Determination of Microbiological Quality of Bread and Sanitation Conditions of Local Bakeries in Aliero Town, Kebbi State

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Abstract

Several variations and changes can occur during bread processing and storage that affect the quality and safety characteristics and acceptability of the bread. The study aimed to assess the microbiological quality of bread and the hygienic conditions of local bakeries in Aliero, Kebbi State. A total of 16 bread samples were obtained (3 from each bakery). Data on socio-demographics and sanitary conditions were collected through interviews and an observation checklist. Standard microbiological methods were used to enumerate, isolate and identify bacteria and fungi. The result of sanitary conditions shows that four bakeries (66%) use boreholes as a source of water supply, two (33%) use well and none of them use pipe borne. In addition, zero (0%) use a flush system of latrine, 66% have a pit latrine while 33% have no latrine facility. All (100%) have stored refuse also and adequately use open surfaces/ditch as a waste disposal method. The result of the study indicated the mean bacterial count (CFU/g) range is 7.4×10^5- $14.4x10^5$, while the mean fungal count (CFU/g) range is 9.0×10^3- 1.1×10^3. Isolated bacteria include Escherichia coli (29.4%), and Pseudomonas spp. (14.7%), Proteus spp. (20.6%), and Bacillus spp. (35.3%). Penicillium spp. (44%), Aspergillus spp. (8%), Rhizopus spp. (28%), Fusarium spp. (20%) were the fungi isolated and identified. Several types of pathogenic and non-pathogenic microorganisms were found to contaminate bread samples in the bakeries. Therefore, relevant regulatory agencies should ensure compliance with the guidelines and requirements for bread manufacturing. In addition, Educational programs have been recommended.

Keywords— Bread contamination, Sanitary condition, Microbiological quality, Total bacterial count

1. INTRODUCTION

Baked products especially bread have been a human diet component from ancient times (10,000 BC) and are among the world's most popular staple foods (de Sousa et al., 2021). Every year, more than 9 billion kg of baked products are produced (Cho & Peterson, 2010), with an average annual consumption of 41 to 303 kg (Nanditha & Prabhasankar, 2008). It is high in protein, complex carbohydrates (mostly starches), fiber, vitamins (particularly B vitamins), and minerals (Cauvain & Young, 2007). All dietary recommendations encourage eating bread, with bread and grain-based foodstuffs being the foundation of the food pyramid (de Sousa et al., 2021).

Bread is a loaf prepared of baking dough made from flour, salt, sugar, yeast, and water. Other components may include fat, milk, milk solids, egg, and anti-oxidants, among others. Bread is an essential staple in Nigeria, with a stable and rising consumption. It is, however, quite costly due to the usage of imported wheat flour(Edema et al., 2004). Bread is widely consumed in both developed and developing cultures(Abdelghafor et al., 2011). Bread has become one of the most popular nonindigenous meals in India, while bread has surpassed rice as the second most popular nonindigenous diet in Nigeria(Das et al., 2012). It is popular in most households, restaurants, and hotels (Emeje et al., 2008). White bread is the most popular bread in Nigeria. This bread is baked

using refined whole wheat flour, commonly known as all-purpose flour, and it has a characteristic white color since the wheat bran has been removed. Protein meals are usually costly and out of reach for the majority of people in developing countries like Nigeria (Malomo et al., 2012).

The safety, acceptability, and quality attributes of the bread can all fluctuate and vary throughout the baking and storage stages. One of these changes is microbial spoilage, such as that caused by mold, bacteria, and yeast. Aspergillus, Fusarium, and Penicillium are the most common molds that spoil bread (Gerez et al., 2009). Other spoilage molds have been described and identified in wheat bread products, including Cladosporium, Mucorales, and Neurospora (Pateras, 2007). The main source of mold rot is post-processing contamination, as loaves of bread are free of mold or mold spores due to heat inactivation shortly after baking (Pateras, 2007; Wang et al., 2017).

