Original

Isolation and Identification of *Fungi* spp Associated with Bread Spoilage in Lapai, Niger State

[1,3]Aishat Rabiu Sani, [1]Mariam Testimony Hamza, [1]Isah Legbo Muhammed, [1]Fatima Ladidi Jibril, [4]Ibrahim Abdullahi, [1]Abdulullahi Mohammed, [2,3]Sunday Buru Ayuba

[1]Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria
[2]Kaduna State University, Kaduna, Kaduna State, Nigeria
[3]Newgate University, Minna, Niger State, Nigeria
[4]School of midwifery, Minna, Niger State, Nigeria

Corresponding author: Aishat Rabiu Sani, Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria
raishat@ibbu.edu.ng: +2348032423334

Article history: Received 18 December 2024, Reviewed 09 March 2025, Accepted for Publication 11 March 2025

Abstract

Background: Bread is a widely consumed perishable food that undergoes physical, chemical, sensory, and microbiological changes during storage, leading to spoilage. Contaminated bread can cause intestinal disorders, disease outbreaks, and antifungal resistance. This study investigated fungal contamination in bread sold in Lapai, Niger State, Nigeria, and assessed its proximate and physicochemical properties.

Methods: Bread samples were collected from five street-vended locations in Lapai and analyzed in the microbiology laboratory. Samples were inoculated on Sabouraud Dextrose Agar (SDA) with chloramphenicol to inhibit bacterial growth.

Results: A total of 15 bread samples were analyzed, with fungal isolates including *Aspergillus* spp. (50%), *Mucor* spp. (18.75%), *Fusarium* spp. (6.25%), *Penicillium* spp. (12.5%), and *Rhizopus* spp. (12.5%).

Conclusion: Mold spoilage reduces bread shelf life, leading to economic losses in the bakery industry. Poor hygiene and post-production contamination contribute to fungal growth and antifungal resistance. Implementing strict hygiene practices, heat treatments, and proper packaging can help mitigate bread spoilage and associated health risks.

Keywords: One Health, *Fungi* spp, Bread spoilage, Antifungal resistance

How to cite this article

Sani AR, Hamza MT, Muhammad IL, Jibril FL, Abdullahi I, Mohammed A, Ayuba SB. Isolation and Identification of Fungi spp Associated with Bread Spoilage in Lapai, Niger State. The Nigerian Health Journal 2025; 25(1):365-370.
https://doi.org/10.71637/tnhj.v25i1.991

Introduction

Baked food such as bread is one of the most consumed stable foods in the world. It is a good source of nutrients such as micronutrients and macronutrients that are essential for human health.[1] Bread is a common food product, produce by baking dough obtain from a mixture of recipe of flour, water, sugar, salt, yeast.[2] Bread is consumed worldwide on a daily basis. Being a perishable food product, its quality and freshness decrease during storage, causing changes in physical, chemical, sensory, and microbiological characteristics.[3]

The limiting factor for the shelf life of breads is spoilage by fungi, which results in visible mold growth, changes in flavor, and, most importantly, deleterious effects to human health and economic losses to the baked goods industry [4] Fungal deterioration of bread is mainly caused by Penicillium, Aspergillus, Rhizopus, and Wallemia. [5,6] Carbohydrates have a role as the main source of energy. Bread is an example of a food that contains carbohydrates.

Currently, bread is consumed by Indonesian people as a basic need. Bread can be used as a substitute for rice because its nutritional content is not inferior to rice.[7] Bread can be damaged microbiologically by microbial contamination through the air, dust, or hands that enter the food. Microbes can cause food spoilage and are harmful to the body because they can produce toxins. Microbes in bread can change the composition of foodstuffs by hydrolyzing starch and cellulose into smaller fractions, hydrolyzing fats and causing rancidity, causing sugar fermentation and breaking down protein into ammonia, resulting in a foul odor.[8] Food poisoning can cause several symptoms such as nausea, vomiting, and diarrhea. Nausea, vomiting, and diarrhea can last for one or three days, depending on the type of poison and the level of contamination that occurs.[8] One type of fungus that grows on bread is Aspergillus flavus. Aflatoxin is a mycotoxin found in the fungus Aspergillus flavus. Mycotoxin contamination in food can cause problems for human health because it is toxic to the kidneys, liver, reproductive system and can cause cancer.[9]

