Products in Warri Metropolis.

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Abstract- ***Bakery products such as meat-pie, cakes, bread etc. are staple foods consumed by people from all works of life. However, these products are susceptible to fungal contamination, which can lead to spoilage. This study aimed to isolate and enumerate the various fungi correlated with bakery products in Warri Metropolis, Nigeria. The research involved collection of bakery products from three different locations (Hospital Road - location A; Okoloba Road - location B; and Erukaye Road – location C) in Warri Metropolis, Delta State, Nigeria for enumeration, isolation and identification of various fungi that cause spoilage of the bakery products. Location A (with mean values of 1.80±0.55, 3.45±1.21, 3.42±1.27, 1.46±0.35 and 4.03±1.23 for Bread, Doughnut, Cake, Biscuit and Meat-pie respectively was observed to have the lowest count followed by Location C (with mean values of 2.93±0.66, 4.20±0.93, 4.07±0.78, 3.80±2.56, and 5.43±0.95 respectively), While Location B (with mean values of 3.17±0.53, 4.90±0.86, 4.43±1.09, 3.13±2.91 and 5.80±0.83) recorded the highest count. Different species of fungi were identified, belonging to the genera: Aspergillus, Fusarium, Penicillum, Mucor, Eurotium, Chladosporium, Alternaria, and Rhizopus. The genus Aspergillus was the most prevalent. Statistically, there was a significant difference comparing the growth of the organisms in location A, location B and Location C. It was observed that location A had the lowest counts of the fungi. There was an increased count in location B as well as location C compared to location A. Therefore, location A < location B ≥ location C based on the analysis of variance. The study highlights the importance of proper handling and storage of bakery products to prevent fungal contamination.***

Index Terms- ***Isolation, Enumeration, Bakery products, Warri Metropolis***

I. INTRODUCTION

Bread and other bakery products are significant food items consumed globally, serving as a vital source of nutrition for people across all socioeconomic backgrounds and geographical locations (Parra *et al.,* 2004). However, these products such as bread are susceptible to deterioration due to physical, chemical and microbial factors (Moore *et al.,* 2008).

Bread and other bakery products can be spoiled due to various microbial activities. Some of these microbes includes species of *Rhizopus, Mucor, Penicillum, Eurotium, Aspergillus and Monillia* (Saranraji *et al*., 2012). These organisms can produce toxins, leading to food poisoning. Consuming these contaminated bakery products can cause widespread gastrointestinal illness, resulting in stomach upset and other health related issues (Ribes *et al.,* 2017). To prevent this, it is essential to implement a standardized food safety system such as Hazards Analysis Critical Control Point (HACCP) (Hogue *et al.,* 2017). This method is now generally considered as a choice for ensuring safety of foods. Hazard Analysis Critical Control Point involves identifying potential hazard in the production process and implementing monitoring procedures to prevent contamination. Regular testing for microorganisms is also crucial to ensure food safety (Magan *et al.,* 2006).

Bread spoilage is usually caused by mould contamination after baking. Although the heat from baking kills mould spores, bread becomes vulnerable to contamination during cooling, slicing, packaging, and storage, when mould spores in the air settle on

the loaves (Marin *et al.,* 2003). Mould growth is the primary cause of microbial spoilage. Research shows that bread molds, particularly *Mucor* and *Rhizopus*, are usually the first to appear during the spoilage, followed by other fungi like *Aspergillus, Penicillum*, and *Fusarium* species. (Prescott *et al.,* 2008).

Mycotoxins, produced by fungi as secondary metabolites, can contaminate foods like bakery products, posing a significant public risk. These toxins have the potentials to cause diseases in human, animals, and livestock (Magan *et al.,* 2006). Environmental factors such as agricultural practices, geographical location, and commodity susceptibility, influenced mycotoxin production, leading to varying levels of contamination during storage and processing. (Aleruchi *et al*., 2019).

Examples of mycotoxins causing human and animal illness include aflatoxin, citrinin, flumonisins, ochratoxin A, patulin, trichothecenes, ergot alkaloids (Brenier-Pinchart *et. al*., 2006).

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 (Williams *et al.,* 2020).

