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Determination of Proximate Composition and the Microorganisms Associated with Spoilage of Bread Sold within Ukwuani, Delta State

Akpeji C. S.[1], Nwabugwu C.[1] Bosah B. O.[1], Otali C.C.[2, 3]., Ossai C. O.[2]

[1]Department of Biological Sciences, Novena University, Ogume, Delta State, Nigeria

[2]Department of Agronomy, Delta State University, Abraka, Delta State, Nigeria

[3]Department of Science Laboratory Technology, Federal Polytechnic, Orogun, Delta State.

Abstract

Bread is an important staple food widely consumed across Nigeria; however, its high moisture content and nutrient-rich composition make it highly susceptible to microbial spoilage arising from post-production contamination, poor handling practices, and inadequate storage conditions. This study evaluated the microbiological quality of different commercial bread brands marketed within Ukwuani, Delta State, and identified the fungal species responsible for their spoilage. A total of five (5) bread brands were randomly collected from different locations within Ukwuani and subjected to standard microbiological analyses to determine bacterial and fungal loads. Isolated microorganisms were identified based on their characteristic colony morphology and microscopic features. In addition, proximate analysis was conducted to assess the nutritional composition of the bread samples and examine its relationship with the predominance of spoilage fungi among the different brands. The results revealed substantial microbial contamination across the bread samples, with fungal counts ranging from 1.71×10^8 cfu/g to 1.0×10^9 cfu/g, the highest fungal load being recorded in sample A2. The predominant fungal isolates associated with bread spoilage were Aspergillus niger, Mucor spp., Penicillium notatum, and Rhizopus stolonifer, while the bacterial isolates comprised Escherichia coli, Bacillus spp., Pseudomonas aeruginosa, and Staphylococcus aureus. The proximate composition varied among the bread brands and provided useful information on the prevalence of specific spoilage fungi in relation to nutrient composition. The findings demonstrate that bread sold within Ukwuani is susceptible to contamination by potentially pathogenic and spoilage microorganisms, highlighting the need for improved hygienic production practices, proper handling, effective storage conditions, and enhanced quality control measures. These interventions will improve microbial safety, preserve product quality, extend shelf life, and protect public health.

Keywords

Bread, fungal, *Aspergillus niger*, Microbiological spoilage, Microorganisms

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Faculty of Science, ATBU Bauchi

Corresponding Author: Akpeji C. S., Department of Biological Sciences, Novena University, Ogume, Delta State, Nigeria

Email: akpejistephanie@gmail.com

INTRODUCTION

Bakery products such as bread are highly perishable and are prone to spoilage by microorganisms. Bread is one of the most widely consumed staple foods globally, providing a rich source of carbohydrates, protein, and other nutrients essential for human health. Its popularity stems from its affordability and versatility. However, the shelf life of bread is significantly affected by its nutrient composition and vulnerability to microbial spoilage. The spoilage of bread not only results in economic losses but also poses possible health risks to consumers due to the proliferation of pathogenic microorganisms.

The proximate value of bread plays a vital role in determining its nutritional value and constancy. For example, high moisture content, creates a conducive environment for microbial growth, quickening spoilage. The microorganisms commonly associated with bread spoilage include fungi, such as *Aspergillus, Penicillium*, and *Rhizopus* species, as well as bacteria, such as Bacillus Spp. These microorganisms result to visible mold growth, off-flavors, and structural degradation of bread.

In regions like Ukwuani, Delta State, where bread is a dietary staple, checking the proximate composition and classifying the microorganisms accountable for its spoilage is vital for ensuring food safety and quality. This study focuses on bread sold within Ukwuani, Delta State, a region in Nigeria with a specific socio-economic context. In Ukwuani, bread is a readily accessible and affordable food item, particularly for low-income households. However, the conditions under which bread is produced, stored, and sold in the region may not always adhere to optimal food safety standards. Factors such as inadequate storage facilities, high humidity, and improper handling practices could contribute to increased microbial contamination and accelerated spoilage. Furthermore, the use of preservatives may be limited or inconsistently applied, further exacerbating the problem.Understanding the factors contributing to bread spoilage is crucial for ensuring food safety and minimizing waste.

