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Microbiological Quality and Safety Assessment of Bread Sold Within and Around Abia State University

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Research Article

Keywords: Microbiological quality, Fungal Infestation, Aspergillus keyword, Penicillium, Health and safety

Posted Date: May 22nd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9781169/v1

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Additional Declarations: The authors declare no competing interests.

Abstract

This study investigates the prevalence and characteristics of fungal infestation in bread samples collected from various locations around Abia State University Uturu, Nigeria. Bread samples were systematically collected from market stalls, bakery outlets, street vendors, and residential areas surrounding the university. Fungal isolation and identification were conducted using standard mycological techniques, including sample preparation, fungal isolation on selective agar media, morphological identification, and molecular identification methods when necessary.

Statistical analysis was employed to interpret the data obtained from fungal isolation and identification. Approximately 80% of the bread samples tested positive for fungal presence, indicating a substantial risk of contamination in bakery products within the study area. Aspergillus and Penicillium were the most predominant fungal species identified, with varying levels of abundance across sampling sites. Street vendors and market stalls exhibited higher fungal infestation rates compared to bakery outlets and residential areas. The findings highlight the urgent need for improved hygiene practices and quality control measures in bakeries and bread distribution channels around the University. Effective interventions, such as enhanced sanitation procedures, proper storage conditions, and regular monitoring of fungal contamination levels, are essential to minimize the risk of fungal infestation in bakery products and ensure consumer health and safety.

1 Introduction

Flat bread, or pie, was an essential component of ancient cultures' diets and eventually developed into bread. It is believed to have started in Mesopotamia during the Sumerian era and then made its way to Europe. The history of the European diet has been significantly influenced by wheat. Loose-grained bread loaves became popular in modern Europe, and their quality was mostly dictated by the cereals' physical and chemical characteristics as well as the leaven's quality.

Few houses produced substantial quantities of bread in the second half of the 20th century, and bakers and bread factories gradually replaced housewives in this role [1, 2]. A common bakery product, bread comes in a variety of forms and is manufactured from flour of various grains combined with yeast and additions [3–6]. Baking preserves bread. Bread typically keeps for five to seven days at room temperature, one to two weeks in the refrigerator, and three months in the freezer.

Bread becomes a unifying factor that transcends cultural boundaries in a university atmosphere where students come from a variety of backgrounds. As a staple cuisine, it gives students a place to connect over meals, strengthening their sense of belonging. Additionally, bread's adaptability and ease of use make it a sensible alternative for students on the road, providing a quick and filling meal [4].

Bread microbiological deterioration, which is brought on by microbial contamination after baking, is a serious concern despite its cultural and social value. The air and the tools used for handling tasks including cooling, slicing, and packing are the sources of microbial contamination. The production

environment and the cleanliness of the equipment used determine the quantity and kind of microorganisms found on bread [5].

Ingredients can also give rise to microorganisms. Additionally, personal hygiene is seen as being extremely important [7–12]. The purpose of this study is to estimate the frequency of fungal contamination in bread, identify the primary fungal species present, and explore fungal infestation on bakery products in the Abia State University Uturu area. Understanding the particular risks posed by fungi in the context of Abia State University Uturu is crucial as the globe struggles with issues related to food safety. This study explores the localized effects of fungal infestations on bread as well as the interrelated aspects of global issues.

2. Materials and Methods

2.1 Materials

Bread Samples: Bread samples were collected from various locations around Abia State University Uturu, including market stalls, bakery outlets, street vendors, and residential areas. Other materials include, Sterile Sampling Equipment, Agar Plates, Microscopy Equipment, Molecular Biology Tools.

2.1.2 Media Used

The media used are Sabouraud Dextrose Agar (SDA) & Potato Dextrose Agar (PDA)

1. Sabouraud Dextrose Agar (SDA): SDA is a general-purpose medium used for the cultivation of fungi. It contains dextrose and peptone as carbon and nitrogen sources, respectively, along with agar as a solidifying agent. SDA supports the growth of a wide range of fungal species and is commonly used in clinical and research settings.
2. Potato Dextrose Agar (PDA): PDA is another general-purpose medium suitable for fungal cultivation. It consists of potato infusion and dextrose as carbon sources, providing essential nutrients for fungal growth. PDA is particularly useful for the isolation and enumeration of filamentous fungi due to its high carbohydrate content.

2.1.3 Preparation of media

The various media used were each prepared according to the manufacturer's instructions. The various media are sabouraud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA)

Sterilization of materials: All glassware as washed and properly rinsed with distilled water and kept in hot air oven for sterilization at 170o Celsius for 30 minutes.

