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Research Article

Fungal Species Associated with Spoilage of Bakery Products in Dutse Metropolis, Nigeria

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ABSTRACT

Bakery products are easily prone to fugal contamination because they are rich source of sugar and can easily hold moisture when in poly bags, this situations or conditions are favourable to fungal growth and multiplication. The study aimed to identify the bakery products fungal contamination rate in the study area. As methodology, three types of bakery products (bread, doughnut and meat pie) were collected for the research. They were analysed using microscopy and culture methods for the identification of possible fungal species associated with spoilage of these important products. The finding showed that, *Aspergilus flavus* was the highest contaminant across the three products (5 isolates), followed by *Aspergilus fumigatus* (3 isolates), *Aspergilus niger* (2 isolates), *Mucor heimalis* (3 isolates), *Penicilium* species (2 isolates), while *Rhizopus stolonifera* had (only 1 isolates), Comparison of the contamination rate of the products showed that bread was the most readily spoiled by fungi while meat pie had the least spoilage rate. The percentage contamination of the products showed that bread had 56.25%, doughnut 25.0% while meat pie had 18.75%. We can base on this research finding conclude that bakery products are prone to contamination by variety of fungal species and bread is easily contaminated than other products.

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INTRODUCTION

Bakery products like bread, doughnut and meat pie are significant food items people acknowledged world over, serving as an exceptionally helpful food types wanted by large categories of human population including the rich and poor in both the rural and urban areas. Bread and other pastries or bakery products undergo different deterioration issues, whether (physical, chemical or microbial). Various organisms are involved in meat pie, bread, dough nut, and other bakery products spoilage (Olufunmilayo *et al*., 2020).

Bakery products are the important staple foods in most countries and cultures. Among them breads, doughnut, and meat pie are among the commonness. Mycotoxins are among the common secondary metabolites of fungi associated with human, which are cheaply found in foods such as bakery products. They also have the potential to cause diseases in humans. The mycotoxins causing human and animal diseases include citrinin, aflatoxin, flumonisins, ochratoxin A, ergot alkaloids, patulin, and trichothecenes (Olufunmilayo *et al*., 2020). Microorganisms usually plays a vital role in baked products flavoring, but at the same time play major role in the spoilage of these products if proper preparation is not observed (Saeed *et al*., 2019).

The fungal spoilage is a key issue in bakery products, although its impact from the safety point of view is relatively shallow, but still the mere presence of visible colonies on the products affect the image of the product leading to economic losses in both the medium and long term intervals (Marín *et al*., 2007). Bread making is regarded as dynamic system that under goes various modifications linked to: chemical (rancidity, changes in nutraceutical value); physical (staling, moisture redistribution); and microbiological (yeast, mold and bacterial spoilage) which induce the staling process. Staling limits bread shelf life (length of time, under defined storage conditions, during which food remains "acceptable" for the consumer). Acceptable reflects to not only being safe, but also being able to retain desired physical, sensory, chemical, and biological characteristics it originally possesses (Patil and Kukade, 2020).

Food industries has developed strategies including the use of high-quality raw materials and the prevention of post-baking contamination because fungal colonies can appear in bread and meat pie and other baked foods formulations, causing consumers rejections which leads to economic losses (Smith *et al*., 2004).

For human health, fungal component of concern in food, is the possibility of mycotoxins, as such consumers removing visibly moldy part of spoiled bread to consume the rest of the product are making serious mistake, this is because the hyaline mycelium of the fungi may have penetrated in the interior of the loaf, releasing secondary metabolites which may include mycotoxins and other molecules with negative biological activity to human (Garcia *et al*., 2019).

Many filamentous fungi such as *Rhizopus, Mucor, Aspergillus* and *Fusarium* are involved in spoilage of bakery products due to improper handling, improper sanitation, and the losses of these products due to these fungi vary between 1.5% depending on seasons, type of products and methods of processing (Patil and Kukade, 2020). There is lack or low level of research on these organisms associated with bakery products in Dutse metropolis.

MATERIALS AND METHODES

Study Area

This research was conducted in Rasheed Shekoni Federal University Teaching Hospital Dutse. The hospital is located within Dutse metropolis, Jigawa State which has the population of 4,361,002 and has the latitude: 11^0 45' 22.25''N and longitude: 9^o 20' 20.26''E (Ado, 2009).