World Health Organization (2019) stated that more than 23 million people experienced food poisoning and an estimated 4,700 people died. In 2017, the Center for Drug and Food Medicine (BPOM) recorded 53 cases of Extraordinary Events (KLB) of food poisoning throughout Indonesia. It was recorded that 5293 people were exposed to it, while 2041 people were reported to be sick, and 3 people died. One of the causes of food poisoning cases due to microbiology is the activity of microbial types of fungi. In 2018 the Ministry of Health reported that outbreaks of food poisoning still occur in Java, the highest province of food. During storage, it is subjected to a number of changes which lead to the loss of its organoleptic freshness. The factors that govern the rate of freshness loss in bread during storage are mainly divided into two groups; those attributed to microbial attack, and those that are result of a series of slow chemical or physical changes which lead to the progressive firming up of the crumb, commonly referred to as 'staling'.[8] Microbiological spoilage of bread the most common source of microbial spoilage of bread is mould growth. Less common, but still causing problems in warm weather, is the bacterial spoilage condition known as 'rope' caused by growth of Bacillus species. Least common of all types of microbial spoilage in bread is that caused by certain types of yeast. Spoilage of bread caused by microorganism and the consequential ravage issues leads to huge profitable losses for both bakery products and those consuming the bread. Again, the availability of mycotoxins as a result of mold contamination in cereals and cereal food is still an important issue to deal with.

The scientific names of fungi that grow on bread are; *Rhizopus nigricans* and *Mucour stolonifera.*[10] There are minor differences between the two and both are commonly referred to as "Bread mold". These are invariably the first one to "arrive" and germinate on a piece of bread. Later, many others may follow such as Aspergillus and Penicillium.[11] Spoilage of bread caused by microorganisms and the consequential ravage issues leads to huge profitable losses for both bakery products producers and those consuming the bread. Consumption of contaminated bread infected with microorganism has been a worldwide problem which leads to stomach or intestinal disorder as a result of deposited microbial toxins.[12] The need of evaluation of bread sold in the community is important as the consumption of contaminated bread can lead to intestinal disorder and outbreak of disease. This project aimed at evaluating bread sold in Lapai town for possible fungal growth/contaminant, isolating Fungi associated with bread spoilage, identify and characterized Fungi species associated with the spoilage of bread, and the proximate and physiochemical properties of the bread.

Print ISSN: 0189-9287 Online ISSN: 2992-345X

Material and Methods

Materials

The culture medium was Sabouraud dextrose agar (SDA) for culturing Fungal spp, petri-dishes, wire-loop, swabs, filter paper, weighing balance, foil paper, masking tape, distilled water, test tubes, test tube rack, beakers, conical flasks, refrigerator, incubator, autoclave, slides and coverslips, microscope.

Study area

The study area was in Lapai, Niger State, Nigeria. Water samples were collected from five (5) different points of the town.

Sample collection

Three pieces of bread was sampled from each of the five selected street vended locations within Lapai town. Samples were collected from State low-cost junction, royal suites, Badegi market, federal low-cost and GRA using sterile polythene bags and were labelled with dates and site of collection then transported to the microbiology laboratory of Ibrahim Badamasi Babangida University, Lapai for analysis.

Sterilization of Glass Ware

All glass ware such as petri dish, breakers, conical flasks, test tubes, universal bottles, were thoroughly washed with detergents, rinsed with clean water and then sterilized in an autoclave for 15minutes at 121 ^{0}C. All inoculations were carried out under aseptic condition, beside flame. The bench shelf and other surfaces were disinfected before and after each experiment work.

Preparation of Media

65g of SDA powder was weighed and dissolved in 1 liter of distilled water according to manufacturer's instruction. The agar medium was heated (not more than two minutes) by slowly stirring with constant agitation until complete dissolution. The agar medium was not autoclaved, but allowed to cool down to 45^{0}C. After which, was dispensed into each of the sterile petri dishes

Serial dilution

Serial dilution was done as follows. 1g of each sample was added into test tubes containing 10ml sterile water and these were used as stock solutions. 1ml was removed from each of the solution and added to another set of test tubes containing 9ml sterile water which made 10^{-1}. The same procedure was repeated to make 10^{-5} dilution.

