ISOLATION AND IDENTIFICATION OF FUNGAL FROM BREAD PRODUCED IN SOME CALABAR BAKERIES

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ABSTRACT

Bread is a staple food in the developed and developing world it is widely accepted and consumed by people of all ages and nationalities, including Nigeria. Bread is a staple food that can be susceptible to microbial spoilage due to mold growth and fungi. The so aim is to determine the identification and isolation of fungi from bread produce in some Calabar Bakeries. The results reveal the presence of four fungi species., Rhizophus sp (21%), Penicillium sp (25%), Aspergillus sp (31%), Mucor sp (23%) the number of isolates varies across samples with Aspergillus sp and Penicillium sp showing the highest level of contamination respectively 31% and 25%, while Mucor sp and Rhizopus sp has the lowest count with are 23% and 21%. The observed fungi contamination suggests four hygiene practices during production and post production, as well as suboptimal storage conditions. The study findings review that bread samples distributed in some Calabar bakeries in cross rivers state exhibited contaminations with a diverse array of bacterial and fungal species, this contamination poses a significant safety risks to human health, take potentially exposing the population food borne diseases

Key words: Isolation, Identification, Bread, Fungal, Rhizophus sp , Penicillium sp , Aspergillus sp ,Mucor sp , Shelf-life, Mold and Bakeries .

INTRODUCTION

Background of study

Bread is a staple food both in the developed and developing world. It is widely accepted and consumed by people of all ages and nationalities including Nigeria. It is available in different forms and consumed in different ways. It can be taken alone as a snack or with hot beverages (tea, cocoa and coffee), cold nourishing drinks (chocolate drinks, milk shakes, etc.) and refreshing drinks (fruit drinks, fruit juices and carbonated drinks). Bread, may also be consumed with breakfast cereals such as corn and millet porridge, 'tom brown', etc (Adebayo *et al*., 2016).

The use of wheat flour in the Nigerian diet has come to stay as there is no home where wheat product is not consumed. It is consumed in the form of biscuits, breads, noodles, pizzas, ball float ('bofrot' in Ghanaian language), cookies & pastries, and other breakfast cereals. Ghana and Nigeria import over one million tons of American red winter wheat annually, making them one of the largest importers of American red winter wheat in the world (Kuchenmuller *et al*., 2013). This situation has

placed a huge financial burden on the Ghanaian economy, as the government often has to import this commodity in foreign currency. Another challenge that has been associated with the continuous use of wheat in bread making is the development of celiac disease (Khan and Saha, 2011). Cassava (Manihot esculenta Crantz) is one of the most important crops in Africa, and Nigeria is the leading producer globally but in Nigeria it is an important perennial crop with a per capita rate of consumption of 152.9 kg/yr. Cassava tubers can be kept in the ground for up to two years prior to harvesting, but once harvested; they begin to deteriorate because of the high moisture content of the fresh roots. Cassava is traded in its processed form due to its bulkiness and early deterioration. Aside using fresh cassava tubers, the commodity is often processed into intermediate products such as 'gari', 'konkonte' (fermented cassava flour) etc. And recently into High Quality Cassava Flour (HQCF), which is a major intermediate product (Hathout and Aly, 2014). In 2003, a presidential initiative was launched in Nigeria with the aim of adding HQCF (10% w/w) to the wheat flour used in bread. The purpose of this initiative was to restrict the outflow of funds for the importation of wheat and to encourage research on cassava/wheat composite breads. In previously published works, different wheat flours were composited with various proportions of cassava starch and flour. Ezekiel *et al*. (2022) reported that the 30% (w/w) inclusion of cassava flour into wheat flour could yield an acceptable fresh loaf of bread, depending on the source of the