Study Population

The study population/items include, bakery products from Fatima Bread Factory, Hasinah Restaurant and Mai goro Shops within Dutse metropolis, Jigawa State.

Sample size Determination

The sample size of this study was determined using the formula (Cochran, 1977).

$$n = \frac{Z^2 pq}{d^2}$$

Where;

n = sample size

z = statistic for level of confidence at 95% = 1.96

p = Fungal prevalence in bakery products; bread, meat pie and dough nut at 5% (Williams *et al.,* 2020*)*

d = allowable error of 5% (0.05)

q = (1 – 0.05) = 0.95

$$n = \frac{3.8416 \times 0.05 \times 0.95}{0.0025}$$

$$n = 72.96 \sim 73$$

Considering an attrition rate of 30%, the sample size was determined to be 94.8= 95

Sample Collection and Processing

The bakery products were aseptically collected randomly and put into polythene bags from each of the three different locations (Fatima Bread Factory, Hasinah Restaurant and Mai goro Shops) with 30 samples from Fatima bread, and also 30 each from restaurants and mai goro shops, all within Dutse Metropolis.

The samples were placed into sterilized specimens transport bag and been transported to Microbiology Laboratory in Rasheed Shekoni Federal University Teaching Hospital in Dutse metropolis, Jigawa State Nigeria, for analysis.

Macroscopy

The specimens of bread, dough nut and meat pie brought from the selected areas were observed, physically for colour changes of the products, and visible fungal growth.

Microscopy

Direct Gram Staining Technique

Smear preparation

This was done by rubbing surface of the sample with saline wet swab sticks and making a smear on a clean grease free glass slide. It was allowed to air dry before been fixed by passing the slide three times over a bunsen flame with the smear pacing upward. The smear was allowed to cool and stained according to Gram's staining technique (Colco, 2005).

Staining Procedure

The dried smear was placed in horizontal position on the staining rack and was flooded with crystal violet solution for one minute, rinsed with water and then drained. It was flooded with Gram's iodine solution for one minute, rinsed with water and then drained. The preparation was decolorized with acetone briefly, rinsed with water and drained, before been counterstained with neutral red for one minute. It was rinsed with water, then the underside of the slide was cleaned with cotton wool and placed on rack to dry. The slide was examined using (40x) to screen, before using oil immersion objective (100x).

Potassium Hydroxide preparation (KOH)

Procedure

A drop of KOH was placed at the center of a clean dry grease-free glass slide. The samples were placed in the KOH with the use of teasing needles to produce a thin homogeneous KOH preparation. It was covered with a cover slip. The preparations were allowed to stands at room temperature for 15minutes. The slides were heated gently by passing it through a bunsen flame several times but not overheated. The preparations were examine using (10×) and (40×) objectives (Ochei and Kolhatkar, 2005).

Cultural Method of Isolation

This was done by making primary inoculum with wet swab stick containing the specimens rubbed from each product, then followed by streaking using sterile inoculation loop on to the surfaces of Sabouraud Dextrose Streptomycin Agar. The cultured plate was incubated at 28 ºC for 5 days for moulds growth and 37ºC for 24 hours for yeast (Sykes and Rankin, 2014). After incubation period, each colony from the overnight growth was also sub-cultured again onto Sabouraud Dextrose Agar (SDA) and incubated at 37 ^{o}C for 24 hours, for purity plate (Sykes and Rankin, 2014).

Lactophenol Cotton Blue Staining Technique

In this case fungal colonies after incubation were place in few drops of lactophenol cotton blue stain on a clean grease free glass slide, covered with cover slip and were examined for microscopic features of multicellular fungi like *Aspergillus* species (Wanger *et al*., 2017).

Statistical Analysis

Data obtained were subjected to descriptive statistical analysis using SPSS version 23.0 Chi-square, was used to determined association and values obtained were considered statistically significant at $p \leq 0.05$ at 95% confidence interval

RESULTS

Out of 90 samples examined, 16 representing 17.8% prevalence were found to be positive. Table 1, provides information on the fungal species associated with the spoilage of bakery products, presenting both the frequency and percentage of each fungal species observed. No growth accounts for the highest observation, with 74 instances, constituting 82.2% of the total observations. This category represents situation where no fungal growth was observed in the bakery products.