Therefore, this study aims to investigate the proximate composition of bread sold in Ukwuani, Delta State, and to identify the specific microorganisms associated with its spoilage. By determining the moisture content, protein levels, fat content, and other key components, we can gain insights into the nutritional quality and susceptibility to spoilage (Oladapo *et al.*, 2020, Adejumo and Adebayo, 2019). Identifying the spoilage microorganisms will allow for targeted interventions, such as improved hygiene practices, the use of appropriate preservatives, and consumer education, to mitigate the risks associated with bread consumption in the region. This research will contribute to a better understanding of food safety challenges in Ukwuani and inform strategies to improve the quality and safety of bread for the local population.

MATERIALS AND METHODS

COLLECTION OF SAMPLES

Bread samples was purchased from within Ukwuani Local Government, Delta State Nigeria. Ukwuani is a Local Government Area (LGA) located in Delta State, Nigeria, with its administrative headquarters in Obiaruku. Situated in the Delta North Senatorial District, the area is part of the Anioma region, which encompasses several LGAs in the northeastern part of the state. The five brands of bread Samples were taken in a clean

polyethylene bag to the laboratory for further work.

STERILIZATION OF MATERIAL

The glass wares were sterilized in a hot air oven at 160° c for 1 hour. Before sterilization in hot air oven, they were meticulously washed with detergent, rinsed in distilled water. All media used for culturing of the microorganisms were prepared according to the manufacturer's directives and autoclaved at 121°C for 15minutes

ENUMERATION AND ISOLATION OF MICROORGANISMS

Isolation of microorganism from the samples will be done using pour plate method. 10g of the bread each sample was weighed and homogenized and about 90ml of water was added. Serial dilution was carried out by mixing 1 ml of the sample with 9 ml of sterile distilled water to give 1:10 dilution. The dilution was made up to 10^{-10}. Using sterile pipette, 1ml of the diluent was plated out using different media (Harrigan and McCance, 1976).

CULTURE PRESERVATION

The preservation of microbial cultures, including bacteria and fungi, is essential for maintaining their viability and functional properties for future use in research, The pure cultures of the fungi isolates will be sub cultured into slants incubated at 37°C until growth becomes visible. The slants were kept as stock cultures under refrigeration (4°C) for up to four weeks.

CHARACTERIZATION AND IDENTIFICATION OF ISOLATED FUNGI

Microbial identification involves determining the type or species of microbe present. Characterization of obtained from bread samples was carried out by employing morphological, physiological and biochemical tests. Techniques used for identification include:

Macroscopic Examination: The cultural characteristics of each of the isolates was observed. They were grouped based on their colonial characteristics such as elevation, size, shape, surface etc.

Microscopic Examination

Microscopic examination is a technique used to observe objects that are too small to be seen with the naked eye. This method utilizes various types of microscopes to magnify specimens, allowing scientists, researchers, and medical professionals to study their detailed structures.

Biochemical Test: Biochemical tests are laboratory methods used to detect the presence or absence of specific enzymes, metabolites, or other biochemical characteristics in organisms, especially microorganisms. These tests help in identifying microorganisms and understanding their metabolic pathways. The biochemical test carried out on the isolates include; Catalase test which was done to check the presence or absence of catalase enzyme, Oxidase test was done to check the presence of oxidase enzymes, Endospore staining was carried out to check the sporulating bacteria and Citrate Utilization Test to access the ability of the organisms to use citrate (Olutiola *et al.*, 1991).

PROXIMATE COMPOSITION

AOAC, (2000) was used to investigate the proximate composition of the bread.

ANALYSIS OF DATA

Data collected was analysed using analysis of

variance and means will be saperated using duncan multiple range test (DMRT) at 95% level of significance.

RESULTS AND DISCUSSION

Table 1: shows the different fungal associated with the different bread samples. The results obtained showed that the spoilt bread samples had *Rhizopus stolonifera, Aspergillus* sp. as the fungal present. *Rhizopus stolonifera* which was obtained as a Whitish colony at the underlying and later become earthy colored to dark and *Aspergillus niger* which is dark to dim earthy colored with yellow on the opposite. The bread samples obtained had *Aspergillus sp.* as the prominent fungal which was observed to be whitish green which turns dark green with time and has cottony surface. This work established that these bakery products were contaminated by fungi. This is in line with the findings of Williams *et al.*, (2016).

