BACTERIAL AND FUNGAL LOAD OF SLICED BREAD ON SALE IN ILORIN METROPOLIS.

BY

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A PROJECT SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORAT ORY TECHNOLOGY, INSTITUTE OF APPLIED SCIENCES (IAS), KWARA S TATE POLYTECHNIC, ILORIN,

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CERTIFICATION

This is certify that this project is the original work carried out and report ed by **ABDULGANIYU ISLAMIYAT ABIODUN** with matric number **HND /23/SLT/FT/1084** to the Department of Science Laboratory Technology, Microbiology unit, Institute of Applied Sciences (IAS) Kwara State Polyte chnic Ilorin and it has been approved In partial fulfillment of the require ments for the Award of Higher National Diploma (HND) In Science Labor atory Technology

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DEDICATION

I dedicate this my project work to almighty Allah the creator of all univer se, the most beneficient , the most merciful Who has being with me thro ughout the period of my stay in KWARA STATE POLYTECHNIC. His grac e sustained me through every challenges, His light guided every steps I take and His faithfulness brought this journey to completion . I will forev er be grateful to you ALLAH.

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ABSTRACT

This study assessed the bacterial and fungal load of sliced bread sold in Ilorin metropolis to evaluate its microbiological safety. Bread samples la beled A, B, and C were collected from local vendors and analyzed using standard microbiological techniques. Nutrient Agar and Sabouraud Dext rose Agar supported bacterial and fungal growth, while Gram and lactop henol cotton blue staining aided identification. Staphylococcus spp., Ba cillus spp., and Streptococcus spp. were the dominant bacteria, while Sa ccharomyces spp., Rhizopus spp., and Aspergillus spp. were the major f ungi. Sample C exhibited the highest fungal diversity, including toxigenic molds. The findings reflect poor hygiene during production and storage, especially among small-scale vendors. The study recommends routine microbial monitoring, improved packaging, and vendor education to red uce contamination and protect public health.

CHAPTER ONE

1.0 INTRODUCTION

Bread is one of the most commonly consumed staple foods worldwide, appreciated for its affordability, palatability, and nutritional value fig.1 . It is typically made from flour, water, and yeast, and can be enriched with other ingredients to enhance taste and shelf life. Sliced bread, in particul ar, is a widely consumed convenience product, especially in urban areas where fast and easy meals are preferred. However, due to its high moist ure content and neutral pH, bread provides an ideal medium for microbi al growth (Shiferaw et al., 2020). Microbial contamination of bread can o ccur at various stages of production and distribution, especially when hy giene is compromised. This contamination may include both bacterial a nd fungal species, some of which pose serious health risks. In many dev eloping countries, including Nigeria, the risk of microbial contamination i s heightened by poor food handling practices, inadequate storage faciliti es, and limited quality control regulations. Bread sold in open markets, r

oadside shops, and small retail outlets may be more vulnerable to conta mination due to exposure to environmental pollutants and poor packagi ng. Ilorin, the capital of Kwara State in Nigeria, is a fast-growing metropo lis where many residents depend on pre-packaged and sliced bread for daily consumption. The microbial load on bread in this area is of particul ar interest due to varying standards of hygiene among vendors and bak eries.

Fig. 1

Sliced bread

Source: (Shiferaw et al., 2020).

Bacterial contamination in bread can arise from several sources, includi ng contaminated ingredients, utensils, handling by infected persons, or air-borne microorganisms. Common bacterial contaminants found in bre ad include species of *Staphylococcus*, *Bacillus*, and *Escherichia coli*. Th ese organisms can cause gastrointestinal infections, food poisoning, an d other health issues when ingested (Igiebor et al., 2021). The presence of such bacteria in bread suggests a breakdown in hygiene during produ ction or post-production handling. Monitoring these bacteria is essential for consumer safety and public health planning.

Fungal contamination, on the other hand, is mainly attributed to mold gr owth, which becomes evident through visible spoilage signs such as dis coloration and odor. Fungi like *Aspergillus*, *Penicillium*, and *Rhizopus* sp ecies are frequently isolated from spoiled bread (Akinnibosun et al., 202 2). These fungi not only deteriorate the quality of bread but may also pr oduce mycotoxins—harmful metabolites that can cause chronic health c onditions including liver cancer and immune suppression. The warm an

d humid climate of Ilorin further encourages rapid fungal growth, especi ally when bread is not stored under optimal conditions.