For the positive samples, *Aspergillus flavus* was observed in 5 samples, representing 5.6% of the total observations, *Aspergillus fumigatus* accounted for 3.3%, *Aspergillus niger* 2.2%, *Mucor heimalis* 3.3%, *Penicillium notatum* 2.2%, while *Rhizopus stolonifera* had only 1.1%. This finding revealed that, the most commonly observed fungal species associated with spoilage of bakery product is *Aspergillus flavus*, followed by other species (*Aspergillus fumigatus*, *Mucor heimalis*, *Aspergillus niger*, *Penicillium notatum*, and *Rhizopus stolonifera*) respectively.

Table 1: The Fungal species Associated with Spoilage of Bakery Products

Fungal species	NE(90)	NP	Frequency (n)	Percentage (%)
No growth	--	0	74	82.2
Aspergillus flavus	--	5	5	5.6
Aspergillus fumigatus	--	3	3	3.3
Aspergillus niger	--	2	2	2.2
Mucor heimalis	--	3	3	3.3
Penicillium notatum	--	2	2	2.2
Rhizopus stolonifera	--	1	1	1.1
Total	**90**	**16**	**90**	**100**

Key: NE = Number examined NP = Number of positive samples

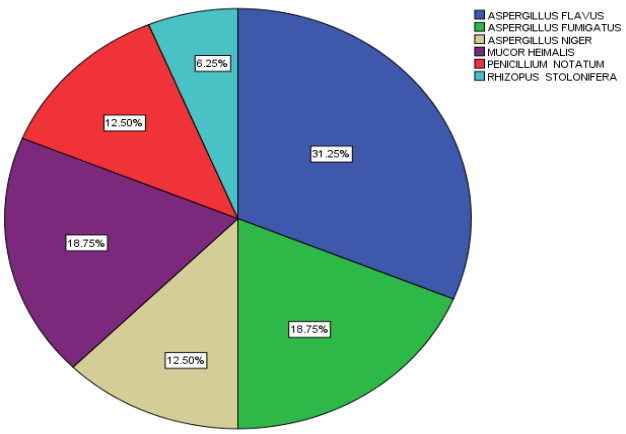

Figure 1: Fungal species associated with spoilage of bakery products

Of the bakery products samples used in the study, bread had 9(30%) positive, meat pie had 3(10%) doughnut had 4(13.3%) of fungal species associated with spoilage of bakery products respectively.

Table 2 presents the percentage occurrence of fungal species associated with the spoilage of bakery products (bread, meat pie, and doughnut). For negative fungal growth on the samples, bread had 70%, while meat pie and doughnut had 90% and 86.7% respectively. About the positive fungal growth, *Aspergillus flavus* were found in 10.0% of bread samples and 6.7% of doughnut samples, *Aspergillus*

fumigatus was found in 6.7% of bread samples and 3.3% of doughnut samples, *Aspergillus niger* was found in 6.7% of bread samples only, *Mucor heimalis* were found in 3.3% each for bread, meat pie, and doughnut samples. *Penicillium notatum* was found in 3.3% of bread and meat pie samples while *Rhizopus stolonifera* were found in 3.3% of meat pie samples only. A p-value of less than or equal to 0.05 was considered statistically significant. In this case, the p-value of 0.396 observed indicates that there is no statistically significant difference in the distribution of fungal species among the three types of bakery products. Overall, the table 2 helps determined the most prevalent fungal species (*Aspergillus flavus*) associated with bakery products spoilage.