Bacillus species, especially Bacillus subtilis and Bacillus licheniformis, can cause bread to become contaminated with bacteria (Cizeikiene et al., 2013). Environmental factors that can encourage bacterial bread contamination include a prolonged cooling period or storage over 25 °C, a pH above 5, a high spore load, and moist bread (Pateras, 2007; Soboleva et al., 2016). Bacterial contamination is usually induced by uncooked dough components, with wheat having the most influence, or by machinery (Pateras, 2007). Bacterial spores have an impact on the quality of the completed bread product since they are difficult to eradicate and can germinate and grow between 36 to 48 hours of baking to generate a soft, stringy, brown dough in the bread that smells like an overripe melon Valerian resembles (Puckova et al., 2005; Soboleva et al., 2016).

Despite significant modernization in bread manufacturing, fundamental problems still exist. This is primarily because bread is perishable, with a short mold-free shelf life of 4 to 10 days that is affected by storage and treatment factors because of its high water activity, which is typically around 0.95 (S. Cauvain, 2012). Although how bread is stored and handled, the state of the environment in which it is processed, and the well-being of industry employees are all vital components in ensuring consumer safety, worries about the safety of bread have grown as a result of the indiscriminate use of potassium bromate (Isong et al., 2013).

Furthermore, despite best efforts to ensure that residents receive quality bread, there are several pathways in the production chain through which bread can become contaminated, particularly during packaging and in the factory by sellers (HPA, 2009). Since improper food handling and poor personal hygiene, particularly during packing, are connected to the majority of foodborne infections, bread contamination, and pathogen proliferation reduce quality and increase the risk of infection for customers (Ehavald, 2009). Food handlers who do not exercise adequate personal hygiene can become a vector for pathogen transmission through their hands, lips, and skin, among other places (HPA, 2009). As a result, the study's objective was to examine the microbiological quality of bread and the sanitary conditions of bakeries in Aliero, Kebbi State.

2. METHOD

2.1. Area of Study

The town of Aliero is located in Kebbi State in northern Nigeria. The coordinates are 12°16′42″N 4°27′6″E in the Kebbi state. The town serves as the administrative center for the Aliero local government region. The town houses the largest onion market in northwest Nigeria and is a significant onion producer in the country.

2.2. Informed Consent

A total of six different bakeries were identified in the town of Aliero. The population for this study consisted of all Aliero bakery businesses, regardless of their legal status. The bakery owners gave their informed consent.

2.3. Samples Collection

Six bakeries provided samples of freshly made bread. The manufacturers provided a total of sixteen bread samples (three each). Bread samples were collected in sterilized polyethylene bags. They were given labels and transported to the lab for microbiological examination.

2.4. Assessment of Managers' Socio-demographics and the Factory's Sanitary State

To obtain data on managers' socio-demographic variables, a structured researcher-administered questionnaire was employed. In addition, an observation checklist was employed to collect data on hygienic conditions. Water supply, toilet availability, waste disposal, refuse management, and waste disposal were all evaluated. The presence relevant item received a value of "1," while its absence received a score of "0."

2.5. Samples Preparation

The stock solution was prepared by weighing one gram (1 g) of each loaf into nine milliliters (9 mL) of sterile distilled water. By adding 1 ml of the stock solution to 9 ml of sterile distilled water, the initial dilution (10-1) was obtained. This process was then repeated until the desired 10-6 dilution was achieved. 0.2 Ml of the dilution factors 10-2 and 10-4 were introduced to sterilized Petri plates.

2.6. Determination of Microbial Load

Using a nutrient agar medium, the spread plate technique was employed to determine the total plate count. After inoculation, the plates were incubated for a 24-hour period at 37 degrees Celsius. Each sample's total number of bacteria cfu/g was calculated and recorded. Additionally, using the spread plate method, the samples were inoculated into an SDA medium enriched with chloramphenicol (40 mg/l). The plates were incubated at 25 degrees Celsius for 5 days. Visible colonies were counted and the total number of fungi was calculated and recorded as cfu/g (Cheesbrough, 2005).

2.7. Identification of Bacterial and Fungal Isolates

Bacterial isolates were characterized using the methods provided by Cheesbrough, (2005) to determine the morphological and biochemical properties of the bacteria. The fungal cultures were subjected to macroscopic and microscopic examinations. The physical properties of mycelia, such as color and structure, were noted. The growing fungus was placed on a slide, stained with lactophenol cotton blue, covered with a coverslip, observed under a microscope, and identified by colony shape and spore characteristics (Humber, 1994; ICMR, 2019).