The microbial quality of bread is often assessed through the enumeratio n of total bacterial and fungal colony counts using standard microbiologi cal techniques. These counts provide an estimate of the overall contami nation level and can serve as an indicator of hygiene practices in bread production and sales. A high microbial load not only reduces the shelf lif e of bread but also increases the likelihood of foodborne illness. Routine microbiological surveillance is therefore necessary to ensure that bread available in markets meets acceptable safety standards (Onifade et al., 2 0 2 3) .

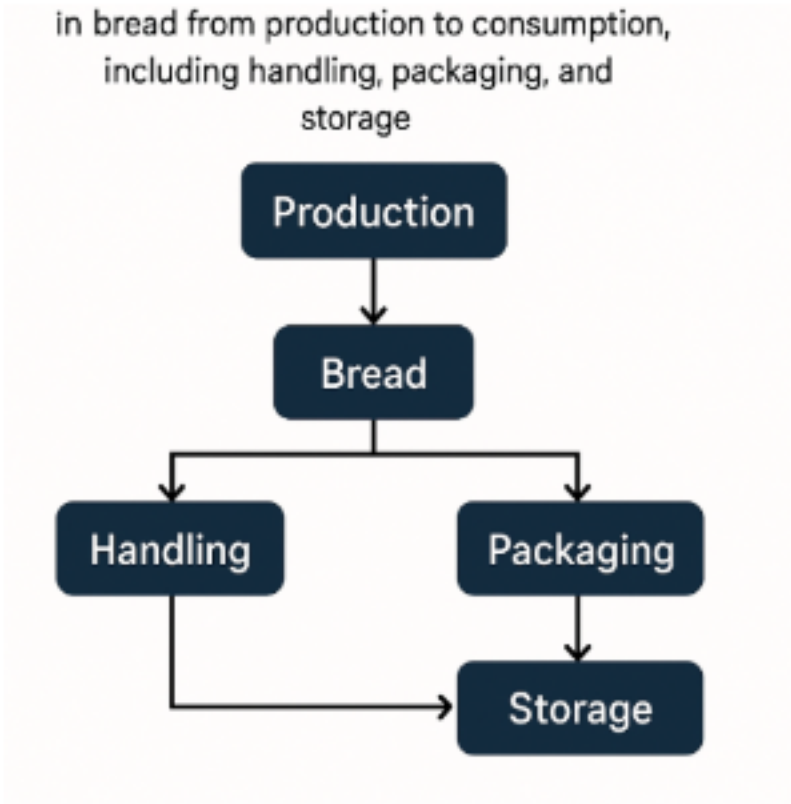
In Nigeria, the National Agency for Food and Drug Administration and C ontrol (NAFDAC) sets guidelines for the microbiological quality of food p roducts. However, enforcement of these regulations is often inconsisten t, especially in informal markets where much of the bread trade occurs. In Ilorin, the sale of bread is typically managed by small-scale retailers w

ho may lack awareness or training in food hygiene practices. This situati on necessitates research to evaluate the actual microbial burden in brea d sold in such environments and determine the associated public health risks (Akinnibosun et al., 2022).

Bread packaging plays a crucial role in determining microbial load. While some brands use airtight plastic wrappers and label their products with production dates, others use loosely sealed polythene bags or even exp ose bread openly for sale. Poor packaging compromises the sterility of b read and facilitates microbial infiltration. In Ilorin, a mix of both packagin g types is observed, often influenced by the bakery's capacity and the v endor’s level of hygiene awareness. The correlation between packaging methods and contamination levels is key to reducing microbial loads.

fig. 2

Flowchart illustrating sources of microbial contamination in bread from production to consumption, including handling, packaging, and storage



Source: (Lateef, 2021).

This visual representation can help depict the possible points of microbial entry and highlight critical control points in bread safety. Such diagrams aid in understanding complex transmission pathways and are essential for designing effective intervention strategies fig. 2.

