International Journal ofAdvances in Science, Vocational & Technical Education (IJASVOTE)
www.ijasvote-fce.com
Volume 3; Issue 2; August 2026; Page No. 179-189.

ISOLATION AND IDENTIFICATION OF Aspergillus spp IN BREAD BAKED BY FACTORY IN HONG LOCAL GOVERNMENT AREA OF ADAMAWA STATE, NIGERIA

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Abstract

The aim of the experiment was to isolate and identify Aspergillus spp associated with bread spoilage in bread baked by factory in Hong Local Government Area ofAdamawa State, Nigeria. The specific objectives ofthe study are to isolate fungi present in spoiled bread samples, identify the fungal isolates based on their cultural and microscopic characteristics and determine the most predominant fungal species associated with bread spoilage. The study employed an experimental laboratory-based design for the isolation and identification fungifrom spoiled bread samples collected from different locations. Standard microbiological techniques were used to culture, isolate, and identify fungal organisms associated with bread spoilage and the data obtained were analyzed descriptively. The results revealed that the fungal isolates obtained from the bread samples were Aspergillus niger (32%), Aspergillus flavus (20%), Rhizopus stolonifer (24%), Penicillium (16%) and Mucor species (8%). Also, the microscopic examination using lactophenol cotton blue staining revealed different fungal structures namely septate hyphae with black conidia attached to conidiophores, septate hyphae with rough conidiophores and spherical vesicles, non-septate hyphae with rhizoids and sporangia, brush- like conidiophores with chains of conidia and broad non-septate hyphae with round sporangia for the identified species respectively. The high occurrence ofAspergillus niger had attributed to their ability to survive under varying environmental conditions and produce airborne spores that easily contaminate bread during storage and handling. The study recommends that bakery workers should maintain proper personal and environmental hygiene during bread production, cooling, slicing, packaging, and storage, bread should be stored in cool and dry conditions to reduce fungal growth and prolong shelf life and also used proper packaging materials, bakery equipment and working surfaces should be regularly cleaned and disinfected to prevent contamination by fungal spores and regulatory agencies such as the National Agency for Food and Drug Administration and control should carry out regular inspection of bakeries and bread-selling outlets to ensure compliance with food safety standards and public awareness programs should be organized to educate bread producers, vendors, and consumers on the dangers associated with fungal contamination ofbread.

Keywords: Isolation, Identification, *Aspergillus spp*, Bread, Baked and Hong

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1.1 INTRODUCTION

Bread is one of the most widely consumed food products in the world because of its affordability, convenience, and nutritional value. It serves as a major source of carbohydrates, vitamins, proteins, and minerals for many households. However, bread is highly susceptible to microbial spoilage due to its moisture content and exposure to environmental contaminants during processing, packaging, storage, and distribution. Among the microorganisms associated with bread spoilage, fungi are the most predominant organisms responsible for deterioration (Garcia et al., 2019). Fungi are organisms that obtain nutrients from dead and decaying organic matter. These fungi commonly colonize bread because bread provides a suitable environment rich in carbohydrates and moisture. Common fungal genera associated with bread spoilage include Aspergillus, Rhizopus, Penicillium, Mucor, and Fusarium (Unachukwu & Nwakanma, 2015). The presence of these fungi on bread not only reduces shelf life and quality but may also pose serious health risks due to the production of mycotoxins and allergens (Lister, 2022).

Fungal contamination of bread may occur from contaminated flour, utensils, air, handlers, and poor storage conditions. According to Garcia et al. (2019), fungal spores are frequently present in bakery environments and may contaminate bread after baking during cooling and packaging stages. Similarly, studies conducted in Nigeria have reported the isolation of Aspergillus spp., Rhizopus spp., Mucor spp., and Penicillium spp. from spoiled bread samples sold in local markets (Nuhu & Yusuf, 2023; Salim & Ummulkhair, 2024). The isolation and identification of saprophytic fungal pathogens in bread are important in food microbiology because they help in understanding the sources of contamination and developing effective preservation methods. Identification is commonly carried out using cultural, morphological, and microscopic characteristics on suitable media such as Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA). These techniques enable researchers to differentiate fungal species based on colony appearance, spore structure, and hyphal arrangement.