Table 2: The Percentage Occurrence of Fungal Species with Respect to the Different Products

Fungal growth	Bread (n, %)	Meat pie (n, %)	Doughnut (n, %)	p-value *
No growth	21 (70.0%)	27 (90.0%)	26 (86.7%)	0.396
Aspergillus flavus	3 (10.0%)	0 (0.0%)	2 (6.7%)	
Aspergillus fumigatus	2 (6.7%)	0 (0.0%)	1 (3.3%)	
Aspergillus niger	2 (6.7%)	0 (0.0%)	0 (0.0%)	
Mucor heimalis	1 (3.3%)	1 (3.3%)	1 (3.3%)	
Penicillium notatum	1 (3.3%)	1 (3.3%)	0 (0.0%)	
Rhizopus stolonifera	0 (0.0%)	1 (3.3%)	0 (0.0%)	
Total	**30 (100.0%)**	**30 (100.0%)**	**30 (100.0%)**	

Key: * = Chi square test, P value ≤ 0.05 is statistically considered significant

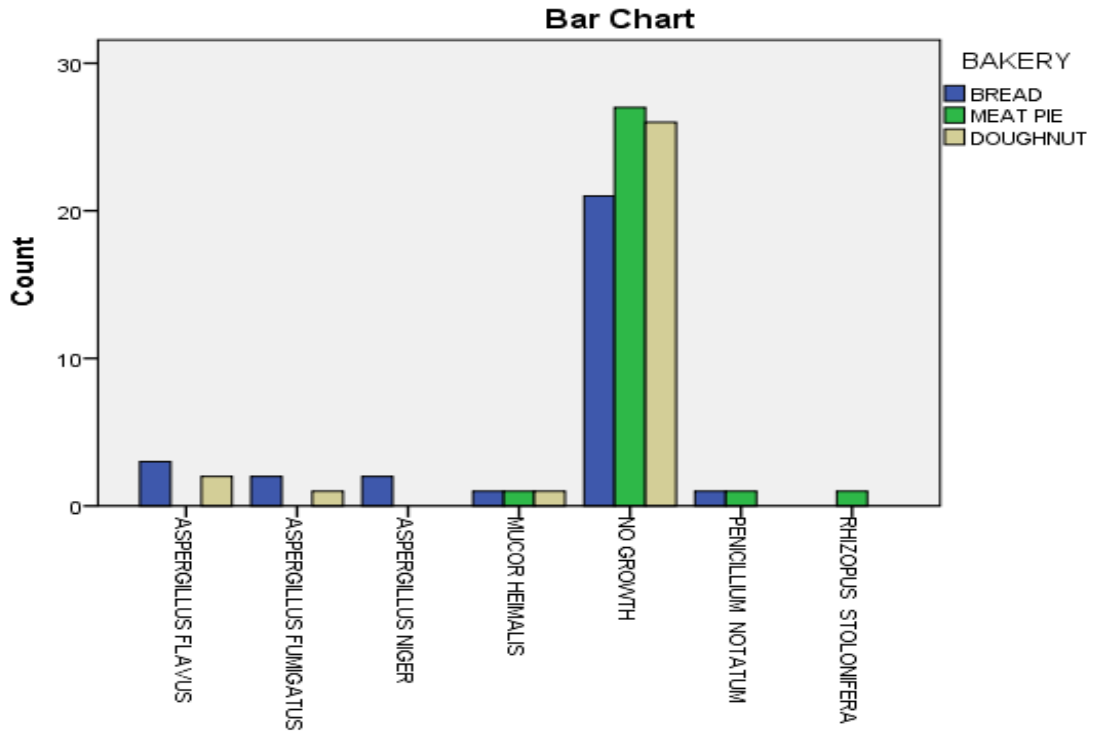

Figure 2: Fungal species associated with spoilage of bakery products

The table 3 presents data on the frequency and percentage distribution of contamination among the selected baked products in the study area. Among them, bread had frequency of contamination of 9(56.25%). Doughnut had 4(25.0%) while meat pie had 3(18.75%).

Table 3: Contamination rate among the selected baked products

Bakery products	NE(90)	NP	Frequency (n)	Percentage (%)
Bread	--	9	9	56.25
Meat pie	--	3	3	18.75
Doughnut	--	4	4	25.0
Total	**90**	**16**	**16**	**100.0**