2.8. Analysis of data

Data were analyzed using SPSS version 16 and presented as mean + SD of determinations made in triplicate. Ms. Excel was used to generate the graphics

Makalah hendaknya memuat tulisan yang berisi 1.Pendahuluan, 2. Metode Penelitian (bisa meliputi analisa, arsitektur, metode yang dipakai untuk menyelesaikan masalah, implementasi), 3. Hasil dan Pembahasan, 4. Kesimpulan dan5. Saran (future works) yg berisi penelitian lanjut di masa mendatang. Pada setiap paragraph bisa terdiri dari beberapa subparagraph yang dituliskan dengan penomoran angka arab seperti yang ditunjukkan section berikut ini. Jumlah halaman minimum 10 halaman dan maksimum 12 halaman ukuran A4.

3. FINDINGS AND DISCUSSION

3.1. Socio-demographic Characteristics of Managers of Bakeries

Table 1 shows the sociodemographic information of the six bakery managers who took part in the study. The findings indicated that all of the managers (100%) are male, with 5 (83%) falling between the ages of 19 and 34, and 1 (16%) falling between the ages of 35 and 50. In addition, four managers (66%) have no formal education, while just two (33%) have tertiary education. Marital status indicated that all of the managers (100%) are married. All of the managers have had no hygiene or sanitation training, and their licenses have not been renewed by an authorized agency.

Table 1: Socio-demographic Characteristics of Bakeries Managers

Variable	**Characteristic**	**frequency (N=6)**	**Percentages (%)**
Sex	Male	6	100
	Female	0	0
Age	19-34	5	83
	35-50	1	16
	>50	0	0
Educational status	No formal education	4	66
	Primary	0	0
	Secondary	0	0
	Tertiary	2	33
Manager's training on hygiene and sanitation	Yes	0	0
	No	6	100
License renewal by the authorized body	Yes	0	0
	No	6	100

3.2 Sanitary Conditions of Bakeries in Aliero

According to the findings, four bakeries (66%) utilize borehole water as a source of water supply, while just two (33%) use well water, and none use piped water. Similarly, none of them utilize a flush kind of latrine, and 66% have pit-latrine facilities, while 33% do not. All (100%) have properly stored their refuse and disposed of it in an open surface/ditch as shown in table 2.

Table 2: Sanitary Conditions of Bakeries in Aliero

Variable	**Characteristics**	**Frequency (N=6)**	**Percentages (%)**
Water supply	a.Piped borne	0	0
	b..Borehole	4	66
	c.Well	2	33
Latrine availability	a.Flush	0	00
	b.Pit latrine	4	66
	c.Not available	2	33
Waste disposal	a.Open surface/ditch	6	100
	b.Septic tank/latrine	0	0
	c.Not available	0	0
Refuse management	a.Proper refuse stored	6	100
	b.Improperly stored	0	0
	c.Not available	0	0
Refuse disposal	a.Munipal container	0	0
	b.Onsite disposal	6	100
	c.Burning	0	0

3.3 Total bacteria count

The total bacterial count of bread sold at Aliero town Kebbi State is given in table 3 below. The result revealed that sample code FB has the lowest bacterial count 7.4×10^5, while the highest bacterial count is $14.4x10^5$.

Table 3: Total Bacterial Count of bread

Sample code	***No.Colonies***	***Cfu/g***	***Mean±SD***
SB	144	14.4×10^5	144±33.94
IB	106	10.6×10^5	106±19.80
HB	108	10.8×10^5	108±50.91
FB	74	7.4×10^5	74±25.46
WB	116	11.6×10^5	116±28.28
AB	112	11.2×10^5	112±45.25

3.4 Total fungal count of bread

Table 4 shows the colony count of fungi isolated from bread sold at Aliero town kebbi state. Based on the result the highest fungal count is 9.0×10^3, while the lowest fungal count is 1.1×10^3.