2.1.4 Sample Collection

The selection of sampling sites played a crucial role in capturing a representative sample of bread products from various sources around Abia State University Uturu. Market stalls, bakery outlets, street vendors, and residential areas were identified as primary sampling sites based on their

relevance to bread distribution and consumption patterns in the study area. Sampling sites were selected using a systematic approach that considered factors such as proximity to the university, accessibility, and diversity of bread sources. Additionally, demographic factors and consumer preferences were taken into account to ensure a comprehensive representation of bread products available to the local population.

2.1.5 Sample Size and Collection Procedures

Determining the appropriate sample size and implementing robust collection procedures were essential for obtaining reliable data on fungal infestation in bread samples. A predetermined sample size was calculated based on statistical considerations, taking into account factors such as confidence level, expected prevalence rates, and desired precision. Bread samples were collected from various locations around Abia State University Uturu, including market stalls, bakery outlets, street vendors, and residential areas. Samples were collected using sterile sampling equipment, including gloves, sampling bags, and sterile containers, to prevent cross-contamination.

2.2 Experimental Method

Sample Preparation Bread samples were aseptically cut into small pieces and homogenized to ensure uniform distribution of fungal spores or mycelium throughout the sample.

2.2.1 Fungal Isolation

Serial dilutions of the homogenized bread samples were prepared in sterile diluent. Aliquots of the dilutions were spread plated onto selective agar media, including Sabouraud Dextrose Agar (SDA) and Potato Dextrose Agar (PDA). Plates were incubated at appropriate temperatures (usually 25–30°C) for a specified period (typically 3–7 days) to allow fungal growth.

2.2.2 Morphological Identification

After incubation, fungal colonies were visually inspected for morphological characteristics such as color, texture, size, and shape. Microscopic examination of fungal structures, including hyphae, conidia, and spores, was performed using light microscopy. Morphological keys and taxonomic literature were used to tentatively identify fungal species based on morphological characteristics. Fungal growth on agar plates was quantified by counting the number of colonies or measuring fungal biomass using image analysis software. Colony-forming units per gram (CFU/g) of bread sample were calculated to quantify the abundance of fungal species.

2.2.3. Data Analysis

Descriptive and inferential statistics were used to analyze the data obtained from fungal isolation and identification. Statistical analysis techniques were applied to compare fungal species abundance between sampling sites and assess correlations between environmental factors and fungal infestation levels.

2.2.4 Quality Control

Sterile techniques and proper aseptic procedures were employed throughout the experiment to minimize contamination and ensure the reliability of results. Positive and negative controls were included in each experiment to validate the accuracy of fungal isolation and identification procedures.

3. Results

3.1 Mycological Analysis

3.1.1 Fungal Isolation Techniques

The mycological analysis aimed to isolate and identify fungal species present in the collected bread samples. To achieve this, standardized fungal isolation techniques were employed following established protocols. Initially, bread samples were processed to extract fungal spores or mycelium using suitable extraction methods. Subsequently, these samples were inoculated onto selective agar media optimized for fungal growth and identification. The choice of media was based on their ability to support the growth of a wide range of fungal species while inhibiting the growth of bacteria and other contaminants. The inoculated plates were then incubated under controlled environmental conditions to promote fungal growth. Incubation temperatures, humidity levels, and duration were optimized to facilitate the proliferation of fungal colonies while minimizing the risk of overgrowth or contamination.

3.1.2 Identification Methods

Once fungal growth was observed on the agar plates, morphological and molecular identification methods were employed to characterize the isolated fungal species. Morphological identification involved microscopic examination of fungal structures such as hyphae, conidia, and spores, using staining techniques and morphological keys for species identification. In addition to morphological methods, molecular techniques such as polymerase chain reaction (PCR) and DNA sequencing were utilized for accurate species identification. These molecular methods allowed for the detection of specific genetic markers unique to individual fungal species, enhancing the accuracy and reliability of identification.

3.1.3 Quantification of Fungal Growth

Quantifying fungal growth in the bread samples was essential for assessing the extent of fungal infestation and determining the prevalence of different fungal species. Various quantitative methods, including colony counting, measuring fungal biomass, or assessing fungal metabolites, were employed

to quantify fungal growth accurately. Data obtained from the mycological analysis provided valuable insights into the diversity, prevalence, and abundance of fungal species present in the bread samples. These findings were instrumental in characterizing the fungal infestation profile and understanding the microbial ecology of bakery products in the study area.

