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Research Article

Comparative Analysis of Shelf Life and Fungal Spoilage of Different Brands of Bread Sold within Dutsin-Ma Metropolis

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ABSTRACT

The study aimed to compare the shelf life and fungal spoilage of different brands of bread sold within Dutsin-Ma Metropolis. Nine brands of bread were purchased from different vendors within Dutsin-Ma metropolis and were transported in sterile polyethylene bags to the Microbiology Laboratory of Federal University Dutsin-Ma for analyses using standard procedures. The bread samples were stored at various storage conditions such as room temperature, sun exposure, and refrigerator, respectively, for a specified period of 7 days from day 1 to day 7, and samples were taken at each interval to measure the quality of the bread. At each interval, the shelf life of the bread was evaluated based on the color, texture, smell, and taste of the bread. Bread samples stored under ambient temperature had the highest contamination, while samples stored under the sun had less fungal growth and samples kept in a refrigerator showed no fungal growth at all. Analysis of different nine (9) brands of bread yielded fungal species such as *Aspergillus niger*, *Rhizopus stolonifer*, *Fusarium* and *Penicillium* species. *Aspergillus niger* was found to have the highest percentage occurrence (28%), while *Fusarium* spp and *Trichophyton rubrum* had the least percentage occurrence (8%). It is recommended that bakery processes should ensure proper hygiene during the manufacturing process to minimize microbial contamination, especially moulds. People are also advised to buy modern-produced breads for their consumption which is thought to have improved quality standards than the locally produced ones.

Keywords: Bakery, Bread, Fungi, Shelf Life, Spoilage, Water Activity

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INTRODUCTION

Depending on national and regional traditions, bread can take many different forms and be consumed by a large number of people in Nigeria and overseas. According to Melini and Francesca (2018), bread can be found in commercially packaged sliced bread or freshly prepared daily by bakers. According to the Australian Bureau of Statistics, bakery products include cakes, biscuits, gingerbread, pies, crumpets, and breads (both leavened and unleavened).

Nigerian bread production and consumption habits varies greatly from one another (Association of Plant Bakers, 2017). In most Northern states of Nigeria, such as Katsina, Kano, Kaduna, etc., where bread is primarily consumed as "gurasa" (locally baked bread), baked bread account for the largest portion of the country's food traditions. Countless industrial bakeries have also emerged in response to the growing demand for sliced and wrapped bread, providing a significant source of income for many of their patrons. According to the International Association of Plant Bakers (AIBI, 2018), bread is readily ruined, and as it is preserved, its quality and palatability decline along with its physiological, biochemical, sensory, and microbiological properties. According to Garcia *et al*.'s 2019 research, bread and other products' elevated water activity and slightly acidic pH are important elements that are closely linked to fungal deterioration and restrict the growth of other microorganisms. Mold and fungal degradation are the primary causes of significant

financial losses in packaged bread goods. They might also contain mycotoxins, which would be extremely detrimental to bakeries' profits and risk to public nbfc.vu83wklskae38mhealth. Penicillium (Penicillium *chrysogenum*, *Chrysonilia sitophila, Penicillium brevicompactum), Aspergillus, Eurotium, Penicillium roqueforti, Monilla, Wallemia,* and other common molds like *Rhizopus* and *Mucor* are the main fungal genera that cause bakery products to spoil (Rahman *et al*., 2022; Saranraj and Geetha, 2012). Yeasts can also be the source of the "chalk mold" issue; *Saccharomycopsis fibuligera* and *Hyphopichia burtonii* are the main culprits (Garcia *et al*., 2019).

The apparent development of mold, the invisible generation of mycotoxins, and the formation of off-flavors, which may occur even before visible fungal outgrowth, are the three main signs of microbiological spoilage caused by fungi (Melinie *et al*., 2017). The issue of spoiled bread is concerning since it leads to significant food waste (5–10% of global bread production losses), financial losses for the bakery sector and consumers (Melikoglu *et al*., 2011) and intoxication of humans from fungal mycotoxins. In actuality, these latter are frequently linked to some acute and chronic illnesses in people (Oliveira *et al*., 2014).

There is a shortage of data regarding a comparative analysis of the shelf life and susceptibility to fungal spoilage among various brands of bread sold particularly in Dutsin-Ma and Katsina State at large. This implies that there is limited or insufficient knowledge about how different bread brands are consumed in the Dutsin-Ma metropolis in terms of their longevity and vulnerability to fungal deterioration. This study was, therefore, conducted to address this void and to provide valuable insights into the shelf life and fungal spoilage characteristics of different bread brands sold in Dutsin-Ma as well as to help bread industries in making informed decisions about the quality of various bread and other bakery products available in the town, and their patronage by consumers.

MATERIALS AND METHODS

Study Area

The study area of research was Dutsin-Ma Metropolis, Katsina State. Dutsin-Ma Local Government lies between the latitude of 12°17.00N' to 12°17.84 and longitude 007°26'E.