Table 4: Total fungal count of bread

Sample code	***No.Colonies***	***Cfu/g***	***Mean±SD***
SB	90	9.0×10^3	90±7.0
IB	15	1.5×10^3	15±35.35
HB	70	7.0×10^3	70±2.21
FB	12	1.2×10^3	12±2.28
WB	11	1.1×10^3	11±3.35
AB	13	1.3×10^3	13±1.14

3.5 Identification of Bacterial and Fungal Isolated from the Bread

As shown in figure 1, the bacteria identified include *Escherichia coli* (29.4), and *Pseudomonas spp.* (14.7), *Proteus spp.* (20.6), and *Bacillus spp.* (35.3). In addition, figure 2

shows fungi isolates that include: Penicillium spp. (44%), Aspergillus spp. (8%), Rhizopus spp. (28%), Fusarium spp. (20%).

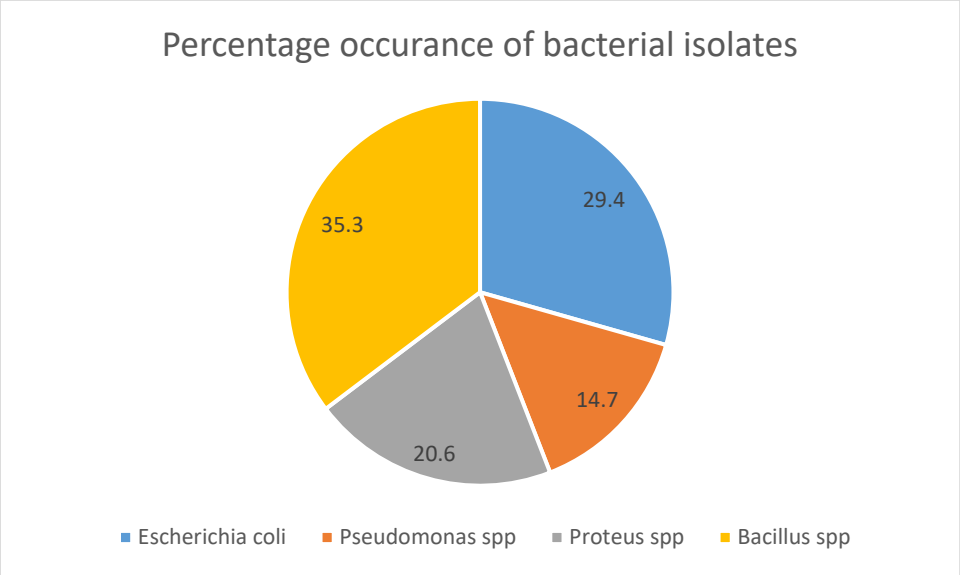

Figure 1. Bacterial isolates by the percentage of occurrence

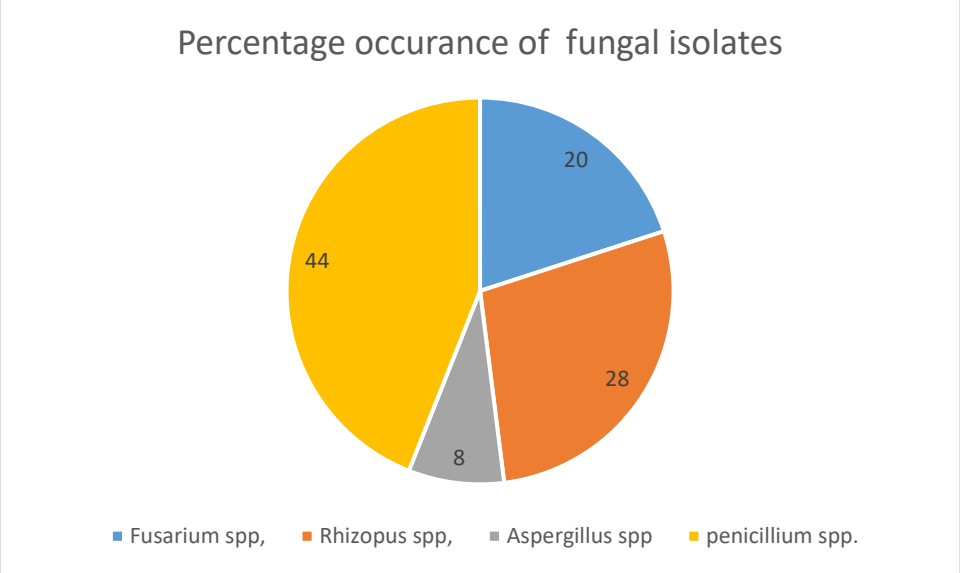

Figure 2. Fungal isolates by the percentage of occurrence

This study deals with the microbiological quality of bread and the hygienic conditions of bakeries in the city of Aliero, Kebbi State, Nigeria. Socio-demographic information from 6 bakery managers who took part in the study revealed that all managers are men (100%). Cultural orientation frequently has an impact on gender's role in socioeconomic growth, and it differs geographically. Furthermore, 4 (66%) of managers have no formal education, while only 2 (33%) have tertiary education. All employees involved in bread-making must have appropriate basic education, and training/experience (Attahiru et al., 2016; NAFDAC & FSAN, 2018)

The results of this study also revealed that no managers had received any training in sanitation and hygiene, even though managers and trained food handlers can lower the risk of foodborne illness and raise standards for cleanliness and hygiene in food establishments (Boro et al., 2014). The cause might be that the responsible authority did not conduct a hygiene and sanitation training program in the studied area.

According to the study's findings, 0% of bakeries had formal licensing certificates that had been renewed. The refusal of managers to renew their licenses might be a secondary reason, with the ineffectiveness of the regulators' efforts being the primary one. This is in contrast to the results of Girmay et al., (2020) who reported that 28.1% of food establishments had a renewed formal license certificate. In addition, the study results showed that all bakeries are missing very important sanitary parameters (lack of running water and on-site waste disposal). It has been suggested that food should be prepared in places far from sources of contamination such as garbage, sewage, and animals (NAFDAC & FSAN, 2018).