Storage temperature and duration also significantly influence microbial growth on bread. Bread stored at ambient temperatures, especially in w arm regions like Ilorin, can develop fungal growth within a few days. Refr igeration can delay spoilage, but not all vendors or consumers have acc ess to cold storage. Bread that remains on shelves for extended periods without refrigeration is more likely to harbor large populations of microor ganisms, particularly fungi. It is therefore important to assess storage c onditions during microbial analysis. Consumer habits and preferences c an also influence the safety of bread. In Ilorin, affordability often takes p recedence over quality, leading consumers to purchase bread from vend ors with questionable hygiene practices. Moreover, because some cons umers do not check expiration dates or storage conditions, bread may b e consumed well past its safe shelf life. This practice not only increases the risk of foodborne illness but also underscores the importance of con sumer education in food safety practices (Raji et al., 2025). The objectives of this study are to isolate and identify bacterial and fung

al species present in sliced bread sold in Ilorin metropolis, determine the ir load, and assess the hygienic conditions of the sales environment. Thi s study also seeks to evaluate whether the observed microbial loads ex ceed the recommended safety limits, and suggest appropriate interventi ons. Such findings can guide public health authorities and food safety a gencies in improving bread hygiene standards across the city. To achiev e these objectives, this study adopts standard microbiological technique s including serial dilution, plating on selective media, colony count deter mination, and biochemical and morphological identification of isolates. T hese methods are widely used in food microbiology and provide reliable results when properly executed. The study area, Ilorin metropolis, includ es multiple districts where bread is sold in large quantities, offering a re presentative sample for investigation (Akinnibosun et al., 2022).

Previous studies conducted in other Nigerian cities have reported conce rning levels of microbial contamination in bread. For instance, research i n Lagos and Ibadan indicated the presence of pathogenic microorganis

ms in several bread brands sold in local markets (Oladipo & Adeleke, 20
20). However, limited data exists on the microbial quality of bread in Ilori
n, which has a unique combination of urban and semi-urban bread distri
bution networks. Filling this gap is critical to understanding regional vari
ations in food hygiene (Lateef, 2021). Ultimately, this research highlights
the need for integrated food safety systems that encompass producers,
vendors, regulatory agencies, and consumers. The findings may also se
rve as a basis for awareness campaigns aimed at improving hygiene pr
actices in bread production and sales. With increasing urbanization and
population growth in Ilorin, demand for bread is expected to rise, further
necessitating stringent safety measures. The health implications of con
suming contaminated bread must not be underestimated.
Bread is a vital part of the Nigerian diet, and ensuring its microbiological
safety is essential for public health. The current study aims to shed light
on the bacterial and fungal loads present in sliced bread sold in Ilorin me
tropolis. By doing so, it seeks to provide evidence-based recommendatio

ns for improving bread safety and reducing the burden of foodborne dis eases in the region. This study underscores the interconnectedness of f ood hygiene, public health, and consumer awareness (Raji et al., 2025).

1.1 Literature review

Several studies have documented the microbial contamination of bread and its potential health risks to consumers. Bread is susceptible to cont amination due to its high moisture content, nutrient composition, and ex posure to the environment during handling and storage. According to Sh iferaw et al. (2020), bread sold in open markets in Addis Ababa exhibite d high microbial counts, mainly due to poor storage conditions and unhy gienic handling by vendors. These findings highlight that contamination can be introduced during post-baking processes, emphasizing the need for good hygiene practices in the supply chain.

Research conducted in Nigeria has revealed the presence of pathogenic

and spoilage organisms in commercially available bread. In a study by Ig iebor et al. (2021), samples of sliced bread from retail outlets in Delta St ate were found to contain high levels of *Staphylococcus aureus*, *Bacillus cereus*, and various molds, especially when stored at room temperature. The study emphasized the risk of foodborne illnesses associated with c onsuming such contaminated bread, and advocated for improved packa ging and labeling of bread products to reduce consumer exposure.