Sample Collection

A total of nine (9) different brands of bread were purchased from different vendors within Dutsin-Ma metropolis, Katsina State and transported in a sterile polyethylene bag to the Microbiology Laboratory of Federal University Dutsin-Ma for analyses.

Determination of Shelf Life of Bread Samples by Organoleptic Observation

Different brands of bread purchased were examined organoleptically to determine their shelf life based on their various conditions of storage. Each of the bread samples was stored at three (3) different storage conditions for a period of seven (7) days. The storage conditions were Sun exposure, ambient temperature exposure, and refrigeration. Samples were analyzed at each day of storage till day seven (7) to assess the shelf-life of the breads.

The observation was done using the sensory organs like smell, texture, and colour change after each day of storage, the texture, visible growth/colour, and the odour of the bread samples were observed and recorded respectively, according to guidelines described by Alexander (1999) and Dubey and Maheshawi (2004).

Isolation of Fungi

A normal saline solution was prepared and 9ml of this solution was dispensed into separate test tubes. Next, 0.5 grams of each bread sample was homogenized in 5 ml of prepared normal saline solution. These homogenized samples were then serially diluted using the Dubey and Maheshawi (2004) method, generating dilutions of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}, respectively.

The lowest dilution (10^{-5}) was swabbed unto prepared Sabouraud Dextrose Agar (SDA) plates for growth, while the 10^{-4} was inoculated on SDA agar plates for colony count which was used to assess the level of contamination of the bread samples.

Identification of Fungal Isolates

Both macroscopic and microscopic methods were employed to identify the fungal isolates. A drop of lactophenol blue stain was applied to the inoculum on a grease-free, clean slide; a small amount of the fungal culture was emulsified on the slide and covered with a coverslip to prevent bubbles; the slide was then examined under a microscope; additionally, a slide culture of the fungal isolate was made and observed under a microscope using the low power

objective (10X) and high-power objective (40X). The cultural characteristics observed were compared with those found in the color atlas of Kaminski (2009) and Sarah *et al*. (2016).

RESULTS

Table 1 shows organoleptic observation of the bread samples. Bread sample stored under the sun and in ambient temperature showed visible fungal growth from day four (4) and changes in both odor and texture while bread samples stored in the refrigerator revealed no visible fungal growth down to day seven (7) which signifies that refrigeration is the best temperature of storage for extension of bread shelf life.

Table 2 shows shelf life of bread under different storage conditions, it revealed that refrigeration has the highest shelf life compared to those stored under the sun and in room temperature. Signifying that refrigeration has the highest shelf life of bread samples.

The examination of each bread sample for evidence of fungal growth revealed a progressive deterioration pattern. As the storage period increased, there was a corresponding increase in fungal load (count), indicating worsening spoilage. Notably, by the seventh day of storage, all examined samples exhibited their highest fungal counts. This trend persisted across samples subjected to different storage conditions, from refrigeration to exposure to sunlight, with those stored at room temperature exhibiting the highest contamination levels. The level of deterioration for each bread sample is presented in Table 3.

Table 4 shows fungal organisms isolated and their cultural and morphological characteristics. The distribution of fungal isolates in different brands of bread is presented in Table 5. *Aspergillus niger* had highest percentage occurrence (28%), followed by *Aspergillus flavus* and *Penicillum* spp. (20%), while *Trichophyton rubrum* and *Fusarium* spp. had the lowest incidence frequency (8%).

Table 1: Organoleptic Observation at Different Storage Conditions

Condition of storage	Day1	Day2	Day3	Day4	Day5	Day6	Day7
Ambient temperature	No growth, no change in odour and texture	No growth, no change in odour and texture	Growth, slight change in odour and texture	Growth, change in odour and texture	Growth, change in odour and texture	Growth, change in odour and texture	Growth, change in odour and texture
Under the sun	No growth, no change in odour and texture	No growth, no change in odour and texture	No growth, no change in odour and texture	Growth, change in odour and texture	Growth, change in odour and texture	Growth, change in odour and texture	Growth, change in odour and texture
Refrigeration	No growth, no change in odour and texture	No growth, no change in odour and texture	No growth, no change in odour and texture	No growth, no change in odour and texture	No growth, no change in odour and texture	No growth, no change in odour and texture	No growth, no change in odour and texture

Table 2: Shelf Life of Bread under Different Storage Conditions

Storage Condition	No. and Percentage of Samples Showing Growth (%)						
	Day1	**Day2**	**Day3**	**Day4**	**Day5**	**Day6**	**Day7**
Ambient temperature	1(11.1)	2(22.2)	6(67.0)	8(89.0)	9(100.0)	9(100.0)	9(100.0)
Under the sun	0(00)	1(11.1)	5(55.5)	9(100.0)	9(100.0)	9(100.0)	9(100.0)
Under refrigeration temperature	(00)	(00)	(00)	(00)	(00)	(00)	0(00)