WHO also isolated similar fungal from bread samples. These fungi that contaminated the food samples are of public health importance because they have the capacity to cause infections like aspergillosis, peniciliosis, fusariosis, zygomycoses etc *Aspergillus niger* and *Aspergillus flavus* are wide spread in nature and these are generally found in foods such as bakery products. This is in line with the works of Chozhavendhan *et al.*, 2014 who earlier reported Aspergillus *niger and Aspergillus flavus* in food and are capable of producing toxins which can cause food intoxication in human. These organisms, for example, *Aspergillus* as well as other fungi can produce mycotoxins causing diverse illnesses in humans when consumed.

Rhizopus is a mold and a common saprophytic fungus on plants and specialized parasites on animals. *Rhizopus* species are found on numerous natural substances including natural foods grown from the ground, peanuts, cowhide and bread including other pastry items, for example, such as doughnut, cake, biscuit, meat pie etc. A portion of the *Rhizopus* species, for example, *Rhizopus stolonifer* (dark bread form) is an perceptive specialist of ailments. Zygomycosis (fungal infection) is the primary illness that may be brought about by this organism, *R. stolonifer.* This ailment is extremely dangerous and may cause death (Pitt and Hocking, 2015).

Table 1: Macroscopic and microscopic characteristics of the fungal isolates

Isolates	Colonial/ Structural morphology	Microscopic morphology	Probable organism
1. A1	Whitish colony at the underlying. Later become earthy colored to dark.	Non-septate mycelia, with sporangiousphore, ovoid fit as a fiddle with sub-globose columella	*Rhizopus stolonifer*
2. A2			*Aspergillus niger*
3. A3	Whitish green which turns dark green with time, has	Hyphae is septate, unbranched conidiophores	*Aspergillus flavus*

		cottony surface.	expanded at the tip. The pialids that produce conidia is available	
3.	A4	Dark to dim earthy colored with yellow on the opposite.	Phialids orne straightforwardly on the globes vesicle sclerotia and erect conidiopores and are septate.	*Aspergillus niger*
4.	A5	Dark to dim earthy colored with yellow on the opposite.	Phialids orne straightforwardly on the globes vesicle sclerotia and erect conidiopores and are septate.	*Aspergillus niger*

Table 2 shows the microbial count of the bread samples. The viable count extended between $1.0x\ 10^3 \pm 0.30^e$ and $4.4x10^3 \pm 0.20^a$. The least viable count was seen in sample A1 while A2 accounted for the highest viable count. The total coliform ranged amid $0.8x10^3 \pm 0.00^c$ and $1.6x10^3 \pm 0.10^a$. The total mold count ranges from $0.3x10^5 \pm 0.10^d$ - $3.7x10^5 \pm 0.10^{a.}$ The lowest mold count was recorded in sample A1 while the highest mold count was seen in A2. The outcomes of this study show the class B food grade class due to more coliform in the samples, this may specify little level of sanitation (FEHD, 2002; Abena *et al.*, 2022). $0.3x10^5 \pm 0.10^d$ – $3.7x10^5 \pm 0.10^a$ was the mold count obtained.

The lowest count was recorded in Sample A1 while A2 had the highest mold. This result is in line with the account of Abdalla *et al.*, (2009), who reported that the overall phase of insufficient hygiene and sanitation might result in microorganisms in food.

The outcomes in this research are similar to the report of Isong, (2013) that the occurrence of huge microorganisms in some foods is owing to unsanitary practices by food handlers. Samples A1, A2, A3, A4 and A5 varies significantly at $P \leq 0.05$ (Feltes *et al.*, 2017).

Many microorganisms had been observed to proliferate in food readily at an extensive range of temperatures. The Frequency occurrence of bacteria isolated from bread samples as shown in figure 1 revealed the fungi were *S. cerevisiae, R. stolonifer, Mucor sp., and A. niger*. This may be due to inadequate hygiene and sanitation during processing (FEHD, 2002). The distribution of bacteria contaminant as shown in figure 2 reveled *E.coli* (24%), *Bacillus* spp (29%), *Pseudomonas aeruginosa* (18%) and *Staphylococcus aureus* (29%) are the major bacteria isolated from the bread samples.