Composite flour used. Bread from various flour composites from roots and tubers such as cassava and sweet potato have also been shown to have higher yield and profit margins making it a viable product to improve livelihoods and boost national economies. The International Institute of Tropical Agriculture (IITA) recounted that 40% cassava flour bread that possessed comparable eating qualities to 100% wheat flour bread was produced (Artun et al., 2012). Previous investigations into the incorporation of HQCF into wheat flour for bread making have concentrated on the acceptability of the composite bread to consumers. Ezekiel *et al*. (2022) recounted that bread stakeholders gave numerous explanations for the slow adoption of HQCF in bread because of the life span of cassava bread owing to its high microbial load and moisture content. The authors correlated the microbial count of 100% wheat bread with substitution levels of 10%, 20%, 30% and 40% cassava composite bread and found high microbial counts in the cassava composite breads. Balkan and Ertan (2015) stated that with the exceptions of wine and cheese, the sensory characteristics of most foods tend to decline throughout storage. However, most consumers tolerate this change provided the foods remain safe. The Institute of Food Science and Technology (IFST) Guidelines states that, "shelf life is the period of time during which a food product will remain safe, be sure to retain desired physical, sensory, chemical and microbiological characteristics and also conform to any label declaration of nutritional data when stored under the recommended conditions. This definition takes into consideration the storage conditions on the shelf-life of products. Hathout and Aly (2014) that the storage characteristics of most food products are usually measured in controlled environmental condition but this is seldom done in practice after the product has left the producer to the retail point.

According to Jard *et al*. (2021) factors that affect the shelf-life of food products can be classified as extrinsic and intrinsic. Stored bread undergoes changes such as redistribution of moisture, starch retrogradation, loss of flavour and aroma and increase in product firmness. Microbial spoilage of bakery products is mostly characterized by the onset of staling and ropiness and these indicate the end of the shelf life. A lot of methods have been applied in the estimation of shelf life of food products. Regression models can be applied to products stored at given condition. The authors recounted also that predictive models give the best shelf life estimation than other applied shelf estimation models due to its accuracy by taking into consideration the decrease or increase in the quality of a product with respect to time. This study seeks to apply predictive shelf life models in the estimation of the shelf life of composite bread stored under the different conditions by stakeholders in the bread value chain in Nigeria (Hassan *et al.*, 2014).

Some factors that can contribute to mold growth in bread include: Long storage, Moisture condensation on the bread surface, and the ability of mold to synthesize proteolytic and amylolytic enzymes.

Fungal have received considered research due to global consumer concerns that affect the shelf-life of food products, Fungi isolation is the process of separating and studying individual fungal organisms for research purposes, and this fungal are the catalyst for food spoilage. Fungal found in bread are: Aspergillus niger, Aspergillus spp, Rhizophus sp, Penicillium sp, Aspergillus flavus, and Aspergillus fumigatus.

Statement of Research problem

Variety of bakery products are available in the market. Earlier bakery products were considered as a sick man's diet or poor man's diet. It has now become the essential food item for a vast majority of the whole population (John and Mishra, 2017). Bread becomes contaminated after baking, from the mold spores present in the atmosphere surrounding loaves during cooling, slicing, packaging and storage. Most common source of microbial spoilage is due to mold growth. According to the previous studies (Amadi *et al.*, 2014) bread molds like Mucor and Rhizopus are found to grow first during bread spoilage. This is followed by some other fungi like Aspergillus, Penicillium and Fusarium spp.

This research will be carried out to determine the molds that cause bread to spoil, Bread is a staple food that can be susceptible to microbial spoilage. This research is also carried out to possibly combat the possible problems associated with isolation and identification of fungal isolations from bread.

Justification

Usually bakery products are packed in plastic films after baking and cooling and they consumed within 1 or 2 months. Post process contamination is unavailable. Contamination by fungal organisms

in these kind of products usually comes from the post baking cooling period as the cooking temperature is normally enough to eliminate previous contamination. This study will focus on the fungal spoilage of bakery products, its effect on the shelf life of the bread and the identification of fungal found.

Aim

The aim is to determine the isolation and identification of fungal from bread produced in Calabar bakeries

Objectives

The objectives will be achieve through the following specific objectives;

1. Identifying the types of fungi that germinates on stale breads
2. To determine the fungal genera of public health important associated with sampled bread
3. To ascertain the shelf life of the sampled breads

Figure 1: Slide Showing the Texture of Normal Bread
Source: google image

MATERIALS AND METHODS

Experimental Location

The Study Area for this project work is in Calabar city, Calabar Municipality Local Government Area, Cross Rivers State. Is located on the latitude 4.982873'Nand longitude 8.334503'E of the Greenwich Meridian. It is bounded by Odukpani Local Government Area in the North-East by the great Kwa River. Its Southern shores are bounded by the Calabar river and Calabar South Local Government Area.