Fungal spoilage is particularly common in bread due to the proliferation of molds under humid conditions. A study by Akinnibosun et al. (2022) in Benin City found that bread samples from roadside vendors exhibited vi sible mold growth within 48 hours of purchase. Isolated fungi included *A spergillus niger*, *Penicillium* spp., and *Rhizopus stolonifer*. These fungi n ot only reduce the aesthetic and nutritional value of bread but may also produce mycotoxins, posing serious long-term health risks. The microbial quality of bread is also influenced by the type of packagin g used. Onifade et al. (2023) evaluated different packaging methods an

d found that bread stored in loosely sealed polythene bags had significa ntly higher microbial counts compared to those in vacuum-sealed plasti c. This suggests that airtight packaging acts as a physical barrier agains t environmental contaminants, thereby extending the shelf life of bread. The study recommended that manufacturers adopt modern packaging t echniques to mitigate microbial infiltration.

In an assessment of microbiological safety of bread in southwestern Ni geria, Oladipo and Adeleke (2020) discovered that even well-packaged b read samples carried high bacterial loads when hygiene was not maintai ned during production. Their study emphasized the importance of regul ar inspection of bakeries by food safety authorities and recommended t he implementation of Good Manufacturing Practices (GMP) across bake ries in Nigeria. This is crucial for ensuring that the microbial loads in bre ad products remain within permissible safety limits. Consumers' bread-handling practices can also contribute to microbial co ntamination. Umeh et al. (2021) reported that many consumers in Niger

ia store bread at room temperature, often in unsealed conditions, which accelerates fungal growth. The study recommended public health campaigns to educate consumers about the importance of refrigeration and clean handling practices to preserve bread quality. It also highlighted that vendors often fail to inform buyers about proper storage methods.

A recent study in Kwara State by Yusuf et al. (2023) evaluated the microbial profile of bread from both large-scale bakeries and small-scale vendors. The results showed a significantly higher microbial load in bread sold by small-scale vendors, including isolates of *E. coli*, which suggest fecal contamination. The study attributed this to inadequate handwashing facilities and poor hygiene awareness among local bread handlers. These findings reinforce the need for training programs for vendors and stricter local health inspections.

The presence of antibiotic-resistant bacteria in bread has also been identified as an emerging concern. A study by Ogundipe et al. (2022) reporte

d the isolation of multidrug-resistant *Staphylococcus aureus* and *Entero bacter* species from packaged bread sold in Ibadan. These resistant stra ins pose serious public health threats, especially in communities with li mited access to healthcare. The authors emphasized the importance of antimicrobial resistance monitoring in foodborne pathogens and recom mended routine microbial testing for bakery products.

1.2 Statement of problem

- **Poor Hygiene During Bread Production and Handling:** Despite being a staple food in many Nigerian households, sliced b read is often produced and handled in unhygienic environments. I n Ilorin metropolis, several small-scale bakeries and street vendor s lack proper sanitary practices, which may lead to bacterial and f ungal contamination of bread. This raises public health concerns a

s consumers may unknowingly ingest harmful microorganisms.

- **Lack of Microbiological Surveillance and Regulation Enforcement:**

  There is a significant gap in routine microbiological testing and enforcement of food safety standards for bakery products in Ilorin. Regulatory agencies often focus on large-scale producers, neglecting smaller vendors where most of the population buys their bread. This creates an unchecked risk of foodborne illnesses linked to contaminated sliced bread sold in the area.

- **Limited Public Awareness on Safe Bread Storage and Consumption:**

  Many consumers in Ilorin metropolis are unaware of proper bread storage methods or the implications of consuming bread past its shelf life. Due to warm temperatures and poor packaging, fungal spoilage is common, yet bread continues to be consumed even whe

n visibly contaminated. This highlights the urgent need for public heal th education on microbial safety in ready-to-eat foods.

1.3 AIM

The aim of this study is to determine the bacterial and fungal loads pres ent on sliced bread sold in Ilorin metropolis, in order to assess its microb iological quality, identify potential public health risks, and recommend ap propriate hygienic and regulatory measures for safer consumption.

1.4 Objectives

- To isolate and identify the bacterial and fungal species present on sliced bread sold in Ilorin metropolis.
- To determine the bacterial and fungal load (colony-forming units) on sliced bread samples from different vendors in Ilorin.
- To assess the hygienic conditions and packaging methods used b y bread sellers in Ilorin metropolis.