Mould exposure can have severe consequences, particularly for individuals with pre-existing lung conditions (Hong *et al.,* 2012). Even healthy people can develop respiratory tract issues, such as upper respiratory tract problems, coughing, wheezing and shortness of breath, after mould exposure is linked to the development of respiratory illnesses like asthma, especially in susceptible individuals, including healthy children (Ribes *et al.,* 2017). For those already suffering from asthma, mould exposure can exacerbate symptoms and trigger asthma attacks (Loannis *et al.,* 2011).

II. MATERIALS AND METHODS

Sample Collection

Various bakery products including biscuit, doughnut, bread, cake, and meat-pie, were randomly collected aseptically from three different locations in Warri Metropolis. The samples were gathered into sterilized bags and transported into the Biology/Microbiology Laboratory of Science Laboratory Department, Federal Polytechnic Orogun, Delta State, Nigeria, for Microbiological analysis.

A. Preparation of Culture Medium.

Sabouraud Dextrose Agar (SDA) was utilized for the study. Ten grams of the samples was dissolved in 9 ml of normal saline. It was prepared according to the Manufacturer's instructions, with the addition of 500mg concentration of chloramphenicol (antibiotic) to the medium before pouring the plates to inhibit bacterial contaminants. The plates were allowed to dry and were inoculated with the solution containing the bakery products and were aseptically spread on the surface and were incubated at 27±2°C for 5 days (Douglas, 2018).

B. Characterization and Identification of Fungal Isolates

The fungal isolates were characterized by examining their colonial and cellular structure, including shape, colour, spores production and surface texture characteristic of the isolates were identified macroscopically. A few drops of Lactophenol cotton blue (LPCB) were placed on a clean grease-free microscope slide, and a small fragrant of the fungus was added gently and carefully spread using a sterilized needle. It was properly covered with a cover slip avoiding air bubbles. The slide was then examined under a microscope using x40 and x100 objectives respectively (Williams and White, 2016).

III. RESULTS

Plate 1: Bread Contaminated with fungi

Plate 2: Doughnut contaminated with fungi

Table 1: Frequency occurrence of identified isolated fungi from the bakery products in fungal count.

Moulds in bakery products	Frequency occurrence
Aspergillus sp	82
Rhizopus sp	24
Cladosporium sp	17
Mucor sp	28
Fusarium sp	22
Eurotium sp	17
Penicillium sp	12
Alternaria sp	7
Total	209

Table 2: Total Heterotrophic Fungal Count (cfu/g x10^7) in the Different Locations

Food sample	Bread	Doughnut	Cake	Biscuit	Meat-pie
Location A	1.80± 0.55^a	3.45± 1.21^a	3.42± 1.27^a	1.46± 0.35^a	4.03± 1.23^a
Location B	3.17± 0.53^b	4.90± 0.86^b	4.43± 1.09^a	3.13± 2.91^a	5.80± 0.83^b
Location C	2.93± 0.66^b	4.20± 0.93^c	4.07± 0.78^a	3.80± 2.56^a	5.43± 0.95^a

*Means with same superscript across the column shows no difference at ($p \geq 0.05$

Table 3: ANOVA Table for Total Heterotrophic fungal count (cfu/gx10^7) in the different zones

			Sum of Squares	Df	Mean Square	F	Sig.
Bread * Food sample	Between Groups	(Combined)	6.413	2	3.207	9.419	.002
	Within Groups		5.107	15	.340		
	Total		11.520	17			
Doughnut * Food sample	Between Groups	(Combined)	6.310	2	3.155	3.078	.076
	Within Groups		15.375	15	1.025		
	Total		21.685	17			
Cake * Food sample	Between Groups	(Combined)	3.181	2	1.591	1.401	.277
	Within Groups		17.035	15	1.136		
	Total		20.216	17			
Biscuit * Food sample	Between Groups	(Combined)	17.333	2	8.667	1.714	.214
	Within Groups		75.827	15	5.055		
	Total		93.160	17			
Meat-pie * Food sample	Between Groups	(Combined)	10.431	2	5.216	5.000	.022
	Within Groups		15.647	15	1.043		
	Total		26.078	17			

IV. DISCUSSION

The study revealed fungal contamination in the analyzed bakery products. Table 1 shows and identifies the associated fungi: including *Aspergillus sp, Rhizopus sp, Cladosporium sp, Mucor sp, Fusarium sp, Eurotium sp, Penicillium sp and Alternaria sp*. This is consistent with the findings by Nirmala *et al*., (2016). These fungi pose health concerns, as they can cause infections such as aspergillosis, peniciliosis, fusariosis, zygomycoses etc (Hong *et al.,* 2012).