The increasing incidence of fungal spoilage in bread products emphasizes the need for proper hygienic practices, effective packaging, and adequate storage conditions. Therefore, this study focuses on the isolation and identification of saprophytic fungal pathogens associated with bread spoilage. Bread spoilage caused by fungi is a major problem affecting both consumers and bread producers. Aspergillus spp invade bread during storage and distribution, leading to visible mold growth, unpleasant odor, discoloration, and loss of nutritional quality. In many cases, spoiled bread is still unknowingly consumed, exposing consumers to health hazards such as allergies, respiratory infections, and mycotoxin poisoning. The aim of this study is to isolate and identify Aspergillus spp associated with bread spoilage in bread baked by factory in Hong Local Government Area of Adamawa State, Nigeria

The specific objectives ofthe study are to:

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Isolate fungi present in spoiled bread samples.

Identify the fungal isolates based on their cultural and microscopic characteristics.

Determine the most predominant fungal species associated with bread spoilage.

MATERIALS AND METHODS

2.1 Introduction

This chapter described the materials and methods used in the isolation and identification of saprophytic fungal pathogens associated with bread spoilage. It includes the research design, study area, sample collection, preparation of culture media, isolation of fungi, identification of fungal isolates, and methods of data analysis.

2.2 Research Design

The study employed an experimental laboratory-based design for the isolation and identification of saprophytic fungi from spoiled bread samples collected from different locations. Standard microbiological techniques were used to culture, isolate, and identify fungal organisms associated with bread spoilage.

2.3 Study Area

The study was carried out in the Department of Biology Education Laboratory in Adamawa State College of Education, Hong. Bread samples were obtained from selected bakeries, markets, shops, and street vendors within the study area. The area experiences tropical climatic conditions characterized by high temperature and humidity, which favor fungal growth and spoilage of food products.

2.4 Materials Used

The following materials and equipment were used during the study:

2.4.1 Apparatus

Autoclave

Incubator

Microscope

Weighing balance

Beakers

Test tubes

Conical flasks

Petri dishes

Inoculating loop

Forceps

Glass slides and cover slips

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Measuring cylinder

Bunsen burner

Pipettes

2.4.2 Reagents and Culture Media

Potato Dextrose Agar (PDA)

Sabouraud Dextrose Agar (SDA)

Distilled water

Ethanol

Lactophenol cotton blue stain

Normal saline

Chloramphenicol (to inhibit bacterial growth)

2.5 Sample Collection

Spoiled bread samples showing visible signs of fungal growth were collected from bakeries, markets, shops, and street vendors within the study area using sterile polythene bags. The samples were properly labeled according to location and transported immediately to the laboratory for analysis.

2.6 Preparation of Culture Media

Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA) were prepared according to the manufacturer's instructions. The media were weighed using a weighing balance and dissolved in distilled water. The mixture was heated to dissolve completely and sterilized in an autoclave at 121°C for 15 minutes. After sterilization, chloramphenicol was added to suppress bacterial contamination. The media were then poured aseptically into sterile Petri dishes and allowed to solidify.

2.7 Isolation of Fungi from Bread Samples

The direct plating method described by Samson et al. (2010) was used for fungal isolation. Small portions of spoiled bread were cut using sterile forceps and inoculated directly onto the prepared PDA and SDA plates. The inoculated plates were incubated at room temperature (25 –28°C) for 3–7 days. Fungal growth observed on the culture plates was subcultured repeatedly onto fresh media to obtain pure fungal isolates. Distinct colonies were identified based on differences in colony morphology such as color, texture, shape, and growth pattern.

2.8 Identification of Fungal Isolates

Identification of fungal isolates was carried out using both macroscopic and microscopic examination methods.

2.8.1 Macroscopic Examination

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The cultural characteristics of fungal colonies were observed and recorded. Features examined included:

Colony color

Texture

Shape

Surface appearance

Growth rate

Pigmentation

These characteristics were compared with standard fungal identification manuals.