- To evaluate whether the microbial loads on sliced bread exceed r ecommended safety standards and suggest measures to reduce contamination.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Materials Used

The materials employed for this study included various laboratory equip ment and consumables necessary for microbial analysis. These include d conical flasks, measuring cylinders, Bunsen burner, fire gauze, weighin g balance, inoculating loop, microscope slides, Petri dishes, stirring rods, and syringes. Bread samples labeled A, B, and C were obtained and use d for microbial load testing. Additional items such as paper tape for labe ling, as well as sterile containers for handling the bread samples, were a lso used during the experiment.

2.1.1 Chemicals and Reagents

The chemicals and reagents used in this study were of analytical grade. They included Nutrient Agar (NA) for bacterial culture, Sabouraud Dextr ose Agar (SDA) for fungal growth, and Tryptone Soya Broth (TSB) as a g eneral-purpose enrichment medium. Ciprofloxacin (antibiotic) was used

to inhibit bacterial contamination in fungal culture. Distilled water was u sed throughout the preparation processes. Amoxicillin (1g) and ciproflox acin were dissolved in 10 ml of distilled water, and 1 ml of the reconstitu ted solution was added to the SDA to prevent bacterial interference.

2.2 Preparation of Sample

The bread samples A, B, and C were soaked in clean table water for a fe w minutes to allow microbial detachment from the surface and interior. After soaking, the water from each sample was carefully withdrawn and used as the inoculum. The withdrawn solution was introduced into the r espective prepared culture media for incubation and microbial growth a ssessment.

2.3 Preparation of Media

All culture media were prepared according to manufacturer instructions. Nutrient Agar (NA), Sabouraud Dextrose Agar (SDA), and Tryptone Soya Broth (TSB) were accurately weighed using a digital balance. The appro priate quantities of media powder were dissolved in distilled water, trans

ferred into conical flasks, stirred thoroughly using a glass rod, and sterili zed by autoclaving at 121°C for 15 minutes. After cooling, SDA was sup plemented with 1 ml of reconstituted ciprofloxacin solution to suppress bacterial growth. The media were poured into sterilized Petri dishes and allowed to solidify. Once solidified, the Petri dishes were inverted and la beled using paper tape for proper sample identification.

2.4 Inoculation and Incubation

After preparation, the water withdrawn from each soaked bread sample was poured aseptically into the respective media. For bacterial growth, t he inoculated NA plates were incubated at 37°C for 24 hours. For fungal growth, the SDA plates were incubated at room temperature (25–28°C) for 5 days. The plates were turned upside down during incubation to avo id condensation on the media surface.

2.5 Bacterial Analysis and Staining

After 24 hours of incubation, bacterial colonies were observed on the N A plates. A loopful of growth from each sample was taken and smeared

on a clean microscope slide for Gram staining. Microscopic examination revealed that Sample B showed perfectly shaped cocci, while cocci wer e also observed in Samples A and C, although not as distinct as in B.

2.6 Fungal Analysis and Staining

Following 5 days of incubation, fungal growth was observed prominently on all SDA plates. Sample C exhibited the highest growth, with visible m ucous colonies and multiple fungal types. Sample A also produced muc ous but had fewer colonies compared to C. A small portion of the fungal colony was picked using an inoculating needle, streaked on a clean slid e, stained, and observed under a microscope for morphological characte ristics.

CHAPTER THREE

3.0 RESULTS

The results obtained from the microbiological analysis of sliced bread samples labeled A, B, and C. Both bacterial and fungal growth were observed and analyzed through culturing and microscopy.

3.1 Fungal Observation (Lactophenol Cotton Blue Staining)

Fungal growth was assessed based on the colony morphology on Sabouraud Dextrose Agar and microscopic characteristics using lactophenol cotton blue staining. The results are summarized below:

Table 1: Macroscopic Observation of Fungal Growth on Bread Samples

Sample	**Observation**	**Microscopic Observation**
A	i. Creamy mucoid growth ii. Whitish, cottony growth	Yeast Mold
B	i. Creamy mucoid growth	Mold
C	i. Whitish, cottony growth	Mold