This research was conducted at the Research Laboratory of the Department of Science Laboratory Technology, University of Calabar, Calabar Cross rivers state Nigeria in the year 2025.

Sample collection and Processing

Four (4) bread samples were collected into sterile plastic bags from four (4) different bakery point of sale within Calabar, properly labelled and taken to the laboratory where they were kept and monitored daily until spoilage occurred. Portions of each of the spoilt bread were carefully cut with sterile scalpels and one gram each was enriched in sterile sabouraud dextrose broth for twenty-four hours (24 hours).

Table 1: Bread Samples and Locations

Bread Sample	Location
B1	Etta Agbor
B2	Marian Road
B3	State Housing
B4	Goldie Road

Isolation of Mold

One milliliter (1ml) of each of the enriched samples were serially-diluted and 0.1ml of the dilution (10^3) was used to inoculate duplicate plates of already prepared sterile sabouraud dextrose agar (SDA) containing 0.05mg/ml chloramphenicol to inhibit bacterial growth. The sabouraud dextrose agar (SDA) was prepared according to the manufacturer's instructions. The media was autoclaved for 121°C for 15mins. The spread plate technique was used for the inoculation. The inoculated plates were incubated at room temperature (22-25°c) for 72 hours after which the fungal colonies that developed were counted, purified by repeated sub culturing and were stored in sabouraud dextrose agar (SDA) slants for identification.

Identification of the Isolates

The isolates were identified macroscopically and microscopically. The colony colour, texture and size were observed while the microscopic examination was done using lactophenol cotton blue stain. A drop of the stain was placed on a clean grease-free slide. A small portion of the fungal culture was emulsified on the slide and covered with a coverslip, avoiding bubbles and thereafter viewed under the microscope using 10X and 40X objective lens and compared with mycological atlas.

Statistical Analysis

The occurrence of each fungus species isolated from the test samples was determined as a percentage ratio of their prevalence relative to the total number of samples examined. The formula below was used:

$$\% \text{ Occurrence} = \frac{\text{No. of positive test of fungus}}{\text{Total Number tested}} \times \frac{100}{1}$$

The total occurrence of fungi in each bread sample was determined as a percentage ratio of their prevalence in each sample relative to the total number of samples examined in all breads.

$$\% \text{ Occurrence} = \frac{\text{No. of positive test in a bread sample}}{\text{Total Number tested in all breads}} \times \frac{100}{1}$$

Figure 3: Slide showing mold growth of labelled sample bread (B1 – B4)

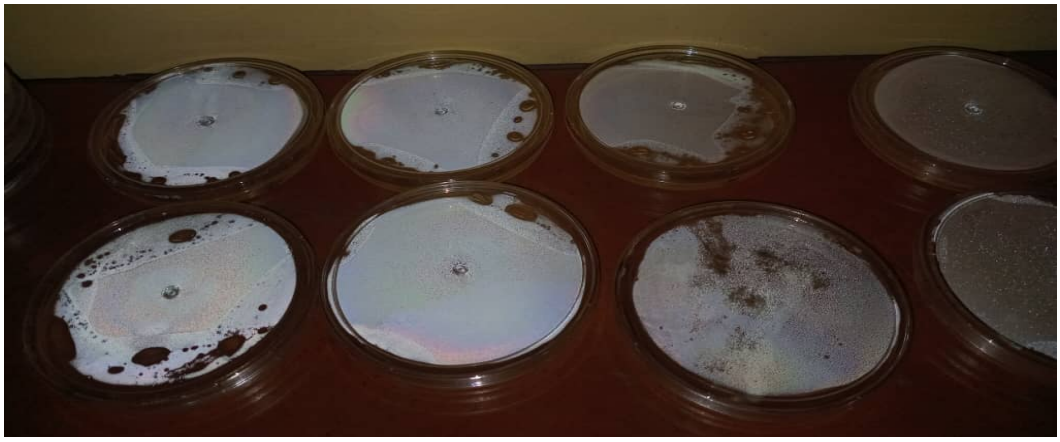

Figure 4: Plate showing fungal growth from isolated mold

RESULTS

Table 2: Shelf Life of Different Bread Samples

This table represent the spoilage intervals when mold was spotted in days. This aid in the identification of the shelf life of the different bread samples.