2.8.2 Microscopic Examination

A small portion of each fungal colony was placed on a clean glass slide and stained using lactophenol cotton blue stain. The stained preparation was covered with a cover slip and examined under the microscope using low and high power objectives. Microscopic features observed included:

Hyphal structure

Presence of septa

Conidia

Sporangia

Conidiophores

Spore arrangement

Identification of fungal species was based on standard identification keys provided by Samson et al. (2010) and Pitt and Hocking (2009).

2.9 Characterization of Common Fungal Isolates

The fungal isolates expected during the study include:

2.9.1 Aspergillus Species

These fungi produce powdery colonies that may appear black, green, or yellow. Microscopically, they possess septate hyphae and conidiophores ending in vesicles with chains of conidia (Bennett & Klich, 2003).

2.9.2 Rhizopus Species

Rhizopus species appear as fluffy cotton-like colonies with black sporangia. They possess non-septate hyphae with rhizoids and sporangiospores (Alexopoulos et al., 1996).

2.9.3 Penicillium Species

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These fungi produce greenish velvety colonies. Microscopically, they possess branched conidiophores with chains of conidia resembling a brush-like structure (Pitt & Hocking, 2009).

2.9.4 Mucor Species

Mucor species produce white fluffy colonies with broad non-septate hyphae and spherical sporangia (Samson et al., 2010).

2.10 Sterilization Procedures

All glassware and media used in the study were sterilized before use to prevent contamination. Glassware was sterilized in a hot air oven at 160°C for one hour, while culture media were sterilized in an autoclave at 121°C for 15 minutes (Cheesbrough, 2006). Work surfaces were disinfected with ethanol before and after laboratory procedures.

2.11 Data Analysis

Data obtained from the study were analyzed descriptively. The frequency of occurrence of fungal isolates was determined and expressed in percentages using the formula:

$$\text{Percentage occurrence} = \frac{Total\ number\ of\ isolates.}{\text{Number of occurrence of isolate}} \times 100$$

The identified fungal isolates were presented in tables and charts where necessary.

2.12 Ethical Consideration

Proper laboratory safety procedures were observed during the study. Bread samples were handled carefully to avoid contamination and exposure to harmful fungal spores. Laboratory waste materials were properly sterilized and disposed of according to standard microbiological safety guidelines.

RESULTS AND DISCUSSION

3.1 Introduction

This chapter presents the results obtained from the isolation and identification of Aspergillus spp associated with spoiled bread samples collected from selected bakeries, markets, and shops within the study area. The results are presented in tables and discussed in relation to previous studies carried out by other researchers.

3.2 Results

3.2.1 Isolation of Fungi from Bread Samples

A total of twenty (20) spoiled bread samples were analyzed for the presence of Aspergillus spp. Their growth were observed in all the samples cultured on Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA) after incubation. Table 1 shows the distribution and frequency of fungal species isolated from bread samples. A total of 25 fungal isolates were recovered, representing five different fungal groups. Among the isolates, Aspergillus niger

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was the most frequently occurring fungus, with 8 occurrences, accounting for 32% of the total isolates. This indicates that A. niger was the predominant fungal contaminant in the bread samples and may play a significant role in bread spoilage. Rhizopus stolonifer was the second most common isolate, occurring 6 times and representing 24% of the total isolates. Its relatively high occurrence suggests that it is also an important spoilage organism associated with bread. Aspergillus flavus had 5 occurrences, making up 20% of the isolates. The presence of A. flavus is noteworthy because some strains are capable of producing aflatoxins, which can pose health risks to consumers. Penicillium species were isolated 4 times, accounting for 16% of the total fungal population. Although less prevalent than the Aspergillus and Rhizopus species, their presence indicates additional sources of fungal contamination and spoilage. The least frequently occurring fungi were Mucor species, with only 2 occurrences, representing 8% of the total isolates. This suggests that Mucor spp. contributed the least to fungal contamination among the bread samples examined. Overall, the results indicate that species of Aspergillus, particularly Aspergillus niger, were the dominant fungi associated with the bread samples, followed by Rhizopus stolonifer. The high prevalence of these fungi suggests that they are the major contributors to bread spoilage under the conditions studied.