Table 3: Fungal Counts of Samples Stored under three Different Conditions

Samples	Mean Fungal Count under the Different Storage Conditions (×10-^{5})		
	Sun	Room temp.	Fridge
Sample 1	122.0	280.0	0.0
Sample 2	93.0	TNTC	0.0
Sample 3	24.0	33.5	0.0
Sample 4	7.5	19.0	0.0
Sample 5	11.5	20.0	0.0
Sample 6	13.0	25.0	0.0
Sample7	15.0	67.0	0.0
Sample 8	11.5	33.0	0.0
Sample 9	5.0	12.0	0.0

Table 4: Cultural and Morphological Characteristics of Isolated Moulds

Cultural characteristics	Morphological characteristics	Isolate
Spore heads are often easily apparent and the spores are puffy, black, and spreading	Conidia covering the top two thirds of the conidiophores are borne in 360 configurations	*Aspergillus niger*
Greenish-olive alongside white	Conidia covering the top two-thirds of the conidiophores are borne in 360 configurations.	*Aspergillus flavus*
Extremely glabrous, downy, white to cream, and white to cream in color, with a little yellow-brown reverse pigment	Most colonies have a white to cream color, are suede-like to downy, and are flat to slightly elevated. The reverse pigment may be yellow-brown to wine-red	*Trichophyton rubrum*
Huge, fluffy, white colonies that eventually turn black as a culture ages	Dark pear-shaped sporangium on hemispherical columella; non-septatehyphal; upright sporangiophore joined by stolon and Rhizopus	*Rhizopus stolonifer*
Huge fluffy white colonies nearly completely round the surface	Conidiophores, which are brownish-black chains of Ceridian produced by non-septate branched hyphae that grow at the apex	*Penicillin* spp.
Wooly grows quickly, only producing lemon and yellow colt	Remarkable sickle-shaped macro conidia that are multicellular	*Fusarium* spp.

Table 5: Distribution of Fungal Isolates in Different Brands of Bread

Isolate	Frequency n	Percentage n (%)
Aspergillus niger	7	7(28.0)
Aspergillus flavus	5	5(20.0)
Rhizopus stolonifer	4	4(16.0)
Trichophyton rubrum	2	2(8.0)
***Fusarium* spp.**	2	2(8.0)
***Penicillium* spp.**	5	5(20.0)

DISCUSSION

In this study, it was established that different storage conditions could influence the spoilage of bread. Samples stored under ambient temperature were observed to have higher contamination due to the facts that fungal growth is most enhanced at room temperature, while those stored under sun had less fungal growth simply because sun is less suitable for fungi growth and samples kept in refrigerator showed no fungal growth at all due to the low temperature of storage making it unsuitable for fungal growth. Analysis of nine different brands of bread revealed *Aspergillus niger*, *Rhizopus stolonifer*, *Fusarium* spp. and *Penicillium*. Among the various species of fungi isolated in the study, *Aspergillus niger* was found to have the highest percentage occurrence (28%), followed by *Aspergillus flavus* and *Penicillum* spp. (20%). The highest percentage occurrence of *A. niger* recorded in the study correlates with the findings of Zainab *et al*. (2022), in their study conducted in Kogi

State, Nigeria. *Trichophyton rubrum* and *Fusarium* spp. had the lowest incidence frequency, at 8%. This is consistent with research done at Hislop College in Nagpur by Meraj-ul-haque*et al*. (2017), who examined bread rotting at various temperatures and following incubation. There was no fungal count on the first two days for the samples that were used, according to the total fungal counts of bread samples over a seven-day storage period. Some samples that were kept in the refrigerator on the third day of the investigation did not contain any fungi. In Enugu State, Nigeria, a study by Unachukwu and Nwakanma (2015) identified organisms linked to bread rotting, including *Rhizopus* species, *Aspergillus* species, *Mucor* species, *Penicillium* species, and *Fusarium* species. When compared to samples kept outside under the sun, it was discovered that samples kept at ambient temperature exhibited fungal growth that was comparable to what their investigations had shown. This may be because Katsina State receives more sunlight than Enugu State does. *Penicillium chrysogenum, Rhizopus stolonifer, Aspergillus niger, Fusarium oxysporum,* and *Mucorhiemalis* were the isolated organisms.

CONCLUSION

Based on the findings of the study, it can be concluded that the storage conditions significantly impact the susceptibility of bread to fungal spoilage. *Aspergillus niger* was found to be the main spoilage fungus of bread sold within Dutsin-Ma metropolis. The results demonstrated that bread stored under ambient temperature exhibited a higher vulnerability to fungal growth, with positive results observed from the fourth to the seventh day, while bread stored under sunlight showed lower susceptibility to fungal spoilage compared to ambient conditions. The refrigerator storage condition proved to be the most effective in inhibiting fungal growth, as evidenced by the absence of visible growth up to the eighth day, thus, it happened to be the most preferred storage condition, providing the longest shelf life of bread of approximately 7 days.

Author's Contribution

The study was conceived by Mzungu and Ogbo, and the experiments were designed and performed by Ogbo. Data analysis and interpretation was done by Umar and Mzungu. Umar drafted the manuscript. The final manuscript draft was read and approved by all the authors.

Conflict of Interest

The authors declared that there was no conflict of interest.

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