TABLE 2: MICROBIAL COUNT OF BREAD SAMPLES

Sample	Total Viable Count (cfu/ml)	Coliform (cfu/ml)	Mold count (sfu/ml
A1	$1.0x10^3 \pm 0.30^e$	$0.8x10^3 \pm 0.00^c$	$0.3x10^5 \pm 0.10^d$
A2	$4.4x10^3 \pm 0.20^a$	$1.6x10^3 \pm 0.10^a$	$3.7x10^5 \pm 0.10^a$
A3	$3.5x10^3 \pm 0.10^b$	$1.4x10^3 \pm 0.30^b$	$2.9x10^5 \pm 0.10^b$
A4	$1.6x10^3 \pm 0.20^d$	$0.8x10^3 \pm 0.20^c$	$0.7x10^5 \pm 0.20^c$
A5	$1.7x10^3 \pm 0.20^c$	$0.8x10^3 \pm 0.20^c$	$0.7x10^5 \pm 0.20^c$

The proximate analysis of bread is shown in Table 3. The protein ranged amid 8.22 ± 0.09^c and 8.75 ± 0.11^a. A1 had the highest protein content however, there were no significant difference between the protein obtained in sample A3 and A5 respectively. This may be as a result of the brand of flour used in producing the bread. Protein is an effective nutrient which aids as strength and body builder. Sample A3 and A5 were not significantly different while sample A1 and A2 differs significantly at $P \leq 0.05$. The proportion of fat in sample A2 and A5 differs significantly.

Sample A2 had the highest fat content while sample A4 and A5 had the least fat content among all the samples tested. The decrease in fat content of bread produced from sample A4 and A5 might be due to reduction in fat applied during preparation. The fat content ranged between 1.66 ± 0.04^c and 4.40 ± 0.12^a Sample A4 and A5 were not significantly different while sample A2 and A3 differs significantly at $P \leq 0.05$. The crude fibre content ranged between 0.10 ± 0.02^c and 0.22 ± 0.01^a . The crude fibre content of bread produced in sample A2 had the highest crude fibre while sample A3 had the least among the samples. Fibre is known to help in lowering of serum cholesterol.

Samples A2 and A5 differs significantly at $P \leq 0.05$. The ash content of bread samples ranged between 1.38 ± 0.03^a and 1.77 ± 0.02^c as the bread produced in sample A4 and A5 had the highest ash content more than the bread from the other bakeries. This infers that the mineral content of the wheat flour used in making sample A4 and A5 is higher than those used in other bakeries. Also, the other ingredients in the recipe for example, salt, differ from one bakery to another and may also contribute to the mineral level.

Sample A1 and A3 were not significantly different while sample A2 and A4 differs significantly at $P \leq 0.05$. The moisture content ranged between 18.98 ± 0.29^a and 18.06 ± 0.21^b. There was no significant difference between sample A2, A3, A4, and A5. This implies that bread with highest moisture content can easily

get spoilt or deteriorate. The carbohydrate content of the samples ranged between 57.22 ±0.02[b] and 60.40 ±0.27[d] . Sample A1 had the highest carbohydrate content while sample A2 had the least carbohydrate content among all the samples tested. The difference in carbohydrate content may be due to the varied flour used in the different. All samples A1, A2, A3 A4 and A5 differs significantly at P≤ 0.05 in carbohydrate content.

TABLE 3.0. PROXIMATE COMPOSITION OF BREAD SAMPLES

Sample	Moisture(%)	Ash(%)	Protein(%)	Fat(%)	Fibre(%)	CHO(%)
A1	16.21±0.39[b]	1.38±0.04[c]	8.78±0.11[a]	1.66±0.04[c]	0.15±0.03[b]	60.40±0.27[a]
A2	18.45±0.38[a]	1.60±0.09[b]	8.28±0.06[c]	4.40±0.12[a]	0.22±0.01[a]	58.89±0.50[c]
A3	18.89±0.27[a]	1.32±0.03[c]	8.56±0.06[b]	2.40±0.06[b]	0.10±0.02[c]	59.63±0.32[b]
A4	18.98±0.29[a]	1.77±0.02[a]	8.22±0.09[c]	1.54±0.04[d]	0.10±0.01[c]	57.22±0.02[d]
A5	18.06±0.21[a]	1.77±0.02[a]	8.22±0.09[b]	1.54±0.04[d]	0.10±0.01[c]	57.22±0.02[d]