Table 2: Shelf Life of Different Bread Samples

Bread Sample	Spoilage Interval/when mold was spotted (in days)
BI	4
B2	3
B3	6
B4	2

Table 4: Number of Isolates from Each Bread Sample:

The table presents the number of fungal isolates identified from different bread samples (labeled B1, B2, B3 and B4). Four fungal species were observed: Rhizopus sp, Penicillium sp, Aspergillus sp, and Mucor sp.

Sample B1 contained 2 isolates of Rhizopus, 3 of Penicillium, 3 of Aspergillus, and 1 of Mucor.

Sample B2 had higher counts, with 4 isolates of Rhizopus, 3 of Penicillium, 4 of Aspergillus, and 5 of Mucor.

Sample B3 contained no Rhizopus, 3 of Penicillium, 3 of Aspergillus, and 1 of Mucor.

Sample B4 had the highest counts: 5 Rhizopus, 6 Penicillium 7 Aspergillus and 4 Mucor isolates

In total:

Aspergillus sp was the most frequently isolated fungus (16 isolates),

followed by Penicillium sp (13 isolates),

Rhizopus sp (11 isolates), and

Mucor sp (12 isolates).

Among the four types of fungi isolated, **Aspergillus sp** was the most frequently found, followed by **Penicillium sp, Mucor sp,** and **Rhizopus sp.** This indicates that Aspergillus may be the most dominant mold present in the sample bread

Table 4: Number of Isolates from each bread samples

	Rhizopus sp	*Penicillium* sp	*Aspergillus* sp	*Mucor* sp
B1	2	3	3	1
B2	4	3	4	5
B3	-	1	2	2
B4	5	6	7	4
Total	**11**	**13**	**16**	**12**

Table 5: Frequency of Fungal Isolates Identified in Bread Samples

The table shows the frequency of different fungal species identified in bread samples.

Aspergillus species were the most frequently isolated, found in 16 samples, representing 31% of the total.

Penicillium species followed, occurring in 13 samples, which accounts for 25%.

Mucor species were identified in 12 samples, making up 23% of the total.

Rhizopus species had the lowest occurrence, found in 11 samples, representing 21%.

Table 5: Frequency of Fungal Isolates Identified in Bread Samples

Fungal Species	Number of Samples Isolated	percentage Occurrence (%)
Rhizopus sp.	11	21%
Penicillium sp.	13	25%
Aspergillus sp.	16	31%
Mucor sp.	12	23%

Discussion

In this present research, the isolated and identified microorganisms have been determined to be the causative agents of certain diseases. These findings support earlier evidence that foodborne illnesses are prevalent worldwide and contribute to approximately one-third of global mortality (Khanom et al., 2016). Bread's nutrient-rich composition fosters an environment conducive to microbial growth and metabolism, making it perishable. This creates the potential for harmful microorganisms like Aspergillus and Bacillus to persist within the bread matrix, leading to spoilage and rendering the product unsuitable for consumption. Implementing hygienic processing practices is essential to mitigate the risk of contamination and food poisoning, safeguarding consumers' health (Hathout and Aly, 2014). Our findings align with another study conducted in Bori Metropolis (River States, Nigeria), where Aspergillus spp., Penicillium spp., and Rhizopus spp. Were also identified in bread samples. This underscores the presence of common foodborne microorganisms in bread and highlights the need for vigilant monitoring and control measures (Ezekiel et al., 2022). Of particular concern is the prevalence of Rhizopus contamination in the food chain. Rhizipus species are naturally occurring in the flora of both animals and humans, and they can easily be transmitted from handlers and animals in the food preparation area to the food products. This can lead to potential health risks if contaminated bread is consumed. Addressing the issue of unhygienic practices in bread production is of paramount importance. For instance, bread vendors and hawkers may inadvertently contaminate the equipment used in Bakeries and points of distribution, allowing contaminants to enter the bread during various stages of handling and transportation (Jard et al., 2021). By proactively addressing unhygienic practices and adopting stringent control measures, the risk of contamination and associated health hazards can be significantly reduced, safeguarding consumers and maintaining the quality and safety