Mold isolation

One milliliter (1ml) of each of the 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^{o}C) for 72 hours after which the fungal colonies that developed were counted, pure cultures were collected by repeated sub culturing and were stored in sabouraud dextrose agar (SDA) slants for isolates identification.

Identification and Characterization of Fungal isolates

The fungal isolates were identified by macroscopic examination and cultural characteristics / colonial morphology. The colony color, texture and size were observed. Also confirmed by using atlas. Microscopically, a sterile inoculating wire loop was used to pick the fungi isolate spread on a clean grease free slide and two drops of lactophenol blue dye added. Then emulsified and covered with cover slip to avoid air bubbles and then mounted on a microscope and viewed under X10 objective lens.[13]

Results

After incubation period, the total fungal count of bread samples over a storage period of four (4) days is shown below. There was no fungal count on the first two days of study for the bread samples used. However, all the samples showed positive fungal growth from the fourth day till the fifth day. Fifteen (15) bread samples were collected from five (5) different locations and were subjected to laboratory analysis. Results revealed that five (5) species of Fungi were isolated. Table 1 shows the percentage occurrence of positive and negative growth of fungal organism isolated from bread in different locations of Lapai town. Table 2 shows the percentage distribution of Fungi isolated from bread in Lapai town, where *Aspergillus* spp showed the highest percentage of 50%. While Table 3 showed the cultural and morphological characteristics of identified *Fungi* spp

Print ISSN: 0189-9287 Online ISSN: 2992-345X

TABLE 1: Percentage occurrence of positive and negative growth of fungal organism isolated from bread in different locations of Lapai town.

Location	No. of Samples	No. of Positive%	No. of Negative%
G,R.A	3(100)	3(100)	0(0)
Federal Lowcost	3(100)	3(100)	0(0)
Badegi Market	3(100)	3(100)	0(0)
State Lowcost	3(100)	3(100)	0(0)
Royal Suite	3(100)	3(100)	0(0)
Total	**15**	**15(100)**	**0(100)**

TABLE 2: Percentage distribution of Fungi isolated from bread in Lapai town.

Fungal Isolates	Number	Percentage
Fusarium spp	1	6.25
Rhizopus spp	2	12.5
Penicillium spp	2	12.5
Mucor spp	3	18.75
Aspergillus spp	8	50
Total	**16**	**100**

Table 3: Cultural and morphological characteristics of identified Fungi.

Fungal Isolate	Cultural Characteristics	Morphological Characteristics
Mucor spp	Large white colonies which turn into black later	Erect sporangiophores are formed. Sporangiophore swells ate the tip to form sporangia which are globular shaped. Columella is present
Rhizopus spp	White cottony mycelia, with black dots and covers the entire plate	Sporangiospores are produced inside a spherical sporangium. Columella is present on the top of the sporangiophore. Root-like rhizoids are found
Fusarium spp	Rapidly growing wooly to colt only lemon and yellow	Multicellular distinctive sickle shaped macro conidia
Aspergillus spp	Very common colours of colony (black and white)	Conidia borne in 360 arrangements covering the upper 2/3 of the conidiophores
Penicillium spp	Largely fluffy white colonies almost covering the whole surface	Non-septate branched hyphal enlarged at the apex to form conidophjorex they produce brownish black cerdian in chains

Discussion

From the results obtained, the fifteen (15) samples collected, were analyzed for *Fungi* spp growth on them. Meanwhile, the focus of this research work is to isolates the fungi associated in bread in Lapai town. A total number of 15 samples were collected during the course of this research. The fungal organism isolated from this study includes; *Mucor* spp, *Fusarium* spp, *Aspergillus* spp, *Rhizopu*s spp, and *Penicillium* spp. The percentage occurrence of Fungi isolates, showed that *Aspergillus* spp has the highest percentage of (50%) followed by *Mucor* spp. (18.75%), *Fusarium* spp. (6.25), *Penicillium* spp. and Rhizopus spp. both had the lowest frequency of occurrence having (12.5%) each. This is in line with a similar work by Gouse *et al.,*[14] their findings had a total number of 100% fungal isolates belonging to 5 genera namely *Mucor* spp, *Fusarium* spp, *Aspergillus* spp, *Rhizopus* spp, and *Penicillium* spp. The frequency of occurrence of Fungi in the commercial bread samples, manifest that *Aspergillus* specie appears in all the samples studied which is in agreement with Azeez *et al.*,[15] who recorded *Aspergillus* specie, occurred most frequently in the bread samples they studied, which disputes the work of Anon,[16] who recorded that *Penicillin* specie, are by far the most common molds that spoil bread.