KEY: NE = Number examined, NP = Number of positive samples

DISCUSSION

According to the findings, the overall contamination rate of the bakery products by fungal species was 16(17.8%). This is not in agreement with the findings of Patil and Kukade, (2020), conducted in Akola Maharashtra State, India. Who reported nineteen 19(82.6%), contamination rate by fungal species. The variation may be due to the different in samples size, method employed or due to geographical differences. The most commonly observed *fungal* species associated with spoilage of bread were *Aspergillus flavus* (10.0%), *Aspergillus fumigatus* (6.7%), and *Mucor heimalis* (3.3%). *Aspergillus flavus* (10.0%), emerged as the predominant species, followed by *Aspergillus fumigatus* (6.7%), *Mucor heimalis* (3.3%) and *Penicillium notatum* (3.3%). This is in agreement with the findings of Olufunmilayo *et al*. (2020) in Port Harcourt Metropolis Nigeria who reported *Aspergillus flavus, Aspergillus fumigatus, Mucor heimalis* and *Penicillium notatum* among the fungal species associated with spoilage of bread, as the most contaminated bakery product in their study.

These findings are consistent with other previous researches indicating that *Aspergillus* species are frequently implicated in the spoilage of bakery products due to their ability to produce toxins and degrade food components. It is also, in agreement with the findings of Okonkwo *et al*. (2020) conducted in Enugu state, Nigeria who reported *Aspergillus* species (15.15%), *Penicillium* species (9.09%), and *Mucor* species (33.33%) among the fungal isolates associated with spoilage of breads in their study.

Interestingly, a substantial proportion of samples showed no fungal growth, suggesting either low levels of contamination or other factors influencing spoilage in bakery products. This variability in contamination levels underscores the importance of implementing stringent hygienic practices and monitoring protocols in bakery facilities to mitigate the risk of fungal contamination and ensure food safety. This is in line with the findings of Uraz, in (2020), who earlier reported that *Aspergillus niger* and *Aspergillus flavus* among others produce mycotoxins which can cause food intoxication in human beings and animals.

The commonly observed fungal species associated with the spoilage of meat pie in the study were *Mucor heimalis* (3.3%) *Penicillium notatum* (3.3%) and *Rhizopus stolonifera* (3.3%) which are not in agreement with the findings of Mitchell *et al*. (2022), conducted in Remo local government areas of Ogun State, Nigeria, who reported *Aspergillus* species (12.5%), *Altenaria* species (4.2%) and *Fusarium solani* (4.2%), as their fungal isolates and others bacterial species associated with spoilage of meat pie in their study area.

Is also not in agreement with findings of Fletcher *et al*. (2023), in Lafia Nasarawa State, Nigeria who conducted their research on microbial spoilage of meat pie, and found *Aspergillus* species (30%) and *Penicillium chrysogenum* (26.7%), as their only fungal isolates.

On species contamination rates, *Aspergillus flavus* was found in 6.7% of doughnut samples, *Aspergillus fumigatus* in 3.3% and *Mucor heimalis* also in 3.3% of the samples. Therefore, doughnut was the second most contaminated bakery products in the study area. This is in agreement with findings of Okafor *et al*. (2022) in Lagos State, Nigeria who reported *Aspergillus flavus* (5.0%) and *Aspergillus fumigatus* (4.5%), among the fungal isolates and others bacterial species associated with spoilage of snacks such as dough nut among other samples of the research they conducted in their study area.

Furthermore, the analysis of contamination patterns among different types of bakery products revealed that bread was the most contaminated with (56.25%), followed by doughnut with (25.9%) and meat pie with (18.75%). While bread exhibited the highest

frequency of contamination, doughnut and meat pie also showed significant levels of fungal growth, but to a lesser extent. These findings highlight the need for targeted interventions to address contamination risks specific to each type of bakery product. This could include optimizing storage conditions and implementing sanitation protocols tailored to their production processes.

Conclusion

We can conclude based on the results that different *fungal* species (from both moulds and yeasts) are associated with contamination of bakery products in the study area. This provides valuable data on organisms associated with spoilage of bakery products in the study area.

Recommendations:

The primary outcome of the result show a low overall contamination rate with *Aspergillus flavus* as the most frequent contaminant. Also due to the following limitations (small sample size, limited geographic scope, inappropriate incubation temperature, reliance only on morphological identification), there is need for cautious interpretation of this results, as such future studies with larger samples and molecular confirmation would greatly assist. As such we recommend (improved hygiene in bakeries, routine monitoring, and better storage practices).

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