of bread products (Amadi et al., 2014). The utilization of preservatives has emerged as an alternative approach to extend the shelf life of bakery products, despite not being favored by consumers. Commonly employed in the baking industry are propionates, sorbates, and sometimes benzoates (Hassan et al., 2014). However, despite their effectiveness, certain fungal species have displayed resistance even at the permissible maximum concentrations allowed in food. Notably, Fusarium species is one such resistant species, known to exhibit an extended lag phase followed by rapid multiplication, which could be a key factor contributing to its prevalence in deteriorated samples examined in this study (Khanom et al., 2016). Moro, Mukunda et al. (2022) demonstrated the resistance of Fusarium and Penicillium species isolated from moldy bread to propionic acid concentrations permitted for use in bread, as well as Aspergillus resistance to sorbic acid. Additionally, the same study revealed that both Aspergillus and Rhizopus species displayed resistance to elevated concentrations of acetic acid. The selection of sanitization agents to combat spoilage fungi is a critical factor as the effectiveness of the process relies on the susceptibility of the fungi to the active components and the concentration of the sanitizer applied. A recent study by Oluwajoba et al. (2022) revealed variations in sanitizer sensitivity among strains of the same fungal species and even among different species isolated from spoiled baked goods. This underscores the significance of assessing the sensitivity of spoilage fungi causing losses in each individual bread industry.

Another crucial measure to prevent fungal spoilage is adopting a hygienic layout for buildings and facilities. This includes segregating post-baking areas (cooling and packing) from the bread making processing area, thereby reducing the influx of spores from raw materials that may contaminate freshly baked bread slices (Shephard, 2018). Additionally, minimizing the time of cooling and packaging can positively impact shelf life. By reducing the exposure of bread to contaminated air, the chance of spore deposition on the product surface is diminished, leading to an extended shelf life.

Conclusion

The study findings revealed that bread samples distributed in some Calabar bakery in Cross River State exhibited contamination with a diverse array of bacterial and fungal species. This contamination poses a significant safety risk to human health, potentially exposing the population to food-borne diseases. Bread is abundant in carbohydrates, serving as a primary energy source for the body. Moreover, it acts as a crucial fuel for essential organs such as the brain, kidneys, heart muscles, and central nervous system. Additionally, the presence of such contaminants indicates inadequate hygienic practices carried out by the handlers involved in the bread's production and distribution.

Recommendations

By addressing the issues related to handwashing, promoting hygienic handling practices, and embracing GHP and HACCP principles, the risk of contamination in bread can be significantly reduced, leading to safer food consumption and improved public health. Based on the finding of this study the following recommendations were made;

1. Frequent and sufficient hand washing, this suggests that the hands of individuals involved in food handling, such as vendors and consumers, may carry harmful microorganisms that can contaminate the food, including bread.
2. To mitigate the risk of contamination, both bread vendors and consumers are strongly advised to follow hygienic practices while handling bread. This includes maintaining cleanliness during storage, distribution, and consumption.
3. It is recommended to buy bread from reputable and approved sources that adhere to proper processing and packaging practices.

4. Bread and other foods producers should implement Good Hygienic Practice (GHP) and Hazard Analysis and Critical Control Point (HACCP) principles

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CONTRIBUTION OF AUTHOR

The author faithful, onyinyechi uche solely conceptualized and designed the study on the isolation and identification of fungi from bread produced in some bakeries in Calabar. All bread samples were personally purchased from selected bakeries within Calabar metropolis using proper sampling techniques. The author carried out the preparation of culture media, sterilization of equipment, and inoculation of samples under aseptic conditions. Laboratory procedures including serial dilution,

plating, incubation, and microscopic examination were performed by the author to isolate and identify fungal species. Relevant literature was reviewed, and data obtained from laboratory analyses were compiled, statistically analysed, and interpreted by the author. The author also prepared the entire project manuscript, including discussions, conclusions, and recommendations, without the contribution of any co-author.

Data Availability:

The data supporting the findings of this study, including raw laboratory results, photographs of fungal colonies, and statistical analyses, are available from the author upon reasonable request.

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Conflict of interest:

The author declares no conflict of interest**.**

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