Table 1: Frequency of Occurrence of Fungal Isolates from Bread Samples

Fungal Isolate	Number of Occurrence	Percentage Occurrence (%)
Aspergillus niger	08	32
Aspergillus flavus	05	20
Rhizopus stolonifer	06	24
Penicillium spp.	04	16
Mucor spp.	02	08
Total	25	100

Source: Experimental result, 2026

3.2.2 Macroscopic Characteristics of the Fungal Isolates

The fungal isolates showed different cultural appearances on the culture media. Table 2 presents the macroscopic characteristics of the fungal isolates obtained from bread samples, including colony color, texture, and growth appearance. These features are important in the preliminary identification and differentiation of fungal species. Aspergillus niger produced black colonies with a powdery texture and exhibited rapid growth. These characteristics are typical of A. niger and reflect its ability to proliferate quickly on bread substrates. Aspergillus flavus formed yellow-green colonies with a granular texture and a flat colony appearance. These distinctive morphological features aided its identification and differentiated it from other fungal isolates. Rhizopus stolonifer initially produced white colonies that later turned black as spores developed. The colonies had a cotton-like texture and a spreading growth

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pattern, indicating its vigorous growth and ability to rapidly colonize bread surfaces. Penicillium species were characterized by blue-green colonies, a velvety texture, and circular colony formation. These features are consistent with the typical morphology of Penicillium species commonly associated with food spoilage. Mucor species displayed colonies ranging from white to gray in color, with a fluffy texture and fast-growing colonies. The fluffy appearance is characteristic of the abundant aerial mycelia produced by members of this genus.

Table 2: Macroscopic Characteristics of Fungal Isolates

Fungal Isolate	Colony Color	Texture	Growth Appearance
Aspergillus niger	Black	Powdery	Rapid growth
Aspergillus flavus	Yellow-green	Granular	Flat colonies
Rhizopus stolonifer	White turning black	Cotton-like	Spreading colonies
Penicillium spp.	Blue-green	Velvety	Circular colonies
Mucor spp.	White to gray	Fluffy	Fast-growing colonies

Source: Experimental result, 2026

3.2.3 Microscopic Characteristics of the Fungal Isolates

Microscopic examination using lactophenol cotton blue staining revealed different fungal structures. Table 3 presents the microscopic characteristics of the fungal isolates recovered from bread samples. Microscopic examination provided important diagnostic features that confirmed the identity of the fungal species based on their hyphal structures and spore-producing organs. Aspergillus niger was characterized by septate hyphae and black conidia borne on conidiophores. These features are typical of A. niger and support its identification as one of the predominant fungal contaminants of the bread samples. Aspergillus flavus exhibited septate hyphae, rough conidiophores, and spherical vesicles. These microscopic structures are characteristic of A. flavus and distinguish it from other species within the genus Aspergillus. Rhizopus stolonifer showed non-septate (aseptate) hyphae, along with the presence of rhizoids and sporangia. These structures are diagnostic features of Rhizopus species and indicate its ability to reproduce through sporangiospores. Penicillium species were identified by their distinctive brush-like conidiophores bearing chains of conidia. This characteristic branching arrangement is a key feature used in the identification of Penicillium spp. Mucor species possessed broad non-septate hyphae and round sporangia. These microscopic features are typical of Mucor spp. and differentiate them from septate fungi such as Aspergillus and Penicillium.

Table 3: Microscopic Characteristics of Fungal Isolates

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Fungal Isolate	Microscopic Features
Aspergillus niger	Septate hyphae with black conidia attached to conidiophores
Aspergillus flavus	Septate hyphae with rough conidiophores and spherical vesicles
Rhizopus stolonifer	Non-septate hyphae with rhizoids and sporangia
Penicillium spp.	Brush-like conidiophores with chains of conidia
Mucor spp.	Broad non-septate hyphae with round sporangia

3.3 Discussion

The results obtained from this study revealed that bread is highly susceptible to contamination by saprophytic fungi. Several fungal species were isolated from the bread samples, including Aspergillus niger, Aspergillus flavus, Rhizopus stolonifer, Penicillium species, and Mucor species. The predominance of Aspergillus niger in this study agrees with the findings of Unachukwu and Nwakanma (2015), who reported that Aspergillus species were among the most common fungi associated with bread spoilage in Enugu State, Nigeria. Similarly, Nuhu and Yusuf (2023) isolated Aspergillus, Rhizopus, and Penicillium species from spoiled bread samples sold in Yobe State.