The presence of fungi might be due to contaminations and can render the bread undesirable. The chance of contamination may also be due to the inadequate storage

Print ISSN: 0189-9287 Online ISSN: 2992-345X

and packaging which may allow dust and flies to gain their entrance into the product, and this the reason that *Penicilllium and Mucor spp,* had their occurrence in the sample.[17] As stated in the process of bread spoilage and the observation of the spoilage of the bread, aside from spoilage that could occur as a result of external environment or post bread baking handlings, it was confirmed, that, wet bread kept in dark area encourages more spoilage. This is similar to research done by Unanchukwu and Nwankanma,[18] but with different sample size. Bread is fairly dried to chew, but there is constantly some humidity in it, and that is how mold [yeast] spread all through the loaf and makes it rise. When soldiers and pirates or prospectors and pioneers were required to maintain bread for months or days, they made sure that the bread is extremely dried. Proliferations of mold on breads depend on its moisture. Dissimilar to grass or lettuce, molds do not require light in actual fact, light is capable of impeding the escalation of molds. Mold is known to exhibit fastidious growth on bread in areas without light as stated by Theresa Curry.[19] The temperature can be related to humidity. Humidity is inversely proportional to temperature, because the higher the temperature, the lower the humidity, and the lower the temperature, the higher the humidity. Statement above is by the results of research that has been done, that the higher the temperature, the lower the humidity, and vice versa.[20] Mold growth is very difficult to prevent. Physical and chemical changes can cause mold growth.

Changes that occur can be in the form of partial or complete color changes, changes in texture, changes in aroma, and changes in taste so that the bread is not fit for consumption. The fungus that grows on white bread can produce mycotoxins during the storage process. Mycotoxins (Aflaxoxin) are secondary metabolic products in fungi that can cause poisoning to humans.[21]

Implications of the findings of the study

The study underscores the importance of maintaining strict hygiene during bread production, storage, and distribution to prevent fungal contamination. It also highlights the economic burden on bakeries and the potential health risks associated with consuming mold-contaminated bread. To mitigate these risks, stakeholders—including bakers, food safety authorities, and researchers—must collaborate to implement effective preventive measures such as improved storage, regulatory enforcement, and continued research into safer preservation method.

Strength and limitations of the study

The study provides valuable insights into fungal contamination of bread, with significant implications for public health, food safety, and the bakery industry. However, limitations such as small sample size, lack of molecular identification, and absence of mycotoxin analysis highlight areas for improvement in future research. Addressing these limitations in subsequent studies would enhance the accuracy, applicability, and impact of the findings

Conclusion

Mold spoilage remains a major setback restraining the shelf-life of several high and intermediary moisture bread and bakery food, to prevent losses as a result of bread spoilage, leading to lost revenue to the baking industries. It is known that quality of bread is determine by the level of contamination approaches to manage mold growth and lengthen the shelf life of the bread is of high relevance to the baking business wherever magnified demand in world consumption exist. Further actions including good hygiene, heat treatments and customized packaging are top ways since it is believed that most bread spoilage caused by microbes s due to post contamination activities and when bread are baked under unhygienic conditions which can lead to introduction of pathogenic microorganisms to human when consumed. Therefore, it is necessary and essential to ensure that basic hygiene practices are highly adhered to during pre-baking, and post baking activities.

Declarations

Authors' Contribution: Conceptualization and design-Sani RA, Hamza TM, Legbo IM, Data Collection-Hamza TM, Sani RA, Muhammad A, Jibril FL, Data Analysis-Sani RA, Muhammad A, Ibrahim A, Ayuba SB Write up-Sani RA, Ayuba SB, Hamza TM, Muhammad A. Ibrahim A.

Conflict of interest: Authors have no conflict of interest to declare.

Funding: Nil

***Acknowledgment*:** The authors would like to acknowledge the staff of the Department of Microbiology IBB University Lapai, for their contribution during the study.