The study revealed a prevalent presence of *Aspergillus* species in most cultured plates. Notably, *Aspergillus*, *Mucor, Penicillum* and other fungi can produce mycotoxins, which can cause a range of illnesses in humans upon consumption (Saranraj, *et al.,* 2012).

The study revealed the Fungal Counts of the various locations within Warri Metropolis, including Hospital Road - location A, Okoloba Road - location B and Erukaye Road – location C. The Hospital Road – Location A (with mean values of 1.80±0.55, 3.45±1.21, 3.42±1.27, 1.46±0.35 and 4.03±1.23 for Bread, Doughnut, Cake, Biscuit and Meat-pie respectively) was observed to have the lowest count, followed by Erukaye Road – Location C (with mean values of 2.93±0.66, 4.20±0.93, 4.07±0.78, 3.80±2.56, and 5.43±0.95 respectively), While Okoloba Road – Location B (with mean values of 3.17±0.53, 4.90±0.86, 4.43±1.09, 3.13±2.91 and 5.80±0.83) recorded the highest count. The findings indicate that the bakery products collected from Okoloba Road were the most contaminated during the study. This could be attributed to the exposure of these products to atmospheric fungal spores, allowing fungi to colonized and proliferate on the products to a greater extent than those from other locations. The study revealed that *Aspergillus* species was the most dominant fungus present. To prevent contamination and proliferation of *Aspergillus* and other fungi, it is essential to properly protect and preserve bakery products.

The Analysis of Variance (ANOVA) at 5% confidence level demonstrated that significant differences in fungal growth was observed among samples compared across the different locations.

Statistical analysis revealed significant differences in fungal growth among samples from Hospital Road (Location A), Okoloba Road (Location B) and Erukaye Road (location C). Hospital Road (Location A) had the lowest fungal count. In contrast, Okoloba Road (location B) and Erukaye Road (Location C) showed increased fungal counts. The analysis of variance ranked the locations as follows: Hospital Road < Okoloba Road ≥ Erukaye Road. Environmental factors, such as varying atmospheric fungal spore concentrations, may have contributed to these differences (Magan *et al*., 2006).

CONCLUSION AND RECOMMENDATIONS

A. Conclusion

The study highlights that bakery products can be contaminated with fungi, even when prepared hygienically and stored properly. The isolation, enumeration, and identification of various pastry items underscore the importance of consuming bakery products promptly, rather than storing them for extended periods, to mitigate potential public health risks. It is therefore recommended that, in order to minimize contamination, pastry items should be protected from atmospheric exposure, as fungal spores present in the air can easily contaminate them. Additionally, the preparation and packaging environment should be rigorously cleaned and sanitized to reduce the risk of contamination to the lowest possible level.

B. Recommendations

Based on the research findings, the following recommendations are made:

i. To reduce fungal contamination, bakeries should follow stringent hygiene procedures when preparing, baking, chilling, and packaging their goods.
ii. Since airborne fungal spores are a major source of contamination, it is best to utilize packaging materials that are both airtight and moisture-resistant to avoid exposure to the atmosphere.

iii. To prevent fungal growth, bakery items should be kept in a controlled environment with regulated humidity and temperature. Particularly for perishable goods like meat pies and cakes, refrigeration is recommended.
iv. To lower the danger of spoiling and potential health risks, consumers should be urged to eat bakery items within the suggested shelf life and refrain from storing them for an extended period of time.
v. To reduce the chance of cross-contamination, the preparation and sales area, including surfaces and equipment, should be cleansed and disinfected on a regular basis.
vi. Health organizations and pertinent parties should educate bakers, suppliers, and customers about the dangers of fungal contamination and the importance of following food safety regulations.
vii. Government organizations like NAFDAC and public health authorities ought to keep an eye on and enforce bakers' and vendors' adherence to food safety laws.
viii. In other regions of Delta State and throughout Nigeria, more thorough research on the fungal contamination of bakery goods should be conducted. Molecular techniques should be used to identify the fungal species more precisely.

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