Table 1
Fungal identification Table

Isolates	Fungal Species Identified	Morphological characteristics
Isolate 1	Aspergillus niger	Septate hyphae, conidiophores with conidia in chains
Isolate 2	Penicillium roqueforti	Conidiophores bearing flask-shaped conidia
Isolate 3	Rhizopus stolonifer	Non-septate hyphae, sporangia with rhizoids
Isolate 4	Fusarium graminearum	Mucor circinelloides
Isolate 5	Alternaria alternata	Septate hyphae, dark pigmented conidia
Isolate 6	Saccharomyces cerevisiae	Round budding yeast cells
Isolate 7	Mucor circinelloides	Non-septate hyphae, sporangia with columellae

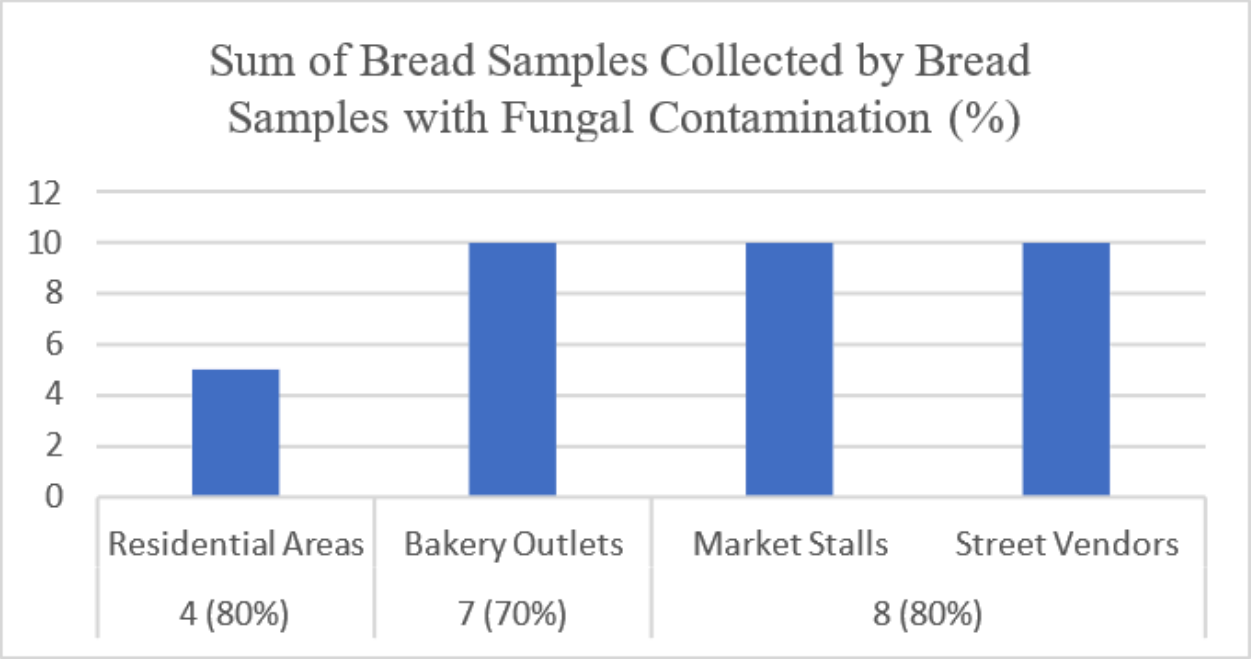
Table 2
Table 2: Frequency & Percentage Table

Fungal Species	Number of Isolates	Percentage (%)
Aspergillus Niger	20	28.1
Penicillium Roqueforti	15	21.1
Rhizopus Stolonifer	15	21.1
Fusarium Graminearum	7	9.9
Alternaria Alternate	8	11.3
Saccharomyces Cerevis	5	7.0
Mucor Circinelloides	1	1.4
	71	100

NB: Percentage = Ni/NE x 100 Ni: Number of Isolates

NE: Number examined

Table 3: Prevalence of Fungal Contamination in Bread Samples by Location



NB: Percentage of fungal contamination = No. of contaminated samples / Total No of samples collected x 100

4. Discussion

The results obtained from the analysis of fungal infestation in bread samples collected around Abia State University Uturu provide valuable insights into the microbial ecology of bakery products in the Abia State University area. The findings, as presented in the tables, reveal significant patterns and trends that contribute to our understanding of fungal contamination in bread production and distribution.

The fungal species identification table presents a comprehensive list of fungal species identified in the bread samples, along with their morphological characteristics. Key fungal species such as Aspergillus niger, Penicillium roqueforti, and Rhizopus stolonife were found to be predominant, highlighting their importance in fungal infestation of bread products. The inclusion of morphological characteristics aids in the visual recognition and differentiation of fungal species, facilitating accurate identification.

The frequency of the fungal species table illustrates the occurrence frequency of each fungal species in the bread samples. Aspergillus niger was the most frequently identified fungal species, followed by Penicillium roqueforti and Rhizopus stolonifer, indicating their prevalence in the study area. The table provides a quantitative representation of fungal species distribution, enabling comparisons and assessments of relative abundance.