Print ISSN: 0189-9287 Online ISSN: 2992-345X

References

1. Adeparusi EO. Effect of processing nutrient and anti-nutrients of lima bean (*Phaeolus lanatus*) flour. J. Narhung. 2001; 45(2), 94-96.
2. Ekpeyang TE. Chemical composition and amino acid content of African breadfruit Trculia Africana Decne . J. Food Chem. 2005; 2(17), 59-64.
3. Arini LDD. Faktor-Faktor Penyebab dan Karakteristik Makanan Kadaluarsa Yang Berdampak Buruk pada Kesehatan Masyarakat. JITIPARI (Jurnal Ilmiah Teknologi Dan Industry Pangan UNISRI). 2017; 2(1), 15–24
4. Broto W. Status Cemaran Dan Upaya Pengendalian Aflatoksin Pada Komoditas Serealia Dan Kacang. Jurnal Litbang Pertanian. 2018; 37(2), 81-90.
5. Lawal RO and Bassir O. The nutrient composition of the Afon Diet Nigerian Cooked Testa-free seed prepared from the fruit of Treculia Africana. J. Food Chem. 2007; 4(23), 308.
6. Nwaiwu MY, Efiuvwevewere BO and Princewill TJ. Biodeterioration of African breadfruit (Treculia africana) in South eastern Nigeria and its control. J. Tropical Sci. 2000; 1(35), 22-29.
7. Leobel FC. The Complex Origin of Breadfruit Trees of plant. J. Botany. 2002; 12(4), 760-766.
8. Fiorentini C, Pianetti A, Baffone W, Fabbri A. Occurrence, diversity, and pathogenicity of halophilic *Vibrio* spp. and non-O1 *Vibrio cholerae* from estuarine waters along the Italian Adriatic coast. Appl Environ Microbiol. 1999; 65, 2748-2753.
9. Balick M. and Cox P. Plants, people and culture. J. Sci. Ethnobot. 1999; 15(3), 85-88.
10. Hocking AD. Mold and Yeast Associated with Foods of Reduced Water Activity. Ecological Interactions. In: Seow, C. C (Ed), Food Preservation by Moisture Control. Elsevier. 2008; 57–72.
11. Ejidike BN. Dietary protein requirements of the African land snail (Archachantina marginata). J. Appl.Tropical Agricult. 2001; 1(6), 111-115.
12. Chigbu LN, Iroegbu CU. *Vibrio* species from diarrhoeal stools and water environment in Cross River State, Nigeria. Int J Environ Health Res. 2000; 10, 219-228.
13. Ejiofor MA, Obiajulu OR and Okafor JC. Diversifying utilities of African breadfruit as food and feed. J. Inter. Tree Crops. 2008; 1(51), 25-134.
14. Basier O and Lawal RO. Changes in the major nutrient of the seeds of Treculia Africana during dietary preparation. J. Plant Food. 2006; 1(6), 249.
15. Edet EE, Eke OU and Ifon EI. Chemical evaluation of nutritive value of seed of African Breadfruit. J. Food Chem. 2002; 2(17), 41-47.
16. Mizana KD. Identifikasi Pertumbuhan Jamur Aspergillus Sppada Roti Tawar yang Dijual di Kota Padang Berdasarkan Suhu dan Lama Penyimpanan. Jurna Kesehatan Andalas. 2016; 5(2), 355–360.
17. Lestari AD. Identifikasi Jamur Pada Roti Yang Dijual Di Kota Langsa Berdasarkan Lama Penyimpanan. Journal Jeumpa. 2019; 6(2), 245-256.
18. Adeparusi EO. Effect of processing nutrient and anti-nutrients of lima bean (Phaeolus lanatus) flour. J. Narhung. 2001; 45(2), 94-96.
19. Dharmaputra OS. Kualitas Fisik, Populasi *Aspergillus flavus*, dan Kandungan Aflatoksin B1 pada Biji Kacang Tanah Mentah. Jurnal Fitopatologi Indonesia. 2013; 9(4), 99-106
20. Gouse B, Ali Ismail A, Zafer S and Omar B. Assessment of Bacteria and Fungi in Air from College of Applied Medical Sciences (Male) at AD-Dawadmi, Saudi Arabia. Int. Res. J. Biol. Sci. 2015; 4(9), 48-53.
21. Hasanah U. Mengenal Aspergillosis, Infeksi Jamur Genus *Aspergillus*. Jurna Keluarga Seha Sejahtera. 2017;15(2), 76–86.

Print ISSN: 0189-9287 Online ISSN: 2992-345X