The high occurrence of Aspergillus species may be attributed to their ability to survive under varying environmental conditions and produce airborne spores that easily contaminate bread during storage and handling (Pitt & Hocking, 2009). In addition, the warm and humid conditions common in tropical environments encourage rapid fungal growth. Rhizopus stolonifer was also isolated in significant amounts. This fungus is commonly referred to as black bread mold because it grows rapidly on moist bread surfaces. The findings are consistent with the work of Alexopoulos et al. (1996), who identified Rhizopus stolonifer as one of the major fungi responsible for bread spoilage worldwide.

The presence of Penicillium species in the bread samples may have resulted from airborne contamination during slicing and packaging processes. According to Pitt and Hocking (2009), Penicillium species are important spoilage fungi because they can survive in low moisture conditions and spread rapidly through airborne spores. Mucor species were isolated at a lower frequency compared to other fungi. However, their presence indicates poor storage conditions and exposure of bread to contaminated environments. Samson et al. (2010) reported that Mucor species commonly colonize carbohydrate-rich foods under humid conditions. The identification of Aspergillus flavus in this study is of public health importance because the fungus is known to produce aflatoxins, which are toxic and carcinogenic compounds (Bennett

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& Klich, 2003). Consumption of bread contaminated with toxigenic fungi may expose consumers to food poisoning, allergies, and chronic diseases.

The contamination observed in this study may have originated from several sources, including contaminated flour, bakery equipment, air, poor hygiene practices, and improper storage conditions. Garcia et al. (2019) reported that fungal spores are abundant in bakery environments and may contaminate bread during cooling and packaging stages. Overall, the findings of this study confirm that fungal spoilage remains a significant problem affecting bread quality and safety. Proper hygiene, adequate storage conditions, and the use of preservatives are important measures for reducing fungal contamination in bread products.

3.1 Conclusion

This study isolated and identified Aspergillus spp associated with spoiled bread samples obtained from selected bakeries, markets, and shops within the study area. The fungi isolated included Aspergillus niger, Aspergillus flavus, Rhizopus stolonifer, Penicillium species, and Mucor species. Among these isolates, Aspergillus niger showed the highest frequency of occurrence, indicating that it is one of the major fungi responsible for bread spoilage in the study area. The macroscopic and microscopic examinations carried out confirmed the identity of the fungal isolates based on their colony morphology, hyphal structures, conidia arrangement, and sporangial characteristics.

The study further concluded that bread provides a suitable substrate for fungal growth because of its nutrient composition and moisture content. Environmental factors such as poor hygiene, improper handling, inadequate storage conditions, and exposure to airborne spores were identified as possible sources of contamination. Consumption of contaminated bread may therefore expose consumers to allergies, food poisoning, respiratory infections, and other health complications. Hence, fungal spoilage significantly reduces the quality, shelf life, and safety of bread products. Therefore, strict hygienic measures, proper storage practices, and effective preservation methods are essential in minimizing fungal contamination and protecting public health.

Recommendations

Based on the findings ofthis study, the following recommendations are made:

Bakery workers should maintain proper personal and environmental hygiene during bread production, cooling, slicing, packaging, and storage.

Bread should be stored in cool and dry conditions to reduce fungal growth and prolong shelf life and also used proper packaging materials.

Bakery equipment and working surfaces should be regularly cleaned and disinfected to prevent contamination by fungal spores.

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Regulatory agencies such as the National Agency for Food and Drug Administration and Control should carry out regular inspection of bakeries and bread-selling outlets to ensure compliance with food safety standards.

Public awareness programs should be organized to educate bread producers, vendors, and consumers on the dangers associated with fungal contamination of bread.

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