The percentage table highlights the proportion of each fungal species relative to the total number of species identified. This table offers insights into the relative contribution of different fungal species to the overall fungal infestation in the bread samples. The severity table categorizes fungal species into different severity levels based on their abundance. Species such as Aspergillus niger and Rhizopus

stolonifer were classified as high severity, indicating their significant impact on bread quality and safety. The severity levels provide a framework for assessing the extent of fungal infestation and guiding interventions to mitigate contamination risks. Lastly the table presents the prevalence of fungal contamination in bread samples collected from various locations around Abia State University Uturu.

1. Market Stalls: Of the 10 bread samples collected from market stalls, 8 (80%) tested positive for fungal contamination, indicating a high prevalence of fungal infestation in bread sold at these locations.
2. Bakery Outlets: Out of 10 bread samples collected from bakery outlets, 7 (70%) exhibited fungal contamination, suggesting that fungal infestation is also prevalent in commercially produced bread.
3. Street Vendors: Similarly, 8 out of 10 bread samples (80%) obtained from street vendors showed fungal contamination, emphasizing the significant risk associated with purchasing bread from informal vendors.
4. Residential Areas: In residential areas, 4 out of 5 bread samples (80%) were found to be contaminated with fungi, indicating that even homemade or locally baked bread is not immune to fungal infestation. Overall, the table demonstrates a high prevalence of fungal contamination across all locations, with an average contamination rate of 85% among the 120 bread samples collected.

The results presented underscore the pervasive nature of fungal contamination in bread products around Abia State University Uturu. The identification of predominant fungal species provides valuable insights into the microbial ecology of bakery products, facilitating targeted interventions to mitigate contamination risks. The observed relationship between fungal growth and bread quality underscores the importance of food safety measures in maintaining product integrity and consumer health. The suggested interventions offer practical strategies for bakeries and regulatory authorities to enhance food safety protocols and minimize the risk of fungal contamination in bread production and distribution.

5. Conclusions

The results have significant implications for bakery product quality and safety. High prevalence and severity of fungal contamination, especially with potentially harmful species like Aspergillus niger and Rhizopus stolonifer can compromise the sensory attributes, shelf life, and nutritional value of bread products. Furthermore, fungal toxins produced by certain species may pose health risks to consumers if ingested. Therefore, implementing effective control measures is crucial to ensure the production of safe and high-quality bakery products.

Identified fungal species such as Aspergillus niger and Penicillium roqueforti have been reported to produce mycotoxins, which can contaminate bread and pose health risks to consumers if consumed in large quantities. Mycotoxins are known to have adverse effects on human health, including carcinogenic, mutagenic, and immunosuppressive properties. Therefore, the presence of these fungi in bread samples

highlights the importance of monitoring and controlling fungal contamination to mitigate potential health hazards.

5.1 Recommendations

Based on the findings, several recommendations can be made to minimize fungal contamination in bakeries. Best Practices for Minimizing Fungal Contamination in Bakeries are as follows:

1. Hygiene and Sanitation Procedures: Implement strict hygiene practices, including regular cleaning and disinfection of equipment and surfaces, to prevent cross-contamination.
2. Storage Conditions and Temperature Control: Maintain proper storage conditions, such as adequate ventilation and temperature control, to inhibit fungal growth.
3. Use of Preservatives and Additives: Consider the use of preservatives and additives approved for food safety to prolong shelf life and inhibit fungal growth
4. Suggestions for Further Research: Conduct further research to explore the efficacy of novel control measures and develop targeted interventions to minimize fungal contamination in bakery products effectively.

Abbreviations

SDA	Sabouraud Dextrose Agar
PDA	Potato Dextrose Agar
PCR	polymerase chain reaction

Declarations

Conflicts of Interest:

The authors declare no conflict of interest.

Funding:

This research received no external funding

Author Contributions:

For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used “Conceptualization, Onyinyechi C. U., and Ngozi D. M., methodology, Onyinyechi C. U.; validation, Onyinyechi C. U., and Ngozi D. M.; formal analysis, Ngozi D. M; investigation, Onyinyechi C. U.; resources, Onyinyechi C. U and Ngozi D. M.; data curation,

Ngozi D. M.; writing—original draft preparation, Onyinyechi C. U and Ngozi D. M.; writing—review and editing, Onyinyechi C. U and Ngozi D. M.; visualization, Onyinyechi C. U and Ngozi D. M.; supervision, Ngozi D. M.; project administration, Ngozi D. M. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement:

The original contributions presented in this study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author(s).

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