The mean total bacterial count (7.4×10^5-14.4×10^5) in the present study is higher than that reported by Daniyan & Nwokwu, (2011) between 4.5 and 6.8 log CFUg-1 minced bread. Bread sellers, bakeries, and transportation materials' poor sanitation may be to blame for the excess of total bacterial counts in bread samples (Adesetan et al., 2013). Ehavald, (2009) also reported that more than 90% of bread contamination occurs during the chilling, transport, slicing, and packaging processes. Even though the organisms are not known to be pathogenic, processed foods with high populations of mesophilic aerobic microbes are generally regarded as hazardous (Sudershan et al., 2009). The mean range of fungal counts in the present study was 9.0×10^3–1.1×10^3. This is higher than in the studies by Shiferaw et al., (2018) who reported mean counts (log CFUg-1) of molds and yeasts of 4.0 and 3.0, respectively. Molds and yeasts are the predominant microbial group contaminating bread during processing and handling (Lai & Lin, 2007). The presence of a high fungal count could be due to litter in the environment. Although it is challenging to prevent mold formation in food, its prevalence can be decreased by maintaining sanitary conditions throughout food preparation and storage. Mold spores can be carried by the wind and thus easily find their way into the food sample(Viljoen & von Holy, 1997).

The microbiota that was identified in this study was *Bacillus spp*. followed by *Escherichia coli*, *Proteus spp.,* and Pseudomonas spp. This is consistent with the report by Shiferaw et al., (2018) in which *Bacillus spp*. *Pseudomonas spp.* have been isolated, but differ in isolation from *Staphylococcus spp*., *Micrococcus spp.*, *Acinetobacter spp*., and *Aeromonas spp*. The prevalence of *Bacillus spp*. among isolates could be due to the ability of Bacillus species to resist desiccation, allowing them to survive in dry products such as grain and flour. *Bacillus cereus* is distributed widely in nature and may be isolated from soil, water, and plants (Adams et al., 1995). Even though vegetative bacteria, molds, and viruses are easily removed during baking, it is still possible for contamination to occur after baking through the air, equipment, and handling devices (Dale, 2003). One weakness of this study is that it did not take into account the food handling aspects of food processors in the bakery business

4. CONCLUSION

Different types of pathogenic and non-pathogenic microorganisms were found to contaminate bread samples in bakeries. Therefore, regulators, state departments of health, and local environmental health units must ensure that bakeries comply with regulations and guidelines, and requirements for breadmaking. In addition, educational programs aimed at changing the attitudes of food managers and processors have been recommended. Future studies should focus on the count of bacteria from food utensils, food processors, and bacteriological testing of water used to bake bread.

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