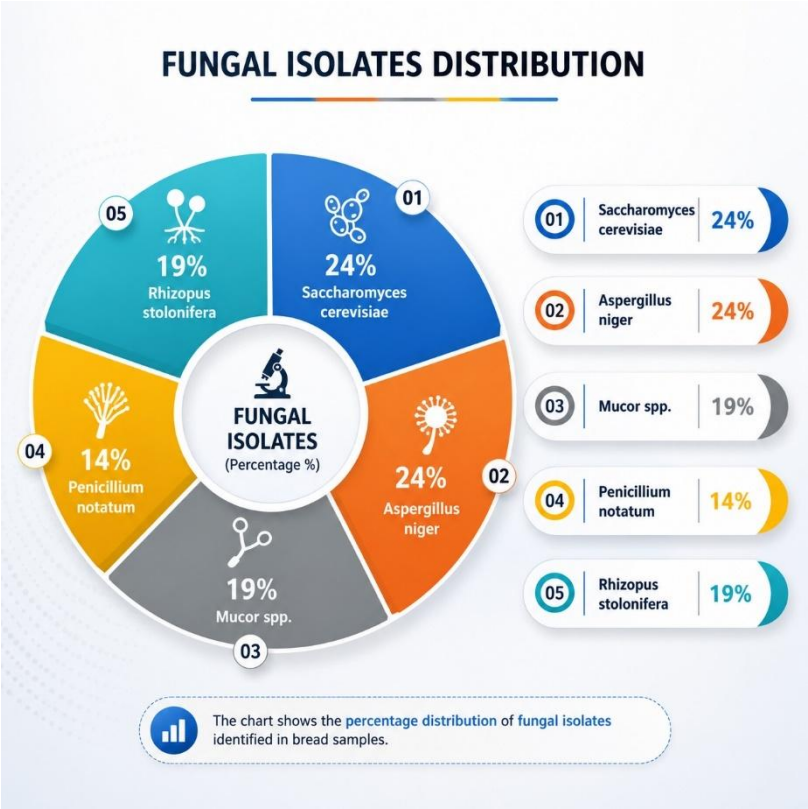

Figure 1: Frequency Occurrence of Fungi Isolated from Bread Samples

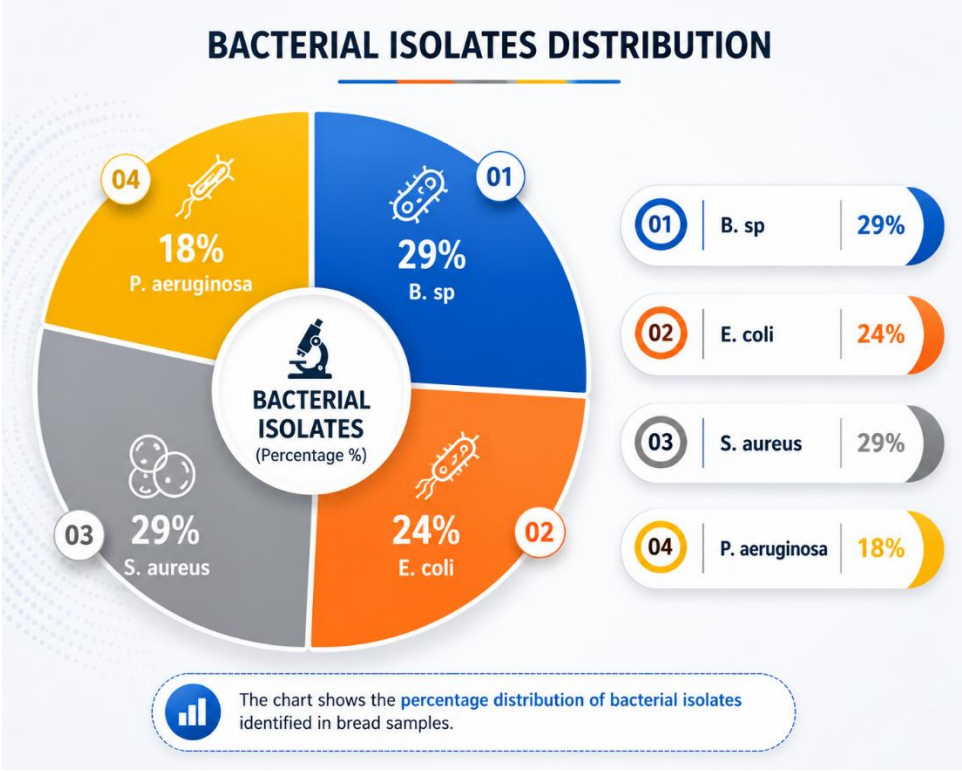

Figure 2: Frequency Occurrence of Bacteria Isolated from Bread Samples

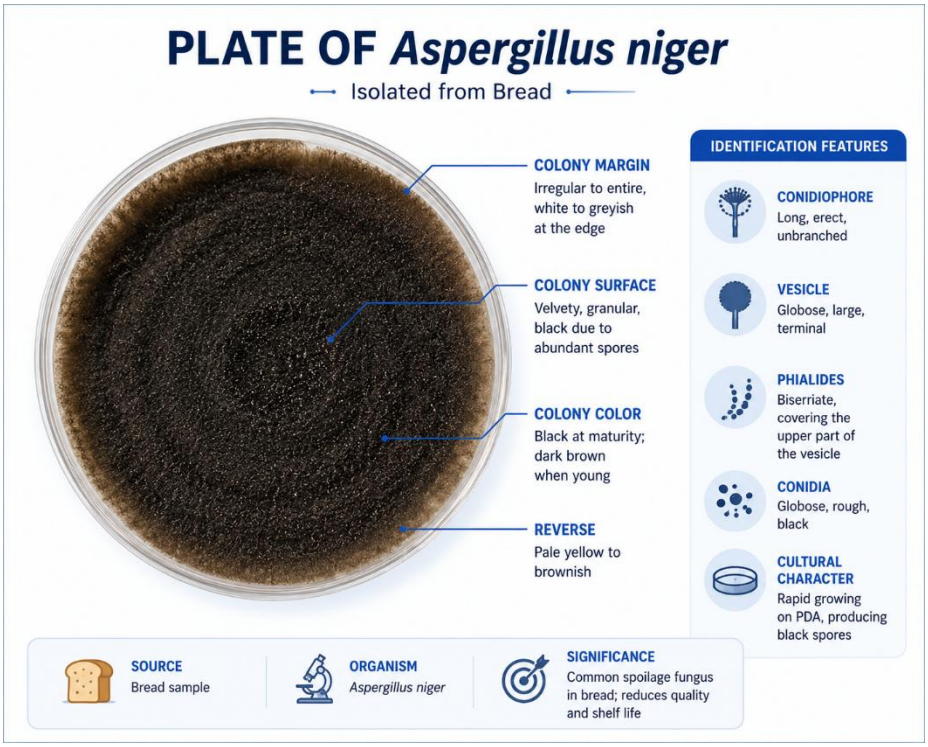

FIGURE 3: Plate of *Aspergillus niger* Isolated from Bread

Conclusion and Recommendations

This study successfully evaluated the proximate composition and identified the predominant microbial contaminants associated with the spoilage of commercially available bread sold within Ukwuani, Delta State. The proximate analysis demonstrated noticeable variations in moisture, ash, crude protein, crude fat, crude fibre, and carbohydrate contents among the different bread brands.

These compositional differences are likely attributable to variations in raw material quality, formulation, manufacturing processes, and post-production storage conditions. Since proximate composition influences microbial growth and product stability, these variations may also contribute to differences in the susceptibility of the bread samples to microbial deterioration.

Microbiological analyses revealed that bread spoilage was predominantly associated with fungal isolates, including *Aspergillus niger*, *Penicillium notatum*, *Rhizopus stolonifer*, and *Mucor* spp., together with bacterial contaminants such as *Bacillus* spp., *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The occurrence of these microorganisms indicates possible contamination during processing, handling, packaging, transportation, and storage.

The isolation of potentially pathogenic bacteria further raises significant food safety concerns and underscores the need for strict adherence to Good Manufacturing Practices (GMP), Good Hygienic Practices (GHP), and Hazard Analysis and Critical Control Point

(HACCP) principles throughout the bread production and distribution chain.

Based on the findings of this study, it is recommended that bakery operators implement stricter sanitation protocols, maintain hygienic processing environments, and adopt improved packaging technologies capable of minimizing post-production contamination. Appropriate storage conditions, including effective temperature and humidity control, should also be maintained to inhibit microbial proliferation and prolong product shelf life. Furthermore, routine microbiological quality assessment and proximate composition analysis should be incorporated into quality assurance programmes to ensure compliance with established food safety standards.

Regulatory agencies should strengthen monitoring and enforcement of food safety regulations through regular inspections and surveillance of bakery products. In addition, sustained public awareness campaigns should be undertaken to educate producers, vendors, and consumers on proper bread handling and storage practices. The adoption of these measures will significantly improve the microbiological safety, nutritional quality, and shelf life of bread, thereby enhance consumer protection and promote public health in Ukwuani, Delta State, and other similar communities.

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